Biology and Control of Eastern Black Nightshade, Palmer Amaranth, and Common Pokeweed in No-till Systems on the Eastern Shore Regions of Virginia and Maryland

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ABSTRACT

Eastern black nightshade, Palmer amaranth, and common pokeweed are three hard to control weed species on the Eastern Shore regions of Virginia and Maryland. Herbicide resistance and lack of herbicide efficacy further complicate the job of controlling these weeds. Studies were conducted on each of these weeds in order to determine herbicide efficacy and potential herbicide resistance. In addition, the translocation and metabolism of ^{14}C-glyphosate was studied in common pokeweed. This research identified a population of eastern black nightshade that was differentially sensitive to families of ALS-inhibiting herbicides, with tolerance to members of the sulfonylurea family, but controlled with herbicides of the imidazolinone family. A population of Palmer amaranth was found to be glyphosate-resistant, but herbicide programs were identified that could control this biotype in soybean and corn systems. Experiments on the fate of glyphosate in common pokeweed indicated that glyphosate does not readily translocate from treated foliage to other plant parts, which may contribute to shoot regeneration from taproots following glyphosate treatment. Taken together, this research highlights the important weed control issues, including resistant and perennial weeds in agronomic crops that have arisen in Eastern Shore agriculture. This work will help growers to better assess their particular control issues, and take appropriate steps to mitigate any problems.

I dedicate this work to all the teachers and professors who have helped me through my educational journey.

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CHAPTER 1

LITERATURE REVIEW

The Eastern Shore regions comprise the coastal portions of the states of Virginia and Maryland that are surrounded by the Chesapeake Bay and the Atlantic Ocean. Agriculture in the area is devoted to the production of several crops including: barley (*Hordeum vulgare* L.), corn (*Zea mays* L.), cotton (*Gossipium hirsutum* L.), soybean (*Glycine max* (L.) Merr.), tomato (*Solanum lycopersicum* L.), potato (*Solanum tuberosum* L.), and wheat (*Triticum aestivum* L.). In an area devoted to the production of so many crops, efficient and economic weed control is imperative. Three particular weed species, eastern black nightshade (*Solanum ptycanthum* Dun.), Palmer amaranth (*Amaranthus palmeri* S. Wats.), and common pokeweed (*Phytolacca Americana* L.) can be difficult for Eastern Shore growers to control.

Eastern Shore Cropping Systems and Weed Management Issues

The majority of crops on the regions of Virginia and Maryland are grown under no-till systems. In these systems, crops are seeded without any prior loosening of the soil other than shallow disturbances less than 5 cm (Soane et al. 2012). There are several benefits to no-till farming including improved soil conservation and erosion control, improved soil quality, and increased plant water use efficiency (Brainard et al. 2013, Derpsch et al. 2010, Soane et al. 2012). Going from a conventional tillage to a no-till system can also lead to an increase in perennial weeds, such as common pokeweed (Wruke and Arnold 1985). Increased crop residues can intercept preplant and preemergence herbicides before they reach the soil surface, making them unavailable for plant uptake, thus reducing their efficacy (Chauhan et al. 2012). Growers

increasingly rely on use of non-selective POST herbicides, such as glyphosate, often used in conjunction with genetically modified (GM) crops.

The adoption of GM crops has allowed farmers to experience lower production costs due to more flexible and effective weed and insect control, and it has complimented the effects of conservation agriculture by reducing the effects of erosion on soil and water quality (NRC 2010). Glyphosate is the most widely used non-selective herbicide supplied on GM crops with those resistant to the material. Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase in the shikimic acid pathway. In this pathway shikimic acid binds to EPSP synthase. Next, a molecule of phosphoenolpyruvate (PEP) combines with the complex to form 5-enolpyruvylshikimate-3-phosphate. The result is the conversion of carbohydrate precursors from glycolysis and the pentose phosphate pathway to the aromatic amino acids tryptophan, tyrosine, and phenylalanine (Herrmann and Weaver 1999). One of the intermediates in this pathway is shikimate-3-phosphate or shikimic acid. Glyphosate acts as a competitive inhibitor of PEP for the shikimate-3-phosphate/EPSP synthase enzyme complex which leads to a decrease in these aromatic amino acids.

In genetically modified (GM) crops glyphosate resistance is conferred by inserting an insensitive EPSP synthase gene from *Agrobacterium* sp. or *Escherichia coli* commonly known as the CP4 gene. This gene is expressed at high levels in the plant to make copies of the less sensitive enzyme. The GM plant retains both the wild type and CP4 gene. When glyphosate is applied wild type EPSP synthase is inhibited, but not the CP4 EPSP synthase gene which carries on normal production of aromatic amino acids (Kishore et al. 1992, Padgette et al. 1995).

With the development of glyphosate-resistant (GR) crops, use of this herbicide for POST weed control increased from 189.3 lbs. year^{-1} in 1996 to 1208.8 lbs. year^{-1} in 2005 (NASS 2005).

In 2012, herbicide tolerant corn occupied 7.8 million hectares, 35% of the total corn in the world; and herbicide tolerant soybean was the dominant GM crop, occupying 80.7 million hectares and 81% of the soybeans planted in the world (James 2012).

Increased use of glyphosate has led to the development of weeds resistant to this herbicide (Reddy and Norsworthy 2010, Vencill et al. 2012, Werth et al 2013). To date there are 30 different weed species resistant to this particular mode-of-action (Heap 2014). Glyphosate-resistant Palmer amaranth has been confirmed in much of the southern United States including Arkansas (Norsworthy et al. 2008), Georgia (Culpepper et al. 2006), North Carolina (Culpepper et al. 2008), Tennessee (Steckel et al. 2008), and Virginia (Ahmed 2011).

The acetolactate synthase (ALS) inhibiting class of herbicides have provided another mode-of-action for selective POST control without the use of GM crops. These herbicides act by blocking the ALS enzyme, which catalyzes a key step in the formation of the branched chain amino acids valine, leucine, and isoleucine (Senseman 2007). Plant death results from a lack of protein production and wasteful metabolism. This class of herbicides has been widely adopted due to low use rates, high efficacy, a wide variety of crop selectivity, and cost effectiveness (Dobrow 2010, Saari et al. 1994). There have been 145 different weeds species reported with resistance to ALS-inhibiting herbicides (Heap 2014).

Even though resistance to this class of herbicides has been documented, the p-hydroxyphenyl pyruvate dioxygenase (HPPD)-inhibiting class of herbicides could provide Eastern Shore growers with an alternative to glyphosate and ALS inhibiting herbicides. The HPPD class includes the herbicides mesotrione, tembotrione, and topramezone. These herbicides block the conversion of p-hydroxymethyl pyruvate to homogentisate, a key step in the biosynthesis of plastoquinone (Senseman 2007). This causes an indirect inhibition in the

synthesis of carotenoids due to plastoquinone's involvement as a co-factor of phytoene desaturase. Symptoms resulting from the use of these herbicides include bleaching of new growth tissue followed by plant necrosis. These herbicides have been used successfully in controlling several annual grasses and broadleaf species in corn (Johnson et al. 2012, Mitchel et al. 2001, Soltani et al. 2012). In addition, weed control with HPPD-inhibiting herbicides is often enhanced with the addition of atrazine to the tank mix (Walsh et al. 2012, Williams et al. 2011).

Eastern Black Nightshade

Eastern black nightshade (EBN) is an erect, branching annual or short lived perennial herb found throughout the United States and Canada (Bryson and DeFelice 2009). Germination begins in May and can continue throughout the growing season, with each plant producing approximately 50 to 100 berries per plant and 50 to 100 seeds per berry (Bassett and Munro 1985, Ogg et al. 1981, Werner et al. 1998). Nightshades (*Solanum* spp.) are a genus of weeds that are associated with production problems in multiple crops (Ackley et al. 1999, McGiffen and Masiunas1992). Eastern black nightshade seeds are able to germinate late in the growing season and plants can exist under the crop canopy, even after postemergence (POST) herbicide applications (Milliman 2003). The crop canopy tolerance of EBN has been attributed to a tolerance of low light intensities by means of high light absorption efficiencies, low respiration rates, decreased leaf density, and low root to shoot ratio (Stoller and Myers 1981).

Estimated yield losses caused by EBN can be up to 40% at 5 plants m^{-2} in soybean and 7% at 5 plants m^{-2} in corn (Anonymous 2012). In addition to yield losses, EBN interferes with grain quality (McGiffen and Masiunas 1992, Quakenbush and Anderson 1984). Berries remain on the EBN plant during harvest, thus juice from the ruptured berries can stain or cause soil particles to stick to soybean seeds, thereby reducing crop quality (Ogg et al. 1991, Volenberg et al. 2000).

Glyphosate is commonly used to control most weeds POST; however, studies have shown that glyphosate only provides partial control of EBN (Hoss et al. 2003, Scursoni et al. 2006). Historically, the ALS-inhibiting herbicides imazamox and imazethapyr have been used for EBN control in soybean (Ashigh and Tardiff 2006, Milliman et al. 2003). Overuse of this mode-of-action has selected for resistant populations of EBN. ALS-resistant EBN has been observed in several states including Wisconsin, North Dakota, and Illinois (Heap 2014, Millman et al. 2003, Volenberg et al. 2000).

Palmer Amaranth

Palmer amaranth (PA) is a C4 summer annual herb that is native to the Southern Great Plains (Bond and Oliver 2006); however, populations can be found throughout the Southern United States and as far north as Michigan and New York (NRCS 2014). Palmer amaranth can reach approximately 2 m in height (Bryson and DeFelice 2009), and is distinguished from other members of the Amaranthaceae family by leaves that lack hairs, long petioles, and 0.5 m panicles (Bryson and DeFelice 2009, DeFelice et al. 2011). Palmer amaranth is dioecious (DeFelice et al. 2011), with flowering usually occurring from September to October. While present in several counties in Virginia, PA is a recent introduction to the Eastern Shore. The exact source of importation is unknown, but it is believed it came there via non-certified wheat seed.

Major weed problems are caused by PA in corn (Massinga et al. 2001), cotton (Culpepper et al. 2008, Morgan et al. 2001), soybean (Bensch et al. 2003, Klingaman and Oliver 1994), and peanuts (*Arachis hypogaea* L.) (Burke et al. 2007). Due to its rapid growth rate and tall stature, PA is an aggressive competitor in many cropping systems (Culpepper et al. 2008) and has a comparatively faster growth rate compared to other Amaranthus species (Horak and Loughin 2000, Sellers et al 2003,). Palmer amaranth has been found to have a high photosynthetic rate

(Ehleringer 1983). Leaves of this species have been shown to exhibit osmotic adjustment in periods of low leaf water potential (Ehleringer 1983). Regional dispersal of PA is promoted by prolific seed production as each plant can produce well over 100,000 seeds (Sellers et al. 2003). In addition, PA has been reported to have allelopathic effects, reducing the growth of crops such as onion (*Alium cepa* L.), cabbage (*Brassica oleracea* L.), and sunflower (*Helianthus annuus* L.) (Bradow and Connick 1987, Menges 1987, Menges1988). Klingaman and Oliver (1994) reported PA densities of 0.33 to10 plants m^{-1} row of soybean can reduce soybean yields 17 to 68%, and Bensch et al. (2003) reported that yield losses as high as 78% can occur with a single PA per 0.125 m^{-1} row. Densities of 0.5 to 0.8 plants m^{-1} row of corn have been shown to reduce corn yields 11 to 91% (Massinga et al. 2001).

Historically, glyphosate and ALS-inhibiting herbicides have been used for PA control (Vencill et al. 2012), but frequent use has selected for resistance to both modes-of-action. Glyphosate-resistant PA amaranth was first confirmed in Georgia in 2004 (Culpepper et al. 2006), and other populations resistant to this mode-of-action have appeared throughout the southern United States (Culpepper et al. 2008, Nandula et al. 2012, Norsworthy et al. 2008). ALS-resistant PA has been reported in Kansas Illinois, Georgia, Arkansas, Tennessee, North Carolina, South Carolina, and Florida (Dobrow et al. 2011, Heap 2014, Horak and Peterson 1995, Norsworthy et al. 2013, Sprague et al. 1997, Wise et al. 2009). Palmer amaranth populations with multiple-resistance to ALS-inhibiting herbicides and glyphosate have been found in Georgia and North Carolina (Sosnoskie et al. 2009, Whitaker et al. 2010). Current management practices place emphasis on PRE control of PA using residual herbicides such as fomesafen, flumioxazin, pendimethalin, and *s*-metolachor (Everman et al. 2009, Merchant et al. 2014, Myers et al. 2010, Whitaker et al 2010). However, caution should be taken not to select

for resistance to these herbicides as well as PA has also been documented to be resistant to dinitroaniline, triazine, and HPPD-inhibiting herbicides (Heap 2014). Populations of herbicide-resistant PA have been reported, but levels of resistance have not been confirmed on the Eastern Shore Regions of Virginia and Maryland.

Common Pokeweed

Common pokeweed is an erect, freely branching, perennial herb native to the Eastern United States and parts of Canada (Bryson and DeFelice 2009). Mature plants can reach heights of 1.5 to 2.5 m (Bryson and DeFelice 2009). This species is known for its attractive racemic flowers and berries produced in late summer. Common pokeweed populations expand via vegetative reproduction from buds on the taproot, and by seeds that can remain viable for 40 years (Sauer 1952). Much like EBN, this plant also interferes with harvest efficiency and the toxic berries stain crop seed (List et al. 1979).

Common pokeweed has not been reported to be resistant to any herbicide, but it can prove difficult to control in no-till systems. Generally, it is controlled by conventional tillage methods such as disking or mold-board plowing (Refsell 2004), but it is more problematic in no-till systems that rely primarily on herbicides for control. Seedling pokeweed can be relatively easy to control, but perennial pokeweed in no-till systems can be especially difficult to control, especially when soybean is planted as a double crop with small grains later in the season when pokeweed plants are larger and often re-sprout via underground taproots. POST herbicides such as glyphosate, 2,4-D and dicamba have been shown to severely injure pokeweed (Sellers and Ferrell 2006), but applications seldom provide complete control of the underground taproot allowing regrowth to occur later in the season or the next year. Glyphosate is translocated in the phloem after foliar absorption; however, weed control efficacy is often related to other factors

such as plant growth stage and sink-source relationships (Li et al. 2005, Sandberg et al. 1980). Therefore, glyphosate is often used as the primary means of pokeweed control in-crop, but is ineffective at providing season-long control. Dicamba and 2,4-D are not currently in soybean, and are only available for limited use in corn. However, GM crops tolerant to dicamba and 2,4-D, have been developed and should be commercially available within the next few years.

Collectively, EBN, PA, and common pokeweed represent a serious challenge to Eastern Shore producers. The presence of ALS-resistant EBN could further limit control options available to Eastern Shore producers. Due to PA's fairly recent introduction and its documented resistance to multiple herbicide modes-of-action, it is important to determine the susceptibility of this weed to different herbicide modes-of-action. Although herbicide resistant pokeweed has not been reported, more research is needed on how this weed can be controlled with glyphosate and other herbicides. The objectives of these studies were 1) to examine cases of herbicide resistance in EBN and PA; 2) evaluate alternative modes-of-action, in particular the HPPD-inhibiting class of herbicides, for control of these weeds; and 3) examine the physiological effects of glyphosate application to pokeweed at different growth stages.

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CHAPTER 2

EASTERN BLACK NIGHTSHADE TOLERANCE TO SULFONYLUREAS AND OTHER HERBICIDES

Abstract

Eastern black nightshade is a problematic weed in many agronomic and vegetable cropping systems in the Eastern Shore Regions of Virginia and Maryland. Field studies were conducted in 2010 and repeated as greenhouse studies in 2011 and 2012 to evaluate the possibility of ALS-resistance in a population of eastern black nightshade and to evaluate alternative herbicides to this mode-of-action for control. Under greenhouse conditions, bromoxynil, dicamba, and the HPPD-inhibiting herbicides topramezone, tembotrione, and tembotrione + thiencarbazone-methyl controlled EBN 95% or greater by 4 WAT. Injury symptoms were observed with sulfonylurea herbicides nicosulfuron, rimsulfuron, and thifensulfuron earlier in the study, they failed to provide control by 3 WAT. In contrast, the imidazolinone herbicide imazamox, controlled EBN at least 90%. This research helps to confirm that EBN on Virginia's Eastern Shore is tolerant to the sulfonylurea family of ALS-inhibiting herbicides, but may still be susceptible to the imidazolinone family of ALS-inhibiting herbicides. It also shows that the HPPD-inhibiting herbicides can provide a viable alternative for POST control of EBN.

Nomenclature: eastern black nightshade, *Solanum ptycanthum* Dun.; bromoxynil; dicamba; imazamox; mesotrione; nicosulfuron; rimsulfuron; thifensulfuron; tembotrione; thiencarbazone-methyl, topramezone;

Key Words: acetolactate synthase, herbicide resistant, imidazolinone, sulfonylurea.

Introduction

Eastern black nightshade (EBN) is an erect, branching annual or short lived perennial herb found throughout the United States and Canada (Bryson and DeFelice 2009). Nightshades (*Solanum* spp.) are a genus of weeds that are associated with production problems in multiple crops (Ackley et al. 1999, McGiffen and Masiunas 1992). A single EBN plant can produce several thousand seeds which can germinate between 2 and 4 weeks after anthesis and can remain viable in soil for many years (Ogg et al.1981, Zhou et al. 2005). Eastern black nightshade seeds are able to germinate late in the growing season and plants can exist under the crop canopy, even after postemergence (POST) herbicide applications (Milliman 2003). This ability is due to a tolerance of low light intensities by means of high light absorption efficiencies, low respiration rates, decreased leaf density, and low root to shoot ratio (Stoller and Myers 1981).

Eastern black nightshade can cause yield losses up to 40% at 5 plants m^{-2} in soybean (*Glycine max* [L.] Merr.) and 7% at 5 plants m^{-2} in corn (*Zea mays* L.) (Anonymous 2012). This species not only causes reduction in crop yields, but interferes with harvest quality (McGiffen and Masiunas 1992, Quakenbush and Anderson 1984). Berries remain on the EBN plant during harvest time, thus juice from the ruptured berries of can stain or cause soil particles to stick to soybean seeds, thereby reducing crop quality (Ogg et al. 1991, Volenberg et al. 2000).

The majority of corn and soybean production on the Eastern Shore regions of Virginia and Maryland is conducted under no-till or minimum till systems. In these cropping systems herbicides are relied on almost exclusively for weed control. Nonselective herbicides such as glyphosate are often used in crop. For years, growers on Virginia's Eastern Shore have relied on

the use of genetically modified (GM) crops tolerant to the herbicide glyphosate; however, health concerns have led to intense regulation of GM crops in European and Asian markets (Davison 2010, Kalaitzandonakes et al. 2014), leading many growers to produce non-GM crops for export. This means that growers must rely on alternative herbicide chemistries for postemergence control of EBN.

One option for postemergence control of EBN, primarily in soybean, are the acetolactate synthase (ALS) inhibiting class of herbicides. These herbicides act by blocking the ALS enzyme, which catalyzes a key step in the formation of the branched chain amino acids valine, leucine, and isoleucine (Senseman 2007). Plant death results from a lack of protein production and wasteful metabolism. This class of herbicides has been widely adopted due to low use rates, high efficacy, a wide variety of crop selectivity, and cost effectiveness (Dobrow et al. 2011, Saari et al.1994).

ALS-inhibiting herbicides can be categorized into the following families: imidazolinones, sulfonylureas, triazolopyrimidines, and pyrimidinylthiobenzoates (Senseman 2007). The imidazolinone family includes herbicides such as imazamox and imazethapyr; the sulfonylurea family includes herbicides such as primisulfuron, nicosulfuron, prosulfuron, and rimsulfuron; and the triazoloppyrimidine family includes herbicides such as flumetsulam and cloransulam (Ashigh and Tardif 2006, Senseman 2007).

The ALS-inhibiting herbicides were once used successfully for weed control in various crops including corn, soybean, and wheat. However, resistance to this particular mode have action has occurred in many weed species, including Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Sprague et al. 1997), common ragweed (*Ambrosia artemisiifolia* L.) (Patzoldt et al. 2001, Taylor et al. 2002), and Italian ryegrass (*Lolium perenne ssp. multiflorum* Lam.) (Chandi

et al 2011, Taylor and Coats 1996). The frequent use of imidazolinone herbicides has led to resistant populations of EBN in several areas of the United States including Wisconsin, North Dakota, and Illinois (Heap 2014, Millman et al. 2003, Volenberg et al. 2000), but it has yet to be confirmed on Virginia's Eastern Shore. In recent years the discoveries of ALS-resistant smooth pigweed (*Amaranthus hybridus* L.) (Manley et al. 2008, Whaley et al. 2006) in Virginia, have led to concerns that more weed species may have developed a resistance to this particular mode-of-action. Over the years growers on Virginia's Eastern Shore have been relying on glyphosate tolerant and sulfonylurea tolerant (STS) crops, resulting in heavy use of the herbicides chlorimuron and thifensulfuron. A lack of EBN control has been reported using these herbicides and other members of the sulfonylurea family of ALS-inhibiting herbicides.

An alternative mode-of-action to use for control of EBN would be the p-hydroxyphenyl pyruvate dioxygenase (HPPD)-inhibiting herbicides. This group includes the herbicides mesotrione, tembotrione, and topramezone. These herbicides block the conversion of p-hydroxymethyl pyruvate to homogentisate, a key step in the biosynthesis of plastoquinone (Senseman 2007). This causes an indirect inhibition in the synthesis of carotenoids due to plastoquinone's involvement as a co-factor of phytoene desaturase. Symptoms resulting from the use of these herbicides include bleaching of new growth tissue followed by plant necrosis. These herbicides have been used successfully in controlling several annual grasses and broadleaf species in corn (Johnson et al. 2012, Mitchel et al. 2001, Soltani et al. 2012). The combination of HPPD-inhibiting herbicides and atrazine has also been reported to have synergistic effects in controlling several weed species (Armel et al. 2007, Hugie et al. 2008, Walsh et al. 2012, Williams et al. 2011).

In 2010 a local grower in Eastville, VA reported a lack of EBN control using ALS-inhibiting herbicides. As EBN becomes more of a problem for Eastern Shore growers, it is important to assess which herbicides will provide effective control. The objectives of these studies were to 1) evaluate the response of EBN to various herbicides used in corn, and in particular the ALS-inhibiting herbicides, and 2) evaluate the response of EBN to HPPD-inhibiting herbicides applied alone or in combination with atrazine.

Materials and Methods

EBN response to ALS-inhibiting and other herbicides

Three experiments were conducted between 2010 and 2012 to evaluate EBN response to various postemergence herbicides, including several ALS-inhibiting herbicides. The first trial was established on May 27, 2010 in a corn field in Eastville, VA. Two subsequent trials were conducted in a greenhouse at Painter, VA on June 30, 2011 and June 26, 2012. The trials were arranged as a randomized complete block (RCB) designs with eight treatments (Table 2.1) and four replications. A nontreated check was also included in each trial for comparison. In the trial conducted at the Eastville, VA corn field, plots were 2.5 m wide and 6.1 m long and treatments were applied using a propane-pressurized backpack sprayer with a 1.5 m boom and five XR 11003 flat fan nozzles delivering 186 L ha^{-1}.

Subsequent trials were conducted in the greenhouse due to insufficient EBN population densities in field sites. These studies were arranged in a similar manner to the field studies with the exception that a POST treatment of imazamox applied at 44 g ai ha^{-1} was added to the treatments from the first field study in order to evaluate control differences between two different families of ALS-inhibiting herbicides, imidazolinone and sulfonylurea. All herbicides used in

greenhouse studies were applied using a compressed air, moving nozzle, cabinet sprayer equipped with one 8002EVS nozzle delivering 171 L ha^{-1} at 289 kPa.

Eastern black nightshade seed was collected from the same site in the fall of 2010 for the following years' study. Populations of EBN were established in 53 cm by 27 cm flats then transplanted into 722 cm^3 pots containing a commercial potting mix (Pro Mix BX Mycorrhizae, Premier Tech Horticulture, Quakertown, PA). For the 2012, study the potting mix ratio was changed to 50:50 of field soil to potting mix. The field soil was the Bojac sandy loam characterized to have 55% sand, 37% silt, and 8% clay with < 2% organic matter and pH of 5.5. to Virginia's Eastern Shore. Plants were watered daily as needed and growth occurred under diurnal cycles of light and temperature. Herbicide applications were made to plants averaging 4 to 6 cm in height. Greenhouse plants were watered daily as needed.

For both field and greenhouse studies, visual assessments of EBN control were made on a scale of 0 (no control) to 100 (plant death) 2 and 4 weeks after treatment (WAT). After 4 weeks, plants were harvested and dried on a greenhouse bench for 3 to 4 days in order to obtain total aboveground biomass. Data were analyzed in PROC GLM SAS 9.2 (SAS Institute, Inc. Cary, NC). Data were tested for homogeneity of variance prior to ANOVA and transformed to the arcsine of the square root where needed to stabilize variance. If transformation was needed prior to ANOVA, subsequent means were back transformed for presentation. Sums of squares were partitioned to reflect trial and treatment effects and interactions. Main effects and interactions were tested against the means square error of the appropriate trial interaction. If no trial interactions were significant, data were pooled. Appropriate means were separated using Fisher's Protected LSD test at $P = 0.05$.

Effects of HPPD-inhibitors and atrazine on EBN

Three trials were conducted to evaluate combinations of HPPD-inhibiting herbicides with atrazine for EBN control and biomass reduction. The three trials for evaluating HPPD inhibitors and atrazine were conducted using the same method and on adjacent locations to the field and greenhouse trials mentioned above. Treatments included a factorial arrangement of herbicides (five levels) and atrazine admixture (none and 560 g ai ha^{-1}). The herbicide factor contained the following levels: none, topramezone at 18 g ai ha^{-1}, tembotrione at 92 g ai ha^{-1}, tembotrione at 90 g ai ha^{-1} plus thiencarbazone-methyl at 18 g ai ha^{-1}, and mesotrione at 105 g ai ha^{-1}. Methylated soybean oil (MSO) was applied at 1.0% v/v and UAN at 1.25% v/v to all treatments per label recommendations. Data were assessed at the same times and in the same manner as previously discussed for the ALS-inhibitor studies. Statistical procedures were also similar to those previously discussed except that sums of squares were partitioned to reflect trial effects and the factorial treatment structure. Main effects and interactions were tested against the means square error of the appropriate trial interaction. If no trial interactions were significant, data were pooled. Appropriate means were separated using Fisher's Protected LSD test at $P = 0.05$.

Results and Discussion

EBN response to ALS-inhibiting and other herbicides

The interaction of trial and treatment was significant for EBN control 2 WAT ($P < 0.0001$) and 4 WAT ($P < 0.0001$) so data are presented separately for each site (Table 2.2). These few herbicides controlled EBN consistently between trials but these ?? varied between locations and probably caused the trial interaction (Table??). At both field and greenhouse locations, glyphosate controlled EBN 90% or greater throughout the duration of each study (Table 2.2). Bromoxynil and dicamba controlled EBN 100% by 4 WAT in greenhouse studies, but only

controlled EBN 43% and 50%, respectively in the field study. The ALS-inhibiting herbicides nicosulfuron, nicosulfuron plus rimsulfuron, and rimsulfuron plus thifensulfuron controlled EBN less than 20% EBN by 4 WAT. In contrast imazamox controlled EBN 89 to 98% both years in the greenhouse, and plants yielded no measurable biomass (Table 2.3).

These results indicate that this population of EBN did not exhibit an overall resistance to both families of ALS-inhibiting herbicides; however, this species did exhibit tolerance to the sulfonylurea family. Previous studies have also indicated that EBN is tolerant of chlorimuron, thifensulfuron and rimsulfuron (Simpson and Stoller 1995, Wilcut et al.1989). Carey et al. (1997) also showed that EBN was tolerant to nicosulfuron, but was sensitive to the sulfonylurea primisulfuron. The major difference between the two herbicides was indicated as being a lower enzyme sensitivity to nicosulfuron due to a greater amount of the ALS enzyme and as well as a lower translocation rate of the herbicide.

Effects of HPPD-inhibitors and atrazine on EBN

The interaction of trial by herbicide by atrazine was significant for EBN control 2 WAT ($P < 0.0001$) and 4 WAT ($P < 0.0001$) and for EBN biomass ($P = 0.0015$). At 2 WAT, the addition of atrazine significantly improved EBN control with tembotrione, tembotrione plus thiencarbazone-methyl, and topramezone (Table 2.4). This difference in early control with and without atrazine can be attributed to the synergistic relationship of HPPD-inhibitors and atrazine (Armel et al. 2007, Williams et al. 2011)

Although HPPD–inhibiting herbicides may provide much needed control of EBN, corn is the only crop that is tolerant to this particular mode-of-action. Soybeans genetically modified to tolerate this mode-of-action have been developed, (Dufourmantel et al. 2007, Duke 2012, Siehl et al. 2014), but are currently not available for commercial use. However, Eastern Shore producers that specialize in non-GM soybeans, will have limited options for EBN control as

more weeds develop resistance to ALS inhibitors and other herbicides. A more integrated approach to weed management will be required.

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Table 2.1. List of herbicides used study 1.

Herbicide[a]	Trade Name	Rate	Manufacturer	City	State
		g ai ha^{-1}			
acetochlor	Warrant	1246	Monsanto Company	St. Louis	MO
glyphosate	Buctril	21	Bayer Cropscience	Research Triangle Park	NC
dicamba	Clarity	561	BASF Ag Products	Research Triangle Park	NC
imazamox[a]	Raptor	44	BASF Ag Products	Research Triangle Park	NC
glyphosate	Roundup PowerMax	841	Monsanto Company	St. Louis	MO
nicosulfuron	Accent	35	DuPont Crop Protection	Wilmington	DE
nicosulfuron + rimsulfuron	Steadfast	26 + 14	DuPont Crop Protection	Wilmington	DE
rimsulfuron + thifensulfuron	Resolve Q	16 + 4	DuPont Crop Protection	Wilmington	DE

a. Dicamba, imazamox, nicosulfuron, nicosulfuron + rimsulfuron, and rimsulfuron + thifensulfuron applied along with nonionic surfactant (NIS) at 0.25% v/v and urea ammonium nitrate (UAN) at 0.25% v/v. Acetochlor + glyphosate was applied along with NIS at 0.25% v/v.

[b.] Imazamox applied at 44 g ai ha-1 not used in field studies.

Table 2.2. Influence of ALS-inhibiting and other herbicides on eastern black nightshade visually-estimated control and biomass.

Herbicides [a]	Control 2 WAT			Control 4 WAT			Biomass	
	EV	GH1	GH2	EV	GH1	GH2	GH1	GH2
	——————————%——————————						—— g plant^{-1} ——	
Acetochlor + glyphosate	95	88	67	94	88	96	0.83	0.05
Bromoxynil	43	100	100	30	100	100	0	0
Dicamba	58	99	100	69	100	100	0	0
Imazamox[b]	----	61	41	------	89	98	0.1	0
Glyphosate	93	93	93	93	91	99	0	0
Nicosulfuron	14	4	44	6	0	28	1.58	0.78
Nicosulfuron + rimsulfuron	20	7	48	15	5	43	0.9	0.55
Rimsulfuron + thifensulfuron	7	5.2	2	4	0	0	1.1	0.88
LSD	30.8	13.5	16.0	29.8	8.38	11.9	0.78	0.63

[a.] Acetochlor plus glyphosate, bromoxynil, dicamba, glyphosate, imazamox, nicosulfuron, nicosulfuron plus rimsulfuron, and rimsulfuron plus thifensulfuron applied POST at 1246 + 841, 21, 561, 841, 44, 35, 26 +14, and 16 +4 g ai ha^{-1}, respectively. Acetochlor + glyphosate were applied with NIS at 0.25% v/v, other herbicides were applied with both NIS and urea ammonium nitrate at 0.25% v/v.

[b.] Imazamox was not included at EV location.

[c.] Abbreviations: EV, Eastville, VA, GH 1, first greenhouse trial at Painter, VA, GH 2, second greenhouse trial at Painter, VA.

Table 2.3. Control of eastern black nightshade with HPPD-inhibitors with and without atrazine.[a]

	Control 2 WAT						Control 4 WAT					
	with atrazine			without atrazine			with atrazine			without atrazine		
Herbicides[b]	EV	GH1	GH2	EV	GH1	GH2	EV	GH1	GH2	EV	GH1	GH2
	%											
Mesotrione	88	100	100	70	40	100	96	100	100	92	28	100
Tembotrione	93*	100*	100	49*	78*	100	99	100	100	89	99	100
Tembotrione + thiencarbazone-methyl	94	100	100	59	97	100	98	100*	100	91	84*	100
Topramezone	95*	100*	100	53*	80*	100	98*	100	100	91*	98	100
None	43	55	100*	0	0	0*	55*	49	100*	0*	0	0*
LSD (0.05)	26.0	34.1	ns	23.2	26.8	0	19.5	38.8	ns	12.6	35.8	0

[a.] Means followed by an asterix (*) are significantly different according to Fisher's LSD procedure at P≤0.05.

[b.] Mesotrione, tembotrione, tembotrione plus thiencarbazone-methyl, topramezone, applied POST at 105, 92, 92 + 22, and 18.4 g ai ha^{-1}, respectively alone or in combination with atrazine applied at 560 g ai ha^{-1}. Methylated soybean oil (MSO) (1.0% v/v) and UAN (1.25% v/v) were added to all treatments per label recommendations.

[c.] Abbreviations: EV, Eastville, VA, GH 1, GH 1, greenhouse study in 2011, GH 2, greenhouse study in 2012, HPPD, p-hydroxyphenyl pyruvate dioxygenase.

Table 2.4 Biomass of eastern black nightshade following the application of HPPD-inhibitors with and without atrazine.

	Biomass			
	with atrazine		without atrazine	
Herbicides[b]	GH1	GH2	GH1	GH2
	g			
Mesotrione	0	0	0	0
Tembotrione	0	0	0	0
Tembotrione + thiencarbazone-methyl	0	0	0	0
Topramezone	0	0	0	0
None	0.9	0*	1.3	0*
LSD (0.05)	0.7	ns	0.4	ns

[a.] Means followed by an asterix (*) are significantly different according to Fisher's LSD procedure at $P \leq 0.05$.

[b.] Mesotrione, tembotrione, tembotrione plus thiencarbazone-methyl, topramezone, applied POST at 105, 92, 92 + 22, and 18.4 g ai ha^{-1}, respectively alone or in combination with atrazine applied at 560 g ai ha^{-1}. Methylated soybean oil (MSO) (1.0% v/v) and UAN (1.25% v/v) were added to all treatments per label recommendations.

[c.] Abbreviations: GH 1, GH 1, greenhouse study in 2011, GH 2, greenhouse study in 2012, HPPD, p-hydroxyphenyl pyruvate dioxygenase.

CHAPTER 3

MANAGEMENT OF PALMER AMARANTH IN SOYBEAN

Abstract

Field studies were conducted in Hebron, MD in 2011 and 2012 to evaluate different herbicide modes-of-action alone or in tank mix combinations for control of a suspected glyphosate resistant Palmer amaranth population. PRE applications of flumioxazin, flumioxazin + chlorimuron, chlorimuron, chlorimuron + thifensulfuron, sulfentrazone + imazethapyr, pendimethalin, *s*-metolachor, metribuzin, sulfentrazone + metribuzin, linuron, fomesafen, and *s*-metolachor + fomesafen provided over 89% control of Palmer amaranth by 14 DAIT. By 42 DAIT, PRE control declined and chlorimuron, flumioxazin + chlorimuron, fomesafen, sulfentrazone + imazethapyr, imazethapyr, and sulfentrazone + metribuzin controlled palmer amaranth 94% or greater. POST applications of glyphosate failed to improve control each year, while POST applications of imazamox provided 98% control. While several PRE control options are available, effective POST treatments are still needed for season long weed control. ALS-inhibiting herbicides, such as imazamox, remain effective POST options; however, mixing different herbicide modes-of-action will be an essential management strategy in preventing additional resistance issues in the area.

Nomenclature: chlorimuron; flumioxazin; fomesafen; glyphosate; imazamox; imazethapyr; metribuzin; linuron; *s*-metolachor; sulfentrazone; thifensulfuron; Palmer amaranth, *Amaranthus palmeri* S. Wats.; soybean, *Glycine max* L. Merr.

Key Words: acetolactate synthase, genetically modified, glyphosate-resistant, herbicide resistant.

Introduction

Palmer amaranth (PA) is a C4 summer annual herb that can reach approximately 2 m in height (Bryson and DeFelice 2009, DeFelice MS et al. 2011) This species can be distinguished from other members of the Amaranthaceae family by its glabrous stems, long leaf petioles, and a 0.5 m panicle (Bryson and DeFelice 2009). This species is dioecious, producing male and female flowers on separate plants (DeFelice et al. 2011), with flowering usually occurring from September to October. This species is native to the Southern Great Plains (Bond and Oliver 2006), and populations can be found throughout the Southern United States (NRCS 2014). While present in many counties in Virginia, this species was only recently reported on the Eastern Shore regions of Virginia and Maryland in 2011. The exact source of importation is unknown, but it is believed it came there via non-certified wheat seed (H. Wilson personal communication)

Palmer amaranth is a major weed problem in corn (*Zea mays* L.) (Massinga et al. 2001), cotton (*Gossipium hirsutum* L.) (Culpepper et al. 2008, Morgan et al. 2001), soybean (Bensch et al. 2003, Klingaman and Oliver 1994), and peanuts (*Arachis hypogaea* L.) (Burke et al. 2007). Due to its rapid growth rate and tall stature, PA is extremely competitive in many cropping systems (Culpepper et al. 2008) and has a comparatively faster growth rate (Horak and Loughin 2000) compared to other *Amaranthus* species (Sellers et al 2003). Palmer amaranth has been found to have a high photosynthetic rate (Ehleringer 1983). Leaves of this species have been shown to exhibit osmotic adjustment in periods of low leaf water potential (Ehleringer 1983). Palmer amaranth is a prolific seed producer, producing well over 100,000 seeds per plant (Sellers et al. 2003). In addition, PA has been reported to have allelopathic affects, reducing the growth of crops such as onion (*Alium cepa* L.), cabbage (*Brassica oleracea* L.), and sunflower

(*Helianthus annuus* L.) (Bradow and Connick 1987, Menges 1987, Menges1988). These characteristics result in PA being highly competitive with other crop species. Klingaman and Oliver (1994) reported PA densities of 0.33 to 10 plants m^{-1} row of soybean can reduce soybean yields 17 to 68%, and Bensch et al. (2003) reported that yield losses as high as 78% can occur with a single PA per 0.125 m^{-1} row.

The majority of soybean production on the Eastern Shore of Virginia and Maryland is conducted under no-till or minimum till systems, making herbicide application the primary means of weed control. Although, growers are relying more on herbicides such as flumioxazin for preemergence (PRE) weed control, glyphosate-based weed control systems are still common (Johnson et al. 2012, Stewart et al. 2011, Whitaker et al. 2010).

Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase. The shikimic acid pathway converts carbohydrate precursors from glycolysis and the pentose phosphate pathway to aromatic amino acids (Herrmann and Weaver 1999). One of the intermediates in this pathway is shikimate-3-phosphate. One of the intermediates in this pathway is shikimate-3-phosphate. EPSP synthase binds to shikimiate-3-phosphoate. Next a molecule of phosphoenolpyruvate (PEP) combines with the complex to form 5 enolpyruvylshikimate-3-phosphate. Glyphosate acts as a competitive inhibitor of PEP for the shikimate-3-phosphate/EPSP synthase enzyme complex which leads to a decrease in the production of tryptophan, tyrosine, and phenylalanine. Plant death results from a lack of protein production and wasteful metabolism.

Glyphosate is a non-selective herbicide, and its use in-crop requires the crop itself to be genetically modified (GM) to be resistant to the herbicide. In GM crops glyphosate resistance is conferred by inserting an insensitive EPSP synthase gene from *Agrobacterium* spp. or

Escherichia coli commonly known as the CP4 gene. This gene is expressed at high levels in the plant to make copies of the less sensitive enzyme. The GM crop retains both the wild type and CP4 gene. When glyphosate is applied, wild type EPSP synthase is inhibited, but not CP4 EPSP synthase which carries on normal production of aromatic amino acids (Kishore et al. 1992, Padgette et al. 1995). Resistance in PA has been attributed to amplification of the EPSP synthase gene (Gaines et al. 2010). Similarly, more copies of EPSP synthase are able to compete with the glyphosate molecules.

In 2012, herbicide tolerant soybean was the dominant GM crop, occupying 80.7 million hectares and 81% of the soybeans planted in the world (James 2012). Overuse of these GM crops, specifically glyphosate-resistant (GR) crops, has selected for the presence of GR-resistant PA throughout many parts of the southern United States including Arkansas (Norsworthy et al. 2008), Georgia (Culpepper et al. 2006), North Carolina (Culpepper et al. 2008), Tennessee (Steckel et al. 2008), and Virginia (Ahmed 2011). Despite its recent introduction, several PA populations on the Eastern Shore regions are suspected to be GR, and other methods of control need to be explored.

The acetolactate synthase inhibiting class of herbicides provides another mode-of-action for postemergence (POST) control. However, a similar species, smooth pigweed (*Amaranthus hybridus*), has been documented as being ALS resistant on Virginia's Eastern Shore (Manley et al. 1998, Poston et al. 2002, Whaley et al. 2006). ALS-resistant PA has also been documented in several states including Arkansas, Georgia, Florida, North Carolina, Tennessee, and mainland Virginia (Ahmed 2011, Heap 2014, Wise et al. 2009). This is of particular concern since resistant biotypes of Amaranthus species can cross-pollinate with herbicide susceptible species (Franssen et al. 2001, Tranel et al. 2002, Wetzel et al.1999). Palmer amaranth with multiple

resistance to ALS-inhibiting herbicides and glyphosate has been found in Georgia and North Carolina (Sosnoskie et al. 2009, Whitaker et al. 2007). Palmer amaranth has also been documented to be resistant to dinitroanilines, triazines, and HPPD- inhibiting herbicides (Heap 2014).

Due to the possibility of PA being resistant to more than one herbicide, the objectives of this study were to 1) evaluate different herbicide modes-of-action alone or in combination for PRE control of a PA population in soybean, and 2) confirm the presence of GR-PA in a particular soybean field.

Materials and Methods

A field experiment was conducted between 2012 and 2013 to evaluate PA response to various PRE and POST herbicides commonly used in soybean. Field trials were established in Hebron, MD. Soybean, variety AG4232, was planted on May 23, 2012 and May 24, 2013 into wheat stubble. Preemergence applications were made on May 24, 2012 and May 29, 2013, respectively. Postemergence applications were made 14 days after the initial treatment (DAIT). The soil type was a Runclint loamy sand characterized to have 80% sand, 12% silt, and 5% clay with < 3% organic matter and a pH of 4.5.

Trials were arranged in randomized complete block designs with 13 treatments (Table 3.1) and 3 to 4 replications. A nontreated check was also included in each trial for comparison. Corn field plots were 2.5 m wide and 6.1 m long and treatments were applied using a propane-pressurized backpack sprayer with a 1.5 m boom and five XR 11003 flat fan nozzles delivering 186 L ha^{-1}.

Visual assessments of PA control were made on a scale of 0 (no control) to 100 (plant death) 28 and 42 DAIT. Data were analyzed in PROC GLM SAS 9.2 (SAS Institute, Inc. Cary, NC). Data were tested for homogeneity of variance prior to ANOVA and transformed to the arcsine of the square root where needed to stabilize variance. If transformation was needed prior to ANOVA, subsequent means were back transformed for presentation. Sums of squares were partitioned to reflect trial and treatment effects and interactions. Main effects and interactions were tested against the means square error of the appropriate trial interaction. If no trial interactions were significant, data were pooled. Appropriate means were separated using Fisher's Protected LSD test at P = 0.05.

Results and Discussion

In June of 2013 the experiment site received 25.2 cm of rainfall, causing flooding issues (Table 3.2). This resulted in the average soybean canopy coverage to be 27% at 42 DAIT in 2013 compared to 77% at the same rating date in 2012. The interaction of trial and treatment was not significant for PA control (P = 0.10), so data was pooled over both years. Count data yielded a significant treatment by year interaction 14 DAIT (P = 0.002); however, there was no significant treatment (P = 0.23) or location by treatment interaction (P = 0.26) means for 42 DAIT. Therefore, only data from 14 DAIT is presented.

All treatments controlled PA 89% or greater 14 DAIT (Table 3.3). The level of control declined for most treatments by 42 DAIT, but the following herbicides controlled PA 94% or greater: chlorimuron, flumioxazin + chlorimuron, fomesafen, sulfentrazone + imazethapyr, imazethapyr, and sulfentrazone + metribuzin. This was significantly greater than control with flumioxazin, pendimethalin, and *s*-metolachor. The loss in control over time is common with these three herbicides, and previous studies have indicated that these herbicides will provide

varying levels of PA control. Whitaker et al. (2010) reported that pendimethalin controlled PA up to 12% and *s*-metolachor controlled PA up to 15 % in two GR-PA populations within 30 days of the application. Ahmed and Holshouser (2012) reported that flumioxazin controlled PA 80 to 91%, pendimethalin controlled PA 20 to 43%, and *s*-metolachor controlled PA 33 to 65% 30 days after planting. Everman et al. (2009) also reported that pendimethalin controlled PA 74% in cotton.

POST applications of glyphosate failed to provide adequate control of emerged PA indicating resistance to this herbicide. The development of herbicide resistance is often the result of selection pressure caused by the use of one herbicide over another (Vencill et al. 2012). However, since it this species has only recently been reported in the area, it is possible that GR-PA was already present at the time of its introduction.

Although yield data were not taken, all herbicides used in these studies are registered at the rates applied. Additional studies have also indicated that the herbicides used in this study provide no adverse harm to the crop yields (Johnson et al. 2012, Ovejero et al. 2013). Mahoney et al. (2014) reported that *s*-metolachor, sulfentrazone, and flumioxazin caused 1, 18, and 3% injury 4 weeks after soybean emergence, but plants eventually grew out of the injury and there was no negative impact on yield.

Despite good control, these plots were not completely free of PA by 42 DAIT. Count data were confounded by the presence of emerged seedlings and did not accurately reflect levels of control (Table 3.4). However, average PA counts, including those in the untreated check, appeared lower than other studies done in areas where PA had been established for a number of years (Bensch et al. 2003, Meyers et al. 2010, Steckel et al. 2008). However, even at low

densities PA can still cause a reduction in yield (Bensch et al. 2003, Klingaman and Oliver 1994).

With such a small population, eradication efforts will be essential before more plants set seed. Even though several treatments controlled PA 90% or greater, any escapes surviving subsequent applications could still pose a threat. PA seed has the potential to germinate throughout the growing season (Keeley et al. 1987, Jha et al. 2010, Steckel et al. 2004). Norsworthy et al. (2014) reported that a single PA escape, could infest 95 to 100% of a field within three years of its introduction. With this in mind, growers will have to consider controlling PA before, during, and after their crops have been harvested.

There are very few herbicides registered in soybean that will provide the same desired level of PA control that glyphosate does late in the growing season. Although, the ALS-inhibiting herbicide imazamox provided over 98% control by 42 DAIT in 2012 and 2013, growers cannot continue to rely on this mode-of-action alone for fear of developing resistance to this mode-of-action as well. Recently, a population of PA in a separate field adjacent to our test site was reported to have multiple-resistance to both glyphosate and the ALS-inhibiting herbicide imazethapyr (Heap 2014, Mark VanGessel personal communication). Long term control of this weed on the Eastern Shore regions will ultimately rely on applying multiple herbicides with different modes-of-action. Given that environmental conditions are seldom favorable to support season-long control with PRE herbicides, additional research also needs to be performed using alternative modes-of-action for effective POST control.

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Table 3.1. List of Herbicides used in chapter 3

Herbicide[a]	Trade Name	Rate	Manufacturer	City	State
		g ai ha^{-1}			
Chlorimuron	Classic	22	DuPont Crop Protection	Wilmington	DE
Chlorimuron + thifensulfuron	Synchrony XP	11 + 4	DuPont Crop Protection	Wilmington	DE
Flumioxazin	Valor SX	72	Valent USA Corporation	Walnut Creek	CA
Flumioxazin + chlorimuron	Valor XLT	91 + 31	Valent USA Corporation	Walnut Creek	CA
Fomesafen	Reflex	420	Syngenta Crop Protection	Greensboro	NC
Glyphosate	Roundup PowerMax	841	Monsanto Company	St. Louis	MO
Linuron	Lorox DF	560	Tessenderlo Kerley	Phoenix	AZ
Metribuzin	Tricor DF	420	United Phosphorous	King of Prussia	PA
Pendimethalin	Prowl H_2O	1600	BASF Ag Products	Research Triangle Park	NC

S-metolachor	Dual II Magnum	1420	Syngenta Crop Protection	Greensboro	NC
S-metolachor + fomesafen	Prefix	1217 + 266	Syngenta Crop Protection	Greensboro	NC
Sulfentrazone + imazethapyr	Authority Assist	291 + 59	FMC Corporation	Philadelphia	PA
Sulfentrazone + metribuzin	Authority MTZ	303 + 202	FMC Corporation	Philadelphia	PA
Imazamox	Raptor	44	BASF Ag Products	Research Triangle Park	NC

[a.] All herbicides except imazamox applied PRE. Imazamox was applied POST 14 days after planting along with nonionic surfactant (NIS) and urea ammonium nitrate (UAN) applied at 0.25 and 1.25 %v/v. All PRE herbicides were followed by glyphosate applied with 2200 g ha^{-1} ammonium sulfate POST 14 DAIT.

[b.] Abbreviation: preemergence; POST, postemergence.

Table 3.2. Rainfall Data for Hebron MD, 2012-2013.

	Total Precipitation	
Month	2012	2013
	——————cm——————	
April	7.5	9.6
May	6.6	8.4
June	8.9	25.2
July	5.9	9.7
August	10.9	18.5

Table 3.3. Control of Palmer Amaranth in Hebron, MD in 2012 and 2013.[a]

		Palmer amaranth control	
Herbicides[b]	Rate	14 DAIT[c]	42 DAIT
	g ai ha^{-1}	——————%——————	
Chlorimuron	22	99 a	98 a
Chlorimuron + thifensulfuron	11 + 4	99 a	90 abc
Flumioxazin	72	94 b	73 cd
Flumioxazin + chlorimuron	91 + 31	99 a	98 a
Fomesafen	420	98 a	94 ab
Linuron	560	99 a	77 bcd
Metribuzin	420	98 a	80 abcd
Pendimethalin	1600	92 bc	73 cd
S-metolachor	1420	89 c	68 d
S-metolachor + fomesafen	1217 + 266	99 a	91abc
Sulfentrazone + imazethapyr	291 + 59	95 ab	95 ab
Sulfentrazone + metribuzin	303 + 202	99 a	96 ab
Imazamox (POST)	44	0 d	98 a

[a.] Data pooled over years (2012 and 2013). Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD test at $P \leq 0.05$.

[b.] All herbicides except imazamox applied PRE. Imazamox was applied POST 14 days after planting along with nonionic surfactant (NIS) and urea ammonium nitrate (UAN) applied at 0.25 and 1.25 %v/v. All PRE herbicides were followed by glyphosate applied with 2200 g ha^{-1} ammonium sulfate POST 14 DAIT.

[c.] Abbreviation: DAIT, days after initial; PRE, preemergence; POST, postemergence.

Table 3.4 Palmer Amaranth Density 14 DAIT in Hebron, MD after preemergence herbicide application. [a]

		Palmer amaranth density	
Preemergence herbicides		2012	2013
	g ai ha^{-1}	—— counts m^{-1}——	
Chlorimuron	22	0 d	0 b
Chlorimuron + thifensulfuron	11 + 4	0 d	0 b
Flumioxazin	72	0.75 ab	0 b
Flumioxazin + chlorimuron	91 + 31	0 d	0 b
Fomesafen	420	0.17 cd	0 b
Linuron	560	0 d	0 b
Metribuzin	420	0 d	0 b
Pendimethalin	1600	0.92 a	0.25 b
S-metolachor	1420	0.67 abc	1.0 a
S-metolachor + fomesafen	1217 + 266	0 d	0.08 b
Sulfentrazone + imazethapyr	291 + 59	0.42 abcd	0.25 b
Sulfentrazone + metribuzin	303 + 202	0 d	0
untreated		0.67 abc	1.1 a

[a.] Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD test at $P \leq 0.05$.

CHAPTER 4

MANAGEMENT OF PALMER AMARANTH IN CORN

Abstract

In 2012 and 2013 field studies were conducted in Hebron, MD to evaluate HPPD-inhibiting herbicides alone or in combination with atrazine for control of glyphosate-resistant Palmer amaranth in a no-till corn system. Both years isoxaflutole, isoxaflutole + atrazine, isoxaflutole + thiencarbazone, isoxaflutole + thiencarbazone + atrazine, *s*-metolachor + atrazine, and atrazine provided over 95% PRE control of Palmer amaranth 28 DAIT. Both years topramezone + atrazine, tembotrione, and tembotrione + atrazine provided over 98% POST control of Palmer amaranth. Glyphosate failed to control emerged Palmer amaranth. This population was found to have an LD_{50} value of 1429 g ae ha^{-1}, confirming glyphosate resistance in the area.

Nomenclature: atrazine; glyphosate; isoxaflutole; *s*-metolachor; tembotrione; thiencarbazone-methyl; topramezone; Palmer amaranth, *Amaranthus palmeri* S. Wats.; corn, *Zea mays* L.

Key Words: genetically modified, glyphosate-resistant, herbicide resistant

Introduction

Palmer amaranth (PA) is a C4 summer annual herb that can reach approximately 2 m in height (Bryson and DeFelice 2009). This species can be distinguished from other members of the Amaranthaceae by its glabrous stems, long petioles, and 0.5 m panicle (Bryson and DeFelice 2009, DeFelice et al. 2011). This species is dioecious, producing male and female flowers on

separate plants (DeFelice et al. 2011), with flowering usually occurring from September to October. This species is native to the Southern great plains (Bond and Oliver 2006), and populations can be found throughout the Southern United States (NRCS 2014). While found in counties in Virginia, this species was only recently discovered on its Eastern Shore in 2011. The exact source of importation is unknown, but it is believed it came there via non-certified wheat seed (H. Wilson personal communication).

This species is a major weed problem in corn (Massinga et al. 2001), cotton (*Gossipium hirsutum* L.) (Culpepper et al. 2008, Morgan et al. 2001), soybean (*Glycine max* [L.] Merr.) (Bensch et al. 2003, Klingaman and Oliver 1994), and peanuts (*Arachis hypogaea* L.) (Burke et al. 2007). Due to its rapid growth rate and tall stature, PA is an aggressive competitor in many cropping systems (Culpepper et al. 2008) and has a comparatively faster growth rate (Horak and Loughin 2000) compared to other *Amaranthus* species (Sellers et al 2003). Palmer amaranth has been found to have a high photosynthetic rate (Ehleringer 1983). Leaves of this weed species have been shown to exhibit osmotic adjustment in periods of low leaf water potential (Ehleringer 1983). Palmer amaranth is a very prolific seed producer, producing well over 100,000 seeds per plant (Sellers et al. 2003). In addition, PA has been reported to have allelopathic affects, reducing the growth of crops such as onion (*Alium cepa* L.), cabbage (*Brassica oleracea* L.), and sunflower (*Helianthus annuus* L.) (Bradow and Connick, 1987, Menges 1987, Menges1988). These features result in PA being highly competitive with other crop species. Palmer amaranth densities of 0.5 to 0.8 plants m^{-1} row of corn have been shown to reduce corn yields 11 to 91% (Massinga et al. 2001).

The majority of corn production on the Eastern Shore of Virginia and Maryland is conducted under no-till or minimum till systems, making herbicide application the primary means of weed

control. Although there are a variety of herbicide options, growers in Virginia and Maryland continue to rely on weed control programs based on the use of the herbicides atrazine and glyphosate. Although there are a variety of herbicide options, atrazine continues to be one of the most widely used herbicides in corn production systems (Mueller et al. 2010, NASS 2005, Williams et al. 2011).

Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase. The shikimic acid pathway converts carbohydrate precursors from glycolysis and the pentose phosphate pathway to aromatic amino acids (Herrmann and Weaver 1999). One of the intermediates in this pathway is shikimate-3-phosphate. EPSP synthase binds to shikimiate-3-phosphoate. Next a molecule of phosphoenolpyruvate (PEP) combines with the complex to form 5 enolpyruvylshikimate-3-phosphate. Glyphosate acts as a competitive inhibitor of PEP for the shikimate-3-phosphate/EPSP synthase enzyme complex which leads to a decrease in the production of tryptophan, tyrosine, and phenylalanine.

In genetically modified (GM) crops, glyphosate resistance is conferred by inserting an insensitive EPSP synthase gene from *Agrobacterium* spp. or *Escherichia coli* commonly known as the CP4 gene. This gene is expressed at high levels in the plant to make more copies of the less sensitive enzyme. The GM plant retains both the wild type and CP4 gene. When glyphosate is applied, wild type EPSP synthase is inhibited, but not CP4 EPSP synthase, which allows normal production of aromatic amino acids (Kishore et al. 1992, Padgette et al. 1995).

With the development of glyphosate-resistant (GR), corn use of this herbicide for POST weed control increased from 189.3 lbs. year^{-1} in 1996 to 1208.8 lbs. year^{-1} in 2005 (NASS 2005). In 2012, herbicide tolerant corn occupied 7.8 million hectares, 35% of the total corn in the world (James 2012). Overuse of these GM crops, especially GR crops, has selected for the presence of

GR-PA throughout the southern United States including Arkansas (Norsworthy et al. 2008), Georgia (Culpepper et al. 2006), North Carolina (Culpepper et al. 2008), Virginia (Ahmed 2011), and Tennessee (Steckel et al. 2008). Palmer amaranth populations on the Eastern Shores of Virginia and Maryland are suspected of being resistant to glyphosate (VanGessel 2012 personal communication).

Thus far triazine resistant PA has not been reported In Virginia; however, two similar species, redroot pigweed (*Amaranthus retroflexus*) and smooth pigweed (*Amaranthus hybridus*), have been documented as being resistant to atrazine (Heap 2014). Palmer amaranth with triazine resistance has been reported in Georgia, Texas, Kansas, and Nebraska (Heap 2014, Jhala et al. 2014, Prostko 2014).

An alternative mode-of-action for PA control in corn is the inhibition of 4- hydroxyphenyl pyruvate dioxygenase (HPPD), an important enzyme in carotenoid biosynthesis. Affected plants will show bleaching of leaf tissue followed by necrosis (Beltran et al. 2003). These HPPD-inhibiting herbicides can be applied either preemergence (PRE) (isoxaflutole, mesotrione) or postemergence (POST) (mesotrione, topramezone, tembotrione). Therefore, these herbicides may be effective against weeds that may be triazine and/or glyphosate resistant.

The objectives of these studies were to 1) confirm glyphosate resistance based on field applications 2) evaluate different HPPD herbicides with and without atrazine as an alternative mode-of-action for management of PA in corn, and 3) determine the extent of herbicide resistance issues identified by the field studies.

Materials and Methods

Field Studies

A field experiment was conducted between 2012 and 2013 to evaluate PA response to various PRE and POST herbicides commonly used in corn. A second experiment was conducted to evaluate the level of glyphosate resistance in a population. Field trials were established in Hebron, MD. Corn, variety DKC62-58, was planted in VA on April 25, 2012 and MD on April 24, 2012 and April 27, 2013. Preemergence applications were made on Apr 25, 2012 and May 1, 2013, respectively. Postemergence applications were made 28 days after the initial treatment (DAIT). The soil type was a Runclint loamy sand characterized to have 80% sand, 12% silt, and 5% clay with < 3% organic matter and a pH of 4.5.

Trials were arranged in randomized complete block designs with 11 treatments (Table 4.1) and 3 to 4 replications. A nontreated check was also included in each trial for comparison. Corn field plots were 2.5 m wide and 6.1 m long and treatments were applied using a propane-pressurized backpack sprayer with a 1.5 m boom and five XR 11003 flat fan nozzles delivering 186 L ha^{-1}.

Visual assessments of PA control were made on a scale of 0 (no control) to 100 (plant death) 28 and 42 DAIT. Data were analyzed in PROC GLM SAS 9.2 (SAS Institute, Inc. Cary, NC). Data were tested for homogeneity of variance prior to ANOVA and transformed to the arcsine of the square root where needed to stabilize variance. If transformation was needed prior to ANOVA, subsequent means were back transformed for presentation. Sums of squares were partitioned to reflect trial and treatment effects and interactions. Main effects and interactions were tested against the means square error of the appropriate trial interaction. If no trial

interactions were significant, data were pooled. Appropriate means were separated using Fisher's Protected LSD test at P = 0.05.

Glyphosate Rate Titration Study

Herbicides used in greenhouse studies were applied using a compressed air, moving nozzle, cabinet sprayer equipped with one 8002EVS nozzle delivering 171 L ha^{-1} at 289 kPa. Glyphosate-resistant PA seed was collected from the field site in 2011 and from another site in King William, VA in 2012. These populations were compared to an herbicide susceptible population obtained from Azlin Seed Service (AZ) (Leland, MS). Populations of PA were established in 53 cm by 27 cm flats then transplanted at the 2 to four leaf stage into 722 cm^3 pots containing a commercial potting mix (Pro Mix BX Mycorrhizae, Premier Tech Horticulture, Quakertown, PA). Seedlings were established in 53 cm x 27 cm flats containing commercial potting mix (Pro Mix BX Mycorrhizae, Premier Tech Horticulture, Quakertown, PA). Plants were watered daily as needed and growth occurred under diurnal cycles of light and temperature. Herbicide applications were made to plants averaging 4 to 6 cm in height.

Trials were arranged in randomized complete block designs with 7 treatments and 10 replications. Glyphosate (Roundup PowerMax, Monsanto, St. Louis, MO) was applied at 217; 433; 870; 1,730; 3,470; 6,940; and 13,900 g ae ha^{-1} in combination with 2,243 g ha^{-1} ammonium sulfate (AMS), with 870 g ae ha^{-1} corresponding to the recommended field application rate. Plant mortality (dead or alive) was recorded 28 days after treatment. The lethal dose necessary to kill 50% (LD_{50}) population was determined using probit maximum likelihood analysis in ARM 8 (Gylling Data Management, Inc, Brookings SD)

Results and Discussion

Field Studies

In June of 2013 the MD site received 25.2 cm of rainfall, compared to 8.9 cm in 2012 (Table 4.2). The interaction of trial and treatment was significant for PA control 28 DAIT ($P = 0.001$) and 41 DAIT ($P < 0.0001$), as well as PA density 28 DAIT ($P < 0.0001$) and 41 DAIT ($P < 0.0001$). Therefore, data is presented individually for both years. Atrazine, isoxaflutole, isoxaflutole + atrazine, isoxaflutole + thiencarbazone-methyl, and *s*-metolachor + atrazine controlled PA greater than 95% 28 DAIT both years (Table 4.3). POST only applications of tembotrione, tembotrione + atrazine, and topramezone + atrazine controlled PA 98% or greater 41 DAIT. Both years, a decline in PRE control was observed between 28 and 41 DAIT. POST glyphosate applications to these plots did not improve control levels. In 2012, all treatments controlled PA 94% or greater control 41 DAIT, but in 2013 isoxaflutole, s-metolachor, and atrazine controlled PA 63, 67, and 75%.

The decline in the efficacy of the PRE herbicide treatments is not uncommon, as soil properties can greatly affect these herbicides. Preemergence herbicides require irrigation or an activating rainfall within 7 to14 days after application in order to be incorporated into soil water and made available for plant uptake. Therefore, inadequate or delayed precipitation can reduce herbicide efficacy, and high amounts of precipitation can cause the herbicide to leach out of the zone of germination (Stewart et al. 2012).

In soil, water, and vegetation, isoxaflutole is converted into an herbicidally active diketonitrile derivative (DKN) (Beltran et al. 2003, Rice et al. 2004). This conversion has been shown to increase with moisture content (Beltran et al. 2003), which affects DKN availability in

the plant. However, higher amounts of rainfall, such as those observed in June 2013 also have the potential to leach herbicides out of the zone where weed germination occurs (Kloppel et al. 1997, McGrath et al. 2010, Ulrich et al. 2013). Whether or not tillage was implemented can have a compounding effect on herbicide loss. Hall et al. (1991) reported greater leaching losses of atrazine in no-till systems vs. conventional systems, due to the greater number of macropores in non-tilled vs. tilled soils.

The lack of POST control with glyphosate indicates the presence of GR-PA. However, the HPPD-inhibiting herbicides topramezone and tembotrione when applied with or without atrazine provided excellent control. The use of these herbicides can also provide some residual activity for late season PA control. Applications of POST herbicides with residuals have been shown to be as or more effective than POST applications alone (Lindsey et al. 2012). *S*-metolachor is another herbicide that has been recommended as a tank-mix partner for POST applications to help control late emerging weeds (Holshouser et al 2008, Steckel 2012). However, given the lack of control with *s*-metolachor throughout the course of this experiment, it is doubtful that this herbicide will provide sufficient control without an effective POST herbicide. Similar studies suggest that *s*-metolachor will not provide over 90% control of PA (Everman et al. (2009, Holshouser et al. 2009),

Although yield data was not collected several studies have reported significant yield increases when using an HPPD or HPPD atrazine combination compared to nothing at all (Armel et al. 2003, Stephenson et al. 2004, Stephenson and Bond 2012). Johnson et al. (2002) reported that mesotrione applications with or without atrazine provided 0-15% injury 7 DAT, but 0% injury was observed at 28 DAT. Similarly, Armel et al. (2003) found that corn injury from

mesotrione and atrazine applications was less than 4% 21 DAT, except when heavy rainfall occurred within 7 days of a PRE application.

Glyphosate Rate Titration

The populations from MD, VA, and AZ had LD_{50} values of 1429 g ae ha^{-1} (Figure 4.1), 2783 g ae ha^{-1} (Figure 4.2), and 843 g ae ha^{-1} (Figure 4.3) respectively. The LD_{50} of the MD population compared to the LD_{50} of the VA population and the LD_{50} of the AZ population confirms the presence of glyphosate resistance in the area. Studies have shown that different populations of PA can have varying levels of resistance. Results for the MD population are similar to that of Ahmed (2011) in which a population from Greensville, VA had an LD_{50} of 1,470 g ae ha^{-1}, and results for the VA population are similar to that of Norsworthy et al (2008) in which a population in Mississippi County Arkansas had an LD50 of 2,820 g ae ha^{-1}.

It is possible that both the Virginia and Maryland populations have different mechanisms of resistance, and further research is needed to determine the exact mechanism. When glyphosate is applied to a susceptible weed species, EPSP synthase is inhibited, resulting in the accumulation of shikimate. Culpepper et al. (2006) reported a lack of shikimate accumulation in the presence of glyphosate in a GR population in Georgia indicating that EPSP synthase is not inhibited. Gaines et al. (2011) reported that the mechanism of resistance in this Georgia population was due to an amplified EPSP synthase gene. Therefore, EPSP synthase is produced at elevated levels such that activity of the shikimate pathway is normal despite the presence of glyphosate.

In contrast, Steckel et al. (2008) reported shikimate accumulation in a resistant population in Tennessee, indicating normal function of the shikimate pathway. Gene expression studies have found the gene coding for EPSP synthase to be most highly expressed in meristems (Shaner 2009, Weaver and Hermann 1997). Therefore glyphosate needs to translocate to growing points

to be effective. Shaner (2009) reported that in some GR weeds, glyphosate moves into the leaf via the transpiration stream, and becomes trapped in the distal portion of the leaf instead of being loaded into the phloem. This indicates that a lack of translocation to the meristems can also impart glyphosate resistance.

Due to the nature of these populations, higher application rates of glyphosate may still be used to control other weed species, but it will be necessary to combine glyphosate with another POST herbicide to control PA. The lack of differences between atrazine alone and HPPD-inhibitors applied PRE suggests that HPPD-inhibitors be used as a POST alternative or tank mix partner to glyphosate.

Overall, control of PA can still be achieved at these sites using an atrazine or HPPD-based herbicide program. However, growers need to be aware that caution should be taken not to overuse these two modes-of-action, thus selecting for multiple resistance.

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Table 4.1. Herbicides used for control of Palmer amaranth in corn

Herbicide[a]	Trade Name	Rate	Manufacturer	City	State
		g ai ha^{-1}			
Atrazine	Aatrex 4L	1121[a] 560[b]	Syngenta Crop Protection	Greensboro	NC
Glyphosate	Roundup PowerMax	1060	Monsanto Company	St. Louis	MO
Isoxaflutole	Balance Flexx	53	Bayer CropScience	Research Triangle Park	NC
Isoxaflutole + thiencarbazone	Corvus	55 + 22	Bayer CropScience	Research Triangle Park	NC
S-metolachor	Dual II Magnum	1420	Syngenta Crop Protection	Greensboro	NC
S-metolachor + atrazine	Bicep II Magnum	875 + 1130	Syngenta Crop Protection	Greensboro	NC
Tembotrione	Laudis	92	Bayer CropScience	Research Triangle Park	NC
Topramezone	Impact	18	AMVAC Chemical Corp	Los Angeles	CA

[a.] Rate of atrazine applied for PRE control in conjunction with isoxaflutole and isoxaflutole plus thiencarbazone-methyl.

[b.] Rate of atrazine applied for POST control in conjunction with tembotrione and topramezone.

Table 4.2. Selected rainfall data from Hebron, MD in 2012 and 2013.

Month	Total Precipitation	
	2012	2013
	————cm————	
April	7.5	9.6
May	6.6	8.4
June	8.9	25.2
July	5.9	9.7
August	10.9	18.5

Table 4.3. Control of Palmer Amaranth in Maryland Using HPPD-inhibiting Herbicides with and without atrazine in 2012 and 2013.[a]

		Palmer amaranth control			
		28 DAIT[c]		41 DAIT	
Herbicides[b]	Rate	2012	2013	2012	2013
	g ai ha^{-1}	%			
Atrazine	1121	99 a	99 a	99 a	75 c
Isoxaflutole	53	99 a	95 b	97 ab	63 c
Isoxaflutole + atrazine	53 + 1121	99 a	99 a	99 a	95 a
Isoxaflutole + thien	55 + 22	98 ab	99 a	97 ab	92 ab
Isoxaflutole + thien + atrazine	55 + 22 + 1121	96 bc	81 c	94 b	68 c

S-metolachor	1420	96 ab	78 b	94 a	67 cd
S-metolachor + atrazine	875 + 1130	98 ab	99 a	99 a	96 a
Tembotrione	92	0 d	0 d	98 a	99 a
Tembotrione + atrazine	92 + 560	0 d	0 d	99 a	99 a
Topramezone	18	0 d	0 d	99 a	80 bc
Topramezone + atrazine	18 + 560	0 d	0 d	99 a	99 a

[a.] Means within a column followed by the same letter are not significantly different according to Fisher's LSD procedure at $P \leq 0.05$.

[b.] All herbicides except topramezone and tembotrione applied with and without atrazine were applied PRE. Topramezone and tembotrione applied with and without atrazine were applied POST 28 DAIT along with methylated seed oil (MSO) and urea ammonium nitrate (UAN) at 1 and 1.25% v/v. All PRE herbicides were followed by glyphosate applied with 2200 g ha^{-1} ammonium sulfate POST 14 DAIT.

[c.] Abbreviation: DAIT, days after initial; PRE, preemergence; POST, postemergence; thien, thiencarbazone-methyl

Table 4.4. Palmer Amaranth Density in Maryland After the Application of HPPD-inhibiting Herbicides with and without atrazine in 2012-2013.[a]

		Palmer amaranth density			
		28 DAIT[c]		41 DAIT	
Herbicides[b]	Rate	2012	2013	2012	2013
	g ai ha^{-1}	——————————————————m^{-1}——————————————————			
Atrazine	1121	0.03 bc	0.03 bc	0.03 bc	0.03 bc
Isoxaflutole	53	0 c	0 c	0 c	0 c
Isoxaflutole + atrazine	53 + 1121	0 c	0 c	0 c	0 c
Isoxaflutole + thien	55 + 22	0.1 bc	0.1 bc	0.1 bc	0.1 bc
Isoxaflutole + thien + atrazine	55 + 22 + 1121	0.5 abc	0.5 abc	0.5 abc	0.5 abc

S-metolachor	1420	0.4 abc	0.4 abc	0.4 abc	0.4 abc
S-metolachor + atrazine	875 + 1130	0.1 bc	0.1 bc	0.1 bc	0.1 bc
Tembotrione	92	1.3 a	1.3 a	1.3 a	1.3 a
Tembotrione + atrazine	92 + 560	1.7 a	1.7 a	1.7 a	1.7 a
Topramezone	18	0.4 abc	0.4 abc	0.4 abc	0.4 abc
Topramezone + atrazine	18 + 560	0.6 ab	0.6 ab	0.6 ab	0.6 ab
nontreated		1.6 a	1.6 a	1.6 a	1.6 a

[a.] Means within a column followed by the same letter are not significantly different according to Fisher's LSD procedure at $P \leq 0.05$.

[b.] All herbicides except topramezone and tembotrione applied with and without atrazine were applied PRE. Topramezone and tembotrione applied with and without atrazine were applied POST 28 DAIT along with methylated seed oil (MSO) and urea ammonium nitrate (UAN) at 1 and 1.25% v/v. All PRE herbicides were followed by glyphosate applied with 2200 g ha^{-1} ammonium sulfate POST 14 DAIT.

[c.] Abbreviation: DAIT, days after initial; PRE, preemergence; POST, postemergence; thien, thiencarbazone-methyl

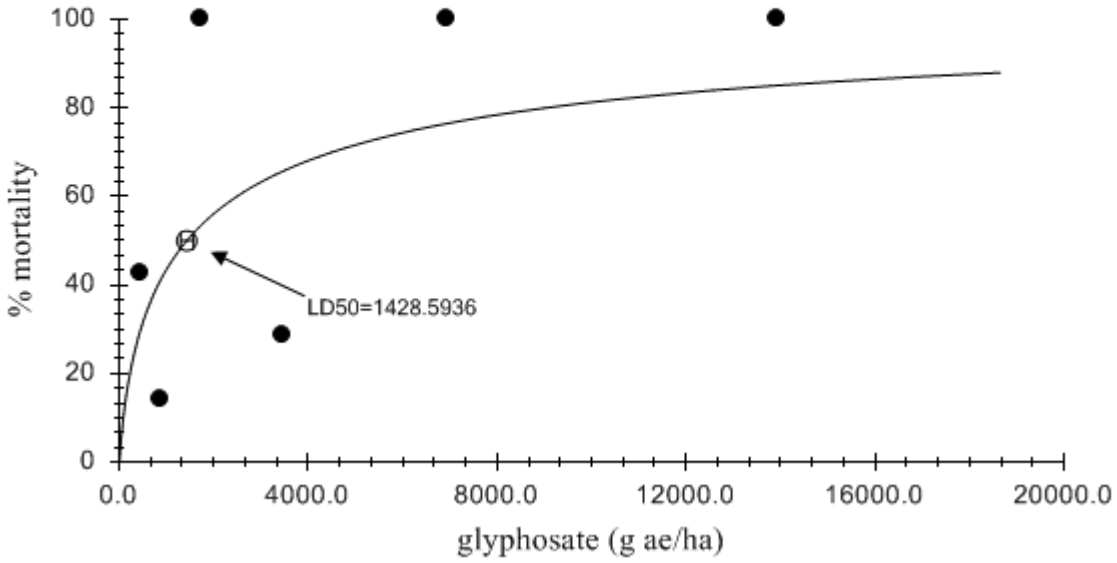

Figure 4.1. Glyphosate-resistant Palmer amaranth control in Hebron, MD.

$y = 1.5x + 1.7$ LD_{50} = 1,429 g ae ha^{-1}

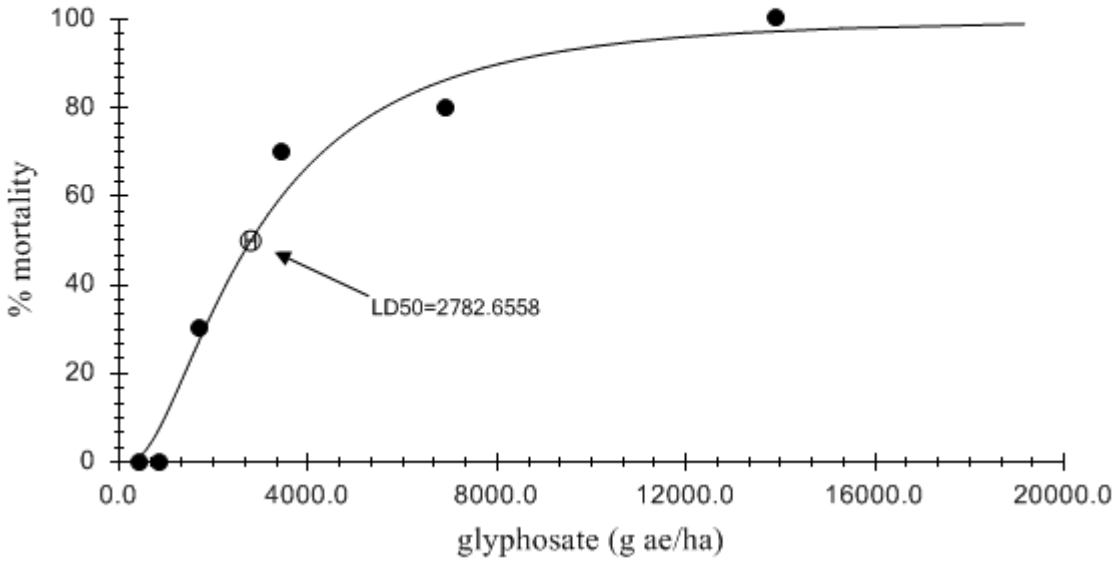

Figure 4.2. Glyphosate –resistant Palmer amaranth control in King William, VA.

$y = 2.8x - 4.6$ LD_{50} = 2,783 g ae ha^{-1}

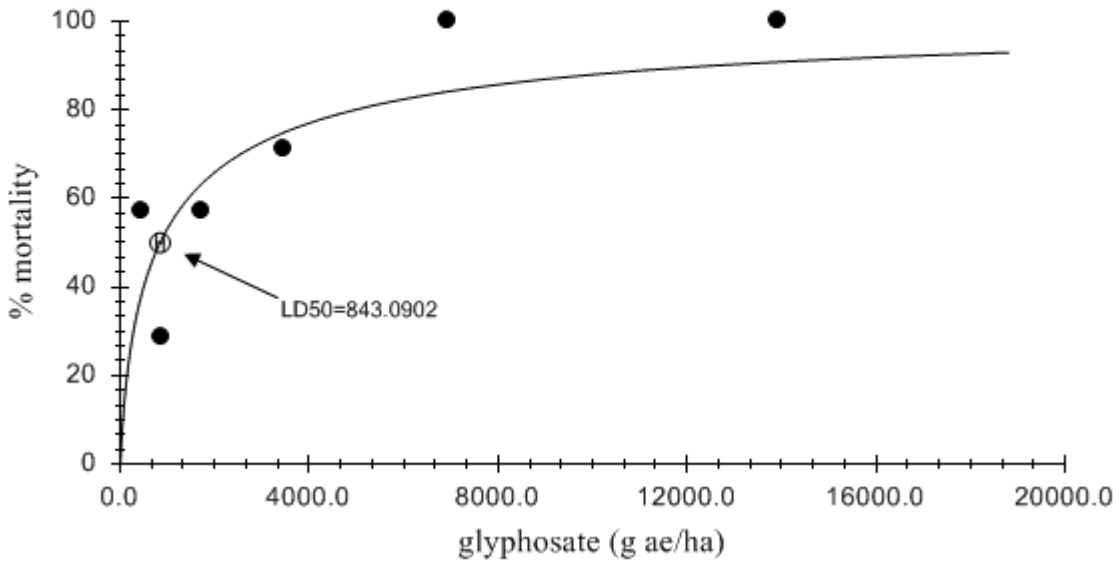

Figure 4.3. Control of glyphosate susceptible Palmer amaranth provided by Azlin Seed Service.

$y = 1.1x + 1.8 \quad LD_{50} = 843 \text{ g ae ha}^{-1}$

CHAPTER 5

ABSORPTION, TRANSLOCATION, AND METABOLISM OF GLYPHOSATE IN COMMON POKEWEED

Abstract

Common pokeweed is a problematic weed in no-till systems due to its perennial nature and associated regrowth following POST herbicide applications. A study was conducted to determine how ^{14}C labeled glyphosate is translocated and metabolized in pokeweed. The majority of absorbed radioactivity was found within treated leaf tissue with less than 5% of applied radioactivity found in non-treated leaves, fruits, stems, and taproots. This indicates that there is little basipetal translocation of glyphosate once it is applied to the plant, and this amount may not be enough to affect regrowth from the taproot.

Nomenclature: glyphosate; common pokeweed; *Phytolacca Americana* L.

Key Words: absorption, ^{14C}, taproot, translocation

Introduction

Mature common pokeweed can be especially difficult to control in no-till systems, especially when crops are planted later in the growing season when pokeweed plants are mature. Herbicides such as glyphosate, 2,4-D and dicamba have been shown to severely injure pokeweed (Sellers and Ferrell 2006), but they seldom provide complete control of the taproot allowing regrowth to occur later in the season or the next year. In addition, 2,4-D and dicamba are not currently available for postemergence control of pokeweed in most crops.

Pokeweed plants possess large taproots that store carbohydrates which can act as reserves during periods of dormancy and from which regrowth can occur (Wyka 1999). These taproots

can be up to 15 cm in diameter and grow to depths of more than 0.3 m (Anonymous 2011). During the growing season carbohydrates and other nutrients are moved from the taproot into new shoots, leaves, flowers and fruits. After fruit has set carbohydrates are translocated back into the taproot. Glyphosate and other foliar applied herbicides are systemic and translocated to sinks along with carbohydrates via the phloem (Grangeot et al. 2006, Kirkwood et al. 2000). If glyphosate is applied at a time when the net flow of carbohydrates is from taproots to foliage or floral structures, glyphosate may not move to the taproot in sufficient quantities to kill the plant.

Transport of glyphosate to the root system has been shown to prevent regrowth of deep-rooted and rhizomatous weeds (Bromilow and Chamerlain 2000). Applications of glyphosate in late summer or early fall have been shown to control perennial weeds such as cogongrass (*Imperata cylindrica*) (Dozier et al. 1998), johnsongrass (*Sorghum halepense*) (Norton and Merkle 1976), and blackberry (*Rubus* spp.) (Yonce and Skroch 1989); therefore, growth stage and timing of herbicide applications are important factors to consider in long term control of perennial plants (Grangeot et al. 2006). Systemic herbicides such as glyphosate or dicamba may more readily affect the taproot if sprayed later in the growing season to taller plants or after fruiting has occurred.

Preliminary studies have indicated that while glyphosate and other herbicides may cause injury to the aboveground pokeweed tissue, but plants are eventually able to recover via new shoot growth from the taproot (Appendices A, B, and C). The objective of this study was to evaluate how glyphosate is translocated and metabolized in pokeweed when applied at two different growth stages.

Materials and Methods

Pokeweed seedlings were collected at Virginia Tech's Glade Road research center in Blacksburg, VA and established under natural light and temperatures in 16.5 cm by 16 cm pots containing commercial potting mix (Pro Mix BX Mycorrhizae, Premier Tech Horticulture, Quakertown, PA) in the greenhouse at the Virginia Tech Eastern Shore AREC in Painter, VA. Plants were returned to Glade Road and allowed to acclimate to greenhouse prior to the onset of the experiment. Plants were watered daily and fertilized with Miracle-Gro All Purpose Plant Food 24-18-6 (The Scotts Miracle-Gro Company, Marysville, OH) every 7 to 14 days prior to experiment initiation.

Absorption and Translocation

Three leaves from each plant were shielded using a 1 cm wide strip of aluminum foil. Plants were sprayed-to-wet with a 2% solution of glyphosate (Roundup Pro Concentrate, Monsanto, St. Louis MO) using a handheld garden sprayer. Immediately following the herbicide application the foil was removed and a total of nine 1 µl droplets of ^{14}C-glyphosate spotting solution (33.3 KBq) were placed on the adaxial surface of the second fully expanded leaf, sixth leaf, and eighth leaf from the top of the plant in non-fruiting plants; and the second, sixth, and eighth leaf below the lowest fruiting structure in fruiting plants. The glyphosate was ^{14}C-phosphonomethyl-labeled (specific activity = 50 mCi/mmol, purity 99% by TLC). It was converted from acid to the isopropylamine salt by combining 500 µl of ^{14}C glyphosate solution with 2 µl isopropyl amine, and 0.8% MON 56164 (Monsanto Company, St. Louis, MO) (nonionic polyethoxylated tallow amine) surfactant.

Plants were harvested 5 and 10 days after treatment. The 1-cm wide treated section of each treated leaf was removed and placed in a scintillation vial containing 3 ml of water and vortexed for 30 seconds, the leaf was then removed and rinsed with 3 ml of methanol using a separate scintillation vial. Following the removal of the leaf, 15 ml of scintillation fluid

(ScinitiVerse LC Cocktail Scintanalyzed, Fisher Scientific, Pittsburgh, PA) was added to each leaf rinsate, and unabsorbed radioactivity quantified by liquid scintillation spectrometry (LSS) (LS 6500 multipurpose scintillation counter, Beckman Coulter Inc, Indianapolis, IN). The remainder of the plant was then divided into treated leaves, non-treated leaves, fruits, stems, and taproots. In order to minimize loss due to glyphosate degradation after harvest samples were kept at -20 C until processed.

Treated Leaf tissue was homogenized in 10 ml 4:1 cold acetonitrile: water using a tissue grinder. Non-treated leaves, fruit, stems, and roots were homogenized in 10 to 60 ml 4:1 cold acetonitrile using a Waring blender (Waring Commercial, Torrington CT). The homogenate was then rinsed into a vacuum filtration apparatus using an additional 10 ml of the same solvent. The residue was then rinsed again using an additional 5 ml of solvent and transferred to a 20 ml scintillation vial. The filtrate was then evaporated to dryness at 100 C using an N-EVAP 112 nitrogen evaporator (Organomation Associates Inc, Berlin, MA). Once dry, samples were re-suspended using 500 µl de-ionized water and vortexed for 30 seconds. A 20 µl aliquot was then combined with 15 ml scintillation fluid and counted using LSS. Dry samples were then stored at 0 C for the metabolism studies.

The residue filter papers of each section were oven dried at 70 C for 48 hours then combusted using a biological oxidizer (OX-700, R. J. Harvey Instrument Corporation, 123 Patterson St., Hillsdale, NJ 07642), and CO_2 trapped in a commercially available liquid scintillation cocktail (Carbon-14 Cocktail, R. J. Harvey Instrument Corporation, Tappan, NY). Combustion efficiency (91%) was determined by adding known amounts of radioactivity to untreated plant tissue prior to combusting. Total percent ^{14}C recovered was calculated by

combining the ^{14}C activity recovered from treated leaf washes, combusted filter paper, and the aliquot taken from the liquid extraction procedure.

Metabolism

100 µl of each sample of treated leaves, non-treated leaves, stems, and taproots from the liquid extraction was spotted on 20 by 20 cm silica gel thin-layer chromatography (TLC) plates. The solvent system consisted of 55 ml ethanol, 35 ml H_2O, 2.5 ml of 15N NH_4OH, 3.5 g TCA, and 2 ml of17 N acetic acid (Sprankle et al. 1978). Plates were partitioned into ten 2-cm wide lanes. A standard consisting of 2 µl of the previously mentioned ^{14}C-glyphosate solution was spotted on the first lane of each plate. The remaining lanes received a single replicate of treated leaf, non-treated leaf, stem, or taproot from each plant in the study. Following development, the plates were then air dried and placed on a Bioscan radiochromatogram scanner (Bioscan System 200 Imaging Scanner, Bioscan, Inc., NW Washington, D.C., 20007, USA) in order to determine radioactive portions and corresponding *Rf* values. Radioactive peaks were integrated using Win-Scan software (Lab Logic Win-Scan Radio TLC Version 2.2(5) 32-bit,BioScan, 4590 MacArthur Boulevard NW, Washington, DC 20007.) Unmetabolized ^{14}C glyphosate was identified by comparing the *Rf* value of the standard.

The study was arranged as a split plot design with harvest time as the main plot and factorial subplots of growth stage and plant parts. Data were converted to percent of applied ^{14}C and subjected to ANOVA in SAS 9.2 (SAS Institute, Inc. Cary, NC) with sums of squares partitioned to reflect a factorial split plot treatment structure and trial effects. Means were separated using Fisher's Protected LSD at p=0.05.

Results and Discussion

There were no significant growth stage by harvest interactions (P = 0.15) but there were significant differences in ^{14}C recovered from each plant section (P = 0.0002). Treated leaves yielded the most radioactivity for metabolism study; however, variance in the data could not be analyzed due to being either 0 or 100% loaded.

Approximately 55% of the ^{14}C applied was unabsorbed, while 25% of the ^{14}C applied was absorbed by the plants (Table 5.1). This level of absorption is typical for this type of study, as past research has shown foliar absorption of glyphosate to be 25-50% of the amount applied (Sandberg et al. 1980). The amount of glyphosate absorbed may be related to the specific leaf characteristics of the species being examined. Epicuticular waxes can pose a barrier to herbicide uptake (Baker et al. 1980, Levene et al. 1995, White et al. 2002). The glyphosate molecule is negatively charged at physiological pH, and does not readily penetrate a waxy layer (Franz 1985). Similarly, Chachalis et al. (2001) reported higher glyphosate efficacy in trumpetcreeper (*Campsis radicans* [L.] Seem. Ex. Bureau) compared to redvine (*Brunnichia ovata* Walt. Shinners) due to the differences in the hydrophobic nature of the epicuticular waxes and the hydrophilic nature of the glyphosate molecule.

Although, 25% of the ^{14}C applied was absorbed, 15% remained in the treated leaf, and 10% was translocated to other structures. This level of glyphosate translocation in pokeweed is similar to that found in old world climbing fern (*Lygodium microphyllum* [Cav] R. Br.) (Hutchinson et al. 2010), and redvine (Reddy 2000) (Table 5.2). The efficiency of glyphosate translocation is dependent on sink-source relationships (Sandburg et al. 1980, Walker and Oliver 2008). Glyphosate is phloem mobile and travels systemically throughout the plant, accumulating in areas of new growth.

The amount of ^{14}C found in the taproot does not suggest that glyphosate moves to the taproot in large quantities. This and preliminary studies suggest that even though glyphosate is effective in killing aboveground tissue, it is not translocated downward where it can affect regrowth from the taproot. This low level of translocation may be species specific as previous studies have indicated higher levels of basipetal translocation of ^{14}C-glyphosate to the taproots of other perennial weeds. Sandberg et al. (1980) were able to recover 59% of foliar applied ^{14}C from the roots of Canada thistle (*Cirsium arvense* L.), and Wyrill and Burnside 1976) were able to recover 28% of the applied ^{14}C in common milkweed (*Asclepias syriaca* L.).

The metabolism data suggested that all ^{14}C recovered was a metabolite of glyphosate. This is unlikely since glyphosate has been found to metabolize slowly in plants (Coupland 1984). In contrast, Sandberg et al. (1980) identified 80% of the ^{14}C recovered from tall morningglory (*Ipomoea purpurea* (L.) Roth) as glyphosate, and Gottrup et al. (1975) found no glyphosate metabolites in Canada thistle.

A total of 80% of the applied ^{14}C was recovered from plant tissue. It is possible that a portion of the ^{14}C applied was exuded by the roots, metabolized by soil organisms, and released as $^{14}CO_2$. Previous studies have reported that glyphosate is metabolized to CO_2 by microbial populations following glyphosate inactivation and binding to soil particles (Haney et al. 2000, Sprankle et al. 1975, Rueppel et al. 1977) Although, soil samples were combusted to determine radioactivity, they did not yield sufficient amounts of radioactivity to account for the losses observed. In addition, as aboveground tissue became necrotic it is possible that some ^{14}C was lost as $^{14}CO_2$ due to the process of tissue decay. Additional studies need to be performed to confirm whether this minute amount of ^{14}C in the taproot is indeed glyphosate, whether or not it

has an effect on bud mortality, and how much $^{14}CO_2$ is lost via tissue decay or by exudation into the rhizosphere.

While glyphosate may be effective in eliminating the aboveground biomass of pokeweed, this study shows that there is little basipetal translocation of the herbicide once it is absorbed by leaf tissue. This low level of glyphosate translocation indicates that the herbicide may not be effective in controlling the taproot. With the taproot intact, the plant will continue to recover following a single glyphosate application. Therefore, multiple applications may be needed to both eliminate aboveground biomass and exhaust carbohydrate reserves provided by the taproot.

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Table 5.1. Percentage of applied radioactivity recovered from plants after foliar treatment of ^{14}C-glyphosate, averaged over all trials.[a]

Recovery area or description	%
Nonabsorbed	55 a
Treated Leaves	15 b
Non-treated Leaves	5 c
Fruit	2 c
Stem	2 c
Taproot	1 c
Recovery	80

[a.] Data pooled over growth stage (non-fruiting and fruiting). Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD test at $P \leq 0.05$.

Table 5.2. Comparison of ^{14}C glyphosate recovered from five weed species.

Species	Absorbed	Leaf Wash	Treated Leaf	Translocated	Citation
			%		
Pitted morningglory	44	56	19	25	Kroger and Reddy 2005
Pokeweed	25	55	15	10	Vollmer et al. 2014
Old world climbing fern	31	34	25	6	Hutchinson et al. 2010
Sicklepod	68	32	53	16	Walker and Oliver 2008
Redvine	22	78	14	8	Reddy 2000

Appendix A

Pokeweed Control 2010

Materials and Methods

Two field studies were conducted in non-crop areas to evaluate various corn and soybean herbicides for POST control of common pokeweed. The first trial was established in Machipongo, VA on May 5, 2010 and a second complimentary trial was established in Painter, VA on May 7, 2010. The soil was a Bojac sandy loam characterized to have 55% sand, 37% silt, and 8% clay with < 2% organic matter and pH of 5.5.

Trials were arranged as a completely randomized design with 7 treatments (Table A.1) and 4 replications with a single plant representing a replication. A nontreated check was also included in each trial for comparison. Applications were made using a propane-pressurized backpack sprayer with a 1.5 m boom and three XR 11003 nozzles flat fan nozzles delivering 186 L ha^{-1} to mature, non-flowering plants averaging 40 cm in height.

Visual assessments of pokeweed control were made on a scale of 0 (no control) to 100 (plant death) 3 and 4 weeks after treatment (WAT). Data were analyzed in PROC GLM SAS 9.2 (SAS Institute, Inc. Cary, NC). Data were tested for homogeneity of variance prior to ANOVA and transformed to the arcsine of the square root where needed to stabilize variance. If transformation was needed prior to ANOVA, subsequent means were back transformed for presentation. Sums of squares were partitioned to reflect trial and treatment effects and interactions. Main effects and interactions were tested against the means square error of the appropriate trial interaction. If no trial interactions were significant, data were pooled. Appropriate means were separated using Fisher's Protected LSD test at $P = 0.05$.

Results and Discussion

The interaction between location and treatment was significant ($P < 0.0001$), so data is presented separately for both years. Glyphosate controlled pokeweed 95% at the Painter site 4 WAT (Table A.2). All other treatments provided less than 65% control by 4 WAT regardless of site. Although, injury symptoms were observed with each herbicide application, most plants showed signs of recovery and had begun to flower by 5 WAT.

Nolte et al. (2002) reported glyphosate controlled pokeweed 88% at the end of the growing season, regardless of application rate and timing. However, the use of glyphosate in-crop is dependent on the crop itself being glyphosate resistant. The ALS-inhibiting herbicides halosulfuron, chlorimuron, and cloransulam are often used in corn and soybeans and can be used as an alternative to glyphosate. This study shows that while these compounds do cause some injury to pokeweed, mature plants are able to recover from herbicide injury. Sprague (2007) reported poor to fair control with chlorimuron, fair control with chlorimuron + thifensulfuron, and poor control with cloransulam. One explanation may be that the pokeweed may have been too large to be effectively injured by these ALS-compounds. VanGessel (1999) reported that cloransulam controlled seedling pokeweed 96% 5 WAT. However, Hoss et al. (2003) showed that the ALS-inhibiting herbicide imazethapyr was less effective in controlling weeds larger than 8 cm in height, and Shaw et al. (1990) showed that johnsongrass control was less than 64% when imazethapyr applied to plants greater than 30 cm. Another caveat is that these herbicide applications may be sufficient in controlling above-ground biomass, but do nothing to control the perennial taproot from which new plants can emerge. Even plants treated with glyphosate were able to recover before the end of the season.

Table A.1. Herbicides used for pokeweed control in Machipongo, VA and Painter, VA.

Herbicide	Trade Name	Rate	Manufacturer	City	State
		g ai ha^{-1}			
Chlorimuron	Classic	125	DuPont Crop Protection	Wilmington	DE
Chlorimuron + flumioxazin + thifensulfuron	Envive	16 + 45 +6	DuPont Crop Protection	Wilmington	DE
Chlorimuron + thifensulfuron	Synchrony XP	16 + 2	DuPont Crop Protection	Wilmington	DE
Cloransulam	FirstRate	18	Dow AgroSciences	Indianapolis	IN
Glyphosate	Roundup PowerMax	841	Monsanto Company	St. Louis	MO
Halosulfuron	Sandea	527	Gowan Company	Yuma	AZ
Thifensulfuron	Harmony Extra SG	3	DuPont Crop Protection	Wilmington	DE

Table A.2 Pokeweed Control in Machipongo, VA and Painter, VA in 2010.[a]

		Pokeweed control			
		3 WAT[c]		4 WAT	
Herbicides[b]	Rate	MP	PT	MP	PT
	g ai ha^{-1}	%			
Chlorimuron	125	48 ab	49 c	44 bc	48 c
Chlorimuron + flumioxazin + thifensulfuron	16 + 45 +6	55 a	56 b	63 a	60 b
Chlorimuron + thifensulfuron	16 + 2	48 ab	45 cd	44 bc	48 c
Cloransulam	18	5 d	35 e	3 d	30 d
Glyphosate	841	55 a	90 a	61 a	95 a
Halosulfuron	527	36 c	46 cd	35 c	44 c
Thifensulfuron	3	44 bc	43 d	45 b	46 c

[a.] Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD test at $P \leq 0.05$.

[b.] Chlorimuron plus flumioxazin plus thifensulfuron, cloransulam, and halosulfuron were applied with nonionic surfactant (NIS) at 0.25% v/v. Chlorimuron, chlorimuron plus thifensulfuron, and thifensulfuron were applied with NIS and urea ammonium nitrate at 0.25% and 1.25% v/v, respectively

[c.] Abbreviation: MP, Machipongo, VA; PT, Painter, VA; WAT, weeks after treatment.

Appendix B

Pokeweed Recovery from PRE and POST-BLOOM Glyphosate Applications

Materials and Methods

A field study was conducted to evaluate recovery of perennial pokeweed after glyphosate applications at two growth stages. The trial was established in a non-crop area in Painter, VA where pokeweed had been present in the same location for a number of years. The study consisted of a completely randomized design with two treatments (non-fruiting and fruiting growth stages) and 8 replications. A 2% solution of glyphosate (Roundup PowerMax, Monsanto, St. Louis, MO) was applied using a solo backpack sprayer (Solo USA, Newport News, VA) to mature pokeweed plants on August 14, 2012 before fruit set and again in November 12, 2012 after fruit set. All plants were flagged in order to mark their location for the following year. These areas were then evaluated the following spring for signs of recovery based on whether or not regrowth occurred at each location.

Results and Discussion

The majority of aboveground plant tissue became necrotic within 2-3 weeks of glyphosate application (Figure B.1). However, by spring of 2013, all pokeweed was able to recover regardless of application timing (Figure B.2), and showed no signs of glyphosate injury from the previous year (Figure B.3). Therefore, it is possible that while glyphosate may be successful in controlling the aboveground portion of pokeweed, it will not successfully in controlling the underground taproot in perennial pokeweed. In contrast, Patches and Curran (2014) reported that applications of glyphosate on pokeweed after mid-June provided at least 93% control 8

WAT and at least 80% control the following spring. Our study indicates that a single glyphosate application may be ineffective against perennial pokeweed regardless of growth stage. Therefore, multiple applications at different growth stages may be needed to effectively kill the underground taproot.

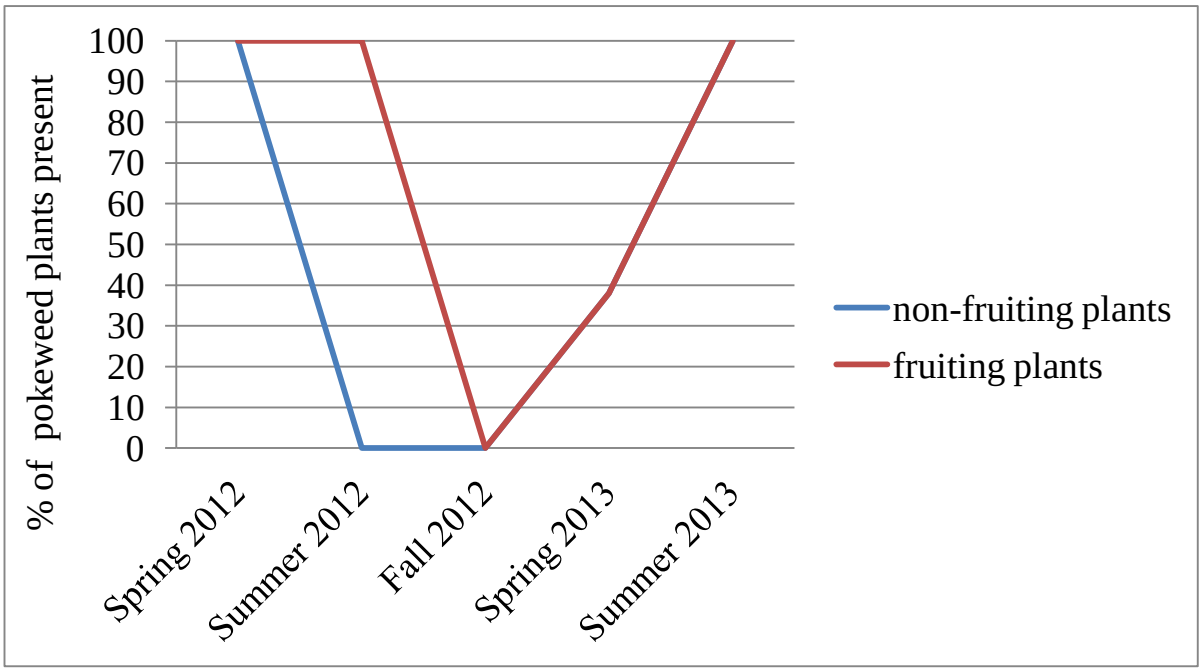
Figure B.1. Pokeweed response to glyphosate 3 WAT for (A) non-fruiting and (B) fruiting plants.

A) non-fruiting pokeweed B) fruiting pokeweed

Figure B.2. Recovery of pokeweed 1 year after glyphosate treatment (A) before fruiting and (B) after fruiting.

A) non-fruiting pokeweed B) fruiting pokeweed

Figure B.3. Pokeweed recovery from glyphosate application (2012-2013)



Appendix C

Pokeweed Taproot Response to Glyphosate under Greenhouse Conditions

Materials and Methods

A study was conducted to evaluate the response of pokeweed taproots over time to a single application of glyphosate. Mature, fruiting pokeweed plants were established in the same way as the translocation and metabolism study. The study consisted of a completely randomized design with two plants per harvest and six harvests. A non-treated check was included at each harvest time for comparison. The trial was initiated on August 8, 2014 by applying a 2% glyphosate solution (Roundup Pro Concentrate, Monsanto Company, St. Louis, MO) to all pokeweed plants in the study except the non-treated control. Plants were harvested 5, 10, 15, 20, 25, and 30 days after treatment (DAT). Fresh weights of taproots were recorded at each harvest. Data were tested for homogeneity of variance prior to ANOVA. Sums of squares were partitioned to reflect trial and treatment effects and interactions. Main effects and interactions were tested against the means square error of the appropriate trial interaction. If no trial interactions were significant, data were pooled. Appropriate means were separated using Fisher's Protected LSD test at P = 0.05.

Results and Discussion

The interaction of harvest date and treatment was not significant for pokeweed fresh weight (P = 0.16), so data was pooled over each harvest time. Visible chlorosis of aboveground tissue was observed in all plants at all rating dates, with all aboveground tissue being dead by 15 DAT. There were no significant differences in the fresh weights of treated and nontreated plants (Table C.1). However, the taproots of treated plants began to exhibit signs of decay by 15 DAT compared to the non-treated plants (Figure C.1). This could indicate that over a longer period of

time the underground portion of the pokeweed will may die off as well. However, these plants were less than a year old and acclimated to greenhouse conditions, so glyphosate may not be as effective on larger pokeweed in the field where the taproot has been established for a longer period of time.

Table C.1. Fresh weights of pokeweed taproots following glyphosate application averaged over 30 days.[a]

	Pokeweed Fresh Weight
	g
Treated	35.1 a
nontreated	45.6 a

[a.] Means with the same letter in a column are not significantly different according to according to Fisher's LSD test at $P \leq 0.05$.

Figure C.1. Comparison of nontreated (A) and treated (B) 15 DAT

A) Non-treated B) Treated