PALMER AMARANTH CONTROL IN ESTABLISHED ALFALFA AND DOCUMENTATION OF GLYPHOSATE-RESISTANT *AMARANTHUS* SPECIES IN KANSAS

by

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Abstract

Palmer amaranth is a troublesome weed that competes for water, nutrients, and sunlight in many cropping systems throughout the United States. It is a serious production problem for alfalfa growers in the southern Great Plains region because of extended germination and impact on forage quality and yields. Glyphosate has been used extensively to control Palmer amaranth but control has become difficult. The objectives of this research were to (1) evaluate various herbicide treatments for Palmer amaranth control in established alfalfa, (2) confirm the presence and scope of glyphosate-resistance in common waterhemp and Palmer amaranth populations in eastern Kansas, and (3) to characterize glyphosate-resistance in two Palmer amaranth populations from south central Kansas. Residual Palmer amaranth control in alfalfa varied among herbicide treatments. The best late season Palmer amaranth control was accomplished with sequential treatments that included flumioxazin at 140 g ha^{-1} or diuron at 2,690 g ha^{-1} as dormant applications followed by a between cutting treatment of flumioxazin at 70 g ha^{-1}, which was still providing 85 to 96% control in late summer. Several other treatments provided good early season Palmer amaranth control, but control diminished as the season progressed. Palmer amaranth emerges throughout the growing season and therefore, sequential herbicide treatments with good residual activity may be necessary for season-long control. Greenhouse studies indicated that glyphosate-resistant common waterhemp is present throughout eastern Kansas with several populations that survived glyphosate up to two times the suggested use rate. Glyphosate-resistant Palmer amaranth was documented in several populations collected from various counties throughout Kansas. Two populations collected in south central Kansas in 2011 survived up to eight times the typical field use rate of glyphosate. Six more populations collected in 2012 displayed similar resistance characteristics with three populations surviving up

to four times the typical rate of glyphosate. Shikimate assays on susceptible and resistant Palmer amaranth biotypes confirmed resistance to glyphosate.

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Chapter 1 – Review of Literature

Introduction

Weeds have the potential to cause billions of dollars' worth of crop yield loss each year in the United States (Bridges 1992). Pigweeds *(Amaranthus* spp.) are common broadleaf weeds that infest field crops throughout the United States and the world (Gossett and Toler 1999). Research has shown that significant yield reductions in soybean [*Glycine max* (L.) Merr.], corn (*Zea mays* L.), grain sorghum [*Sorghum bicolor* (L.) Moench ssp. Bicolor], and peanut (*Arachis* hypogea L.) have been a direct result of Palmer amaranth interference (Bensch et al. 2003; Burke et al. 2007; Klingaman and Oliver 1994; Massinga et al. 2001; Moore et al. 2004). However, losses in yield and forage quality occur in hay crops around the world as well. Data generated from Swanton et al. (1993) showed that millions of dollars in annual losses were caused by weeds in 58 different commodities in Canada; approximately 50% of total loss occurred in hay crops in Eastern Canada.

Palmer Amaranth *(Amaranthus palmeri* (S.) Wats.*)*

There are nearly 75 species in the genus *Amaranthus*, part of the Amaranthaceae family, worldwide (Steckel 2007). The word describing the genus *Amaranthus* is derived from the Greek word "amarantus," which means "everlasting" or "never failing flowers" (Steckel 2007). Within this large genus, there is a group of 10 species that are dioecious. Dioecious refers to species having male and female reproductive organs on separate plants. Unlike the monoecious *Amaranthus* spp., the dioecious *Amaranthus* spp. are all native to North America. The dioecious *Amaranthus* spp. all share a combination of characteristics that occur in only a small number of monoecious species, which are a pentamerous staminate flower together with complex terminal

inflorescences, often called spikes (Steckel 2007). One of the most competitive and highly invasive pigweeds in the United States is Palmer amaranth (*Amaranthus palmeri*).

Palmer amaranth is a serious production problem for many growers in the southern Great Plains region because of its competitiveness and effect on agricultural production. Palmer amaranth can germinate throughout the growing season and thus, result in multiple flushes of new seedlings. Because of multiple Palmer amaranth flushes through the growing season, season-long control is difficult. This weed grows rapidly and can reach two meters or more in height (Horak and Loughin 2000). According to Ehleringer (1983), Palmer amaranth has a high photosynthetic capacity and utilizes the C_4 photosynthetic pathway. In addition to this, leaves of Palmer amaranth have the ability to solar track (Ehleringer 1983). This indicates that under low water stress conditions, the plant maintains high photosynthetic rates because of the high photosynthetic capacity. Although, many pigweed species are found in wetland areas, Palmer amaranth can survive in many dry areas as well. It is suspected that there are three types of adaptions to these dry conditions. Broadly categorized, these would include 1) rapid germination characteristics, 2) rapid growth characteristics, and 3) drought tolerance mechanisms (Ehleringer 1983). These features make Palmer amaranth a fierce competitor in our crop production systems.

Palmer amaranth is commonly confused with other pigweed species and can be difficult to distinguish in the seedling stage, causing identification and communication problems (Horak and Peterson 1995). Under favorable conditions, Palmer amaranth can germinate early in the growing season, grow very rapidly under a long germination window, and produce a vast amount of seed that is transported very easily. Keeley et al. (1987) explored the growth and seed production of Palmer amaranth and reported female plants produced 62,000 to 600,000 seeds

when grown without competition. Since female Palmer amaranth plants can produce this many seeds, good weed management strategies are critical.

Palmer amaranth competes effectively for light, water, and nutrients (Massinga et al. 2001) and can significantly reduce crop yields. Two factors that influence the competition of Palmer amaranth on field crops are 1) weed density and 2) time of emergence. These two factors can ultimately lower crop yields and impede crop harvest. Bensch et al. (2003) found that the weed density-yield loss response of soybean was greatest for Palmer amaranth compared to common waterhemp, redroot pigweed, and prostrate pigweed and that pigweeds emerging with soybean were more competitive than pigweed plants emerging later. Palmer amaranth that emerged with the soybeans caused 79% yield reductions at 8 plants m^{-1} of row density (Bensch et al. 2003) and closely relates to the research findings from Klingaman and Oliver (1994) who reported soybean yield reductions ranging from 17 to 68% for Palmer amaranth densities of 0.33 to 10 plants m^{-1} of row. This is further supported by the research findings from Massinga et al. (2001) who showed that Palmer amaranth emerging with corn reduced yield from 11 to 91% as density increased from 0.5 to 8 plants m^{-1} of row (Massinga et al. 2001). Differences in the competitive ability of pigweed species do exist and need to be considered when implementing control measures.

Common Waterhemp *(Amaranthus rudis* Sauer*)*

Common waterhemp is a troublesome weed throughout the Midwestern United States. Common waterhemp can be found from Texas to Maine (Nordby et al. 2007) and is becoming difficult to manage in agronomic crops. Common waterhemp has biotypes with resistance to many common herbicides used in production systems making this pigweed species difficult to

control. The first reported case of common waterhemp resistance to triazine herbicides was in southeast Nebraska in 1990 (Anderson 1996), while resistance to ALS-inhibiting herbicides was first confirmed in a biotype of common waterhemp found in northeast Kansas in 1993 (Horak and Peterson 1995). The introduction of Roundup Ready® soybean in 1996 helped many farmers regain control of waterhemp; however, this weed possesses many traits that continue to make it a formidable foe in any management system (Nordby et al. 2007). Therefore, the recommended management strategies for herbicide-resistant weed populations include an integrated system of crop rotation, rotation of herbicide modes of action, tank-mixes of herbicides with different modes of action, and cultivation (Peterson 1999).

Common waterhemp, as the name implies, thrives in wet areas of fields, but is adapted to a variety of conditions (Nordby et al. 2007). Like Palmer amaranth, common waterhemp is a member of the pigweed family *(Amaranthus* spp.) and has similar distinguishing features that make this weed unique. Common waterhemp is a dioecious plant that can produce up to two million seeds per female plant (Battles et al. 1998). Waterhemp seeds generally germinate early in the growing season, within 305 growing degree days (base temperature of 10 C), but can emerge until fall (Sellers et al. 2003). Waterhemp plants emerge throughout the growing season, and a higher percentage of plants emerge later in the season than most other summer annual weeds (Hartzler et al. 1999). Once the plant breaks through the soil surface, the growth rate for common waterhemp can be almost 1 inch per day if conditions are favorable. Research findings from Sellers et al. (2003) showed that common waterhemp began emerging between 14 and 17 days after planting whereas Palmer amaranth emerged within five days after planting. Understanding weed seedling emergence, growth rates, and productivity capabilities can be beneficial when considering a weed management strategy.

Once common waterhemp has emerged and begins to compete with a particular crop, yields can be significantly reduced. Season-long competition by common waterhemp at more than 20 plants per square foot reduced soybean yields 44% in 30-inch rows and 37% in 7.5-inch rows (Steckel and Sprague 2004). Waterhemp that emerged as late as the V5 soybean growth stage reduced yields up to 10% (Nordby et al. 2007). Waterhemp can also reduce yields in corn. Season-long competition of common waterhemp at 82 or less plants/m^2 caused 10% corn yield loss and season-long interference at 369 to 445 plants/m^2 caused 36% yield reduction (Cordes et al. 2004).

Plant Responses to Herbicides

Phytotoxic chemicals that are used for weed control are termed herbicides (Anderson 1996). A herbicide has been defined as "any chemical substance or cultured biological organism used to kill or suppress plant growth" (Anderson 1996). Since their introduction, application of herbicides has been a reliable and economic alternative for weed control (Huarte and Arnold 2003). The shift from conventional tillage to no-tillage systems has increased the reliance on herbicides for weed control. Over the past 50 years, repeated use of the same herbicides with similar modes of action has imposed selection for increased herbicide-resistance within or among species that had been susceptible (Holt and LeBaron 1990).

There are three types of plant responses to applied herbicides, and they are typically characterized as: 1) susceptibility, 2) tolerance, and 3) resistance (Anderson 1996). Susceptibility is the lack of capacity to withstand herbicide treatment so that the plant is damaged by herbicides (Holt and LeBaron 1990). Anderson (1996) described susceptibility as a positive response to an applied herbicide and the degree of the response was a measure of a

plant's susceptibility to the applied herbicide, under the conditions involved. Smaller plants are generally more susceptible than taller plants because older, taller plants have the ability to metabolize the herbicide before injury occurs.

The terms tolerance and resistance have been used interchangeably because both describe a condition whereby a plant withstands an herbicide. A working definition of tolerance is the ability of a crop plant to withstand a predetermined dosage of an herbicide, which may be overcome by higher dosages (Anderson 1996). The term tolerance was most often used to designate crop response to an herbicide, and a "tolerant crop" was one that was not significantly injured by an herbicide applied at a recommended dosage (Anderson 1996). Herbicide resistance refers to a plants inherited ability to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild biotype (WSSA 2013). However, resistance is dependent on the selection or evolution of a mutant mechanism within a biotype that allows it to withstand repeated exposure to an herbicide (Anderson 1996; Holt and LeBaron 1990). The widespread distribution of herbicide resistance is a growing concern for farmers throughout the United States and the world.

Glyphosate

Since its introduction in mid-1970, glyphosate (N-(phosphonomethyl) glycine) has provided a broad-spectrum of weed control at a cost effective rate for many farmers. The introduction of genetically modified (GM) crops in 1996 containing genes for glyphosate-resistance differed from conventional crops in that glyphosate herbicide could be applied for in-crop weed control. This was a beneficial addition for farmers because glyphosate was better at controlling larger weeds, has no soil activity (allowing for flexible crop rotations), and has low

environmental and human health risks (Boerboom and Owen 2006). Over the years, glyphosate use has increased the number of acres planted to Roundup Ready® crops; however, it has also increased the potential for selecting glyphosate-resistant (GR) weed biotypes throughout the world.

Glyphosate is an inhibitor of the shikimic acid pathway. The shikimic acid pathway is crucial for the production of three essential amino acids: tryptophan, tyrosine, and phenylalanine. The mechanism of action is also unique in that the binding site for glyphosate is reported to closely overlap with the binding site of PEP (Dill et al. 2010, Franz et al. 1997). Glyphosate has the ability to translocate to growing meristematic tissues and affect underground meristems, corms, rhizomes, and other potential vegetative structures, which regenerate when only upper vegetative material is killed (Dill et al. 2010). Glyphosate binds tightly to soil through chelation and therefore, has no soil activity. Glyphosate has primarily been adopted because of effectiveness, low cost, and simplicity. However, resistance to glyphosate is becoming a growing concern.

History of Glyphosate Resistance

Roundup Ready® soybean and corn acreage has been steadily increasing since their introduction and the number of resistance issues concerning glyphosate applications has increased as well. There are currently 24 weed species that have developed resistance to glyphosate (Heap 2013). Confirmation of glyphosate-resistant Palmer amaranth and common waterhemp has been documented since 2005 in the United States (Heap 2013). Palmer amaranth and common waterhemp are among the most resistant-prone dicots, with resistance now confirmed to four herbicide modes of action in the United States (Norsworthy et al. 2008, Heap

2013). In 1998, one waterhemp population was confirmed with multiple resistances to both acetolactate synthase (ALS) - and photosystem II (PSII)-inhibiting herbicides (Boerboom and Owen 2006). A waterhemp biotype in Kansas was documented to be approximately 34, 82, 8, and 4 times more resistant than a susceptible common waterhemp biotype to acifluorfen, lactofen, fomesafen, and sulfentrazone, respectively (Shoup et al. 2003). More recently, waterhemp garnered the distinction of being the first U.S. weed to develop multiple resistances: these combinations include resistance to ALS-, PSII-, and Protoporphyrinogen oxidase (PPO)-inhibiting herbicides; and glyphosate, ALS-, and PPO-inhibiting herbicides (Boerboom and Owen 2006). This genetic diversity causes severe management issues in agronomic systems.

A weed's potential for developing glyphosate resistance is primarily guided by three factors: weed biology, intensity of glyphosate use, and glyphosate rate (Boerboom and Owen 2006). Glyphosate has been an alternative herbicide that easily controls Palmer amaranth, including those plants resistant to ALS-inhibiting herbicides, making glyphosate ideal for Palmer amaranth control in glyphosate-resistant crops (Norsworthy et al. 2008). Glyphosate-resistant Palmer amaranth was confirmed in Arkansas in 2005. The resistant biotype had an LD_{50} (lethal dose of herbicide needed to kill 50% of the plants) of 2,820 g ha^{-1} glyphosate, which was 79- to 115-fold greater than that of susceptible biotypes and 3.4 times a normal glyphosate-use rate of 840 g ha^{-1} (Norsworthy et al. 2008). This research conducted by Norsworthy et al. (2008) confirms that a Palmer amaranth biotype from Mississippi County, AR, had evolved resistance to glyphosate and that glyphosate alone was no longer a viable option for control of this resistant biotype.

Further research was conducted by Sosnoskie et al. (2011) confirming multiple resistance in Palmer amaranth to glyphosate and pyrithiobac in Georgia. Glyphosate at 6,930 g ha^{-1} and pyrithiobac at 420 g ha^{-1} applied alone provided no more than 89 and 65% control 1 to 8 weeks after treatment (WAT), respectively. The dose-response analyses developed from greenhouse data indicated that the estimated glyphosate rates required to cause 50% injury and reduce plant fresh weights by 50% relative to the non-treated control in a suspected glyphosate- and ALS-resistant Palmer amaranth biotype were 12 and 14 times greater, respectively, than the estimated values for the susceptible (S) biotype (Sosnoskie et al. 2011).

A more diagnostic screening method for documenting glyphosate resistance involves extracting EPSPS (5-enolpyruvoylshikimate-3-phosphate synthase), which is the site of action of glyphosate (Shaner et al. 2005; Steinrucken and Amrhein 1980). When glyphosate inhibits EPSPS there is an accumulation of shikimate, the dephosphorylated substrate of the enzyme (Amrhein et al. 1980; Shaner et al. 2005). This method has been used to detect resistance in crops and also used to detect resistance in GR-weeds (Shaner et al. 2005). Shikimate did not accumulate in a GR-rigid ryegrass population treated with glyphosate (Simarmata et al. 2003) or in a GR-horseweed population treated with a sublethal rate of glyphosate, although the same rate did cause shikimate accumulation in a susceptible horseweed population (Feng et al. 2004). Steckel et al. (2008) documented Palmer amaranth in Tennessee where shikimate accumulated in both resistant and susceptible plants, indicating that 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) was inhibited in both types. The results suggest that an altered target site is not responsible for glyphosate resistance in these Palmer amaranth biotypes. The shikimate assay procedure requires access to biochemistry lab equipment that is difficult to access or often times unavailable at the field level. Multiple methods of assessing glyphosate resistance help

confirm the occurrence of glyphosate-resistant weeds and provide insight on the mechanism of resistance.

Alfalfa (*Medicago sativa* L.)

Weeds are a primary production factor that can decrease the quality of alfalfa, lower crop yields, interfere with harvest, and decrease the profitability of the crop. Weeds interfere with alfalfa during establishment and throughout the duration of its life, reducing dry matter yields, and plant persistence by competing for light, water, and nutrients (Arregui et al. 2001). Because of its importance among forage crops, alfalfa is referred to as the "Queen of Forages." Alfalfa can be one of the most profitable agronomic crops based on the quality of the harvested crop. Alfalfa is a high-yielding, perennial legume that is well-suited to hay, silage, or pasture production and produces an excellent quality, high-protein forage (Hancock et al. 2005). However, alfalfa can be a difficult crop to establish especially if weeds are present and environmental conditions are not favorable.

There are many factors that can affect alfalfa production. Spring regrowth is an important process for alfalfa production, but can vary based on environmental conditions and primary establishment of the alfalfa plant itself. An alfalfa plant will become dormant as the temperatures become colder to ensure winter survival. Most winter-hardy alfalfa varieties have several nodes placed below the soil surface which aids in winter survival by providing soil insulation for the perennial over-wintering crown structures (Meyer 1999). "Green-up" occurs when the buds located on the crown begin to grow in response to warm spring temperatures (Undersander et al. 2011). The first cutting harvest will usually yield more tonnage per hectare because there is less stress early in the growing season. Heat stress, drought, weed competition,

and lack of nutrients decrease alfalfa growth. Drought reduces stem growth more than leaf growth, causing shortened plants that are low-yielding but generally high in quality because of the increased leaf-to-stem ratio (Undersander et al. 2011).

There are several other factors that can affect an alfalfa stand. Increasing age of stand, too many cuttings during the growing season, untimely fall harvesting, and overuse by grazing animals often result in one or all of the following: reduced yields, limited root growth, increased winterkill and/or injury, thinning of stands, grass and weed invasions, and increased disease susceptibility (Meyer and Helm 1994). A six year study conducted in Fargo, ND to evaluate stand age effects on alfalfa productivity showed that forage yields averaged 12 tons of dry matter ha^{-1} during the first harvest year, 10 tons ha^{-1} in the second year, and 9 tons ha^{-1} in the third year, a decrease of 3 tons ha^{-1} between first and third production years (Meyer and Helm 1994). Tonnage can be important when maximum yield is the main goal however, quality can decrease with increased tonnage.

The number of cuttings obtained from an alfalfa stand depends on the available soil water for regrowth (Meyer and Helm 1994). The first crop should be harvested by the 10% bloom stage (late bud to early bloom), whereas the third cutting should be harvested at 10 to 50% bloom to allow buildup of root reserves to aid in overwintering, and forage will be of high quality (Meyer and Helm 1994). Each cutting should be harvested at about 5 cm from the soil surface to ensure that there is enough foliage left for regrowth.

Weeds affect alfalfa yields and quality, so chemical weed control is widely used in alfalfa production. Wilson Jr. (1981) found that controlling weeds in established alfalfa significantly increased the estimates of protein and total digestible nutrients produced per hectare each

growing season compared to the weedy check. Time of application of herbicides is important for increasing weed control in alfalfa. Timing of application of herbicides in alfalfa are preplant, postemergence to alfalfa and preemergence to weeds, postemergence to both, and dormant (DOR) to alfalfa (Thompson et al. 2013). Dormant applications are made after alfalfa goes dormant in the fall and before the alfalfa plant resumes active spring regrowth and producers will generally see little to no effect on the alfalfa stand or its quality. Dormant herbicides may provide control of existing winter annual weeds as well as residual control of late germinating summer annual weeds. Between cutting (BC) applications can be made after a cutting has been taken from the field. It is crucial to apply the herbicides before the plants produce very much green foliage, otherwise stunting or foliar necrosis can result from the application.

Some herbicides labeled for use in alfalfa have good residual activity on broadleaf and annual grass weeds. Diuron is a dormant season herbicide that has been around for many years. This herbicide provides excellent control of many winter annual broadleaf weeds and good to excellent control of many broadleaf summer annuals (Thompson et al. 2013). Winter annual broadleaf weeds include prickly lettuce, flixweed, tansy mustard, field pennycress, and shepherdspurse. Kochia, common lambsquarters, morningglory species, pigweed species, common ragweed, and Pennsylvania smartweed are summer annual weeds which can be controlled by diuron. Flumioxazin is another herbicide that provides adequate control of broadleaf and annual grass weeds. Like diuron, it provides fair to excellent residual control. Flumioxazin as a DOR or BC application is an herbicide that has recently become labeled in alfalfa production. Flumioxazin will be beneficial for alfalfa growers for maintaining broadleaf and grass weeds in their production systems.

Sulfentrazone is not currently registered for use in alfalfa, but could be an option if alfalfa tolerance is acceptable. Sulfentrazone is labeled for use in soybeans and sunflower in Kansas. In soybeans, it is applied as preplant incorporated or preemergence. It provides fair to excellent control of many annual grasses and provides good control of Eastern black nightshade, common lambsquarters, and pigweed spp. (Thompson et al. 2013). In sunflower, sulfentrazone can be applied as a burndown, preplant, or preemergence. Sulfentrazone plus carfentrazone in the fall can provide residual control of broadleaf weeds into the spring (Thompson et al. 2013).

Alfalfa can be one of the most profitable agronomic crops because of its high energy value and protein content. However, good management and timely crop harvest is critical to the success of alfalfa production and quantity. When well-managed, alfalfa is a high-value crop that can be profitably produced for cash hay market or stored as hay or silage for on-farm use (Hancock et al. 2012).

Common waterhemp and Palmer amaranth are very problematic weeds in crop production systems and control of these pigweed species is difficult. These two species have a wide window for germination, grow rapidly, produce a vast number of seeds, and are developing resistance to multiple classes of herbicides. The objectives of this research were to (1) document the season-long emergence of Palmer amaranth and evaluate Palmer amaranth control in established alfalfa with various labeled and experimental herbicides, (2) document the presence and scope of GR common waterhemp and Palmer amaranth in Kansas, and (3) characterize two GR Palmer amaranth populations in Kansas.

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Chapter 2 - Palmer Amaranth Control in Established Alfalfa

ABSTRACT

Palmer amaranth is a serious production problem for alfalfa growers in the southern Great Plains region. Infestations of Palmer amaranth in alfalfa can reduce yields and lower the quality of the harvested product. Field experiments were conducted near Clay Center, KS in 2011 and 2012 to evaluate various herbicide treatments in alfalfa for crop response and Palmer amaranth control at regular intervals throughout the growing season. Dormant season treatments included labeled rates of several registered herbicides including flumioxazin, hexazinone, diuron, trifluralin, and terbacil. Experimental treatments included sulfentrazone and pyroxasulfone herbicides. Between cutting treatments included flumioxazin, imazethapyr, imazamox, and sulfentrazone. Palmer amaranth emergence was monitored weekly in 2012 to relate emergence patterns through the season to residual herbicide activity. In 2011, several treatments provided early season Palmer amaranth control, but the best late season control of Palmer amaranth was from treatments that included flumioxazin at 140 g ha^{-1} either as a dormant or between cutting treatment, or a dormant treatment of diuron at 2,690 g ha^{-1}. Palmer amaranth control on September 19 was 82% for the between cutting treatment of flumioxazin, 60% for the dormant treatment of flumioxazin, and 76% for the dormant treatment of diuron. All other treatments provided no more than 10% Palmer amaranth control by September 19. In 2012, the best late season Palmer amaranth control of 85 to 96% was achieved with sequential treatments that included flumioxazin at 140 g ha^{-1} or diuron at 2,690 g ha^{-1} as dormant applications followed by a between cutting treatment of flumioxazin at 70 g ha^{-1}. Several other treatments provided good early season Palmer amaranth control, but control diminished as the season progressed. Palmer amaranth began emerging May 1, 2012 with 20% cumulative emergence by June 3 (33 d), 80%

cumulative emergence by July 8 (35 d later), and 100% emergence by August 5. Palmer amaranth emerged throughout the growing season and therefore, sequential herbicide treatments with good residual activity may be necessary for season-long control.

INTRODUCTION

Alfalfa is one of the most important forage crops in the United States. In 2011, total hay production in the United States was 22.8 million ha (Anonymous 2012). In Kansas, alfalfa hay production was 2.25 million Mg produced in 2011 and is the 4th most widely grown agronomic crop in the state (Anonymous 2012). It is grown on a variety of soil types and across many different climates around the world. Alfalfa is highly beneficial for livestock producers because of its forage quality.

Alfalfa establishment can be difficult due to certain competition factors. Weeds compete with alfalfa for sunlight, water, and nutrients. Moyer (1985) has shown that weed control during alfalfa establishment is required to prevent crop yield losses in subsequent years. Ott et al. (1989) reported that volunteer wheat (*Triticum aestivum* L.) emerging with seedling alfalfa in the fall could reduce first-cutting alfalfa yields by more than 80%. Furthermore, Pike and Stritzke (1984) observed that cheat (*Bromus secalinus* L.) infestations reduced first-cutting yields 60 to 85% when not controlled in the fall and total alfalfa yield was reduced 25 to 35% across all cuttings. Weeds can also reduce alfalfa quality (Cords 1973; Cosgrove and Barrett 1987). If weeds are not adequately controlled during establishment, a poor stand will result. Weeds are also problematic in established alfalfa as well, resulting in reduced quality and yield (Cords 1973; Kapusta and Strieker 1975; Smith 1969).

Since their introduction, herbicides have been widely adapted into agricultural production systems for weed control. When alfalfa stands deteriorate as a result of winter killing, disease, etc., weeds become established and compete for growth resources (Robison et al. 1978). Several herbicides are commonly used in alfalfa, such as hexazinone, metribuzin, paraquat, pronamide, and terbacil (Kapusta and Strieker 1975; Peters et al. 1984; Wilson 1989). In established stands

of alfalfa grown for hay, pasture, dehydration, or seed, producers often prefer a residual herbicide such as terbacil or hexazinone, as opposed to a short-lived POST herbicide such as 2, 4-DB [4-(2, 4 –dichlorophenoxy) butanoic acid] because a broader spectrum of weeds is controlled by a combination of immediate and residual herbicide actions (Malik et al. 1993). Many weeds in alfalfa are better controlled with soil-applied as opposed to foliar-applied herbicides; however the cost per acre has been a deterrent to acceptance by farmers (Robison et al. 1978).

Excellent weed control can be achieved in alfalfa with flumioxazin and diuron (Thompson et al. 2013). Furthermore, a postemergence (POST) herbicide that has been effective at controlling summer annual grass and broadleaf weeds has been paraquat (Thompson et al. 2013). However, Peters et al. (1984) showed that paraquat was less effective for controlling weeds when applied in late March than when applied in February. A study conducted by Malik et al. (1993) showed that hexazinone provided the most consistent weed control in established alfalfa grown for seed. Average control of Canada thistle (*Cirsium arvense* (L.) Scop.), catchweed bedstraw (*Galium aparine* (L.)), dandelion (*Taraxacum officinale* (G.H.) Weber *ex* Wiggers), perennial sowthistle (*Sonchus arvensis*), quackgrass (*Elymus repens* (L.) Gould), Russian pigweed (*Axyris amaranthoides* (L.), and scentless chamomile (*Matricaria perforate*) was 80%. Alfalfa seed production was 33% greater than other herbicide treatments average across all sites (Malik 1993). Although a number of other herbicides are currently labeled for weed control in alfalfa, all products have limitations because of low soil activity on some weed species, potential crop safety concerns, or other deficiencies (Curran et al. 2008; Hagood et al. 2009; Hahn 2010).

The objectives of this study were to evaluate alfalfa crop tolerance and Palmer amaranth control with various labeled and experimental herbicide treatments under dryland and irrigated conditions and to document season-long emergence patterns of Palmer amaranth.

MATERIALS AND METHODS

Field experiments were conducted near Clay Center, KS to evaluate Palmer amaranth control in established alfalfa under dryland conditions in 2011 and 2012 and irrigated conditions in 2012. The soil for both experiments was a Muir Sandy loam (fine-silty, mixed, mesic, Pachic Haplustolls) with pH of 6.4, organic matter of 0.8% and soil texture was 74% sand, 20% silt, and 6% clay. The alfalfa was established in 2004 resulting in a timeworn, thin, and highly variable stand by 2011 and allowing for Palmer amaranth infestation. In 2012, the irrigated experiment received water 2 to 3 times per week beginning in April through to September with 2.5 cm of water applied each time. The dryland experiment received natural precipitation both years.

Labeled and experimental herbicide treatments were used to evaluate season-long Palmer amaranth control. Herbicide applications included dormant (DOR) and between cutting (BC) treatments between the first and second harvest either alone or sequentially. Dormant season treatments included labeled rates of several registered herbicides, including flumioxazin, hexazinone, diuron, trifluralin, and terbacil. Experimental treatments included sulfentrazone and pyroxasulfone herbicides. Between cutting treatments included flumioxazin, imazethapyr, imazamox, and sulfentrazone. A non-treated plot was included for comparison. Additional herbicide treatments were added in 2012 in response to the results of 2011. Treatment formulations, timings, and rates are shown in Tables 2-1 and 2-2. All herbicide treatments were applied using a CO_2 back-pack sprayer delivering 140 L ha^{-1} at 193 kPa through TurboTee[1]

110015 wide angle flat fan spray tips. Weather data for both application timings and years are shown in Table 2-3.

Alfalfa injury and Palmer amaranth control were evaluated at regular intervals throughout the growing season. Visual ratings of alfalfa injury and Palmer amaranth control were recorded one week after treatment and throughout the duration of the growing season on a scale of 0 to 100, where 0 equals no effect and 100 equals plant mortality or complete weed control.

Alfalfa was harvested by clipping the alfalfa 8 cm above the crown in a 0.25 m^2 quadrat, weighed, and then dried at 50 C for 7 days. Once dried, the samples were weighed and submitted to a commercial laboratory[2] for quality analyses. Alfalfa yield, forage quality, and forage quality analyses were then analyzed for statistical differences.

In 2012, Palmer amaranth emergence was monitored in 0.25 m^2 quadrats at the four corners of each experiment by removing emerged plants and recording them at weekly intervals throughout the season. A three-parameter logistic model was fit to Palmer amaranth emergence data based on Julian days, such that May 1 is day 122, June 3 is 155, July 8 is 190, and August 5 is 218. The emergence model was:

$$y = \frac{\mathrm{a}}{1 + (\frac{X}{\mathrm{E50}})^{b}}$$

where *y* is the cumulative % emergence, *X* is cumulative growing degree days (GDD), a is the maximum % emergence, E_{50} is the inflection point (GDD) of curve, and *b* is the slope of the curve at the inflection point.

The experimental design for each experiment was a randomized complete block with three replications, and 3 by 9 m plots. Data were subjected to analysis of variance using PROC GLIMMIX in SAS 9.2[3], and means were separated using Fisher's protected LSD at $P \leq 0.05$.

Alfalfa injury and Palmer amaranth control were then compared to the untreated check using contrasts and pairwise comparisons to determine if significant differences were observed at $P \leq 0.05$.

RESULTS AND DISCUSSION

Alfalfa Injury

Several treatments injured the alfalfa at both application timings for both the dryland and irrigated experiments. Injury was observed 7 and 14 DAT in 2011 (Table 2-4) and 7, 14, 21, and 28 DAT in 2012 (Table 2-5). Injury symptoms from these herbicide treatments consisted of leaf chlorosis, necrosis, and general stunting. The substantial injury caused in 2012 was likely enhanced by the warm weather, alfalfa coming out of dormancy, and addition of crop oil concentrate (COC) with one of the herbicide formulations.

In 2011, some DOR applications significantly injured the alfalfa 7 DAT but injury was not evident by 14 DAT (Table 2-4). A DOR premix of sulfentrazone & carfentrazone at 140 & 15 g ha^{-1} + COC at 1% v/v caused 6% injury while all other DOR treatments did not significantly affect the alfalfa (Table 2-4). Much of these injury symptoms were general stunting with some foliar necrosis to the trifoliate leaf tips. Significant foliar necrosis was also observed from seven BC treatments 7 DAT but only two treatments had a significant effect on the alfalfa 14 DAT. By 21 DAT, injury was not evident for any of the herbicide treatments (Table 2-4).

In 2012, three herbicide treatments caused significant chlorosis and necrosis to the alfalfa in the dryland experiment. Dormant applications of sulfentrazone & carfentrazone at 140 & 15 + COC and 280 & 30 g ha^{-1} + COC at 1% v/v, and sulfentrazone & carfentrazone at 140 & 15 g ha^{-1} caused 47, 83, and 13% injury to the alfalfa 7 DAT, respectively, but new alfalfa growth was not affected as symptoms subsided with time and were no longer evident by 28 DAT (Table 2-5).

Alfalfa injury was not evident following BC applications (data not presented). Alfalfa injury was substantially less for the same treatments in the irrigated experiment and all chlorosis, necrosis, and stunting had diminished by first harvest.

Alfalfa yield and quality

In 2011, first cutting alfalfa yields from plots treated with DOR herbicide applications ranged from 4,390 to 5,130 kg ha^{-1} (Table 2-6). Alfalfa yields from the herbicide-treated plots were not different from the untreated check. The DOR application of flumioxazin at 140 g ha^{-1} followed by BC imazethapyr at 70 g ha^{-1} + COC at 1% v/v had higher alfalfa yield when compared to the untreated check for the second cutting, but the difference was probably due to variable alfalfa stands and not treatment effect. All other herbicide treatments were not different from the untreated check at a significance level of 0.05 (Table 2-6). In 2012, a DOR application of flumioxazin & pyroxasulfone at 140 & 180 g ha^{-1} in the irrigated experiment and a DOR application of sulfentrazone at 280 g ha^{-1} in the dryland experiment both yielded lower when compared to the untreated check (Table 2-7). Less yield was recorded from plots treated with a BC application of sulfentrazone & carfentrazone at 140 & 15 g ha^{-1} in the dryland experiment when compared to the untreated check (Table 2-7). However, alfalfa yield did not differ among for any other herbicide treatments or application timings for both the dryland and irrigated experiments, respectively. Alfalfa stands were variable for the dryland and irrigated experiments which may have had an effect on yield data. Alfalfa forage qualities were not different from the untreated check. Crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), total digestible nutrients (TDN), calcium (Ca), phosphorus (P), potassium (K), Magnesium (Mg), and relative feed value (RFV) were unaffected by all herbicide treatments and timings (data not

shown). Weed competition with alfalfa was minimal for the first two alfalfa cuttings and the herbicide treatments appeared to have minimal negative effect on alfalfa yield and quality.

Palmer amaranth control

Several herbicide treatments provided early season Palmer amaranth control, but the best late season control was provided by treatments that included flumioxazin or diuron alone or in combination with another herbicide in 2011 (Table 2-8). A BC application of flumioxazin at 140 g ha^{-1} provided the best late season control of Palmer amaranth with 82% control by the end of the season. A dormant application of diuron at 2,690 g ha^{-1} provided 77% control late in the season, which was significantly better than the DOR applications of flumioxazin which provided 60% control. The BC application of flumioxazin would persist later into the summer than the DOR treatments. This provides better control of later germinating weeds. A dormant application of flumioxazin & pyroxasulfone at 140 & 180 g ha^{-1} provided 65% control and a sequential application of flumioxazin at 140 g ha^{-1} followed by imazethapyr at 70 g ha^{-1} + COC at 1% v/v provided 68% control of Palmer amaranth late into the season. All other herbicide treatments provided little to no Palmer amaranth control by the end of the season (Table 2-8).

Palmer amaranth control in 2012 was similar to 2011. All herbicides provided fair to good control of Palmer amaranth early in the season but control diminished for many of the treatments towards the end of the season. The best late season control of Palmer amaranth was achieved with treatments that included a DOR application of flumioxazin or diuron, followed by a BC application of flumioxazin. These treatments were added in 2012 based on the 2011 results in an attempt to achieve better late season Palmer amaranth control. Flumioxazin at 140 g ha^{-1} followed by flumioxazin at 70 g ha^{-1} provided 88 and 87% control for the dryland and irrigated experiments, respectively. Diuron at 2,690 kg ha^{-1} followed by flumioxazin at 70 g ha^{-1}

provided 96 and 88% for the dryland and irrigated experiments, respectively (Tables 2-9 and 2-10). Late season Palmer amaranth control with DOR applications of flumioxazin and diuron or BC treatments with flumioxazin were better than the other treatments, but not as good as the sequential treatments. Control varied 44-85% for DOR and BC applications of flumioxazin and diuron for the dryland and irrigated experiments (Tables 2-9 and 2-10).

Palmer amaranth emergence

Palmer amaranth began emerging early in the season and continued throughout the duration of the growing season. In 2012, Palmer amaranth began emerging May 1, with 20% cumulative emergence by June 3 or 33 days after weed emergence began, 80% cumulative emergence by July 8, and emergence stopped by August 5 (Figure 2-1). The greatest emergence was between June 3 and July 8. Total Palmer amaranth emergence was greater on the dryland experiment than the irrigated experiment with 436 and 136 plants m^{-2}, respectively, but emergence patterns were similar. Palmer amaranth germinates throughout the growing season and herbicides with long residual soil activity may be necessary to achieve acceptable season-long control.

Several treatments provided early season Palmer amaranth control and the best late season control of Palmer amaranth was provided by treatments that included a DOR application of flumioxazin or diuron, followed by a BC application of flumioxazin. Therefore, sequential herbicide treatments with good residual activity may be necessary for season-long control of Palmer amaranth.

SOURCES OF MATERIALS

[1]Teejet Spraying Systems, Wheaton, IL 60189-7900.

[2]SDK Laboratories, Inc. 1000 Corey Road Hutchinson, KS 67501.

[3]SAS version 9.2, SAS Institute Inc., 100 SAS Campus Drive, Cary NC 27513.

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Table 2-1. Herbicide treatments, timings and rates for dryland experiment at Clay Center, KS in 2011.

Herbicide[a]	Timing[b]	Rate
		(g ai ha^{-1})
Flumioxazin	DOR	140
Diuron	DOR	2,690
Flumioxazin&pyroxasulfone	DOR	140&180
Hexazinone	DOR	560
Trifluralin	DOR	2,240
Terbacil	DOR	900
Hexazinone&diuron	DOR	590&710
Sulfentrazone	DOR	280
Sulfentrazone&carfentrazone+COC	DOR	140&150
Flumioxazin/imazethapyr+COC	DOR/BC	140/170
Sulfentrazone&carfentrazone/ sulfentrazone&imazethapyr+COC	DOR/ BC	140&15/ 130&29
Flumioxazin	BC	140
Sulfentrazone	BC	140
Sulfentrazone	BC	280
Sulfentrazone&carfentrazone	BC	140&15
Sulfentrazone&imazethapyr+COC	BC	150&30
Sulfentrazone&imazethapyr+COC	BC	290&60

[a]COC = crop oil concentrate at 1% v/v; & = formulated premix; / = sequential application.
[b]DOR = dormant herbicide treatments; BC = between cutting treatments.

Table 2-2. Herbicide treatments, timings and rates for irrigated and dryland experiments at Clay Center, KS in 2012.

Herbicide[a]	Timing[b]	Rate
		(g ai ha^{-1})
Flumioxazin	DOR	140
Diuron	DOR	2,690
Flumioxazin&pyroxasulfone	DOR	140&180
Hexazinone	DOR	560
Trifluralin	DOR	2,240
Terbacil	DOR	900
Hexazinone&diuron	DOR	590&710
Sulfentrazone	DOR	280
Sulfentrazone&carfentrazone+COC	DOR	140&150
Flumioxazin/imazethapyr+COC	DOR/BC	140/170
Sulfentrazone&carfentrazone/ sulfentrazone&imazethapyr+COC	DOR/ BC	140&15/ 130&29
Flumioxazin/flumioxazin	DOR/BC	140/70
Diuron/flumioxazin	DOR/BC	2,690/70
Flumioxazin	BC	140
Imaxamox+COC+UAN	BC	40
Sulfentrazone	BC	140
Sulfentrazone	BC	280
Sulfentrazone&carfentrazone	BC	140&15
Sulfentrazone&imazethapyr+COC	BC	150&30
Sulfentrazone&imazethapyr+COC	BC	290&60

[a]COC = crop oil concentrate at 1% v/v; UAN = urea-ammonium nitrate applied at 2.5% v/v; & = formulated premix; / = sequential application.
[b]DOR = dormant herbicide treatments; BC = between cutting treatments.

Table 2-3. Weather data at time of applications to the dryland and irrigated alfalfa experiments at Clay Center, KS in 2011 and 2012.

	Dryland				Irrigated	
	----------2011----------		----------2012----------		----------2012----------	
Application date	03/10/11	05/23/11	03/09/12	04/23/12	03/09/12	04/23/12
Time of day	5:30 PM	4:30 PM	11:30 PM	7:30 PM	1:30 PM	6:30 PM
Application timing[a]	DOR	BC	DOR	BC	DOR	BC
Air temperature (C)	12	28	22	20	21	22
Relative humidity %	52	45	16	44	17	38
Wind speed (m s^{-1})	1.8	1.8	1.7	2.7	2.0	1.8
Wind direction	S	E	SW	S	SW	SSW
Dew presence	No	No	No	No	No	No
Soil temperature (C)	8	27	8	18	9	16
Soil moisture	Good	Dry	Dry	Dry	Dry	Dry
Cloud cover %	0	0	0	0	0	0

[a] DOR = dormant application, BC = between cutting application

Table 2-4. Visible injury to alfalfa as affected by dormant and between cutting herbicide applications to the dryland experiment at Clay Center, KS in 2011.

			Alfalfa Injury				
Herbicide[a]	Rate	Timing[b]	7 DAT[c]	14 DAT[c]	7 DAT[d]	14 DAT[d]	21 DAT[d]
	(g ai ha^{-1})		(%)				
Flumioxazin	140	DOR	0	0	0	0	0
Diuron	2,690	DOR	0	0	0	0	0
Flumioxazin&pyroxasulfone	140&180	DOR	0	0	0	0	0
Hexazinone	560	DOR	0	0	0	0	0
Trifluralin	2,240	DOR	0	0	1	0	0
Terbacil	900	DOR	0	0	0	0	0
Hexazinone&diuron	590&710	DOR	0	0	0	0	0
Sulfentrazone	280	DOR	2	0	0	0	0
Sulfentrazone&carfentrazone+COC	140&15	DOR	6	0	0	0	0
Flumioxazin/imazethapyr+COC	140&70	DOR/BC	4	0	2	0	0
Sulfentrazone&carfentrazone/	140&15/	DOR/	4	0			
sulfentrazone&imazethapyr+COC	130&29	BC			5	1	0
Flumioxazin	140	BC			20	8	0
Sulfentrazone	140	BC			4	0	0
Sulfentrazone	280	BC			8	4	0
Sulfentrazone&carfentrazone	140&15	BC			4	0	0
Sulfentrazone&imazethapyr+COC	150&30	BC			8	2	0
Sulfentrazone&imazethapyr+COC	290&60	BC			7	0	0
Untreated			0	0	0	0	0
LSD ≤ 0.05			2	0	6	3	0

[a]COC = crop oil concentrate at 1% v/v; UAN = urea-ammonium nitrate applied at 2.5% v/v; & = formulated premix; / = sequential application.
[b]DOR = dormant herbicide treatments; BC = between cutting treatments.
[c]DAT = days after dormant treatments.
[d]DAT = days after between cutting treatments.

Table 2-5. Visible injury to alfalfa as affected by dormant herbicide applications for the irrigated and dryland experiments at Clay Center, KS in 2012.

			-------Irrigated-------		----------Dryland----------			
Herbicide[a]	Rate	Timing[b]	7 DAT[c]	14 DAT[c]	7 DAT[c]	14 DAT[c]	21 DAT[c]	28 DAT[c]
	(g ai ha^{-1})		----------(%)----------					
Flumioxazin	140	DOR	0	0	0	0	0	0
Diuron	2,690	DOR	0	0	0	0	0	0
Flumioxazin&pyroxasulfone	140&180	DOR	0	0	0	0	0	0
Hexazinone	560	DOR	0	0	0	0	0	0
Trifluralin	2,240	DOR	0	1	0	0	0	0
Terbacil	900	DOR	0	0	0	0	0	0
Hexazinone&diuron	590&710	DOR	0	0	0	0	0	0
Sulfentrazone	280	DOR	0	0	0	0	0	0
Sulfentrazone&carfentrazone+COC	140&15	DOR	3	0	47	13	3	0
Sulfentrazone&carfentrazone+COC	280&30	DOR	20	3	83	50	20	3
Flumioxazin/imazethapyr+COC	140&70	DOR/BC	0	0	0	0	0	0
Sulfentrazone&carfentrazone/ sulfentrazone&imazethapyr+COC	140&15/ 130&29	DOR/ BC	0	0	13	3	0	0
Flumioxazin/flumioxazin	140/70	DOR/BC	0	0	0	0	0	0
Diuron/flumioxazin	270/70	DOR/BC	0	0	0	0	0	0
Untreated			0	0	0	0	0	0
LSD ≤ 0.05			2	2	10	3	2	2

[a]COC = crop oil concentrate at 1% v/v; UAN = urea-ammonium nitrate applied at 2.5% v/v; & = formulated premix; / = sequential application.
[b]DOR = dormant herbicide treatments; BC = between cutting treatments.
[c]DAT = days after dormant treatments.

Table 2-6. Alfalfa yields as affected by dormant and between cutting herbicide applications for the dryland experiment at Clay Center, KS in 2011.

Herbicide[a]	Rate	Timing[b]	Alfalfa yield	
			1st cutting	2nd cutting
	(g ai ha^{-1})		-------kg ha^{-1}-------	
Flumioxazin	140	DOR	4390	3090
Diuron	2,690	DOR	4460	2800
Flumioxazin&pyroxasulfone	140&180	DOR	4560	2840
Hexazinone	560	DOR	4730	3080
Trifluralin	2,240	DOR	5280	3080
Terbacil	900	DOR	4860	3280
Hexazinone&diuron	590&710	DOR	4650	2620
Sulfentrazone	280	DOR	4660	3000
Sulfentrazone&carfentrazone	140&15	DOR	4510	2180
Sulfentrazone&carfentrazone+COC	280&30	DOR	4540	2690
Flumioxazin/imazethapyr+COC	140&70	DOR/BC	4730	3370**
Sulfentrazone&carfentrazone/ sulfentrazone&imazethapyr+COC	140&15/ 130&29	DOR/ BC	5130	2870
Flumioxazin	140	BC		2240
Sulfentrazone	140	BC		2600
Sulfentrazone	280	BC		2520
Sulfentrazone+COC	140	BC		2550
Sulfentrazone&carfentrazone	140&15	BC		2860
Sulfentrazone&imazethapyr+COC	150&30	BC		2590
Sulfentrazone&imazethapyr+COC	290&60	BC		2830
Untreated			5480	2260
LSD ≤ 0.05			1180	950

**Indicates significance at $\alpha \leq 0.05$ when compared to untreated check.
[a]COC = crop oil concentrate at 1% v/v; UAN = urea-ammonium nitrate applied at 2.5% v/v; & = formulated premix; / = sequential application.
[b]DOR = dormant herbicide treatments; BC = between cutting treatments.

Table 2-7. Alfalfa yields as affected by dormant and between cutting herbicide applications for the dryland and irrigated experiments at Clay Center, KS in 2012.

			Alfalfa yield			
			Irrigated		Dryland	
Herbicide	Rate	Timing	1st cutting	2nd cutting	1st cutting	2nd cutting
	(g ai ha^{-1})		----------kg ha^{-1}----------			
Flumioxazin	140	DOR	2830	3500	3220	3800
Diuron	2,690	DOR	3000	3450	3340	4500
Flumioxazin&pyroxasulfone	140&180	DOR	1770**	3030	2780	3780
Hexazinone	560	DOR	2690	3300	3540	4370
Trifluralin	2,240	DOR	3080	3230	3400	4300
Terbacil	900	DOR	2520	3350	2830	3640
Hexazinone&diuron	590&710	DOR	3050	3260	3020	4030
Sulfentrazone	280	DOR	3000	3760	3380	2680**
Sulfentrazone&carfentrazone+COC	140&15	DOR	2370	3990	2910	4000
Sulfentrazone&carfentrazone+COC	280&30	DOR	2240	3160	2760	3890
Flumioxazin/imazethapyr+COC	140&70	DOR/BC	2890	3070	2950	4160
Sulfentrazone&carfentrazone/ sulfentrazone&imazethapyr+COC	140&15/ 130&29	DOR/ BC	2070	2960	3460	3430
Flumioxazin/flumioxazin	140/70	DOR/BC	2880	3480	3500	3880
Diuron/flumioxazin	2,690/70	DOR/BC	2860	3390	2900	4150
Flumioxazin	140	BC		3560		4370
Imazamox+COC+UAN	40	BC		3680		3900
Sulfentrazone	140	BC		3710		3870
Sulfentrazone+COC	280	BC		3060		3840
Sulfentrazone&carfentrazone	140&15	BC		3180		3390**
Sulfentrazone&imazethapyr+COC	150&30	BC		2840		3970
Sulfentrazone&imazethapyr+COC	290&60	BC		3320		4100
Untreated			2550	3440	3040	4570
LSD ≤ 0.05			736	828	575	980

**Indicates significance at $\alpha \leq 0.05$ when compared to untreated check.

Table 2-8. Palmer amaranth control for the dryland experiment at Clay Center, KS in 2011.

Herbicide[a]	Rate	Timing[b]	Palmer amaranth control			
			06/07/11	07/06/11	08/16/11	09/19/11
	(g ai ha^{-1})		----------(%)----------			
Flumioxazin	140	DOR	98	98	75	60
Diuron	2,690	DOR	97	97	86	77
Flumioxazin&pyroxasulfone	140&180	DOR	98	98	81	65
Hexazinone	560	DOR	76	0	0	0
Trifluralin	2,240	DOR	58	0	0	0
Terbacil	900	DOR	75	43	13	3
Hexazinone&diuron	590&710	DOR	90	62	23	7
Sulfentrazone	280	DOR	83	0	0	0
Sulfentrazone&carfentrazone	140&15	DOR	78	0	0	0
Sulfentrazone&carfentrazone+COC	280&30	DOR	78	20	0	0
Flumioxazin/imazethapyr+COC	140&70	DOR/BC	98	98	85	68
Sulfentrazone&carfentrazone/ sulfentrazone&imazethapyr+COC	140&15/ 130&29	DOR/ BC	84	45	30	0
Flumioxazin	140	BC	98	98	90	82
Sulfentrazone	140	BC	86	13	7	0
Sulfentrazone	280	BC	83	75	33	8
Sulfentrazone+COC	140	BC	87	45	13	8
Sulfentrazone&carfentrazone	140&15	BC	83	17	0	0
Sulfentrazone&imazethapyr+COC	150&30	BC	82	40	10	9
Sulfentrazone&imazethapyr+COC	290&60	BC	87	82	23	8
Untreated			0	0	0	0
LSD ≤ 0.05			11	30	21	13

[a]COC = crop oil concentrate at 1% v/v; & = formulated premix; / = sequential application.
[b]DOR = dormant herbicide treatments; BC = between cutting treatments.

Table 2-9. Palmer amaranth control for the dryland experiment at Clay Center, KS in 2012.

Herbicide[a]	Rate	Timing[b]	Palmer amaranth control			
			05/14/12	06/23/12	07/23/12	09/18/12
	(g ai ha^{-1})		----------(%)----------			
Flumioxazin	140	DOR	100	100	83	47
Diuron	2,690	DOR	100	100	94	78
Flumioxazin&pyroxasulfone	140&180	DOR	100	100	73	43
Hexazinone	560	DOR	85	42	0	0
Trifluralin	2,240	DOR	73	62	0	0
Terbacil	900	DOR	88	65	10	0
Hexazinone&diuron	590&710	DOR	100	78	20	0
Sulfentrazone	280	DOR	89	58	0	0
Sulfentrazone&carfentrazone+COC	140&15	DOR	95	35	0	0
Sulfentrazone&carfentrazone+COC	280&30	DOR	91	37	7	0
Flumioxazin/imazethapyr+COC	140&70	DOR/BC	100	100	83	67
Sulfentrazone&carfentrazone/ sulfentrazone&imazethapyr+COC	140&15/ 130&29	DOR/ BC	95	92	72	27
Flumioxazin/flumioxazin	140/70	DOR/BC	100	100	96	88
Diuron/flumioxazin	2,690/70	DOR/BC	100	100	99	96
Flumioxazin	140	BC	100	99	89	65
Imazamox+COC+UAN	40	BC	99	92	37	0
Sulfentrazone	140	BC	95	93	87	70
Sulfentrazone+COC	280	BC	95	93	57	30
Sulfentrazone&carfentrazone	140&15	BC	95	87	17	0
Sulfentrazone&imazethapyr+COC	150&30	BC	95	93	60	30
Sulfentrazone&imazethapyr+COC	290&60	BC	100	100	88	72
Untreated			0	0	0	0
LSD ≤ 0.05			13	23	23	30

[a]COC = crop oil concentrate at 1% v/v; UAN = urea-ammonium nitrate applied at 2.5% v/v; & = formulated premix; / = sequential application.
[b]DOR = dormant herbicide treatments; BC = between cutting treatments.

Table 2-10. Palmer amaranth control for the irrigated experiment at Clay Center, KS in 2012.

Herbicide[a]	Rate	Timing[b]	Palmer amaranth control			
			05/14/12	06/23/12	07/23/12	09/18/12
	(g ai ha^{-1})		----------(%)----------			
Flumioxazin	140	DOR	100	99	81	70
Diuron	2,690	DOR	99	99	57	44
Flumioxazin&pyroxasulfone	140&180	DOR	99	100	65	23
Hexazinone	560	DOR	80	40	13	13
Trifluralin	2,240	DOR	75	81	7	0
Terbacil	900	DOR	60	76	23	17
Hexazinone&diuron	590&710	DOR	96	74	55	17
Sulfentrazone	280	DOR	75	47	55	17
Sulfentrazone&carfentrazone+COC	140&15	DOR	83	27	20	0
Sulfentrazone&carfentrazone+COC	280&30	DOR	73	57	20	0
Flumioxazin/imazethapyr+COC	140&70	DOR/BC	100	100	55	23
Sulfentrazone&carfentrazone/ sulfentrazone&imazethapyr+COC	140&15/ 130&29	DOR/ BC	95	96	38	17
Flumioxazin/flumioxazin	140/70	DOR/BC	100	100	90	87
Diuron/flumioxazin	2,690/70	DOR/BC	100	100	90	88
Flumioxazin	140	BC	100	99	90	85
Imazamox+COC+UAN	40	BC	100	92	53	40
Sulfentrazone	140	BC	73	90	62	30
Sulfentrazone+COC	280	BC	92	96	28	23
Sulfentrazone&carfentrazone	140&15	BC	99	99	60	47
Sulfentrazone&imazethapyr+COC	150&30	BC	99	99	30	23
Sulfentrazone&imazethapyr+COC	290&60	BC	100	99	57	47
Untreated			0	0	0	0
LSD ≤ 0.05			13	16	37	34

[a]COC = crop oil concentrate at 1% v/v; UAN = urea-ammonium nitrate applied at 2.5% v/v; & = formulated premix; / = sequential application.
[b]DOR = dormant herbicide treatments; BC = between cutting treatments.

Table 2-11. Palmer amaranth emergence for both the irrigated and dryland experiments at Clay Center, KS in 2012.

	Irrigated				Dryland			
Location[a]	NW	SW	NE	SE	NW	SW	NE	SE
	----------% Cumulative emergence----------							
Julian Days								
122	0	0	0	9	1	0	0	2
129	0	0	0	18	11	11	2	8
136	0	0	0	18	16	14	4	14
151	0	0	0	18	16	14	4	14
158	4	31	13	55	33	31	12	33
165	12	53	13	100	33	32	12	35
178	40	65	31	100	70	58	61	57
185	56	79	69	100	92	59	88	78
198	76	87	69	100	94	60	96	82
205	88	97	97	100	99	96	100	94
218	100	100	100	100	100	100	100	100
226	100	100	100	100	100	100	100	100

[a]Location = quadrat location respective to the experimental plot.

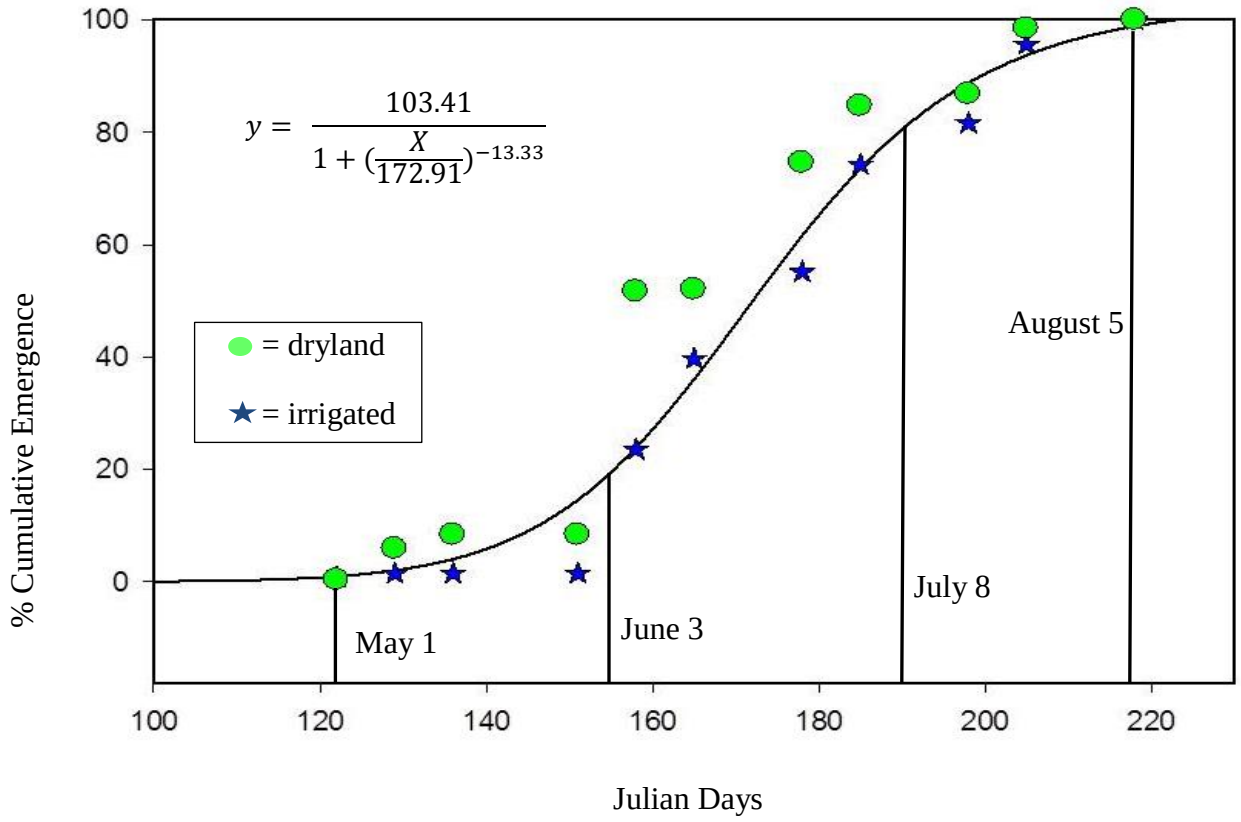

Figure 2-1. Percent cumulative emergence of Palmer amaranth for dryland and irrigated experiments at Clay Center, KS in 2012 such that day 155 is 20% emergence and day 190 is 80% emergence.

Chapter 3 – Occurrence and Distribution of Glyphosate-Resistant Common Waterhemp and Palmer Amaranth in Kansas

ABSTRACT

Common waterhemp (*Amaranthus rudis*) and Palmer amaranth (*Amaranthus palmeri*) are troublesome pigweed (*Amaranthus*) species that can reduce crop yields. Common waterhemp was first confirmed to be resistant to glyphosate in northeast Kansas in 2006. Glyphosate-resistant Palmer amaranth is a major problem in the southeastern United States but has not been previously confirmed in Kansas. The objective of this research was to document the presence and scope of glyphosate-resistant common waterhemp and Palmer amaranth in Kansas. Seed from nine populations of common waterhemp and five populations of Palmer amaranth were collected from soybean and cotton fields throughout eastern Kansas in the fall of 2011 and from 17 populations of Palmer amaranth in the fall of 2012. Plants of each population and species were grown in the greenhouse and treated with glyphosate at 0, 870, 1,740, and 3,480 g ae ha^{-1}, where 870 g ae ha^{-1} is the typical field use rate, when plants were 10 to 14 cm tall and evaluated for control when compared to a known susceptible population of each species. Glyphosate effectively controlled the susceptible population of each species at all glyphosate rates resulting in complete plant mortality. Several populations of common waterhemp survived applications of glyphosate up to two times the typical field use rate with some individuals surviving four times the typical field use rate. Visual injury ranged from 20 to 100% depending on the population and glyphosate rate. Two populations of Palmer amaranth from 2011 and six populations from 2012 showed similar resistance characteristics, with some plants surviving four times the typical field use rate of glyphosate. Glyphosate-resistant common waterhemp is present across much of

eastern Kansas. Glyphosate-resistant Palmer amaranth is now present in central Kansas and will likely become more widespread in the future.

INTRODUCTION

Pigweed (*Amaranthus*) species infest agronomic production fields throughout the United States and have been known to significantly reduce crop yields. The word describing the genus *Amaranthus* is derived from the Greek word "amarantus," which means "everlasting" or "never failing flowers" (Steckel et al. 2008). Common waterhemp is a troublesome weed throughout the midwest United States because of its prolific seed production and rapid growth characteristics (Battles et al. 1998; Bensch et al. 2003). Palmer amaranth is a highly competitive weed and is a growing threat to conservation tillage in the United States. Palmer amaranth is native to North America and is becoming very difficult to control because of its growth characteristics and its evolution of resistance to many classes of herbicides. Identification of common waterhemp and Palmer amaranth is commonly confused with other pigweed species such as redroot pigweed (*Amaranthus retroflexus*). Misidentification of common waterhemp and Palmer amaranth early in the growing season can be detrimental to a grower if proper management is not applied.

Common waterhemp and Palmer amaranth have an extended period of germination and emergence which makes control difficult. They are both dioecious, grow rapidly, and produce an abundant amount of seed. Common waterhemp germinates optimally between 25/20 and 35/30 C (Guo and Al-Khatib 2003), can produce up to 2 million seeds per plant (Battles et al. 1998) and can grow 2 to 3 m tall (Horak and Loughin 2000). Palmer amaranth can grow up to 5 cm per day under optimal conditions reaching heights of 3 to 4 m (Horak and Peterson 1995). Bensch et al. (2003) documented seed production for Palmer amaranth and common waterhemp to be 32,300 and 51,800 seeds m^{-2}, respectively. These characteristics make common waterhemp and Palmer amaranth competitive with agronomic crops.

Weeds resistant to herbicides are among the primary concerns in modern agriculture (Burgos et al. 2013). Glyphosate [*N*-(phosphonomethyl) glycine] is a relatively cheap and effective tool for controlling grass and broadleaf weed species. After the introduction of glyphosate-resistant (GR) crops in 1996, the use of this herbicide increased significantly. It is now estimated that more than 25 million ha of cropland are affected by glyphosate-resistant weed species (Heap 2013).

Common waterhemp was first confirmed resistant to glyphosate in 2005 in Missouri and has been spreading since (Legleiter and Bradley 2008). In 2011, two populations of common waterhemp were confirmed resistant to glyphosate in Texas (Light et al. 2011). They found that the lethal dose (LD_{50}) was 736 g ae ha^{-1} for the susceptible population which was equivalent to 0.9x labeled rate of glyphosate, whereas the resistant lines exhibited a broad range of resistance with LD_{50} values ranging from 3.5 to 59.7x the labeled rate of glyphosate (Light et al. 2011). First report in Kansas of glyphosate-resistant common waterhemp occurred in northeast Kansas in 2006 (Heap 2013). Glyphosate-resistant common waterhemp has spread rapidly; control is becoming extremely difficult and will likely become more problematic in the near future.

Glyphosate-resistant Palmer amaranth was first observed in 2004 in a field in Macon County, Georgia (Culpepper et al. 2006). Glyphosate at 12x the labeled rate of 840 g ae ha^{-1} failed to provide acceptable control of this biotype in the field (Culpepper et al. 2006). Since then, glyphosate-resistant Palmer amaranth has spread through the southeastern part of the United States and has begun to move northward. Norsworthy et al. (2008) initiated an experiment to quantify the level of glyphosate resistance compared to a known susceptible population that had not been previously exposed to glyphosate. Results showed that the resistant biotype had an LD_{50} of 2,820 g ae ha^{-1} glyphosate, which was 79 to 115x greater than that of the

susceptible biotypes and 3.4x a normal glyphosate-use rate of 840 g ae ha^{-1} in Arkansas (Norsworthy et al. 2008).

Glyphosate-resistant Palmer amaranth has not been previously confirmed in Kansas however, several cases of "difficult to control" Palmer amaranth have been reported. The objective of this study was to document the presence and scope of glyphosate-resistant common waterhemp and to determine if Palmer amaranth has evolved resistance to glyphosate in Kansas and document its distribution.

MATERIALS AND METHODS

Common waterhemp and Palmer amaranth seed were collected in October, 2011 from cotton and soybean fields in eastern Kansas where surviving plants remained. Ten to 20 female plants were collected from each field. Wise et al. (2009) documented collecting 10 to 30 females per field of Palmer amaranth and Burgos et al. (2013) believe that five to ten is a sufficient number of plants to represent a sample for cross-pollinated species. A total of nine common waterhemp populations and five populations of Palmer amaranth were evaluated. In September 2012, Palmer amaranth seed were collected in a similar manner from 17 fields throughout central Kansas where glyphosate did not effectively control it. Global positioning system (GPS) coordinates at each location are reported in Tables 3-1, 3-2, and 3-3 and sampling locations by county are represented visually on a map of Kansas (Figure 3-1). Seed from each site was threshed and placed in storage at -5 C until planted. Known glyphosate-susceptible common waterhemp and Palmer amaranth populations collected from the Department of Agronomy Ashland Bottoms Research Farm near Manhattan, KS were included for comparison.

From December to April 2011 and again in 2012, seed for all common waterhemp and Palmer amaranth populations were sown in separate flats, with a volume of 3.4 L and filled with

0.7 kg of Miracle-Gro moisture control potting mix[1]. Plants were grown under greenhouse conditions of 27/24 ± 2 C day/night temperature with a 16/8 h day/night period and light intensity of 80 µmol m^{-2} s^{-1} photosynthetic photon flux. Individual seedlings were transplanted into 0.25-L pots when plants were at the cotyledon stage of growth and watered as needed. The plants were treated when they reached 10 to 14 cm in height. Three treatment levels of glyphosate[2] were applied at 870, 1,740, and 3,480 g ae ha^{-1}, which represents 1, 2, and 4 times the typical field use rate. Ammonium sulfate (AMS) at 1% w/w was added to all herbicide treatments. Glyphosate was applied with a bench-type sprayer[3] equipped with an 80015LP[4] spray tip to deliver 187 L ha^{-1} at 138 kPa.

Percent control was determined 7 and 14 days after treatment (DAT) on a 0 to 100% scale where 0 = no effect and 100 = plant death. The experimental design was a randomized complete block with eight replications and was repeated. Control data were subjected to analysis of variance and pooled across runs because of an insignificant run effect using PROC GLIMMIX in SAS 9.2[5]. A pairwise comparison was conducted using a Dunnett's adjustment to compare all populations by rate against the susceptible population and the adjusted p-value was used for significance at $P \leq 0.05$.

RESULTS AND DISCUSSION

Common Waterhemp

Glyphosate provided 100% control of the known susceptible common waterhemp population at 7 DAT (Table 3-4). Glyphosate caused foliar chlorosis and necrosis to the leaves of plants from other populations, but many plants displayed little to no symptoms by 14 DAT. Some plants were injured, but by 14 DAT had begun to regrow and injury symptoms were less than 5% in many cases. All populations except NM11 were different from the susceptible

population. Population NM11 was sampled from a field in Nemaha County, KS and appeared to be more susceptible to glyphosate than the other eight populations. Percent control of NM11 was 78 and 87% with 870 g ae ha^{-1} at 7 and 14 DAT, respectively (Table 3-4). Control of all other populations was different from the susceptible population with control ranging from 21 to 69% at 14 DAT (Table 3-4). Several populations survived the 2x rate of glyphosate with control ranging from 53 to 74% at 14 DAT, which were different from the susceptible population (Table 3-4). Population FR11(3) differed from the susceptible population when treated with 3,480 g ha^{-1} glyphosate, with control ranging from 73 to 83% at 7 and 14 DAT (Table 3-4). All other populations were not statistically different from the susceptible population when treated with 3,480 g ha^{-1} glyphosate. Light et al. (2011) found that the LD_{50} value for the susceptible population of common waterhemp (736 g ae ha^{-1}) was equivalent to the 0.9x labeled rate of glyphosate, whereas the putatively resistant lines exhibited a broad range of resistance with LD_{50} values ranging from 3.5 to 59.7x the labeled rate of glyphosate. This research showed that four out of nine common waterhemp populations survived a 2x rate and one population survived a 4x rate of glyphosate.

Glyphosate has been used extensively and failure to control common waterhemp with a typical field use rate often results in reapplications with higher rates. The repeated overuse of glyphosate has resulted in the selection of glyphosate-resistant weeds. Some populations of common waterhemp that have been exposed to greater rates of glyphosate for an extended period of time display higher levels of resistance. Selection pressure has increased the number of glyphosate-resistant common waterhemp populations throughout the midwest United States. Glyphosate-resistant common waterhemp is present in several counties across eastern Kansas and control of this pigweed species is an increasing concern.

Palmer Amaranth

In 2011, five populations of Palmer amaranth were collected and analyzed for differences in response to increasing glyphosate rates in comparison to the susceptible population. Control of populations from Clay, Dickenson, and Douglas Counties was 100% for the 1, 2, and 4x rates of glyphosate 7 and 14 DAT and not different from the susceptible population (Table 3-5). However, two different populations collected from Cowley County, KS were not effectively controlled with 870 and 1,740 g ha^{-1} of glyphosate. Control of population CL11(1) ranged from 35 to 41% with 870 g ae ha^{-1} by 7 and 14 DAT and 58 to 68% with 1,740 g ha^{-1} glyphosate by 7 and 14 DAT (Table 3-5). Population CL11(2) showed 40 to 44% control with 870 g ae ha^{-1} by 7 and 14 DAT and from 84 to 89% control with 1,740 g ae ha^{-1} glyphosate by 7 and 14 DAT (Table 3-5). Four times the suggested use rate of glyphosate provided control of 91 to 93% by 7 and 14 DAT which was significantly different from the susceptible population (Table 3-5).

In 2012 the 17 populations of Palmer amaranth showed symptoms such as foliar chlorosis and leaf necrosis but many of the resistant plants outgrew the symptoms by 14 DAT. Seven populations collected from Cowley, Pottawatomie, and Stafford Counties were not effectively controlled with glyphosate at 870 g ha^{-1} by 14 DAT. Three of four populations from Cowley County, KS were not adequately controlled with glyphosate at 870 g ha^{-1} by 14 DAT. Control of population CL12(1) was 43 to 55%, CL12(3) was 56 to 58%, and CL12(4) was 49 to 54% with 870 g ae ha^{-1} glyphosate at 7 and 14 DAT (Table 3-6). Population CL12(2) was different from the susceptible population 7 DAT with control of 77% but did not differ by 14 DAT. However, control was 87% by 14 DAT, indicating some plants were not fully controlled with the 1x rate of glyphosate. Furthermore, two populations collected from Pottawatomie County, KS [PT12(1) and PT12(2)] were not controlled with a 1x rate of glyphosate such that PT12(1) was only

controlled 67 to 66% and control for population PT12(2) was 60 to 58% at 7 and 14 DAT (Table 3-6). Control started to diminish as plants began growing out of their injury symptoms 14 DAT. Many plants treated with 870 g ha^{-1} glyphosate were visually unaffected after 14 DAT indicating differences within populations. Four populations from Stafford County, KS were collected after a report of failed Palmer amaranth control in an irrigated glyphosate-resistant soybean field. Populations SF12(1) and SF12(2) did not differ from the susceptible population 14 DAT across all rates of glyphosate. However, SF12(3) and SF12(4) were only controlled 35 to 36% and 38 to 46% with 870 g ha^{-1} glyphosate by 7 to 14 DAT when compared to the susceptible population. All other populations did not differ from the susceptible population (Table 3-6).

Six Palmer amaranth populations collected in 2012 displayed resistance surviving and application of 1,740 g ae ha^{-1} glyphosate. Populations CL12(1), CL12(3), CL12(4), PT12(2), SF12(3), and SF12(4) survived the 2x rate of glyphosate and were different from the susceptible population. Control for these populations at 14 DAT was 83, 70, 65, 74, 82 and 81%, respectively (Table 3-6). No other populations differed from the susceptible population 14 DAT. CL12(1), CL12(3), and PT12(2) were not fully controlled with 4 times the typical field use rate of glyphosate at 3,480 g ae ha^{-1}. Control was 88, 86, and 94% at 14 DAT which was different from the susceptible population (Table 3-6). All other populations did not differ from the susceptible population.

A wide variety of crops are grown in Kansas and the use of glyphosate-resistant technology is very common (Anonymous 2013). The overreliance on glyphosate to control weeds has led to glyphosate-resistant weed biotypes. In Cowley County in 2011, two populations of Palmer amaranth collected from a cotton and soybean field were not adequately controlled with one and two times the typical field use rate of glyphosate. One of those

populations [CL11(1)] survived a 4x rate of glyphosate. In 2012, six populations were not adequately controlled with a 2x rate and three of those populations were uncontrolled with a 4x rate of glyphosate. Complete control of the susceptible biotype was achieved with glyphosate at 870 g ha^{-1} by 7 DAT, while 1,740 and 3,480 g ha^{-1} were still ineffective at controlling the resistant biotypes. These results mimic research findings of Culpepper et al. (2006) who found that control of a susceptible biotype of Palmer amaranth was noted with 0.6 kg ha^{-1} while a 12-fold increase in glyphosate (7.2 kg ha^{-1}) was necessary for complete control of the resistant biotype. These results are also consistent with Norsworthy et al. (2008), who found a resistant Palmer amaranth biotype in Arkansas had an LD_{50} of 2,820 g ha^{-1} glyphosate, which was 79- to 115-fold greater than that of the susceptible biotypes and 3.4 times a normal glyphosate-use rate of 840 g ha^{-1}. The development and spread of glyphosate resistance in Palmer amaranth has become an issue for crop producers in the United States.

Glyphosate-resistant horseweed, giant ragweed, common ragweed, common waterhemp, and kochia have been confirmed in Kansas (Heap 2013). Several populations of common waterhemp survived a 1x rate of glyphosate (Figure 3-2). This research confirms that glyphosate-resistant common waterhemp is present in several counties in eastern Kansas with several populations surviving two times the typical field use rate of glyphosate. Eight populations of Palmer amaranth survived the 2x rate of glyphosate and were different from the susceptible population 14 DAT (Figure 3-2). Therefore, this confirms that glyphosate-resistant Palmer amaranth is now present in Kansas and glyphosate alone is not a viable option for controlling these pigweed species. Alternative weed management strategies need to be explored for controlling glyphosate-resistant common waterhemp and Palmer amaranth in Kansas crop production systems.

SOURCES OF MATERIALS

[1]Miracle-Gro moisture control potting mix, Scotts Miracle-Gro Products Inc., 1411 Scottslawn Road, Marysville, OH 43041.

[2]Roundup WEATHERMAX®, Monsanto Company, St. Louis, MO 63167.

[3]Research track sprayer, De Vries Manufacturing, RR 1, Box 184, Hollandale, MN 56045.

[4]Teejet Spraying Systems, Wheaton, IL 60189-7900.

[5]SAS version 9.2, SAS Institute Inc., 100 SAS Campus Drive, Cary NC 27513.

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Table 3-1. GPS locations for each population of common waterhemp collected in Kansas during the fall of 2011.

County	Population[a]	GPS coordinates		Elevation
				---m---
Susceptible	S	N 39 07.470	W 96 36.840	311
Crawford	CR11	N 37 23.549	W 94 43.013	277
Franklin	FR11(1)	N 38 36.532	W 95 17.971	279
Franklin	FR11(2)	N 38 36.229	W 95 17.199	274
Franklin	FR11(3)	N 38 34.753	W 95 16.903	280
Franklin	FR11(4)	N 38 32.361	W 95 14.649	300
Franklin	FR11(5)	N 38 32.212	W 95 14.533	300
Marshall	MS11	N 39 50.526	W 96 16.103	413
Nemaha	NM11	N 39 46.135	W 96 08.516	330
Washington	WS11	N 39 50.498	W 96 51.921	410

[a]Population = Kansas county codes followed by the year collected followed by reference number.

Table 3-2. GPS locations for each population of Palmer amaranth collected in Kansas during the fall of 2011.

County	Population[a]	GPS coordinates		Elevation
				---m---
Susceptible	S	N 39 07.470	W 96 36.840	311
Clay	CY11	N 39 19.847	W 97 04.346	356
Cowley	CL11(1)	N 37 14.518	W 97 06.850	374
Cowley	CL11(2)	N 37 11.262	W 97 10.764	349
Dickenson	DK11	N 38 44.705	W 97 16.772	387
Douglas	DG11	N 39 00.023	W 95 13.728	243

[a]Population = Kansas county codes followed by the year collected followed by reference number.

Table 3-3. GPS locations for each population of Palmer amaranth collected in Kansas during the fall of 2012.

County	Population[a]	GPS coordinates		Elevation
				---m---
Susceptible	S	N 39 07.470	W 96 36.840	311
Clay	CY12	N 39 19.155	W 97 02.906	364
Cowley	CL12(1)	N 37 16.843	W 97 08.969	354
Cowley	CL12(2)	N 37 08.381	W 97 07.864	336
Cowley	CL12(3)	N 37 08.381	W 97 07.864	336
Cowley	CL12(4)	N 37 13.832	W 97 05.363	372
Ellsworth	EW12(1)	N 38 46.528	W 98 11.273	551
Ellsworth	EW12(2)	N 38 37.394	W 98 21.870	566
Marion	MN12	N 38 33.085	W 96 57.342	450
Pottawatomie	PT12(1)	N 39 12.831	W 96 14.988	300
Pottawatomie	PT12(2)	N 39 12.831	W 96 14.988	300
Pottawatomie	PT12(3)	N 39 13.010	W 96 15.559	300
Pottawatomie	PT12(4)	N 39 11.132	W 96 02.889	288
Riley	RL12	N 39 18.824	W 96 56.437	391
Stafford	SF12(1)	N 38 12.163	W 98 43.503	572
Stafford	SF12(2)	N 38 12.163	W 98 43.503	572
Stafford	SF12(3)	N 38 06.276	W 98 50.374	600
Stafford	SF12(4)	N 38 05.401	W 98 50.366	597

[a]Population = Kansas county codes followed by the year collected followed by reference number.

Table 3-4. Visible common waterhemp control 7 and 14 days after treatment (DAT) with glyphosate in Kansas in 2011.

	Glyphosate Rate[a]					
	870		1,740		3,480	
Population	7 DAT[b]	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT
	----------(% Control)----------					
S	100	100	100	100	100	100
CR11	28**	33**	74**	80	91	95
FR11(1)	45**	47**	72**	82	91	95
FR11(2)	46**	48**	50**	57**	84**	91
FR11(3)	21**	21**	46**	53**	73**	83**
FR11(4)	29**	40**	68**	73**	88	94
FR11(5)	57**	62**	71**	74**	92	96
MS11	62**	63**	81	84	91	94
NM11	78	87	94	98	96	99
WS11	67**	69**	77	79	89	93

**Indicates significance at $\alpha = 0.05$ when compared to the susceptible population.
[a]Rate = g ha^{-1}; all treatments included AMS at 1% w/w.
[b]DAT = days after treatment.

Table 3-5. Visible Palmer amaranth control 7 and 14 days after treatment (DAT) with glyphosate in Kansas in 2011.

	Glyphosate Rate[a]					
	870		1,740		3,480	
Population	7 DAT[b]	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT
	----------(% Control)----------					
S	100	100	100	100	100	100
CY11	100	100	100	100	100	100
CL11(1)	35**	41**	58**	68**	91**	93**
CL11(2)	40**	44**	84**	89**	96	98
DK11	100	100	100	100	100	100
DG11	100	100	100	100	100	100

**Indicates significance at $\alpha = 0.05$ when compared to the susceptible population.
[a]Rate = g ha^{-1}; all treatments included AMS at 1% w/w.
[b]DAT = days after treatment.

Table 3-6. Visible Palmer amaranth control 7 and 14 days after treatment (DAT) with glyphosate in Kansas in 2012.

	Glyphosate Rate[a]					
	870		1,740		3,480	
Population	7 DAT[b]	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT
	----------(% Control)----------					
S	100	100	100	100	100	100
CY12	100	100	100	100	100	100
CL12(1)	43**	55**	66**	70**	81**	88**
CL12(2)	77**	87	85	91	99	100
CL12(3)	56**	58**	57**	65**	80**	86**
CL12(4)	49**	54**	66**	74**	91	98
EW12(1)	97	99	100	100	100	100
EW12(2)	100	100	100	100	100	100
MN12	100	100	100	100	100	100
PT12(1)	67**	66**	88**	95	99	100
PT12(2)	60**	58**	86**	82**	85**	94**
PT12(3)	98	99	99	100	99	100
PT12(4)	100	100	100	100	100	100
RL12	93	100	100	100	100	100
SF12(1)	99	100	98	100	98	100
SF12(2)	93	94	97	100	98	100
SF12(3)	35**	36**	73**	83**	90	96
SF12(4)	38**	46**	71**	81**	98	99

**Indicates significance at $\alpha = 0.05$ when compared to the susceptible population.
[a]Rate = g ha^{-1}; all treatments included AMS at 1% w/w.
[b]DAT = days after treatment.

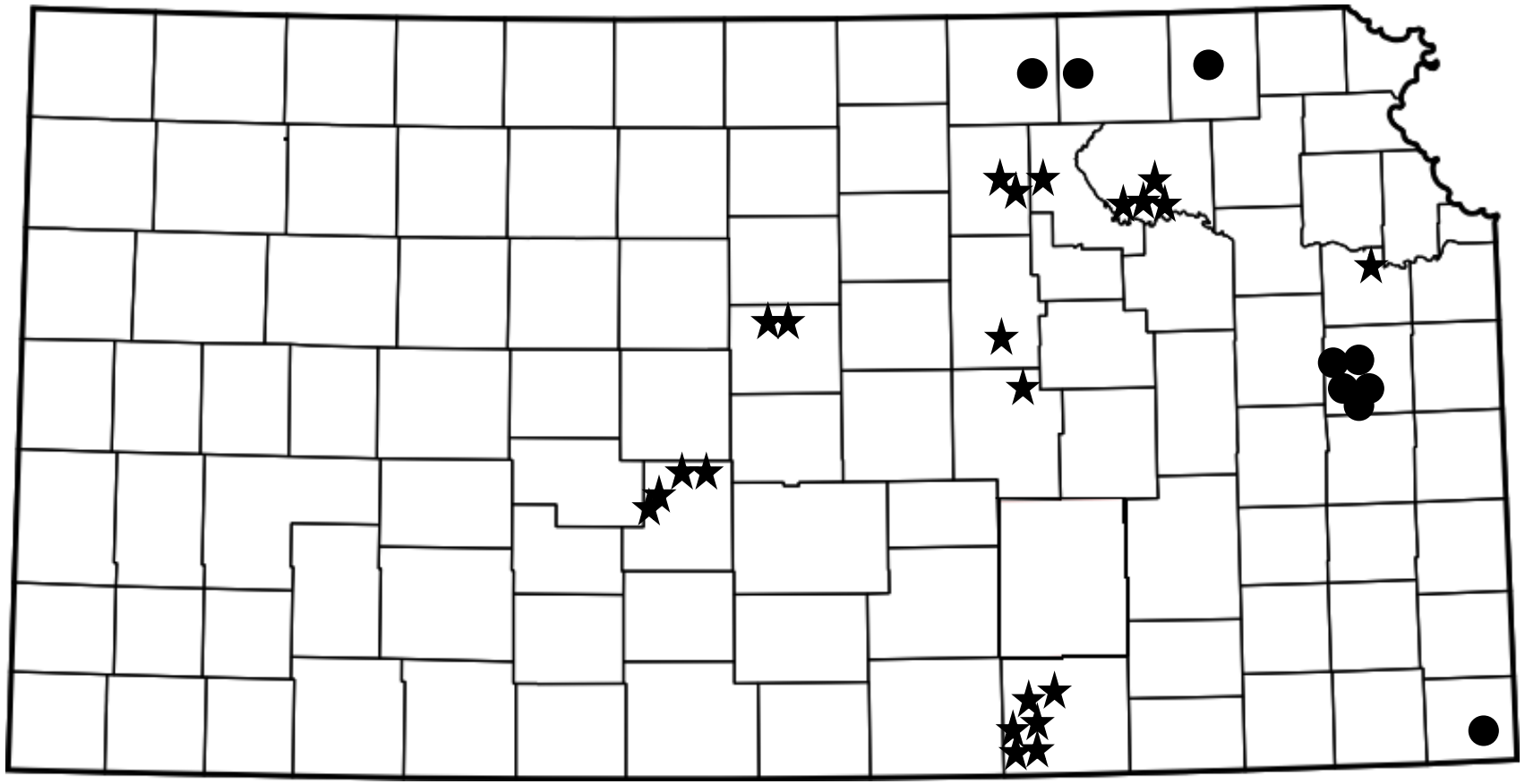

Figure 3-1. Locations of common waterhemp and Palmer amaranth collected in the fall of 2011 and 2012. Circles indicate nine common waterhemp populations and stars represent 22 Palmer amaranth populations in Kansas.

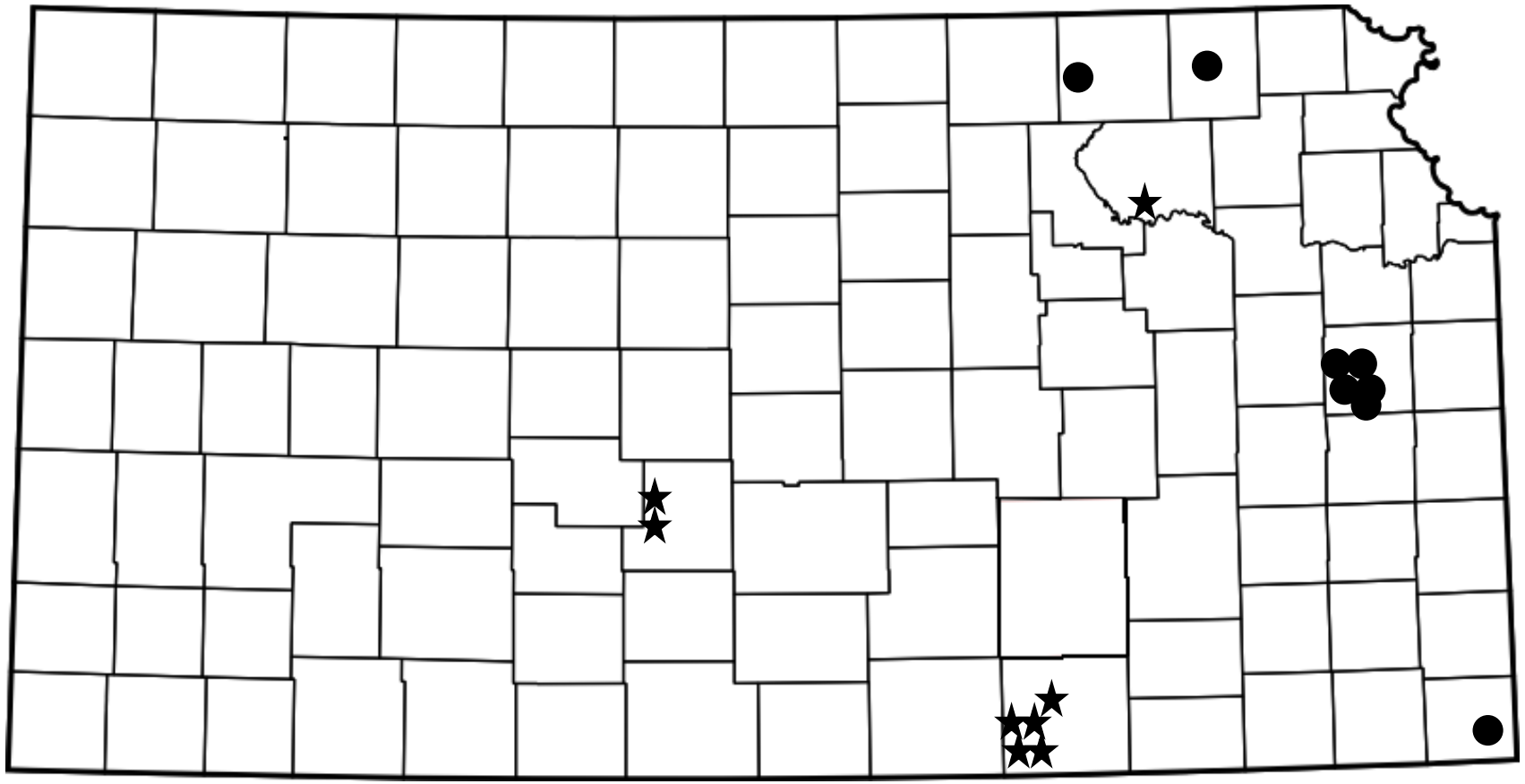

Figure 3-2. Locations of glyphosate-resistant common waterhemp and Palmer amaranth in Kansas. Circles indicate glyphosate-resistant common waterhemp and stars represent glyphosate-resistant Palmer amaranth populations that were confirmed in Kansas.

Chapter 4 – Glyphosate Dose-Response and Shikimate Accumulation Assay of Palmer Amaranth Populations

ABSTRACT

Palmer amaranth is difficult to control because it germinates early in the growing season, grows rapidly, and has evolved resistance to certain classes of herbicides. Two separate experiments were conducted to 1) evaluate the dose response of two Kansas populations of Palmer amaranth suspected to be resistant to glyphosate and 2) conduct shikimate accumulation assays to the 2011 and 2012 populations that survived a 2x rate of glyphosate. The suspected resistant populations were treated with glyphosate rates of 0, 0.50, 1, 2, 4, and 8 times the typical field use rate of 870 g ae ha^{-1} and compared to a known susceptible population that was treated with glyphosate rates of 0, 0.0625, 0.125, 0.25, 0.50, and 1 times the typical use rate. All treatments included ammonium sulfate at 1% w/w. Percent control was evaluated 7 and 14 days after treatment (DAT) on a 0 to 100% scale where 0 is no effect and 100 = plant death. Surviving plants were then harvested and weighed to test for differences in fresh weight biomass between the susceptible and resistant populations. The susceptible population was fully controlled with the typical field use rate of glyphosate. There was variability within the resistant populations however, they behaved similarly. Glyphosate caused chlorosis and necrosis of leaves for both resistant populations 14 DAT with visual injury ranging from 10 to 100% depending on the population and rate. Glyphosate at 870 g ae ha^{-1} did not provide acceptable control of these Palmer amaranth populations. A shikimate accumulation assay was then conducted on two populations from 2011 and eight from 2012 that survived applications of glyphosate. The susceptible population accumulated shikimate when leaf discs were treated with glyphosate. Leaf discs excised from glyphosate-resistant plants did not accumulate shikimate

when treated with 100 µM glyphosate. The assay clearly differentiated between glyphosate-resistant and –susceptible Palmer amaranth plants, in which susceptible plants accumulated greater than 15 ng shikimate μL^{-1} while resistant plants did not accumulate any shikimate. This is the first documented case of glyphosate-resistant Palmer amaranth in Kansas, which will likely become more widespread in the future.

INTRODUCTION

Palmer amaranth (*Amaranthus palmeri* S. Wats.) is a fast growing, prolific seed producer, and has a long germination window, which attributes to its weedy characteristic (Horak and Loughin 2000; Keeley et al. 1987; Steckel et al. 2004). Glyphosate resistance in weeds has increased due to the extensive use of glyphosate and the selection pressure this causes. Over the last decade, glyphosate-resistant (GR) Palmer amaranth has spread throughout the southeast United States and has begun to expand northward. In 2004, glyphosate-resistant Palmer amaranth was first confirmed in a 250-ha cotton field in Macon County, Georgia (Culpepper et al. 2006). Since then, it has become a major concern for conservation farming and for many farmers throughout the United States.

Glyphosate-resistant Palmer amaranth has spread to 18 states in the United States; as far west as California and as far north as Michigan (Heap 2013). Palmer amaranth pollen is wind-mediated and research has shown that long-distance pollen dispersal events occur (Alibert et al. 2005; Hanson et al. 2005; Massinga et al. 2003; Matus-Cadiz et al. 2004; Saeglitz et al. 2000). In 2006 and 2007, studies were conducted in Georgia to determine if glyphosate resistance can be transferred via pollen movement from a GR Palmer amaranth source planted in the center of a 30-ha field to glyphosate-susceptible females planted between 1 and 300 m away (Sosnoskie et al. 2009). Approximately 60% of the offspring derived from susceptible females at the 1-m distance were resistant to glyphosate; at 300-m, approximately 20% of the offspring were resistant (Culpepper et al. 2010). This indicated that glyphosate-resistant pollen can travel long distances, and, breed with other females and thus spread quickly into surrounding fields, making control difficult.

Testing methods for genetically engineered glyphosate-resistant crops are relatively straight forward because the mutations are known and the level of resistance is extremely high (Shaner 2010). However, testing for glyphosate resistance in weed biotypes is more difficult. There are several methods for testing for glyphosate resistance in weedy biotypes. Two common methods for testing resistance to glyphosate are greenhouse dose-response assays and shikimate accumulation assays. The greenhouse dose-response assay requires several herbicide treatment levels and is the most definitive. Treatment levels used for the dose-response will depend on the level of resistance and the inherent sensitivity of the species to glyphosate (Shaner 2010).

Glyphosate resistance in Palmer amaranth was confirmed using glyphosate rate response studies. In greenhouse studies, the original Georgia GR biotype had a glyphosate I_{50} (rate of glyphosate required to reduce shoot fresh weight by 50%) of 1.2 kg ae ha^{-1}, approximately eight times greater than that of the susceptible biotype with an I_{50} of 0.15 kg ha^{-1} (Culpepper et al. 2006). In the field, glyphosate at 1.25, 2.5, 5.0, 7.5, and 10 kg ha^{-1} controlled emerged Palmer amaranth 8, 17, 46, 70, and 82%, respectively (Culpepper et al. 2006). In this experiment, glyphosate at 12 times the recommended rate, or 10 kg ha^{-1}, failed to provide commercially acceptable control of this weed species (Culpepper et al. 2006). Similar dose-response results were seen in Palmer amaranth populations in Arkansas (Norsworthy et al. 2008). The resistant biotype had an LD_{50} of 2.82 kg ha^{-1} glyphosate, which was 79- to 115-fold greater than the susceptible biotypes and 3.4 times the normal-use rate of glyphosate. In Tennessee, GR-Palmer amaranth was also examined. Field and greenhouse research confirmed that two separate populations had reduced biomass sensitivity, 1.5x to 5.0x, to glyphosate compared to susceptible populations, although the level of resistance was higher based on plant mortality response, about

10x (Steckel et al. 2008). Although good results are achieved with these assays, they require a lot of time and can be expensive.

Shikimate accumulation assays are quick alternatives to test for glyphosate resistance in weed biotypes; however, sophisticated laboratory equipment is needed to conduct these experiments. An alternative method to determine whether a suspected GR weed has a resistant EPSPS (5-enolpyruvoylshikimate-3-phosphate synthase) is to monitor the effect of glyphosate on shikimate levels (Shaner et al. 2005). Glyphosate competes with the substrate phosphoenolpyruvate for a binding site on the enzyme 5-enol-pyruvylshikimate-3-phosphate synthase, resulting in uncontrolled flow of carbon and subsequent accumulation of shikimate in affected sensitive tissues (Amrhein et al. 1980). Accumulation of shikimate in glyphosate-treated plants indicates the herbicide is affecting the activity of EPSPS (Mueller et al. 2003).

Research has shown that some GR weed biotypes do not accumulate shikimate. Shikimate accumulation after glyphosate treatment has been used to identify GR-soybean and GR-cotton with a resistant EPSPS (Pline et al. 2002; Singh and Shaner 1998). Similar methods are used to detect resistance in weeds. Shikimate did not accumulate in a GR-rigid ryegrass population treated with glyphosate (Simarmata et al. 2003) or in a GR-horseweed population treated with a sublethal rate of glyphosate, although the same rate did cause shikimate accumulation in a susceptible horseweed population (Feng et al. 2004). Culpepper et al. (2006) detected shikimate in leaf tissue of glyphosate-resistant Palmer amaranth at the lowest concentration of glyphosate at 8.4 mg L^{-1}, and shikimate concentration increased linearly as glyphosate concentration increased. The susceptible population accumulated shikimate across all glyphosate doses, whereas the resistant population did not accumulate shikimate at the highest dose tested (Gaines et al. 2011). This population of Palmer amaranth exhibited no changes in

glyphosate uptake and translocation in comparison to a susceptible population (Culpepper et al. 2006), and no known target-site mutations associated with glyphosate resistance have been identified in the EPSPS gene sequence (Gaines et al. 2010). However, DNA blots provided initial evidence to suggest EPSPS gene amplification was present in the resistant population. The data presented by Gaines et al. (2010) showed that the recent evolution of glyphosate resistance in a Palmer amaranth population is due to EPSPS gene amplification and increased EPSPS expression. The reported data indicate that an EPSPS gene amplification in glyphosate-resistant Palmer amaranth from Georgia results in high levels of EPSPS expression and that the mechanism imparts high-level glyphosate resistance.

After screening for glyphosate resistance in five Palmer amaranth populations collected from central Kansas during the fall of 2011, two populations from Cowley County, KS survived a 2x rate of glyphosate and one of those was uncontrolled with a 4x rate of glyphosate. The objectives of this research were to (1) determine if the two Cowley County populations are resistant to glyphosate, (2) quantify the level of glyphosate resistance compared to a known susceptible population that had not been previously exposed to glyphosate, and (3) to observe whether or not shikimate accumulates in plants from the glyphosate-resistant and -susceptible populations.

MATERIALS AND METHODS

Dose-Response Assay on Two Palmer Amaranth Populations From 2011

Seed from two Palmer amaranth populations from Cowley County, Kansas previously screened for glyphosate resistance were used to quantify the level of resistance to glyphosate. Global positioning system (GPS) coordinates at each location and year are reported in Tables 4-1 and 4-2. Palmer amaranth populations collected from the Department of Agronomy Ashland

Bottoms Research Farm near Manhattan, KS known to be susceptible to glyphosate were included for comparison.

In April to May 2012, Palmer amaranth populations were sown in separate flats, measuring 3.4 L, and filled with 0.7 kg of Miracle-Gro moisture control potting mix[1]. The plants were grown under greenhouse conditions of 27/24 ± 2 C day/night temperature with a 16/8-h day/night period and light intensity of 80 µmol m^{-2} s^{-1} photosynthetic photon flux. Individual seedlings were transplanted into 0.25-L pots when plants were at the cotyledon stage of growth and watered as needed. The experimental design was a randomized complete block (blocked by plant size) with treatments replicated six times, and the experiment was repeated. The plants were treated when they reached 10 to 14 cm in height with glyphosate[2]. The known susceptible population was treated with 54, 109, 217, 435, and 870 g ae ha^{-1} glyphosate whereas the suspected resistant populations were treated with 435, 870, 1,740, 3,480, and 6,960 g ae ha^{-1} glyphosate. The lowest rate corresponds to 1/16 and the highest rate corresponds to 8 times a typical use rate of glyphosate at 870 g ae ha^{-1}. Ammonium sulfate (AMS) at 1% w/w was added to all herbicide treatments. Glyphosate was applied with a bench-type sprayer[3] equipped with an 80015LP[4] spray tip to deliver 187 L ha^{-1} at 138 kPa.

Percent control was determined 7 and 14 days after treatment (DAT) on a 0 to 100% scale where 0 = no effect and 100 = plant death. At the final evaluation, plants were clipped at soil level and shoot fresh weights were determined. Percent control data were subjected to analysis of variance using PROC GLIMMIX in SAS 9.2[5]. Nonlinear regression analysis of the six glyphosate rates were used to determine the rate required to cause 50 and 90% injury (ED_{50} and ED_{90}) as well as 50 and 90% growth reduction (GR_{50} and GR_{90}) values of each accession

and were determined using R statistical software[6]. The control or mortality response, y, herbicide rate, 'x' were:

$$y = \frac{\mathrm{a}}{1 + \exp(-(-\mathrm{x} - \mathrm{ED50})/\mathrm{b}))}$$

where 'a' is the upper limit or 100%, 'b' is the slope. Resistance index (RI) was calculated by dividing ED and GR values of the resistant populations by the ED and GR values of the susceptible population.

In Vivo Shikimate Accumulation Assay

Palmer amaranth populations that survived glyphosate applications during the initial screening process (Chapter 3) were subjected to an in vivo shikimate accumulation assay for identifying glyphosate-resistant plants. Eight individuals from each of the glyphosate-resistant and –susceptible populations were measured for shikimate accumulation. Leaf discs (4-mm diam) were excised from leaves with a modified cork borer equipped with a spring-loaded plunger. One disc was placed in each well of a 96-well microtiter plate[7]. Control wells contained 10 mM ammonium phosphate plus 0.1% (v/v) Tween 80 surfactant. The glyphosate treatment wells contained 100 µl of 10 mM ammonium phosphate (pH 4.4), 0.1% (v/v) Tween 80 surfactant and a single concentration of glyphosate[8] at 100 µM. Plates were wrapped in plastic wrap to minimize evaporation. Plates were then incubated under fluorescent lights (150 mM m^{-2} s^{-1}) at 27 C for 16 h. After incubation, plates were placed in a -20 C freezer until the solution froze and then thawed at room temperature or at 60 C for 30 min. Twenty-five microliters of 1.25 N HCl were pipetted into each well, giving a final concentration of 0.25 N HCl per well. The plates were incubated at 60 C for 15 min. At the end of this incubation, the discs had turned a uniform gray–green color, indicating complete penetration of the tissue by the

acid. Shikimate was determined spectrophotometrically following the procedure of Cromartie and Polge (2000). Aliquots of 25 µl were transferred from each well to another microtiter plate to which 100 µl of 0.25% (w/v) periodic acid[9] /0.25% (w/v) m-periodate[10] was added to each well. This plate was incubated at 60 C for 30 min and then 100 µl of 0.6 N sodium hydroxide/0.22 M sodium sulfite was added to each well. The optical density at 380 nm was measured within 30 min using a microtiter plate spectrophotometer[11]. A shikimate standard curve was developed by adding known amounts of shikimate[12] to wells containing leaf discs not exposed to glyphosate so that shikimate levels could be reported as micrograms shikimate per milliliter HCl solution. Shikimate accumulation greater than 15 ng μL^{-1} is susceptible and no shikimate accumulation are resistant biotypes. This procedure was developed by Shaner et al. (2005).

RESULTS AND DISCUSSION

Dose-Response

Glyphosate at 54 g ae ha^{-1} significantly injured the susceptible population and caused substantial foliar chlorosis and necrosis. Visual control was 89 and 93% 7 and 14 DAT, respectively (Table 4-3). Control increased with increase in rate and 100% control was achieved with glyphosate at 218 g ae ha^{-1} for the susceptible population (Table 4-3). The resistant populations were not adequately controlled with the typical field use rate of 870 g ha^{-1} glyphosate . CL11(1) was only controlled 45 and 48% at 7 and 14 DAT with 870 g ha^{-1} glyphosate and by 14 DAT, many resistant plants resumed growth. Control increased as glyphosate rates increased but 6,960 g ha^{-1} (8 times the typical use rate) did not adequately control all resistant biotypes (Table 4-3). Control was 95 and 98% at 7 and 14 DAT which indicated that some plants were not fully controlled with 8 times the field use rate of glyphosate.

CL11(2) displayed similar resistance characteristics with visual control of 69 and 68% at 7 and 14 DAT with 870 g ha^{-1} glyphosate, respectively. Resistant biotypes were not completely controlled with 6,960 g ha^{-1} 7 and 14 DAT (Table 4-3). Visual control with 8 times the use rate of glyphosate was 92 and 96% indicating some biotypes were severely injured but not completely dead.

Effective dose (ED) and growth reduction (GR) values were calculated and dose-response curves were generated along with resistance indices; the higher the resistance index (ratios of ED_{50} and GR_{50} values relative to that of a susceptible population), the greater the level of resistance. The ED_{50} values for the susceptible and resistant populations were 28, 906, and 353 g ha^{-1} glyphosate for the susceptible, CL11(1), and CL11(2) populations, respectively (Table 4-4). CL11(1) and CL11(2) were 33- and 13-fold more resistant than the susceptible population. Dose-response curves described visual control of both glyphosate-resistant and –susceptible biotypes (Figure 4-1). Furthermore, the ED_{90} values for CL11(1) and CL(2) were 5,240 and 2,945 g ha^{-1} glyphosate whereas it was only 49 g ha^{-1} for the susceptible population. This corresponded to 107- and 60-fold more resistant than the susceptible population, indicating a high level of resistance in these two Palmer amaranth populations (Table 4-4).

It is suspected that 10 g ae ha^{-1} would cause little to no biomass reduction to the glyphosate-resistant Palmer amaranth populations so a data point at 10 g ha^{-1} glyphosate for population CL11(2) was included to sufficiently fit the data to the log-logistic model (Figure 4-2). GR_{50} values were 27, 582, and 145 g ha^{-1}. GR_{90} values correspond with ED_{90} values as they were 68, 5,209, and 2,312 g ha^{-1} glyphosate for the susceptible, CL11(1), and CL11(2) populations (Table 4-5). Both resistant populations were statistically different from the

susceptible population and high rates of glyphosate are required to decrease biomass of these two populations.

These Palmer amaranth populations are highly resistant when compared to the known susceptible biotype with CL11(1) requiring 107 times the dose of glyphosate to achieve adequate control of 90%, where CL11(2) required 60 times the dose of glyphosate compared to the susceptible population. Log-logistic dose-response curves visually explain percent control of glyphosate-resistant and –susceptible biotypes.

The level of resistance reported here is higher than the six- to eight-fold level of resistance reported for Palmer amaranth in Georgia (Culpepper et al. 2006). However, Norsworthy et al. (2008) documented a glyphosate-resistant Palmer amaranth biotype in Arkansas that had lethal dose (LD_{95}) values of 12,500 g ha^{-1}, almost 15 times the normal-use rate of glyphosate. Here, the ED_{90} values for the resistant populations of 5,240 and 2,945 g ha^{-1} are 6 and almost 4 times the typical use rate of glyphosate indicating that increasing the glyphosate use rate may not be a feasible alternative for controlling the resistant biotypes.

In Vivo Shikimate Assay

Glyphosate competes with the substrate phosphoenolpyruvate for a binding site on the enzyme 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS, E.C.2.5.1.19), resulting in uncontrolled flow of carbon and subsequent accumulation of shikimate in affected sensitive tissues (Amrhein et al. 1980; Culpepper et al. 2006). Accumulation of shikimate in glyphosate-treated plants indicates the herbicide is affecting the activity of EPSPS (Mueller et al. 2003). In this experiment, shikimate was detected in leaf tissue of glyphosate-susceptible Palmer amaranth with 100 µM of glyphosate. Susceptible plants were accumulating greater than 15 ng shikimate

μL^{-1}. However, shikimate was not detected in leaf tissue of glyphosate-resistant Palmer amaranth biotypes (Figure 4-3).

This assay can be used to detect glyphosate-resistant weed populations when plants are small and would provide a quick turnaround time for determining if glyphosate resistance is present. The assay is rapid and has high throughput because shikimate is extracted by freeze-thawing and shikimate is detected by an improved spectrophotometric method (Shaner et al. 2005). However, this assay requires the use of sophisticated lab facilities and materials which are not accessable to local growers for use.

Palmer amaranth is already one of the mose troublesome weeds of agronomic crops across the southern United States (Webster 2005). This research confirms that glyphosate-resistant Palmer amaranth is now present and is found in several counties throughout central Kansas. Spread of pollen and the continued reliance on glyphosate will likely result in the spread of glyphosate-resistant genes and a greater number of resistant populations in the near future. Resistance to other herbicides, such as dinitroanilines and acetolactate synthase inhibitors (Heap 2013), limits the options to control glyphosate-resistant Palmer amaranth in Kansas. Glyphosate alone is no longer a viable option for controlling Palmer amaranth where glypohsate-resistant populations are present.

SOURCES OF MATERIALS

[1]Miracle-Gro moisture control potting mix, Scotts miracle-gro products INC., 1411 Scottslawn Road, Marysville, OH 43041.

[2]Roundup WEATHERMAX®, Monsanto Company, St. Louis, MO 63167.

[3]Research track sprayer, De Vries Manufacturing, RR 1, Box 184, Hollandale, MN 56045.

[4]Teejet Spraying Systems, Wheaton, IL 60189-7900.

[5]SAS version 9.2, SAS Institute Inc., 100 SAS Campus Drive, Cary NC 27513.

[6]R Development Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2010.

[7]Nunc Microwell 96-well plate, VWR International Inc., 17750 East 32nd Place, Suite 10, Aurora, CO 80011.

[8]Roundup Ultramax®, isopropylamine salt of glyphosate, Monsanto Co., 800 North Linbergh Boulevard, St. Louis, MO 63167.

[9]Periodic acid, Sigma-Aldrich Co. LLC, St. Louis, MO 63178. http://www.sigmaaldrich.com/customer-service.html.

[10]*m*-periodate, Sigma-Aldrich Co. LLC, St. Louis, MO 63178. http://www.sigmaaldrich.com/customer-service.html.

[11] Epoch Microplate Spectrophotometer BioTek, 100 Tigan Street Winooski, VT 05404.

[12]Shikimic acid, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178.

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Table 4-1. GPS locations for the susceptible and resistant populations of Palmer amaranth from 2011.

County	Population[a]	GPS coordinates		Elevation
				---m---
Susceptible	S	N 39 07.470	W 96 36.840	311
Cowley	CL11(1)	N 37 14.518	W 97 06.850	374
Cowley	CL11(2)	N 37 11.262	W 97 10.764	349

[a]Population = Kansas county codes followed by the year collected followed by reference number.

Table 4-2. GPS locations for each population of Palmer amaranth collected in Kansas during the fall of 2012.

County	Population[a]	GPS coordinates		Elevation
				---m---
Susceptible	S	N 39 07.470	W 96 36.840	311
Clay	CY12	N 39 19.155	W 97 02.906	364
Cowley	CL12(1)	N 37 16.843	W 97 08.969	354
Cowley	CL12(2)	N 37 08.381	W 97 07.864	336
Cowley	CL12(3)	N 37 08.381	W 97 07.864	336
Cowley	CL12(4)	N 37 13.832	W 97 05.363	372
Ellsworth	EW12(1)	N 38 46.528	W 98 11.273	551
Ellsworth	EW12(2)	N 38 37.394	W 98 21.870	566
Marion	MN12	N 38 33.085	W 96 57.342	450
Pottawatomie	PT12(1)	N 39 12.831	W 96 14.988	300
Pottawatomie	PT12(2)	N 39 12.831	W 96 14.988	300
Pottawatomie	PT12(3)	N 39 13.010	W 96 15.559	300
Pottawatomie	PT12(4)	N 39 11.132	W 96 02.889	288
Riley	RL12	N 39 18.824	W 96 56.437	391
Stafford	SF12(1)	N 38 12.163	W 98 43.503	572
Stafford	SF12(2)	N 38 12.163	W 98 43.503	572
Stafford	SF12(3)	N 38 06.276	W 98 50.374	600
Stafford	SF12(4)	N 38 05.401	W 98 50.366	597

[a]Population = Kansas county codes followed by the year collected followed by reference number.

Table 4-3. Visible control 7 and 14 days after treatment (DAT) with glyphosate on 3 Palmer amaranth populations in Kansas in 2011.

Glyphosate	Susceptible		CL11(1)		CL11(2)	
Rate[a]	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT
	----------(% Control)----------					
0	0	0	0	0	0	0
0.0625	89	93	-	-	-	-
0.125	89	99	-	-	-	-
0.25	98	100	-	-	-	-
0.5	97	98	40	35	55	60
1	100	100	45	48	69	68
2	-	-	70	76	93	93
4	-	-	99	98	99	99
8	-	-	95	98	92	96
LSD (0.05)	12	7	21	20	20	19

[a]Rate = rates are the proportion of a typical field use rate of glyphosate in g ha^{-1}; all treatments included AMS at 1% w/w.

Table 4-4. Dose-response summary. Effective dose (ED) values expressed in g ae ha^{-1} and the resistance index (RI) values represent the degree of resistance compared to the susceptible population.

Population	ED_{50}	ED_{90}	RI_{50}	RI_{90}
Susceptible	28	49		
CL11(1)	906	5,240	33	107
CL11(2)	353	2,945	13	60

Table 4-5. Glyphosate rates required to decrease growth (shoot fresh weight) biomass by 50 and 90% expressed in g ae ha^{-1}.

Population	GR_{50}	GR_{90}
Susceptible	27	68
CL11(1)	582	5,209
CL11(2)	145	2,312

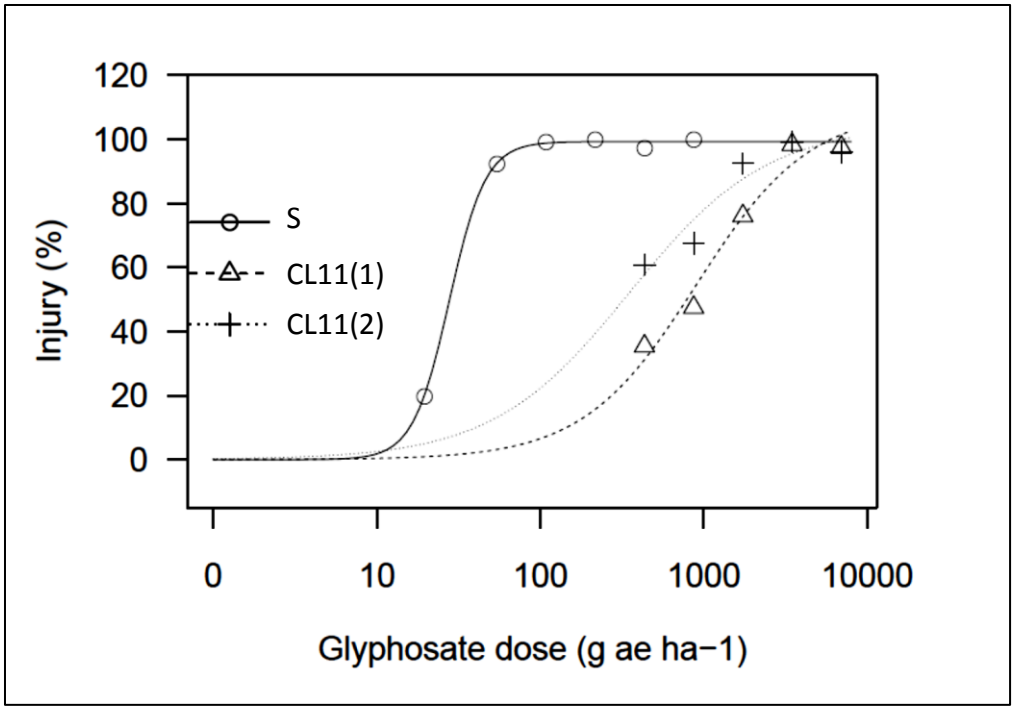

Figure 4-1. Visual injury of glyphosate-resistant and –susceptible Palmer amaranth 14 days after glyphosate application in the greenhouse.

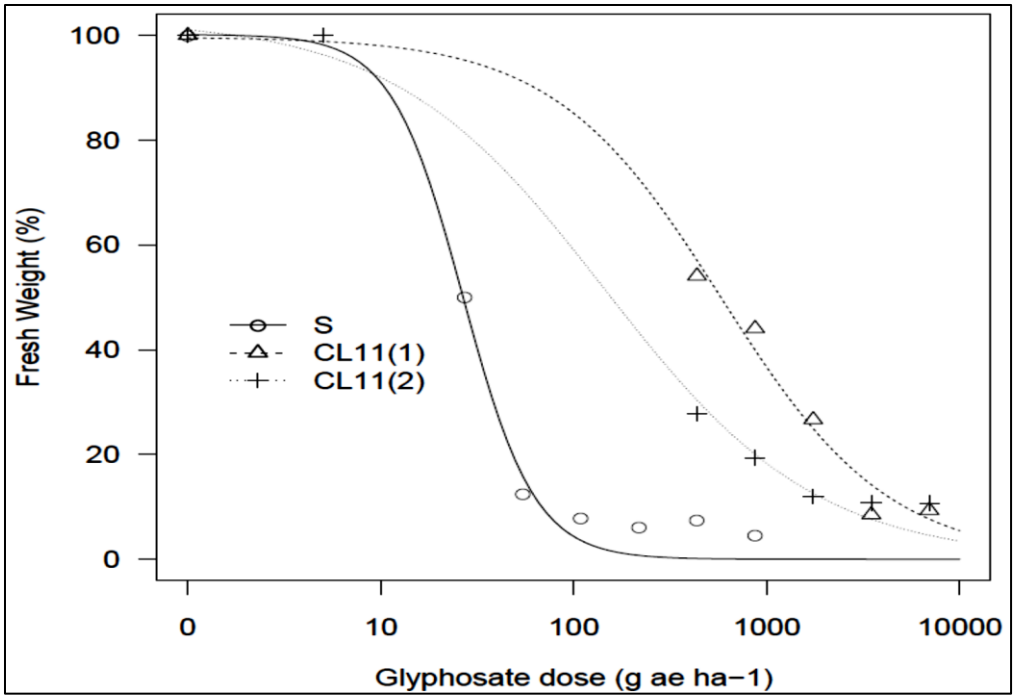

Figure 4-2. Log-logistic dose-response curves of glyphosate-resistant and –susceptible Palmer amaranth shoot fresh weights, as a percentage of the non-treated check, 14 days after glyphosate application.

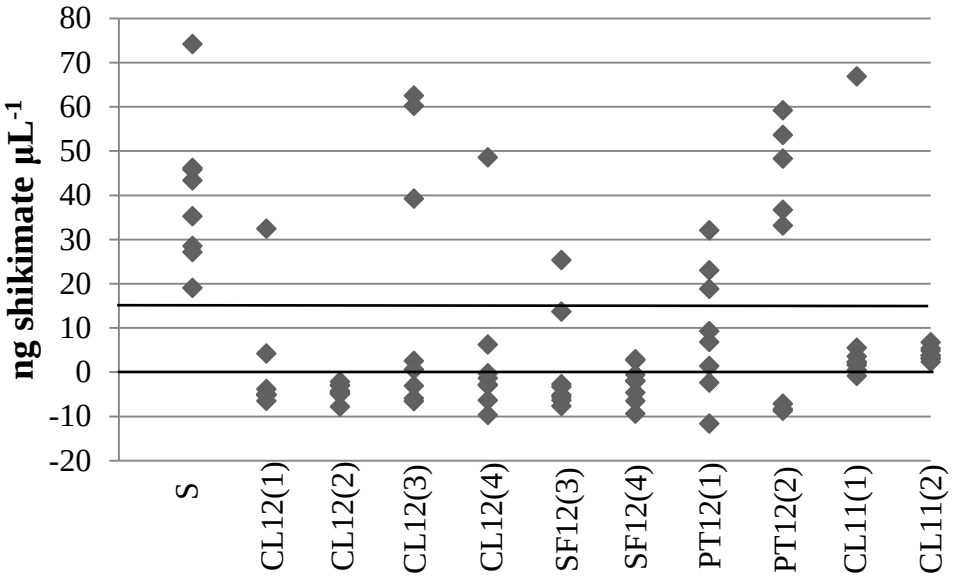

Figure 4-3. Accumulation of shikimate in Palmer amaranth leaf discs excised from 8 different plants per population. Resistant Palmer amaranth plants accumulated little to no shikimate while susceptible plants accumulated greater than 15 ng µL^{-1}.