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Confirmation and management of multiple resistance of horseweed [Conyza canadensis (L.) Cronq.] to glyphosate and paraquat

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CONFIRMATION AND MANAGEMENT OF MULTIPLE RESISTANCE OF HORSEWEED [*Conyza canadensis* (L.) Cronq.] TO GLYPHOSATE AND PARAQUAT

By

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Glyphosate-resistant (GR) horseweed has become a major problem in many row crop production systems in the United States. Horseweed is a winter annual weed common in no-till production systems. Fall-applied herbicides were compared with spring-applied treatments for the control of horseweed. In cotton, fall-applied trifloxysulfuron provided similar or greater control of horseweed when compared to spring-applied treatments of glyphosate + dicamba. Cotton yields with fall-applied trifloxysulfuron, clomazone, and flumioxazin were comparable to or better than spring-applied glyphosate + dicamba both years. Fall-applied cloransulam-methyl, flumetsulam, sulfentrazone, and the combination of chlorimuron-ethyl + metribuzin resulted in horseweed control and soybean yields comparable to spring-applied glyphosate + 2,4-D both years.

Multiple-resistance to glyphosate and paraquat exists in a horseweed population from Mississippi. Herbicide rates of 0.066 kg ae/ha glyphosate and 0.078 kg ai/ha paraquat were required to reduce susceptible horseweed biomass 50%; whereas, rates of 0.78 kg/ha glyphosate and 0.67 kg/ha paraquat were required to reduce biomass of resistant horseweed to a similar intent. This is the first broadleaf weed species reported as exhibiting multiple-resistance to glyphosate and paraquat. The addition of metribuzin to paraquat improved control of paraquat-resistant horseweed. Paraquat at 0.84 kg/ha plus all rates of metribuzin controlled 15-cm tall horseweed at least 90% both years compared to 73% with 0.84 kg/ha paraquat alone.

The addition of 1 and 2% methylated seed oil (MSO) to saflufenacil controlled horseweed 91 and 93%, respectively compared to 78% control with saflufenacil alone. The addition of

saflufenacil to glyphosate improved control of GR horseweed from 50% to 100% at 21 d after treatment; control of horseweed with the combination of saflufenacil + glyphosate was additive. Saflufenacil did not affect absorption of glyphosate in glyphosate-susceptible horseweed; however, absorption increased in GR horseweed from 36 to 44% at 48 h after treatment with the addition of saflufenacil when compared to glyphosate alone treatments. Overall, the addition of saflufenacil reduced glyphosate translocation in horseweed at least 6%; however, due to the exceptional efficacy of saflufenacil on horseweed these reductions did not reduce control of GR horseweed.

DEDICATION

I would like to dedicate this work to my Lord and Savior Jesus Christ who has blessed me so richly with my wonderful wife, Beth and our beautiful children, Adelle and Will. Your love, patience and support have allowed me to achieve my dreams.

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CHAPTER I

INTRODUCTION

Glyphosate is a systemic, non-selective, postemergence herbicide that has been used extensively for the control of troublesome weeds. The effectiveness of glyphosate as an herbicide and the utility of glyphosate-resistant (GR) crops have allowed many producers to reduce labor and input costs as well as implement minimum-tillage and no-tillage practices (Bond et al. 2009; Gianessi 2008; Givens et al. 2009a; Halford et al. 2001). Consequently, this widespread adoption has led to an increase in the number of glyphosate applications made during the growing season and has also increased selection pressure toward the development of GR weeds (Givens et al 2009b; Young 2006). To date, five weed species in Mississippi have been reported as being resistant to glyphosate, including horseweed [*Conyza canadensis* (L.) Cronq.] (Koger et al. 2004), Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] (Nandula et al. 2007a), Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Heap 2010), common waterhemp (*Amaranthus rudis* Sauer) and johnsongrass [*Sorghum halepense* (L.) Pers.] (Vijay Nandula, Miss. State Univ. personal communication).

Horseweed is a member of the Asteraceae family and is classified as a winter or summer annual (Brown and Whitwell 1988). Most winter annuals emerge in the fall, remain vegetative throughout the winter months, and produce seed in the spring to early summer. Research has shown that horseweed may emerge any time of the year but primarily emerges in the spring in more northern latitudes and from early fall through late spring in the South (Bhowmik and Bekech 1993; Buhler and Owen 1997; Eubank et al. 2006; Main et al. 2006; Saphangthong and Witt 2006). This wide window of emergence can potentially cause significant problems in the control of horseweed with postemergence herbicides. The emergence of horseweed is typically greater on or near the soil surface when temperatures are between 10 and 20 C (Eubank et al.

2006; Main et al. 2006; Nandula et al. 2006). Horseweed germinates over a wide range of temperature and environmental extremes (Nandula et al. 2006). Horseweed reproduces only via seed; in the northern temperate zone flowering begins in July and August and ends in October to early November (Holm et al. 1997), whereas in southern latitudes flowering begins in May and June and ends in July and August (personal observation). Horseweed can produce many wind-disseminated seeds with some estimates averaging 50,000 to 200,000 seeds/plant (Bhowmik and Bekech 1993; Holm et al. 1997). Horseweed seed is capable of traveling great distances and is aided in dispersal by means of a pappus (Bhowmik and Bekech 1993; Dauer et al. 2006; Shields et al. 2006). The ease of horseweed spread could pose considerable problems for producers, as the movement of these seed may potentially infest and/or re-infest row crop hectares after control measures have been implemented. Thus, residual herbicides applied in the fall may provide a useful tool in the management and prevention of horseweed.

The majority of row crop hectares in Mississippi are treated with a non-selective herbicide such as glyphosate or paraquat prior to planting to remove winter weeds. Studies have suggested that the early removal of these weeds facilitates rapid drying and warming of the soil, as well as promoting earlier planting (Stougaard et al. 1984). GR weeds such as horseweed and Italian ryegrass have complicated early preplant burndown weed management in Mississippi (Koger et al. 2004; Nandula et al. 2007a). Alternative control measures for GR horseweed have been well documented (Eubank et al. 2008; Steckel et al. 2006). These treatments typically include 2,4-D, dicamba and/or glufosinate for the control of GR horseweed. However, equipment limitations, suitable weather, poor field conditions, and plant-back restrictions may hamper optimum applications of these herbicides. Additional concerns have been raised about the possibility of horseweed developing resistance to phenoxy-type herbicides (Kruger et al. 2008). Many producers in the Mid-South are encountering persistent emergence of horseweed following spring-applied burndown treatments prior to planting (personal observation). The use of fall-applied residual herbicides may prevent or reduce the subsequent emergence of horseweed prior to planting. Stougaard et al. (1984) reported fall applications of the residual herbicide cyanazine prevented emergence of horseweed until planting of no-till soybean (*Glycine max* (L.) Merr.); however, cyanazine is no longer available. Research is needed to evaluate newer residual herbicides on their effectiveness as a means for managing GR horseweed.

Herbicide resistance is becoming a significant problem with over 300 resistant species being documented worldwide (Heap 2010). In North America, horseweed has developed resistance to photosystem II (PSII) inhibitors, bipyridiliums, acetolactate synthase (ALS) inhibitors, ureas, amides, and glycines (Heap 2010; Koger et al. 2004; Smisek et al. 1998; Trainer et al. 2005; VanGessel 2001; Weaver et al. 2004). Horseweed is listed as being a problem weed in many row crop systems including cotton (*Gossypium hirsutum* L.), grain sorghum [*Sorghum bicolor* (L.) Moench], corn (*Zea mays* L.), rice (*Oryza sativa* L.) and soybean (Bond et al. 2009; Brown and Whitwell 1988; Buhler and Owen 1997; Eubank et al. 2008; Mueller et al. 2003; Steckel and Gwathmey 2009; Vencill and Banks 1994; Wiese et al. 1995). Many of the GR horseweed populations identified are from areas of the country where no-till practices have been widely adopted (Koger et al. 2004; Main et al. 2004; VanGessel 2001). No-tillage production systems require the use of a nonselective herbicide application to control weeds prior to planting (VanGessel et al. 2001; Wilson and Worsham 1988). Paraquat has been a complimentary tool in the control of weeds in no-till production systems (Stougaard et al. 1984).

Paraquat is in the bipyridilium class of herbicides that were developed in the 1950's and are described as photosynthetic inhibitors or cell membrane disruptors (Senseman 2007). There are currently two compounds classified as bipyridiliums: paraquat and diquat (Senseman 2007). These herbicides accept electrons in the light reaction cycle of photosystem I (PSI) that occurs in the chloroplast of most plants (Devine et al. 1993a). Once the herbicide intercepts an electron it forms a free radical that reduces oxygen into superoxide radicals, which then go on to produce hydrogen peroxide and hydroxyl radicals. These hydroxyl radicals are highly reactive with unsaturated lipids, which are the primary components making up cell membranes. Lipid cell membranes are rapidly degraded by these hydroxyl radicals leading to their rupture, eventually leading to wilting and desiccation of plant tissues (Devine et al. 1993a; Senseman 2007). Given that photosynthesis is a light-driven process, this reaction is usually very rapid with visual wilting occurring within hours, sometimes within minutes, when ample heat and sunlight are present. Paraquat is a non-selective, foliar-applied herbicide that is very effective in controlling many broadleaf and grassy weed species and is labeled for use in many agronomic, vegetable, fruit, and nut crops (Senseman 2007). Due to the rapid action and effectiveness of paraquat it has been utilized as a burndown herbicide to remove weed species prior to planting as well as a desiccant to facilitate harvesting (Eubank et al. 2008; Ratnayake and Shaw 1992).

Since the commercialization of paraquat, several plants in the genera *Conyza* and *Erigeron* have been reported resistant to paraquat (Kato and Okuda 1983; Watanabe et al. 1982; Heap 2010). Herbicide resistance is the process by which a plant population has inherited the ability to survive herbicide treatment that historically controlled that plant species (Heap 1997; Nandula 2010; Powles et al. 1998). This resistance is thought to occur as the result of random and infrequent mutations within the plant, as there has been no confirmation to date that these mutations are herbicide-induced (Prather et al. 2000). The theory is that a certain small number of plants within a given population hold the capacity to become resistant to a specific herbicide and through the repeated use of that herbicide the susceptible plants are removed and the selection for the resistant species is enhanced. Evolution of herbicide resistance may also be attributed to a lack of herbicide rotation or reliance on a single herbicide mode-of-action (Beckie and Rebound 2009). Possible mechanisms for herbicide resistance may include an altered binding site, enhanced degradation of the active herbicide, reduced uptake and/or translocation of the herbicide to the site-of-action, sequestration or compartmentalization of the herbicide molecule, over-expression or gene amplification of the herbicide target site, or a combination of these mechanisms (Devine et al. 1993b; LeBaron and Gressel 1982; Nandula 2010; Senseman 2007). In the early to mid 1980's *Conyza canadensis* (L.) Cronq, *Conyza sumatrensis* (Retz.), *Conyza bonariensis* (L.) Cronq. and *Erigeron philadelphicus* (L.) were all reported resistant to paraquat (Heap 2010; Kato and Okuda 1983; Watanabe et al. 1982). In most cases resistance occurred where paraquat had been applied multiple times per growing season for consecutive years in orchards and vineyards (Kato and Okuda 1983; Smisek et al. 1998; Watanabe et al. 1982). There are currently 24 species that have been documented resistant to the bipyridiliums chemistry (Heap 2010). Chemical control of horseweed had been successful with glyphosate and paraquat in North America prior to 1998; since then paraquat-resistant horseweed has been documented in Canada and Delaware, and GR horseweed in more than 16 states across the United States, including Mississippi (Koger et al. 2004; Heap 2010; Smisek et al. 1998; VanGessel et al. 2001; VanGessel 2006). *C. canadensis* is the first annual broadleaf to have been documented resistant to glyphosate (VanGessel 2001). Multiple-resistance to both glyphosate and paraquat has not been reported to date. Smisek et al. (1998) reported on the discovery of paraquat-resistant horseweed in Ontario, Canada, where paraquat had been applied 4 to 5 times per year for a 10-year period and exhibited resistance levels 25 to 35 times higher than susceptible populations. These paraquat-resistant plants were also found to have a 7-fold resistance to diquat and a 3-fold resistance to linuron; however, no resistance to more than one mode-of-action was suggested (Weaver et al. 2004).

Numerous studies have been conducted on the mechanisms of resistance occurring in *Conyza* spp. Studies were conducted to evaluate the uptake of paraquat through cuticular waxes (Fuerst et al. 1985). More ^{14}C-paraquat was removed in the aqueous solution from the susceptible than the resistant plants and a negligible amount of ^{14}C-paraquat was harvested in the chloroform wash of either biotype (Fuerst et al. 1985). These findings suggest that paraquat resistance is not a function of reduced uptake, since the resistant biotype took up more paraquat than the susceptible biotype. It has also been suggested that the paraquat cation may be degraded upon entering the chloroplast; however, studies have shown that this hypothesis is false (Fuerst and Vaughn 1990; Jori et al. 2007; Norman et al. 1993). Another study indicated that the resistant population possessed almost three times the amount of a superoxide dismutase which prevented superoxides from developing (Youngman and Dodge 1981). These enzymes help detoxify the effects of superoxides and prevent lipid peroxidative reactions that lead to cell destruction (Youngman and Dodge 1981). Other studies also suggested that paraquat tolerance was due to enhanced activity of enzyme systems that remove toxic free radicals from the plant before damaging levels accumulated (Jansen et al. 1989; LeBaron and Gressel 1982). These enhanced activities of protective enzymes were not supported in other research, however (Shaaltiel and Gressel 1987; Tanaka et al. 1986). Tanaka et al. (1986) excised leaves from susceptible and resistant *C. canadensis* and *C. philadelphicus* under water and then placed the petiole into a solution of ^{14}C-paraquat. Susceptible populations wilted within 48 hours when placed in a solution containing 5 µ*M* paraquat while resistant populations did not wilt after more than 96 hours when placed in a 500 µ*M* paraquat solution. This indicates a nearly 100-fold level of resistance. Autoradiographs were then used to analyze the movement of paraquat in the excised leaves, which showed normal movement of paraquat throughout the leaves of susceptible populations; however, resistant populations seemed to strongly inhibit the movement of paraquat through the vascular system. Tanaka et al. (1986) concluded that resistance was due to reduced translocation of paraquat to sites-of-action, which supported the findings of Fuerst et al. (1985) where paraquat moved through apoplastic tissues, yet was rapidly compartmentalized upon moving out of the xylem tissues. Another study reported finding no differences in uptake of paraquat among resistant and susceptible biotypes (Youngman and Dodge 1981).

In paraquat-susceptible plants, as unsaturated fatty acids are degraded by peroxidation, ethane is evolved. Ethane levels were measured in separate studies and did not increase in paraquat-resistant plants (Shaaltiel and Gressel 1987; Youngman and Dodge 1981). These findings suggest that the active oxygen detoxification pathway in susceptible plants does not prevent photodamage as efficiently as the resistant plants. Shaaltiel and Gressel (1987) also excised chloroplasts from leaf tissues treated with and without paraquat and measured CO_2 fixation. Chloroplasts from paraquat-sensitive plants irreversibly stopped fixing CO_2 within 2 h. Chloroplasts from paraquat-resistant plants, however, were initially inhibited yet fully recovered, indicating that paraquat does reach the chloroplasts of paraquat-treated plants, yet the effects are short-lived.

Polyamines, especially putrescine, levels in resistant horseweed have been shown to have levels two to three times higher than susceptible ones (Racz et al. 2000). Exogenously applied putrescine was applied to paraquat-resistant and -susceptible *C. canadensis*. Polyamines had a minor protective effect on susceptible plants and no effects were seen in resistant biotypes. It was concluded that increased levels of polyamines were in response to stress more so than a mechanism of resistance (Racz et al. 2000). It has also been suggested that there is some unknown mechanism that has a role in the reduction of destructive photooxidants (Amsellem et al. 1993). Recent research has discovered that the ferritin2 gene, which regulates Ferritin: an iron storage protein is upregulated in both susceptible and resistant populations of horseweed (Soos et al. 2006). It was suggested that the enhanced level of expression in this gene are connected with defense reactions in these paraquat-treated plants; however, no elevated levels of superoxide detoxifying enzymes could be detected (Soos et al. 2006). These genes could be coding for proteins that serve as transporters. Studies showed that paraquat was found in the vacuoles and cytosol of resistant horseweed plants up to a month after herbicide treatment (Soos et al. 2006).

Although conclusive evidence does not exist as to the exact mechanism of paraquat resistance, it is generally accepted that it is due to a reduction in translocation of the herbicide to the site-of-action in the chloroplast or some sequestration mechanism of the paraquat cation (Fuerst et al. 1985; Jori et al. 2007; Norman et al. 1993; VanGessel 2006). This reduction in herbicide translocation has also been documented with GR horseweed (Feng et al. 2004; Koger and Reddy 2005). Research focusing on the mechanism of glyphosate resistance has shown that resistance does not appear to be based on the differential uptake of glyphosate, metabolism, differential gene expression of EPSP synthase, or amplification of EPSP synthase; however, one difference was noted between resistant and susceptible plants in that similar amounts of glyphosate were taken up into the treated leaf by each plant, but the susceptible plants translocated about 2-fold more glyphosate into the roots (Feng et al. 2004; Koger and Reddy 2005). These findings suggest that *C. canadensis* has the ability to effectively reduce translocation of both glyphosate and paraquat. This poses the question as to whether *Conyza* spp. could have the propensity to develop multiple resistance to separate modes-of-action such as glyphosate and paraquat simultaneously.

Herbicide-resistant horseweed is a serious concern. GR horseweed has necessitated research to find alternative control measures for this troublesome weed (Eubank et al 2008; Owen et al. 2009; Steckel et al. 2006; Steckel and Gwathmey 2009). Previous research by Eubank et al. (2008) concluded that a horseweed population near Leland, MS was not effectively controlled with either 0.86 kg/ha glyphosate (60 to 65%) or 0.84 kg/ha paraquat (55 to 63%) at 4 wk after treatment (WAT) in separate but adjacent studies. This poses the question as to whether this population exhibits multiple-resistance to glyphosate and paraquat. Interestingly, the addition of metribuzin, a PSII herbicide, to paraquat, improved the level of horseweed control over paraquat alone (Eubank et al. 2008). The synergistic effects of paraquat + PSII inhibitors have been well documented (Colby et al. 1965; Griffin et al. 2004; Putnam and Ries 1967); however, little research has been done to evaluate these chemistries on the control of horseweed. Research investigating alternative methods of control for multiple-resistant horseweed is needed.

Much of the current research in weed science has been focused on investigating GR and alternative means of control for these developing biotypes/populations. Glufosinate effectively controls horseweed (Eubank et al. 2008; Steckel et al. 2006), but control may be reduced with cooler air temperatures at time of application (Owen et al. 2009; Steckel et al. 2006). Preemergence control of horseweed with acetolactate synthase (ALS)-inhibiting herbicides has been effective (Owen et al. 2009); however, widespread development of ALS-resistant horseweed is of concern (Davis et al. 2010; Kruger et al. 2009). Possible control options of GR weeds may include tank-mixing herbicides with glyphosate. The addition of 0.84 kg ae/ha 2,4-D to 0.86 kg/ha glyphosate improved horseweed control to more than 95% compared to glyphosate alone at 65% (Eubank et al. 2008), and Owen et al. (2009) reported that addition of 0.28 kg ae/ha dicamba to glyphosate controlled horseweed 89%. However, there are limitations as to when phenoxy-type products can be applied prior to planting soybean and cotton due to plant-back restrictions and possible crop injury. Additionally, there have been concerns raised over the recent lack of control with spring-applied applications of 2,4-D and dicamba on horseweed (personal observation; Larry Steckel, Univ. of Tenn., personal communication). This raises questions as to the possible development of phenoxy-resistant horseweed. Kruger et al. (2008) reported that some horseweed populations in Indiana exhibited a three-fold tolerance to 2,4-D in a recent greenhouse study. Alternative control options for the postemergence control of paraquat and GR horseweed are needed.

Saflufenacil is a new herbicide currently being developed by BASF Corporation and has shown potential as an alternative means for the control of GR horseweed (Bowe et al. 2009). The mode-of-action of saflufenacil is through the inhibition of protoporphyrinogen oxidase (PPO) activity (Grossman et al. 2010), which ultimately leads to the accumulation of protoporphyrin IX (Proto) (Duke et al. 1991). Proto is a strong photosensitizer that generates high levels of singlet oxygen in the presence of oxygen and light (Duke et al. 1991). These singlet oxygen products lead to the production of hydrogen peroxide, leading to rapid necrosis and wilting of leaf tissues (Grossman et al. 2010). Herbicides that exhibit rapid necrosis of plant tissue may cause the disruption of cell membranes, thereby, inhibiting the uptake and translocation of other herbicides when applied in combination. Reduced glyphosate absorption associated with mixing contact herbicides with glyphosate has been well documented (Hydrick and Shaw 1994; Norris et al. 2001; Starke and Oliver 1998). This raises the question as to whether tank-mixing saflufenacil with a product such as glyphosate may influence the absorption and translocation of glyphosate. In preliminary field studies, a tank-mix of 0.025 kg ai/ha saflufenacil and 0.84 kg/ha glyphosate controlled GR horseweed 97% (Dan Poston, Miss. State Univ., unpublished data). The additive effect that saflufenacil has on glyphosate is unclear. Further research investigating uptake and translocation of a systemic herbicide such as glyphosate as influenced by a contact herbicide such as saflufenacil is needed.

The use of adjuvants to increase herbicide efficacy has been well documented (Hatzios and Penner 1985; McWhorter and Jordan. 1976; Nandula et al. 2007b; Penner 1989; Wanamarta et al. 1989). Preliminary field studies have shown that 0.025 kg/ha saflufenacil alone gave 75% control of GR horseweed while the addition of crop oil concentrate (COC) improved GR horseweed control to 97% (Dan Poston, Miss. State Univ., unpublished data). Further research is needed on the use of adjuvants with saflufenacil.

GR horseweed can pose serious problems for producers, as the movement of wind-blown seed may potentially infest or re-infest row crop hectares after control measures have been implemented. The use of fall-applied residual herbicides may prevent or reduce the subsequent emergence of horseweed prior to planting. Research is needed to evaluate newer residual herbicides on their effectiveness as a means for managing GR horseweed. The objective of this study is to determine if fall-applied residuals are effective in the prevention of horseweed emergence prior to planting cotton and soybean, and if comparable, both efficaciously and economically, to a standard spring-applied burndown treatment.

Paraquat-resistant horseweed has been documented in Canada and Delaware, and GR horseweed in more than 16 states across the United States, including Mississippi (Koger et al. 2004; Heap 2010; Smisek et al. 1998; VanGessel et al. 2001; VanGessel 2006). Herbicide-resistant horseweed is a serious concern and has necessitated research into finding alternative control measures for this troublesome weed (Eubank et al 2008; Owen et al. 2009; Steckel et al. 2006; Steckel and Gwathmey 2009). Previous research by Eubank et al. (2008) concluded that a horseweed population near Leland, MS was not effectively controlled with glyphosate or paraquat. This poses the question as to whether this population exhibits multiple-resistance to glyphosate and paraquat. Paraquat-resistant horseweed is difficult to control with paraquat alone; however, the addition of metribuzin to paraquat has improved the level of horseweed control over paraquat alone (Eubank et al. 2008). Research investigating alternative methods of control for multiple-resistant horseweed is needed. The objective of this study is to determine if multiple-resistance to glyphosate and paraquat exists in horseweed, and if the addition of metribuzin to paraquat improves control of paraquat-resistant horseweed.

Control of GR horseweed can be improved with the addition of 2,4-D or dicamba to glyphosate (Eubank et al. 2008); however, plant-back restrictions and possible crop injury may complicate control measures. Saflufenacil has shown promise as an alternative means of control for GR horseweed. Little research is available on the effects of adjuvants on horseweed efficacy with saflufenacil or the influence on glyphosate absorption and translocation. The objective of this study was to determine the most efficacious adjuvant system for the control of horseweed with saflufenacil, to investigate interactions between saflufenacil and glyphosate mixtures on the control of horseweed, and to determine patterns of uptake and translocation of glyphosate applied alone and in combination with saflufenacil.

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CHAPTER II

HORSEWEED CONTROL WITH FALL-APPLIED PREEMERGENCE HERBICIDES

Abstract

Field studies were conducted in 2006 and 2007 to determine the effectiveness of fall-versus spring-applied herbicides for horseweed control in cotton and soybean. Control of horseweed 4 weeks after treatment (WAT) with spring-applied treatments of glyphosate + dicamba, and glyphosate + dicamba + flumioxazin were 85% and 90%, respectively, in 2006, but fell to 78% and 79%, respectively, in 2007. Fall-applied trifloxysulfuron provided the highest cotton lint yields of 1326 kg/ha and 1127 kg/ha in 2006 and 2007, respectively, and also corresponded to the highest net returns of $336.96 and $285.16 in 2006 and 2007, respectively. Cotton yields averaged across fall-applied herbicides of trifloxysulfuron, clomazone, and flumioxazin were comparable to or better than spring-applied glyphosate + dicamba both years. For soybean, spring-applied glyphosate + 2,4-D provided at least 95% control of horseweed 4 WAT both years. Fall-applied cloransulam-methyl, flumetsulam, sulfentrazone, flumioxazin and the combination of chlorimuron-ethyl + metribuzin, all fall-applied, provided control comparable to spring-applied glyphosate + 2,4-D both years. Soybean yields and net returns with fall-applied flumetsulam, cloransulam-methyl, and the combination of chlorimuron-ethyl + metribuzin, were comparable to that obtained with spring-applied glyphosate + 2,4-D.

Nomenclature: Horseweed, *Conyza canadensis* (L.) Cronq; cotton, *Gossypium hirsutum* L. 'DP 444 BG/RR', 'DP 117 B2RF'; soybean, *Glycine max* (L.) Merr. 'AG 4801 RR' 'DK 4967 RR'.

Key words: Burndown, fall-applied, spring-applied, residuals, resistance.

Introduction

Glyphosate-resistant (GR) crops have been widely adopted throughout the major crop-producing areas of the United States. They have enabled producers to reduce labor and input costs due to the effectiveness of glyphosate in the removal of troublesome weeds (Anonymous 2009; Gianessi 2008). Minimum- and no-tillage production practices have also been more widely adopted (Anonymous 2002, 2009). Many of the GR horseweed [Conyza canadensis (L.) Cronq] populations identified to date occur in areas of the country where no-till practices have been widely adopted (Koger et al. 2004; Main et al. 2004; VanGessel 2001). The majority of row crop hectares in Mississippi are treated with a non-selective herbicide such as glyphosate prior to planting to remove winter weed vegetation. Studies have suggested that the early removal of these weeds facilitates rapid drying and warming of the soil, as well as promoting earlier planting (Stougaard et al. 1984). GR weeds such as horseweed and Italian ryegrass [Lolium perenne L. ssp. multiflorum (Lam.) Husnot] have complicated early preplant burndown weed management in Mississippi (Koger et al. 2004; Nandula et al. 2007). Although current infestations of GR Italian ryegrass are on a smaller number of hectares, GR horseweed has become a significant problem for producers across the Mid-South and is listed as being a problem weed in many row crops including cotton, grain sorghum [Sorghum bicolor (L.) Moench], corn (Zea mays L.), and soybean (Bond et al. 2009; Brown and Whitwell 1988; Buhler and Owen 1997; Heap 2010; Mueller et al. 2003; Steckel and Gwathmey 2009; Vencill and Banks 1994; Wiese et al. 1995). Alternative control measures for GR horseweed have been well documented (Eubank et al. 2008; Steckel et al. 2006). These treatments typically include 2,4-D, dicamba and/or glufosinate for the control of GR horseweed. However, equipment limitations, suitable weather, poor field conditions, and plant-back restrictions may hamper timely applications of these treatments. Additional concerns have been raised over the possible development of horseweed resistant to phenoxy-type herbicides (Kruger et al. 2008).

Horseweed is a winter annual weed which is particularly problematic in no-tillage and minimum-tillage production systems (Barnes et al. 2004; Bruce and Kells 1990). Winter annual weeds in the Mid-South typically emerge in the fall of the year; however, horseweed may emerge in any month of the year (Bhowmik and Bekech 1993; Buhler and Owen 1997; Eubank et al. 2006; Main et al. 2006; Saphangthong and Witt 2006). The emergence of horseweed is typically greater on or near the soil surface and when temperatures are between 10 and 20 C (Eubank et al. 2006; Main et al. 2006; Nandula et al. 2006). Horseweed seeds are wind-dispersed and capable of traveling great distances (Shields et al. 2006). This could pose considerable problems for producers, as the movement of these seed may potentially infest or re-infest row crop hectares after control measures have been implemented. Many producers in the Mid-South are encountering persistent emergence of horseweed following spring-applied burndown treatments prior to planting (personal observation). The use of fall-applied residuals may prevent or reduce the subsequent emergence of horseweed prior to planting. Stougaard et al. (1984) reported fall applications of the residual herbicide cyanazine at 3.4 kg ai/ha prevented emergence of horseweed until planting of no-till soybean; however, cyanazine is no longer commercially available. Research is needed to evaluate modern residual herbicides on their effectiveness as a tool for managing GR horseweed.

The objective of this study is to determine if fall-applied residuals are effective in the prevention of horseweed emergence prior to planting cotton and soybean, and if comparable both efficaciously and economically to a spring-applied burndown treatment.

Materials and Methods

Experiments were established on a producer’s field near the Delta Research and Extension Center, Stoneville, MS (33^{o} 25’09.16” N and 90^{o} 53’09.37” W). The soil type was a Dundee very fine sandy loam (fine-silty, mixed, active, thermic Typic Endoaqualfs) with a pH of 6.1 and organic matter content of 1.2%. Experiments were established following five years of no-tillage GR soybean and were naturally infested with GR horseweed. Plots were 3 m wide x 12 m long, and herbicide treatments were applied with a tractor-mounted compressed-air sprayer calibrated to deliver 140 L/ha at a pressure of 207 kPa using flat-fan nozzles.[1]

Cotton

Field studies were conducted in 2006 and 2007 to determine preemergence (PRE) activity of cotton herbicides on the control of horseweed applied in the fall. Fall treatments were applied on November 2, 2005 and October 24, 2006. Treatments were the maximum labeled rate for the soil type and included: 1.12 kg ai/ha trifluralin, 1.38 kg ai/ha pendimethalin, 1.68 kg ai/ha norflurazon, 0.07 kg ai/ha flumioxazin, 1.68 kg ai/ha fluometuron, 1.12 kg ai/ha diuron, 1.12 kg ai/ha linuron, 0.45 kg ai/ha oxyfluorfen, 0.07 kg ai/ha pyrithiobac, 1.79 kg ai/ha *S*-metolachlor, 1.12 kg ai/ha clomazone, 1.12 kg ai/ha prometryn, and 0.009 kg ai/ha trifloxysulfuron. A nontreated control was also included for comparison of cotton yields. All treatments were applied as PRE treatments with the exception of trifluralin and pendimethalin, which were preplant incorporated (PPI). PPI treatments were incorporated to a depth of five cm with a tractor-mounted pto-driven rotary tiller. A tillage alone treatment was also included for comparison. All plots, including the nontreated, received an application of 0.6 kg ai/ha glufosinate to remove any existing horseweed present and to ensure uniform emergence of horseweed. An incorporating rainfall was recorded within 1 WAT both years. Spring herbicide treatment rates included: 0.86 kg ae/ha glyphosate + 0.28 kg ae/ha dicamba, and 0.86 kg/ha glyphosate + 0.28 kg/ha dicamba + 0.07 kg ai/ha flumioxazin and were applied on March 29, 2006 and March 19, 2007. Horseweed plants were 10- to 15-cm in diameter at time of application of spring treatment. Visual control ratings for fall-applied treatments were determined using a 0 to 100 scale (0, no control; 100, complete control) and were measured at 4 and 24 WAT as percent weed control. Horseweed plant counts and biomass from 2 meter^2 quadrants were recorded at planting or approximately 25 WAT of fall herbicides. Horseweed biomass was obtained by harvesting the uppermost portion of the plant at the soil line and recorded as fresh weight and extrapolated to g/m^2. DP 444 BG/RR

[1] TeeJet XR 11002VS flat-fan nozzle, Spraying Systems Co., North Ave., Wheaton, IL 60189.

cotton was seeded on April 25, 2006 and DP 117 B2RF cotton was seeded on April 19, 2007. Cotton was planted with a four-row planter on 1-m row spacing at a seeding rate of 10 seed/m of row. No additional at-planting burndown herbicides were applied prior to planting cotton. Horseweed was allowed to compete with cotton for approximately 2 wk after planting before in-season herbicides were applied. For in-season weed management, all plots received postemergence (POST) applications of 0.95 kg/ha glyphosate + 1.26 kg/ha *S*-metolachlor and a postdirected layby application of 0.86 kg/ha glyphosate + 1.12 kg ai/ha diuron 2 and 7 wk after planting, respectively. Normal agronomic practices of fertilization, insect control, growth regulators and harvest aides were followed per university recommendations. The center two rows of the four-row plots were harvested using a two-row mechanical spindle picker. Seed cotton was sampled and ginned to determine lint turnout. Lint yield was determined by multiplying the lint turnout percentage by seed cotton weight. Returns above treatment cost were calculated in order to provide economic values of herbicide treatments on weed control. Gross returns were calculated by multiplying cotton lint yields by $0.716/lb (Anonymous 2008a). Herbicide, tillage, and application costs were generated based on University budgets with returns above treatment costs being calculated by subtracting treatment costs from gross returns (Anonymous 2008a).

Soybean

Field studies were conducted in 2006 and 2007 to determine PRE activity of fall-applied soybean herbicides on the control of horseweed. Fall treatments were applied on November 2, 2005, and on October 24, 2006, and were the maximum labeled rate given for the soil type, and included: 0.28 kg ai/ha sulfentrazone, 1.12 kg/ha clomazone, 0.17 kg ai/ha pyroxasulfone, 0.075 kg ai/ha flumetsulam, 0.56 kg ai/ha metribuzin, 0.07 and 0.09 kg ai/ha flumioxazin, 0.044 kg ai/ha cloransulam-methyl, and 0.06 kg ai/ha chlorimuron-ethyl + 0.36 kg/ha metribuzin. A nontreated control was also included for comparison of soybean yields. All fall herbicide treatments were applied as PRE treatments. All plots, including the nontreated, received an application of 0.6 kg/ha glufosinate to remove any existing horseweed vegetation present at time of application of fall treatments and to ensure uniform emergence of horseweed. An incorporating rainfall was recorded within 1 WAT in both years. A spring-applied herbicide treatment of 0.86 kg/ha glyphosate + 0.84 kg ae/ha 2,4-D was included as a comparison. Spring treatments were applied on March 15, 2006 and February 22, 2007. Horseweed plants were 10- to 15-cm in diameter at spring-applied treatment timings. Visual control ratings for fall-applied treatments were determined using a 0 to 100 scale (0, no control; 100, complete control) were collected at 4 and 20 WAT and spring treatments at 4 WAT as percent weed control. Horseweed plant counts and biomass from 2 $meter^2$ quadrants were recorded at planting or approximately 25 WAT of fall herbicides. Horseweed biomass was obtained by harvesting the uppermost portion of the plant at the soil line and recorded as fresh weights and extrapolated to g/m^2. AG 4801 RR soybean was seeded April 12, 2006 and DK 4967 RR soybean was seeded April 17, 2007. Soybean was planted with a seven-row planter on 38-cm row spacing at a seeding rate of 13 seed/m of row. No additional at-planting burndown applications were applied prior to planting soybean. Horseweed was allowed to compete with soybean for approximately 2 wk after planting before in-season herbicides were applied. For in-season weed management, all plots received POST applications of 0.84 kg/ha glyphosate + 1.26 kg/ha *S*-metolachlor and 0.84 kg/ha glyphosate at 2 and 7 wk after planting, respectively. The center five rows of the seven-row plots were harvested with a research combine. Soybean yields and moisture were recorded and moisture was adjusted to 13%. Returns above treatment cost were calculated in order to provide economic values of herbicide treatments on weed control. Gross returns were calculated by multiplying soybean yields by $9.34/bu (Anonymous 2008b). Herbicide and application costs were generated based on University budgets with returns above treatment costs being calculated by subtracting treatment costs from gross returns (Anonymous 2008b).

The experimental design for both studies was a randomized complete block design with four replications. Each experiment was conducted twice. All data were subjected to ANOVA using the general linear model in SAS and means separated using Fisher's Protected LSD at the alpha = 0.05 level of significance. Attempts were made to pool data across years; however, significant treatment by year interactions were detected for all variables; therefore, data are presented separately by year.

Results and Discussion

Cotton

Tillage alone treatments did not reduce horseweed biomass and were not different than the nontreated for all evaluations (data not presented).

Visual control ratings, 4 WAT, confirmed that all emerged horseweed was effectively controlled with fall applications of glufosinate (data not presented). Control of horseweed with spring standard treatments of glyphosate + dicamba and glyphosate + dicamba + flumioxazin were 85% and 90%, respectively, in 2006; however, control ratings fell to 78% and 79%, respectively in 2007 (Table 2.1). The differences in control between years were due to horseweed plants not being completely killed in 2007; however, these plants remained stunted into the season and did not seem to compete with the subsequent cotton crop. Fall-applied trifloxysulfuron and clomazone controlled horseweed at least 96% 20 WAT both years. Control of horseweed with trifloxysulfuron and clomazone were not better than fall-applied diuron in 2006 or 2007, at 84% and 90%, respectively. Trifluralin PPI and pendimethalin PPI controlled horseweed at least 97% in 2006, comparable to trifloxysulfuron; however, control dropped to less than 60% in 2007. This reduction in horseweed control may have been due to the much drier conditions observed in February and March of 2007 than in the previous year. In 2006, horseweed control with flumioxazin was only 80%, 20 WAT, but improved to 90% in 2007. Fluometuron and prometryn controlled horseweed at least 75% comparable to spring-applied treatments each year. Pendimethalin PRE and pyrithiobac controlled horseweed less than 69% both years.

Density and biomass data were missing for 2006 spring-applied treatments of glyphosate + dicamba and glyphosate + dicamba + flumioxazin. Horseweed plant densities in the nontreated plots were 168 and 335 plants/m^2 in 2006 and 2007, respectively (Table 2.1). Differences in horseweed densities between years could be due to population variability or increasing horseweed populations at this location. Variability in horseweed density across years has been reported previously by Davis and Johnson (2008). Trifloxysulfuron applied in the fall prevented horseweed emergence 25 WAT both years. Trifluralin and pendimethalin PPI reduced horseweed populations more than 92% compared to the nontreated, and were comparable to trifloxysulfuron in 2006; however, these treatments reduced horseweed populations less than 22% in 2007, no better than the nontreated. Such a drastic reduction in control was not observed in any of the PRE treatments which would rule out the impact of weather; however the commonality of these treatments being PPI may suggest improper incorporation of the herbicides in 2007. Clomazone only reduced horseweed populations 59% in 2006, but completely prevented horseweed emergence in 2007. As stated previously, clomazone controlled 100% of horseweed 20 WAT; however, 59 horseweed plants/m^2 were present 25 WAT. Spring emerged horseweed between the two observations likely contributed to the discrepancies between visual controls and plant densities of clomazone and was also seen with other herbicides including fluometuron, flumioxazin, diuron, prometryn, and *S*-metolachlor. Spring-applied glyphosate + dicamba and glyphosate + dicamba + flumioxazin did not reduce horseweed populations compared to the nontreated in 2007; however, horseweed plants were severely stunted and biomass was reduced more than 80%, comparable to trifloxysulfuron. Pendimethalin PRE did not reduce horseweed populations either year of the study compared to the nontreated.

Horseweed fresh weights from the nontreated plots averaged 500 and 2100 g/m^2 in 2006 and 2007, respectively (Table 2.1). Pyrithiobac in 2006 actually increased horseweed biomass 61% compared to the nontreated, higher than all other treatments. Horseweed fresh weights were reduced 37% with pyrithiobac in 2007, but were not different from the nontreated. Horseweed biomass with the PPI treatments of trifluralin and pendimethalin were comparable to trifloxysulfuron in 2006; conversely, these treatments were not better than the nontreated in 2007. Pyrithiobac and pendimethalin PRE were not different than the nontreated in either year and pendimethalin PRE had the highest biomass yields among all treatments in 2007, at 2507 g/m^2.

Spring-applied glyphosate + dicamba and glyphosate + dicamba + flumioxazin reduced horseweed biomass by more than 80%, comparable to trifloxysulfuron. The assessment of horseweed biomass may not be an effective means in establishing herbicide efficacy. Single horseweed plants were observed to initiate many tillers and subsequently more biomass per plant in areas with little competition, whereas a predominantly erect stature and lower biomass weights were seen with higher horseweed densities.

Cotton yields for the spring-applied combinations of glyphosate + dicamba and glyphosate + dicamba + flumioxazin in 2006 were 930 kg/ha and 1066 kg/ha, respectively (Table 2.2). Fall-applied trifloxysulfuron provided cotton lint yields of 1326 kg/ha and 1127 kg/ha in 2006 and 2007, respectively, and also corresponded to net returns of $336.96 and $285.16, respectively. No fall-applied herbicides and only the spring application of glyphosate + dicamba + flumioxazin resulted in comparable yields to trifloxysulfuron in 2006. Fall-applied fluometuron, norflurazon, flumioxazin, and clomazone provided cotton yields comparable to the spring-applied treatments of glyphosate + dicamba and glyphosate + dicamba + flumioxazin in 2006. Only the spring application of glyphosate + dicamba + flumioxazin had net returns comparable to trifloxysulfuron in 2006. A few fall-applied herbicides in 2006 had net returns comparable to the spring applications of glyphosate + dicamba and glyphosate + dicamba + flumioxazin and included fluometuron, norflurazon, flumioxazin, and diuron.

Cotton yields for the spring-applied combinations of glyphosate + dicamba and glyphosate + dicamba + flumioxazin in 2007 were 862 kg/ha and 693 kg/ha, respectively (Table 2.2). Cotton injury with spring-applied flumioxazin may have contributed to a reduction in cotton lint yields versus the previous year's results in that visual PPO injury was observed on cotton seedlings (data not presented). The spring-applied combination of glyphosate + dicamba and fall-applied clomazone were the only treatments that had cotton yields and net returns comparable to trifloxysulfuron in 2007. Several fall-applied treatments in 2007 were comparable to or better than the spring-applied standards of glyphosate + dicamba and glyphosate + dicamba + flumioxazin, including trifloxysulfuron, clomazone, oxyfluorfen, flumioxazin, and diuron. Pendimethalin PRE and pyrithiobac resulted in yields comparable to the nontreated plots during both years.

Soybean

Visual control ratings 4 WAT confirmed that all emerged horseweed was effectively controlled with fall applications of glufosinate (data not presented). The spring-applied standard of glyphosate + 2,4-D controlled horseweed at least 95% 4 WAT both years (Table 2.3). In 2006, horseweed control was greater than 80% from all herbicide treatments 20 WAT compared to the nontreated plots. Similar results were seen in 2007 with several treatments being greater than or comparable to 80% control, the exceptions were pyroxasulfone and metribuzin. Fall-applied cloransulam-methyl, flumetsulam, sulfentrazone, 0.09 kg/ha flumioxazin, and the combination of chlorimuron-ethyl + metribuzin controlled horseweed comparable to the spring application of glyphosate + 2,4-D both years.

Horseweed plant densities for the nontreated were 238 and 252 plants/m^2 in 2006 and 2007, respectively, at 25 WAT (Table 2.3). In 2006, all herbicide treatments reduced horseweed populations compared to the nontreated. There were no differences between fall-applied herbicides in 2006 with regard to horseweed populations, and all were comparable to spring-applied glyphosate + 2,4-D. Control differences in horseweed densities were greater in 2007, where only fall-applied sulfentrazone, flumetsulam, the higher rate of flumioxazin (0.09 kg/ha) and the combination of chlorimuron + metribuzin reduced horseweed populations compared to the nontreated. As mentioned previously, the drier than usual months of February and March in 2007 may have contributed to reduced preemergence control of horseweed during this time. All fall-applied treatments were comparable to glyphosate + 2,4-D in reduction of horseweed density with the exception of pyroxasulfone, which only reduced horseweed populations 35% in 2007. The combination of chlorimuron-ethyl + metribuzin prevented emergence of horseweed and reduced horseweed biomass 100% both years.

Horseweed fresh weights from the nontreated plots averaged 1310 and 1880 g/m^2 in 2006 and 2007, respectively (Table 2.3). All herbicide treatments reduced horseweed biomass compared to the nontreated both years. The combination of chlorimuron + metribuzin removed 100% of horseweed biomass both years. In 2006 there were no differences in horseweed biomass between fall-applied chlorimuron-ethyl + metribuzin, cloransulam-methyl, flumetsulam, sulfentrazone, clomazone, and metribuzin, by which horseweed biomass was reduced by more than 93%, comparable to the spring-applied standard of glyphosate + 2,4-D. Pyroxasulfone and flumioxazin at both rates reduced horseweed biomass greater than the nontreated, but less than 74%. There were no differences between any of the herbicide treatments with regards to reduction of horseweed biomass in 2007.

Pyroxasulfone was not commercially available at this writing and was thus omitted from analysis of net returns. Soybean yields averaged 2130 kg/ha in 2006 among treatments. Soybean yields with fall-applied herbicide treatments were better than the nontreated in 2006 and all fall-applied treatments were comparable to the spring-applied application of glyphosate + 2,4-D (Table 2.4). Both fall- and spring-applied treatments improved net returns compared to the nontreated; however, there were no differences in net returns across herbicide treatments in 2006. Soybean yields in 2007 averaged 1910 kg/ha among treated plots. Fall-applied treatments of flumetsulam, cloransulam-methyl, and the combination of chlorimuron-ethyl + metribuzin had soybean yields comparable to the spring-applied treatment of glyphosate + 2,4-D. Yields with cloransulam-methyl were not better than the high rate of flumioxazin. Soybean yields with fall-applied sulfentrazone, clomazone, pyroxasulfone, and metribuzin were not better than the nontreated in 2007. Only the fall-applied treatments of flumetsulam, cloransulam-methyl, and the combination of chlorimuron-ethyl + metribuzin had net returns comparable to the spring-applied treatment of glyphosate + 2,4-D. Treatments of sulfentrazone, clomazone, metribuzin, and flumioxazin at 0.07 kg/ha did not give greater net returns compared to the nontreated. The high variability of soybean yields may have been due to an unusually high amount of rainfall that occurred in July and August of 2007 which caused some seed rot and decay.

In our studies, fall-applied trifloxysulfuron provided consistently high levels of horseweed control, reduction in plant populations and high cotton yields and net returns both years. Owen et al. (2009) reported similar findings where fall-applied trifloxysulfuron controlled horseweed 94% by 25 WAT after application. Cotton yields with fall-applied trifloxysulfuron, clomazone, and flumioxazin were comparable or better to spring-applied treatments of glyphosate + dicamba each year; however, only trifloxysulfuron and flumioxazin gave comparable or better net returns partly due to the high application cost of clomazone. Fall-applied flumetsulam, cloransulam-methyl, and the combination of chlorimuron-ethyl + metribuzin were comparable to the spring-applied standard of glyphosate + 2,4-D in the control and prevention of horseweed, as well as improving soybean yields and net returns both years.

These studies indicate that the use of fall-applied residuals can be as effective as spring-applied herbicides, both efficaciously and economically, for the control and prevention of GR horseweed in cotton and soybean production systems. This is in contrast to the findings of Davis et al. (2009), where spring-applied preplant herbicide treatments were more effective than fall-applied applications in reducing horseweed plant densities. The differences between these studies are likely due to the location of each study. Davis et al. (2009) reported the majority of horseweed emerges in the spring of the year in Indiana, whereas Eubank et al. (2006) have suggested that horseweed emergence occurs primarily in the fall of the year in Mississippi. This could also partly explain the difficulty in controlling well-established horseweed with spring-applied postemergence treatments. Standard spring-applied herbicide treatments containing dicamba or 2,4-D have been generally effective in the removal of horseweed in Mississippi; however, some producers have recently reported problems controlling horseweed with these chemistries (personal observation). The use of fall-applied residuals may alleviate or reduce the need for spring applications to control horseweed in Mississippi. Additionally, horseweed control in the fall may be easier, as plants are small and have a limited root system, whereas plants allowed to overwinter are typically more robust and have a more extensive root system and may be more difficult to control in the spring (Bond et al. 2009).

Management and prevention of herbicide-resistant weeds typically dictates utilizing alternative herbicide modes-of-action, the use of residual herbicides, tillage, crop rotation, and the prevention of seed production strategies should be customized to a particular region, cropping system, or weed species (Beckie and Reboud 2009; Friesen et al. 2000). However, the adoption of fall-applied residuals as a management tool by producers may be slow due to the lack of farmer awareness concerning glyphosate resistance management (Johnson et al. 2009). Additionally, concerns over herbicide runoff and soil erosion have been raised over the use of fall-applied residuals where the ground is void of plant life during a time of year when high rainfall typically occurs. Careful consideration for soil and water quality should be made prior to using fall residuals to prevent contamination of natural resources.

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Table 2.1 Horseweed control 20 WAT, plant density, and horseweed biomass 25 WAT with fall-applied PRE and PPI cotton herbicides[a]

Treatment	Rate	2006 Control 20 WAT	2006 Density 25 WAT	2006 Biomass 25 WAT	2007 Control 20 WAT	2007 Density 25 WAT	2007 Biomass 25 WAT
	kg/ha[b]	—%—	plants/m^2	g/m^2	—%—	plants/m^2	g/m^2
Nontreated		0	168	500	0	335	2100
Trifluralin ppi	1.12	98	14	60	60	310	1370
Pendimethalin ppi	1.38	97	10	50	55	262	1370
Pendimethalin	1.38	55	103	460	44	223	2510
Norflurazon	1.68	80	8	150	84	13	210
Flumioxazin	0.07	80	20	390	90	2	10
Fluometuron	1.68	91	39	130	85	5	80
Diuron	1.12	84	52	380	90	5	40
Linuron	1.12	65	34	360	85	5	260
Oxyfluorfen	0.45	70	46	150	85	4	230
Pyrithiobac	0.07	69	64	810	55	135	1320
S-metolachlor	1.79	80	35	200	83	16	400
Clomazone	1.12	100	59	120	96	0	20
Prometryn	1.12	85	44	370	75	42	500
Trifloxysulfuron	0.009	100	0	0	100	0	0
Glyphosate + Dicamba [c]	0.86 + 0.28	85	.	.	78	210	400
Glyphosate + Dicamba + Flumioxazin [c]	0.86 + 0.28 + 0.07	90	.	.	79	197	410
LSD (0.05)		18	77	260	13	174	880

[a] Abbreviations: PRE, preemergence; PPI, preplant incorporated; WAT, weeks after treatment.

[b] Glyphosate and dicamba treatments in ae/ha all other treatments in ai/ha.

[c] Spring-applied treatments.

Table 2.2 Treatment cost, cotton yields, and net returns for fall-applied PRE and PPI cotton herbicides[a]

Treatment	Rate	Cost	2006 Yield	2006 Net Returns	2007 Yield	2007 Net Returns
	kg/ha	\$/ha[b]	kg/ha	\$/ha[c]	kg/ha	\$/ha[c]
Nontreated		0	0	0	50	12.30
Trifluralin ppi[b]	1.12	5.92	670	167.30	50	7.80
Pendimethalin ppi	1.38	8.13	680	167.40	40	1.70
Pendimethalin	1.38	4.69	180	41.50	60	10.00
Norflurazon	1.68	9.06	840	208.70	340	78.70
Flumioxazin	0.07	4.08	800	204.20	640	162.40
Fluometuron	1.68	6.54	900	225.50	510	125.60
Diuron	1.12	2.38	700	180.40	620	158.60
Linuron	1.12	13.34	550	130.10	540	125.80
Oxyfluorfen	0.45	7.41	550	133.70	740	183.20
Pyrithiobac	0.07	9.80	130	22.80	80	11.30
S-metolachlor	1.79	9.19	500	121.30	370	87.10
Clomazone	1.12	14.37	740	177.50	950	230.50
Prometryn	1.12	3.82	600	150.40	220	53.00
Trifloxysulfuron	0.01	6.40	1330	337.00	1130	285.20
Glyphosate + Dicamba	0.86 + 0.28	6.05	930	234.70	860	217.20
Glyphosate + Dicamba + Flumioxazin	0.86 + 0.28 + 0.07	9.55	1070	266.40	690	170.00
LSD (0.05)			340	86.70	345	89.40

[a] Abbreviations: PRE, preemergence; PPI, preplant incorporated.

[b] Application costs for sprayer 27.5 meter boom \$0.58/ha, 7.3 meter disk & incorporation \$3.43/ha (Anonymous 2008a)

[c] Cotton lint price @ \$0.325/kg (Anonymous 2008a)

Table 2.3 Horseweed control 20 WAT, plant density, and horseweed biomass 25 WAT with fall-applied PRE soybean herbicides[a]

Treatment	Rate	2006			2007		
		Control	Density	Biomass	Control	Density	Biomass
		20 WAT	25 WAT		20 WAT	25 WAT	
	kg/ha[b]	—%—	plants/m^2	g/m^2	—%—	plants/m^2	g/m^2
Nontreated		0	238	1310	0	252	1880
Sulfentrazone	0.28	95	2	10	80	57	280
Clomazone	1.12	99	7	30	76	118	170
Pyroxasulfone	0.17	80	24	340	64	163	360
Flumetsulam	0.08	95	1	0	94	6	10
Metribuzin	0.56	96	35	100	64	132	120
Flumioxazin	0.07	85	27	380	78	102	140
Flumioxazin	0.09	86	15	450	88	40	30
Cloransulam-methyl	0.044	98	2	0	86	151	30
Chlorimuron-ethyl + metribuzin	0.06 + 0.36	99	0	0	99	0	0
Glyphosate + 2,4-D[c]	0.86 + 0.84	95	5	60	95	10	10
LSD (0.05)		13	88	220	15	150	990

[a] Abbreviations: PRE, preemergence; WAT, weeks after treatment.
[b] Glyphosate and dicamba treatments in ae/ha all other treatments in ai/ha.
[c] Spring-applied treatments.

Table 2.4 Treatment cost, soybean yields, and net returns for fall-applied PRE soybean herbicides[a].

Treatment	Rate	Cost	2006 Yield	2006 Net Returns	2007 Yield	2007 Net Returns
	kg/ha[b]	$/ha[c]	kg/ha	$/ha[d]	kg/ha	$/ha[d]
Nontreated		0.00	840	47.10	130	8.80
Sulfentrazone	0.28	11.28	2170	110.60	1170	54.70
Clomazone	1.12	14.37	2310	115.70	1010	42.70
Pyroxasulfone	0.17	.	1760	.	730	.
Flumetsulam	0.08	5.62	2380	128.30	3090	168.40
Metribuzin	0.56	3.58	2130	116.30	800	41.30
Flumioxazin	0.07	4.08	2060	112.10	1290	68.70
Flumioxazin	0.09	4.96	1830	98.00	1560	82.80
Cloransulam-methyl	0.044	8.80	2460	129.60	2420	127.20
Chlorimuron-ethyl + metribuzin	0.06 + 0.36	9.04	2200	114.60	3710	199.70
Glyphosate + 2,4-D[e]	0.86 + 0.84	4.88	2000	107.70	3320	181.90
LSD (0.05)			650	36.30	1110	64.00

[a] Abbreviations: PRE, preemergence; PPI, preplant incorporated.
[b] Glyphosate and 2,4-D treatments in ae/ha all other treatments in ai/ha.
[c] Application costs for sprayer 27.5 meter boom $0.58/ha (2008 MSU Soybean Budget)
[d] Soybean price @ $0.07/kg (2008 MSU Soybean Budget)
[e] Spring-applied treatments.

CHAPTER III

CONFIRMATION AND MANAGEMENT OF MULTIPLE RESISTANCE OF HORSEWEED TO GLYPHOSATE AND PARAQUAT AND ITS CONTROL WITH PARAQUAT AND METRIBUZIN COMBINATIONS

Abstract

Greenhouse studies were conducted in 2007 and 2008 to investigate possible multiple-resistance of horseweed to paraquat and glyphosate. Results indicated that the GR_{50} (herbicide dose required to cause a 50% reduction in plant growth) value for the susceptible population S102 was 0.066 kg ae/ha glyphosate, and for the resistant population MDOT was 0.78 kg/ha glyphosate. The level of glyphosate resistance for MDOT was 12-fold compared with S102. The GR_{50} value for the susceptible population S102 was 0.078 kg ai/ha paraquat, and for the resistant population MDOT was 0.67 kg/ha paraquat. The level of paraquat resistance for MDOT was 9-fold compared with S102. These data suggest that multiple-resistance to glyphosate and paraquat exists in horseweed population from Mississippi. This is the first broadleaf weed species reported as exhibiting multiple-resistance to these chemistries. Field studies were conducted in 2007 and 2008 to evaluate the effect of the addition of metribuzin to paraquat on control of paraquat-resistant horseweed. Paraquat alone at 0.84 kg/ha controlled 10-cm tall horseweed 80 and 40% and 15-cm tall horseweed 73 and 70% in 2007 and 2008, respectively. The addition of metribuzin to paraquat improved horseweed control. Paraquat at 0.84 and 1.12 kg/ha + all levels of metribuzin controlled 10-cm tall horseweed at least 90% in 2007 and 15-cm tall horseweed in 2007 and 2008. Overall, control of 10-cm tall horseweed was poor in 2008 and was likely due to low air temperatures at the time of application. Horseweed plant densities following paraquat alone treatments targeting 10-cm tall were not reduced compared to the nontreated in either year

or the 15-cm tall treatment in 2008. The addition of metribuzin to paraquat reduced 10-cm tall horseweed populations compared to the nontreated by at least 88% in 2007, while no differences were seen in 2008. All treatments effectively reduced horseweed biomass compared to the nontreated in 2007, whereas only paraquat + metribuzin combinations targeting 15-cm tall horseweed reduced biomass in 2008. Among paraquat alone treatments targeting 10-cm tall horseweed, there were no differences in soybean yields compared to the nontreated in 2007; however, all treatments targeting 15-cm tall horseweed improved soybean yields over the nontreated. There were no differences in soybean yields across treated plots for either timing in 2008 due to excessive rainfall at harvest. These findings suggest that the addition of metribuzin to paraquat improves control of paraquat-resistant horseweed.

Nomenclature: Horseweed, *Conyza canadensis* (L.) Cronq.; soybean, *Glycine max* (L.) Merr. 'P 94B73', 'NK S43-B1'.

Key words: Resistance, multiple-resistance, burndown, herbicide efficacy, herbicide mixtures, plant density, plant biomass.

Introduction

Bipyridiliums are a class of herbicides, developed in the 1950's, that are described as photosynthetic inhibitors or cell membrane disruptors (Senseman 2007). There are currently two compounds classified as bipyridiliums: paraquat and diquat (Senseman 2007). These chemistries accept electrons in the light reaction cycle of Photosystem I (PSI) that occurs in the chloroplast of most plants (Devine et al. 1993). This leads to the formation of hydroxyl radicals. Lipid cell membranes are rapidly degraded by these hydroxyl radicals, which results in the rupture of cell membranes and spilling of cell contents, eventually causing wilting and desiccation of plant tissues (Devine et al. 1993; Senseman 2007). Paraquat is a non-selective, foliar-applied herbicide that is very effective in controlling many broadleaf and grassy weed species, and is labeled for use in many agronomic, vegetable, fruit, and nut crops (Senseman 2007). Due to its rapid action and effectiveness, paraquat has been utilized as a burndown herbicide to remove weed species prior to planting, as well as a desiccant to facilitate harvesting (Eubank et al. 2008).

In the early to mid 1980's, *Conyza canadensis* (L.) Cronq*, Conyza sumatrensis* (Retz.)*, Conyza bonariensis* (L.) Cronq. and *Erigeron philadelphicus* (L.) were all reported resistant to paraquat (Kato and Okuda 1983; Watanabe et al. 1982; Heap 2010). In most cases the resistance occurred where paraquat had been applied multiple times per growing season for consecutive years in orchards and vineyards (Kato and Okuda 1983; Smisek et al. 1998; Watanabe et al. 1982). Smisek et al. (1998) reported the discovery of paraquat-resistant horseweed in an orchard in Ontario, Canada, where paraquat had been applied 4 to 5 times per year for a 10-year period and exhibited resistance levels 25 to 35 times higher than that of susceptible populations. These paraquat-resistant plants were also cross-resistant to diquat (Weaver et al. 2004). More recently, VanGessel et al. (2006) documented paraquat-resistant horseweed in Delaware. Paraquat-resistant horseweed had been reported in Mississippi in 1994 (Heap 2010); however, this was simply an observation and definitive testing to prove resistance was not conducted (Kevin Vaughn, USDA-ARS, personal communication). Although conclusive evidence does not exist with regards to the exact mechanism of paraquat resistance, it is generally accepted that it is due to a reduction in movement of the herbicide to the site-of-action in the chloroplast or some sequestration mechanism of the paraquat cation (Fuerst et al. 1985; Jori et al. 2007; Norman et al. 1993; VanGessel et al. 2006). There are currently 23 weed species that have been documented resistant to the bipyridiliums chemistry (Heap 2010).

Conyza species have also been reported resistant to other herbicide modes-of-action, including: Photosystem II inhibitors (PSII), acetolactate synthase inhibitors, glycines, ureas and amides (Heap 2010; VanGessel 2001; Weaver et al. 2004). Glyphosate-resistant (GR) horseweed has become a major problem across much of North America, and has been documented in more than 16 states across the United States, including Mississippi (Heap 2010; Koger et al. 2004; VanGessel 2001). *C. canadensis* is the first annual broadleaf documented resistant to glyphosate worldwide (VanGessel 2001). Similar to paraquat resistance, a reduction in translocation of glyphosate is reported as the primary mechanism for glyphosate resistance in horseweed (Feng et al. 2004; Koger and Reddy 2005). These findings, albeit separate studies, suggest that *C. canadensis* has the capacity to effectively reduce the translocation of both glyphosate and paraquat herbicides within the plant. This poses the question as to whether *Conyza* spp. could have the propensity to develop resistance to both glyphosate and paraquat. Multiple-resistance to both glyphosate and paraquat has been previously reported only in rigid ryegrass (*Lolium rigidum* Gaud.) (Yu et al. 2007).

Herbicide-resistant horseweed is a serious concern. GR horseweed has necessitated research to find alternative control measures for this troublesome weed (Eubank et al 2008; Owen et al. 2009; Steckel et al. 2006; Steckel and Gwathmey 2009). Previous research by Eubank et al. (2008) concluded that a horseweed population near Leland, MS, was not effectively controlled with either 0.86 kg ae/ha glyphosate (60 to 65%) or 0.84 kg ai/ha paraquat (55 to 63%) at 4 weeks after treatment (WAT). Interestingly, the addition of metribuzin, a PSII inhibitor, to paraquat improved the level of horseweed control over paraquat alone (Eubank et al. 2008). Research investigating alternative methods of control for multiple-resistant horseweed is needed.

The objective of this study is to determine if multiple-resistance to glyphosate and paraquat exists in a horseweed population near Leland, MS, and if the addition of metribuzin to paraquat improves control of paraquat-resistant horseweed.

Materials and Methods

Multiple-Resistance Study

Greenhouse studies were conducted to investigate suspected multiple-resistance of horseweed to glyphosate and paraquat. Mature seed from a suspected resistant horseweed population (MDOT) were collected from Washington County, MS, (33^o 25’09.16” N and 90^o 53’09.37” W) that was unsuccessfully controlled by 0.84 kg/ha paraquat and at least two applications of 0.84 kg/ha glyphosate in 2007. Cropping history for the MDOT population was preceded by at least five consecutive years of no-till GR soybean (*Glycine max* (L.) Merr.). A susceptible horseweed population (S102) from Coahoma County, MS (34^{o} 12’07.18” N and 90^{o} 32’09.70” W), was selected for comparison. Horseweed seeds were surface planted into separate 25 x 25 x 6 cm trays containing Jiffy mix potting media.[2] Trays were subirrigated with distilled water and placed in a growth chamber at 24/18 C day/night temperatures with supplemental lighting set to a 14 h photoperiod. When emerged horseweed plants attained at least 3 true leaves in growth, individual plants were transplanted to 10-cm wide x 15-cm deep pots. Pots were then transferred to a greenhouse with natural light supplemented with sodium vapor lamps set to a 14 h photoperiod. Plants were grown at 25/15 C (± 3 C) day/night temperature. Plants were sub-irrigated and fertilized as needed.

Herbicide treatments were initiated when plants uniformly reached 10 to 15-cm in diameter that corresponded to approximately 35 to 40 leaves per plant. Treatments included: potassium salt of glyphosate[3] at 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.3, and 6.7 kg/ha; paraquat[4] at 0.02, 0.03, 0.07, 0.14, 0.28, 0.56, 0.84, 1.12, and 2.24 kg/ha; and a nontreated control. Paraquat treatments included a non-ionic surfactant (NIS) at 0.5% v/v. A commercial glyphosate formulation was used that included an adjuvant system. Treatments were applied using an indoor spray chamber equipped with an air-pressurized flat-fan nozzle[5] calibrated to deliver a spray volume of 140 L/ha at a pressure of 220 kPa. After treatment, plants were returned to the greenhouse, separated to prevent cross-contamination or influence from neighboring plants, and subirrigated as needed without wetting foliage. Horseweed biomass was collected at 3 WAT by harvesting the uppermost portion of the plant at the soil line and recorded as fresh weights. Shoot fresh weights were expressed as a percent of the nontreated control for each population by individual replication.

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[2] Jiffy Products of America Inc. Batavia, IL. 60510.
[3] Roundup WeatherMax®, glyphosate, EPA Reg. No. 524-537, Monsanto Company, 800 N. Linbergh Blvd., St. Louis, MO 63167.
[4] Gramoxone Inteon®, paraquat, EPA Reg. No. 100-1217, Syngenta Crop Protection, Inc., P.O. Box 18300, Greensboro, NC 27419.
[5] TeeJet XR 8002EVS flat-fan nozzle, Spraying Systems Co., North Ave., Wheaton, IL 60189.

Experimental design was a randomized complete block design with four replications of each treatment and population. Each experiment was conducted three times. All data were subjected to ANOVA using the PROCMIXED procedure in SAS with trial as a random variable. Means were separated using Fisher's Protected LSD test at alpha≤ 0.05. Non -linear regression analysis was used to calculate herbicide rates which resulted in a 50% reduction in horseweed biomass (GR_{50}). A sigmoidal log-logistic model (Seber and Wild 1989) would not fit the data due to hormesis effects (Schabenberger et al. 1999). An exponential decay model was used to relate fresh weight reduction as a percent of the nontreated (*Y*) to herbicide rate (*x*) (Y=y0+a*exp(-b*x)) with SigmaPlot software.[6]

Paraquat and Metribuzin Study

Field studies were conducted in 2007 and 2008 to determine if the addition of metribuzin to paraquat improved the control of paraquat-resistant horseweed. Plots were established near the Delta Research and Extension Center, Stoneville, MS (33^o 25'09.16" N and 90^o 53'09.37" W). The soil type was a Dundee very fine sandy loam (fine-silty, mixed, active, thermic Typic Endoaqualfs) with a pH of 6.1 and organic matter content of 1.2%. Plots were established following five consecutive years of no-tillage GR soybean and were naturally infested with horseweed exhibiting resistance to glyphosate and paraquat. The experimental design was a split-plot design with a factorial arrangement of treatments and four replications in a randomized complete block. Split-plot design had one plot being treated when horseweed averaged 10-cm in height and the second plot treated when horseweed averaged 15-cm in height. Plots were 3 x 12 m, and herbicide treatments were applied with a tractor-mounted compressed-air sprayer calibrated to deliver 140 L/ha at a pressure of 207 kPa using flat fan nozzles[7]. Treatments were applied to 10-cm horseweed on February 28, 2007 and March 24, 2008 and to 15-cm horseweed on March 29, 2007 and May 9, 2008. Weather data including average air temperature, solar

[6] SigmaPlot 10.0, SigmaPlot 2006 for Windows, Version 10.0.1.25. SPSS Inc., 444 North Michigan Avenue, Chicago, IL 60611.
[7] TeeJet XR 11002VS flat-fan nozzle, Spraying Systems Co., North Ave., Wheaton, IL 60189.

radiation, relative humidity, and precipitation for 7 d prior and 7 d after postemergence herbicide applications are given in Tables 3.1 and 3.2. Herbicide treatments included: paraquat at 0.56, 0.84, and 1.12 kg/ha alone and in combination with metribuzin at 0.1, 0.2 and 0.4 kg ai/ha. A nontreated control was also included for comparison of soybean yields. All treatments were applied as broadcast foliar-applied treatments and included a NIS at 0.5% v/v. Visual control ratings were determined using a 0 to 100 scale (0, no control; 100, complete control) and were measured at 14 and 28 days after treatment (DAT). Horseweed plant counts and biomass from 2 meter^2 quadrants were recorded at planting or approximately 28 DAT. Horseweed biomass was obtained by harvesting the uppermost portion of the plant at the soil line and recorded as fresh weights and extrapolated to g/m^2. The soybean variety Pioneer 94B73 soybean was seeded April 30, 2007 and NK S43-B1 soybean was seeded on May 9, 2008. Soybean was planted with a seven-row planter on 38-cm row spacing at a seeding rate of 13 seed/m of row. No additional at-planting burndown applications were applied. Horseweed was allowed to compete with soybean for approximately 2 wk after planting (WAP) before in-season herbicides were applied. For in-season weed management, all plots received POST applications of 0.84 kg/ha glyphosate + 1.26 kg ai/ha *S*-metolachlor and 0.84 kg/ha glyphosate alone at 2 and 7 WAP, respectively. The center five rows of the seven-row plots were harvested with a research combine. Soybean yields and moisture were recorded and moisture was adjusted to 13%.

All data were subjected to ANOVA using the general linear model in SAS. Means were separated using Fisher's Protected LSD test at alpha ≤ 0.05.

Results and Discussion

Multiple-Resistance Study

Response of S102 and MDOT populations to increasing glyphosate rate was best fit to an exponential decay model with R^2 values of 0.94 to 0.98, respectively (Figure 3.1). The GR_{50} (herbicide dose required to cause a 50% reduction in plant growth) value for the glyphosate-susceptible population was 0.066 kg/ha, and for the GR population was 0.78 kg/ha. The level of glyphosate resistance for MDOT was 12-fold when compared with S102. This level of glyphosate resistance is similar to those reported previously by Koger et al. (2004) and VanGessel (2001). At 3 WAT, a 40 to 60% reduction in horseweed biomass was realized at the three lowest glyphosate rates (0.025, 0.05 and 0.1 kg/ha) for the S102 population, whereas MDOT averaged a 3% increase in biomass across these same rates. Biomass for the MDOT population was reduced roughly 50% for the normal field use rate of 0.84 kg/ha glyphosate, while S102 was completely controlled at the same rate.

Response of S102 and MDOT populations to increasing paraquat rate was best fit to an exponential decay model with R^2 values of 0.99 and 0.97, respectively (Figure 3.2). The GR_{50} value for S102 was 0.078 kg/ha, and with MDOT was 0.67 kg/ha. The level of paraquat resistance for MDOT was 9-fold when compared with S102. This level of paraquat resistance is much lower than the 22-fold reported by VanGessel et al. (2006), 35-fold by Smisek et al. (1998), and a 100-fold level of resistance in *Conyza bonariensis* by Norman et al. (1993). Horseweed biomass for the MDOT population increased an average of 7% across the three lowest rates of paraquat (0.017, 0.034 and 0.07 kg/ha), while S102 biomass decreased from 15 to 46%. Biomass for the S102 population was reduced more than 90% at the normal field use rate of 0.84 kg/ha paraquat, while MDOT biomass was reduced only 57%.

These data suggest that multiple-resistance to glyphosate and paraquat exists in a horseweed population from Mississippi. This is the first broadleaf weed species reported as exhibiting multiple-resistance to these chemistries, and the first species overall to appear in an agronomic row crop situation. Multiple-resistance to both glyphosate and paraquat has only been previously reported in rigid ryegrass (Yu et al. 2007). The mechanism of resistance within MDOT was not researched in this study; however, a possible reason may be through a rapid sequestration mechanism. Recent studies suggested that glyphosate enters the cytoplasm at the same rate in both glyphosate-resistant and glyphosate-susceptible horseweed; however, within hours glyphosate begins to accumulate in the vacuoles of the resistant, but not in the susceptible plants (Ge et al. 2010). Studies looking into the mechanism of paraquat resistance suggested a sequestration mechanism in that paraquat was found in the vacuoles and cytosol of paraquat-resistant horseweed up to a month after herbicide treatment (Soos et al. 2006). Considering the findings of these separate studies it would seem presumable that a rapid sequestration mechanism may be involved in our multiple-resistance horseweed population.

Of particular interest were the observance of a hormetic response in horseweed biomass for the MDOT population with sub-lethal herbicide rates of both glyphosate and paraquat. Hormesis is the stimulation of plant growth and development with low doses of herbicides (Nandula 2010). Hormetic responses have been reported with glyphosate on barley (*Hordeum vulgare* L.), barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], corn (*Zea mays* L.), conventional soybean, and benghal dayflower (*Commelina benghalensis* L.) (Cedergreen 2008; Tharp et al. 1999; Velini et al. 2008). Hormesis has not been observed with paraquat; however, one study did find hormetic effects on barley with diquat, but this was not repeatable (Cedergreen 2008). In our study, the hormetic effects were repeatedly seen across trials. It is unlikely that hormesis effects in this study were due to plant-to-plant competition, as plants were grown in single species environments and separated after treatment to avoid interference from neighboring plants.

Paraquat and Metribuzin Study

There were interactions by year and by horseweed size, thus data are presented separately. There were no differences between the 14 and 28 DAT control ratings (14 DAT data not presented). Lack of differences between the two observations is likely due to the rapid action of paraquat with visible injury being observed in a matter of days. Paraquat alone at 0.56 kg/ha had the lowest level of 10-cm horseweed control in 2007 (Table 3.3). Increasing paraquat rate improved horseweed control, with the highest rate of paraquat alone controlling horseweed 88%. All levels of paraquat + all levels of metribuzin gave at least 93% control of 10-cm horseweed in 2007. Overall, control of 10-cm horseweed was poor in 2008 compared to 2007. In 2008, the lowest rate of paraquat alone controlled horseweed only 30%, while the highest rate of paraquat alone enhanced horseweed control to merely 50%. Horseweed control in 2008 was improved only with the highest rate of paraquat + all combinations of metribuzin and 0.84 kg/ha paraquat + 0.4 kg/ha metribuzin compared to 1.12 kg/ha paraquat alone. Possible explanations for the reduced levels of control may have been due to a drop in air temperature prior to and following application (Tables 3.1 and 3.2). Low air temperatures have been reported to reduce weed control with paraquat. Purba et al. (1995) reported that the mechanism of paraquat resistance in *Hordeum leporinum* Link. and *H. glaucum* Steud. was temperature-dependent in that resistant plants did not respond to applications of paraquat at 15 C, but were effectively controlled at 30 C. In our studies air temperature did not exceed 10 C for 24 h prior to application and then again 48 h after application of paraquat to horseweed in 2008 (Table 3.2). These cooler air temperatures may have slowed the translocation of paraquat resulting in a reduction in horseweed control. Further research has suggested that lower air temperatures reduced basipetal translocation of paraquat in *Hordeum*. spp. and glufosinate in *Raphanus raphanistrum* (Kumaratilake and Preston 2005; Purba et al 1995).

Visual control ratings of 15-cm horseweed control were lowest with 0.56 kg/ha paraquat, at only 60 and 53% control in 2007 and 2008, respectively, and were lower than all other treatments (Table 3.4). Increasing paraquat rate to 0.84 kg/ha improved horseweed control to 73 and 70% in 2007 and 2008, respectively. In 2007, the addition of metribuzin to paraquat did not improve control of 15-cm horseweed over the highest rate of paraquat alone with the exception of 0.84 kg/ha paraquat + 0.4 kg/ha metribuzin or 1.12 kg/ha paraquat + 0.2 kg/ha metribuzin. The addition of 0.2 kg/ha metribuzin to 0.56 kg/ha paraquat improved control of 15-cm horseweed over the highest rate of paraquat alone in 2008. The medium and highest rate of paraquat + all levels of metribuzin gave at least 93% control of 15-cm horseweed in 2008. These data suggest that the addition of a PSII herbicide, such as metribuzin, to paraquat can improve control of horseweed. Similar findings were reported by Putnam and Reis (1967), where paraquat efficacy of quackgrass (*Agropyron repens* L. Beauv.) was improved with the addition of simazine;

however, these results conflict with the findings of Owen et al. (2009) where 0.84 kg/ha paraquat + 1.12 kg ai/ha prometryn only provided 58% control of GR horseweed of unstated size.

Horseweed plant densities in the nontreated, at the 10-cm growth stage, were 48 and 30 plants/m^2 in 2007 and 2008, respectively (Table 3.3). Horseweed plant densities were not reduced across all levels of the paraquat alone treatments compared to the nontreated in either year. The lowest rate of paraquat alone (0.56 kg/ha) resulted in a net increase in horseweed densities of 52 and 117% in 2007 and 2008, respectively. This was likely due to the removal of competition to horseweed from other weeds such as henbit (*Lamium amplexicaule* L.), common chickweed [*Stellaria media* (L.) Vill.], and annual bluegrass (*Poa annua* L.) within treated plots, allowing emergence of new horseweed plants. Additionally, the addition of metribuzin may have prevented emergence of horseweed after application compared to paraquat alone and may have confounded density results. The addition of metribuzin to paraquat reduced horseweed populations compared to the nontreated, by at least 88%. There were no reductions in horseweed populations across all herbicide treatments in 2008. This is likely attributed to the reduced levels of horseweed control in 2008, as observed previously.

Horseweed plant densities in the nontreated plots, at the 15-cm growth stage, were 44 and 59 plants/m^2 in 2007 and 2008, respectively (Table 3.4). All herbicide treatments reduced horseweed densities compared to the nontreated in 2007. Paraquat alone treatments reduced horseweed densities from 48 to 80%, while the addition of metribuzin to 0.84 and 1.12 kg/ha levels of paraquat reduced horseweed densities from 82 to 100%. Paraquat alone did not reduce horseweed densities compared to the nontreated in 2008. All paraquat + metribuzin combinations reduced horseweed populations compared to the nontreated in 2008.

Horseweed biomass in the nontreated, at the 10-cm growth stage, was 360 and 110 g/m^2 in 2007 and 2008, respectively (Table 3.3). All treatments effectively reduced horseweed biomass compared to the nontreated in 2007. Paraquat alone reduced horseweed biomass from 57 to 88%. The addition of metribuzin to paraquat reduced biomass 98 to 100%; however, these treatments were not better than 0.84 and 1.12 kg/ha paraquat alone. No herbicide treatments reduced horseweed biomass compared to the nontreated in 2008. Several treatments, including all levels of paraquat alone, actually increased horseweed biomass numbers from 124 to 427%. This is likely attributed to the reduced levels of horseweed control in 2008, as stated previously, and the removal of other weed species, allowing remaining horseweed plants to proliferate.

Horseweed biomass in the nontreated, at the 15-cm growth stage, was 1260 and 1140 g/m^2 in 2007 and 2008, respectively (Table 3.4). All herbicide treatments reduced horseweed biomass from 90 to 100% compared to the nontreated in 2007. There were no differences between herbicide treatments in 2007. Paraquat alone treatments did not reduce horseweed biomass compared to the nontreated in 2008. All paraquat + metribuzin treatments improved the reduction in horseweed biomass from 66 to 99% compared to the nontreated.

There were no reductions in horseweed populations and biomass by any herbicide treatments at the 10-cm growth stage in 2008 (Table 3.3). Overall, the combination of reduced horseweed control and a lower number of horseweed plants in the nontreated may have contributed to these differences.

Soybean yields following treatments targeted at 10-cm horseweed in 2007 were 540 kg/ha following 0.56 kg/ha paraquat, 360 kg/ha following 0.84 kg/ha paraquat and 740 kg/ha paraquat following 1.12 kg/ha paraquat, and were not different than the soybean yield of 360 kg/ha from the nontreated (Table 3.3). The addition of metribuzin to paraquat improved soybean yields from 1220 to 2060 kg/ha compared to the nontreated. Soybean yields with the lowest rate of metribuzin + 0.56 and 0.84 kg/ha paraquat at 1220 and 1290 kg/ha, respectively, were the only combinations comparable to the highest rate of paraquat alone at 740 kg/ha in 2007.

Soybean yields following treatments targeted at 15-cm horseweed in 2007 were 1230 kg/ha following 0.56 kg/ha paraquat, 990 kg/ha following 0.84 kg/ha paraquat and 1860 kg/ha paraquat following 1.12 kg/ha paraquat, and were considerably higher than the nontreated yields of 160 kg/ha (Table 3.4). The addition of metribuzin to paraquat did not improve soybean yields compared to 1.12 kg/ha paraquat alone but were higher than 0.84 kg/ha paraquat. There were no differences in soybean yields across treatments for either timing in 2008. This was likely due to below-average rainfall in the months of June and July of 2008 and seed rot due to excessive rainfall in conjunction with Hurricane Gustav two weeks prior to soybean harvest.

The herbicide treatments of paraquat alone at 0.56 and 0.84 kg/ha provided among the lowest levels of horseweed control for either growth stage evaluated each year. The highest rate of paraquat, 1.12 kg/ha + 0.1, 0.2, and 0.4 kg/ha metribuzin gave the highest level of horseweed control each year. In our studies, paraquat alone did not reduce horseweed densities at the 10-cm application timing either year, or the 15-cm timing in 2008 compared to the nontreated, whereas the addition of metribuzin to paraquat reduced horseweed densities at the 10-cm application timing in 2007 and the 15-cm timing both years. All treatments reduced horseweed biomass in 2007; however, only the addition of metribuzin to paraquat reduced horseweed biomass at the 15-cm application timing in 2008. These findings suggest that the combination of paraquat + metribuzin may be additive or possibly synergistic and improve control of paraquat-resistant horseweed. Statistical analysis for the presence of synergism was not conducted in these studies because of the oversight of not including metribuzin alone treatments. The synergistic effects of paraquat + PS II inhibitors have been well documented (Colby et al. 1965; Griffin et al. 2004; Putnam and Ries 1967); however, little research has been done to evaluate these chemistries on the control of horseweed. Kapusta (1979) reported that the addition of metribuzin to paraquat controlled 4- to 6-cm horseweed greater than 95% in no-till soybean; however, a paraquat alone treatment was not included for comparison in this study and several other treatments containing paraquat gave similar results.

Horseweed exhibiting multiple-resistance to glyphosate and paraquat has been confirmed in Mississippi. In our study MDOT survived normal use rates of glyphosate and paraquat. This is the first broadleaf weed species reported as exhibiting multiple-resistance to these chemistries, and the first species overall to appear in an agronomic row crop situation. The use of tank-mixed herbicides, such as the addition of metribuzin to paraquat, will be essential in controlling this troublesome weed.

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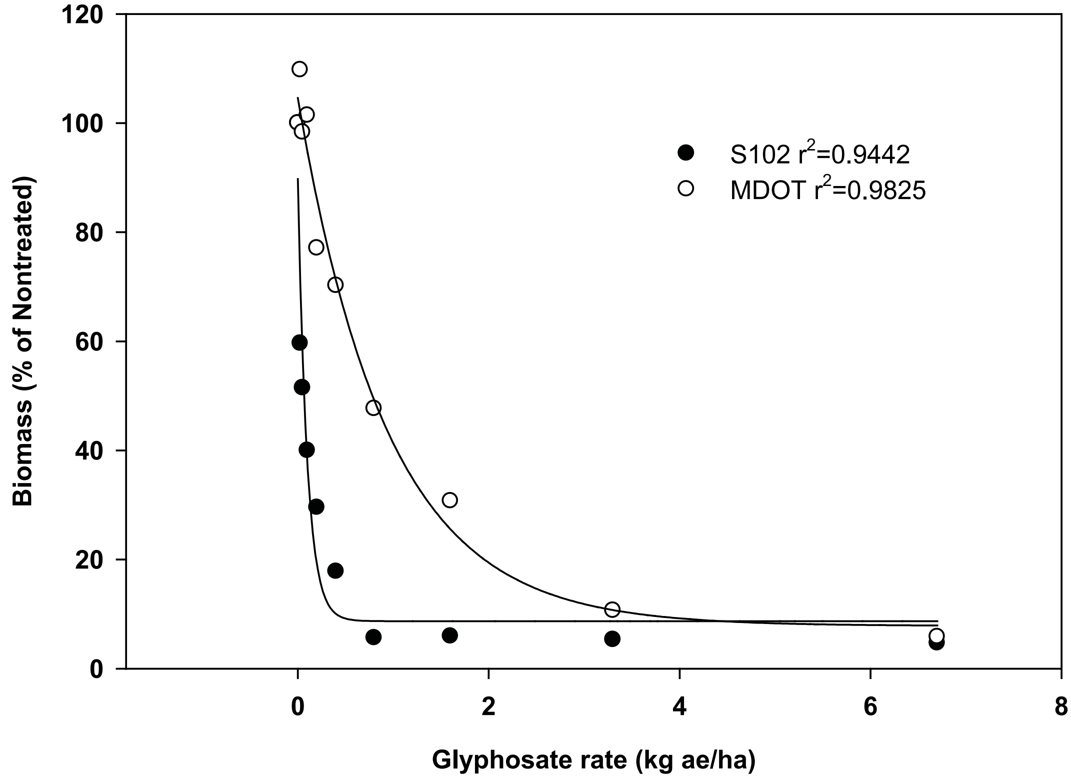

Figure 3.1 Response of suspected glyphosate-resistant (MDOT) and glyphosate-susceptible horseweed (S102) to glyphosate 3 wk after treatment. GR_{50} = 0.066 kg ai/ha for S102. GR_{50} = 0.78 kg ae/ha for MDOT

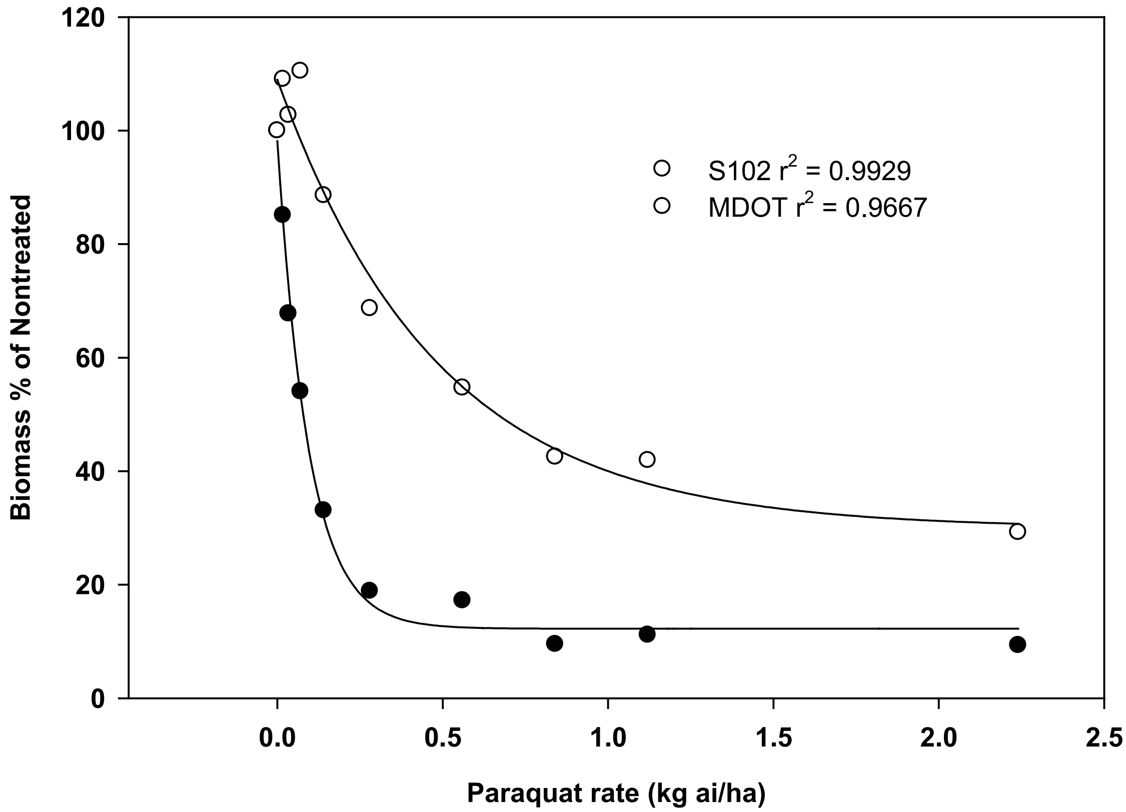

Figure 3.2 Response of suspected paraquat-resistant (MDOT) and paraquat-susceptible (S102) horseweed to paraquat 3 wk after treatment. GR_{50} = 0.078 kg ai/ha for S102. GR_{50} = 0.67 kg ai/ha for MDOT

Table 3.1 Average air temperature, solar radiation, percent relative humidity, and precipitation for 7 d prior and 7 d after postemergence herbicide applications in 2007

Date	Air Temperature	Solar Radiation	Relative Humidity	Precipitation
	C	Langleys/day	%	cm
2/21/07	17	83	87	0.25
2/22/07	17	387	60	0.00
2/23/07	14	465	60	0.00
2/24/07	14	416	50	0.00
2/25/07	16	112	69	2.24
2/26/07	10	487	57	0.00
2/27/07	13	481	60	0.00
2/28/07[a]	13	487	57	0.00
3/01/07	16	250	71	0.00
3/02/07	13	181	57	0.48
3/03/07	11	505	45	0.00
3/04/07	7	413	58	0.00
3/05/07	5	538	57	0.00
3/06/07	10	518	54	0.00
3/07/07	13	450	52	0.00
3/22/07	21	511	59	0.00
3/23/07	22	521	58	0.00
3/24/07	23	508	56	0.00
3/25/07	23	537	53	0.00
3/26/07	23	382	59	0.00
3/27/07	24	370	60	0.00
3/28/07	21	325	68	0.51
3/29/07[b]	21	516	67	0.00
3/30/07	23	492	60	0.00
3/31/07	21	517	59	0.00
4/01/07	11	169	77	0.03
4/02/07	11	392	69	0.03
4/03/07	8	516	63	0.00
4/04/07	5	495	70	0.51
4/05/07	8	473	43	0.00

[a]Date of herbicide application to 10-cm horseweed.
[b]Date of herbicide application to 15-cm horseweed.

Table 3.2 Average air temperature, solar radiation, percent relative humidity, and precipitation for 7 d prior and 7 d after postemergence herbicide applications in 2008.

Date	Air Temperature	Solar Radiation	Relative Humidity	Precipitation
	C	Langleys/day	%	cm
3/17/08	14	473	66	0.00
3/18/08	20	404	58	0.00
3/19/08	22	202	69	0.46
3/20/08	10	147	83	0.15
3/21/08	11	557	65	0.00
3/22/08	16	552	54	0.00
3/23/08	13	559	49	0.00
3/24/08[a]	8	538	60	0.00
3/25/08	8	581	45	0.00
3/26/08	14	578	47	0.00
3/27/08	18	527	60	0.00
3/28/08	22	478	62	0.00
3/29/08	18	289	77	0.18
3/30/08	17	335	74	0.03
3/31/08	21	194	75	0.00
5/02/08	21	498	63	0.00
5/03/08	22	361	75	1.57
5/04/08	16	634	66	0.00
5/05/08	17	618	67	0.00
5/06/08	20	622	61	0.00
5/07/08	21	471	56	0.00
5/08/08	24	484	61	0.56
5/09/08[b]	20	470	75	0.30
5/10/08	23	647	67	0.00
5/11/08	23	321	69	0.08
5/12/08	18	680	49	0.00
5/13/08	18	691	48	0.00
5/14/08	21	198	67	1.14
5/15/08	21	158	83	4.67
5/16/08	20	421	77	1.27

[a]Date of herbicide application to 10-cm horseweed.
[b]Date of herbicide application to 15-cm horseweed.

Table 3.3 Control of 10-cm horseweed, plant density, and horseweed biomass 28 DAT, and soybean yields[a].

		2007				2008			
Treatment	Rate	Control 28 DAT	Density 28 DAT	Biomass 28 DAT	Yield	Control 28 DAT	Density 28 DAT	Biomass 28 DAT	Yield
	kg ai/ha	—%—	plants/m^2	g/m^2	kg/ha	—%—	plants/m^2	g/m^2	kg/ha
Nontreated		0	48	360	360	0	30	110	740
Paraquat[b]	0.56	72	73	150	540	30	65	360	1200
Paraquat	0.84	80	38	90	360	40	71	430	1250
Paraquat	1.12	88	23	40	740	50	59	470	1370
Paraquat + Metribuzin	0.56 + 0.1	93	6	10	1220	40	89	290	1340
Paraquat + Metribuzin	0.56 + 0.2	93	5	10	1490	48	37	180	1570
Paraquat + Metribuzin	0.56 + 0.4	98	2	5	1490	50	47	290	1400
Paraquat + Metribuzin	0.84 + 0.1	93	4	10	1290	48	35	270	1310
Paraquat + Metribuzin	0.84 + 0.2	100	1	0	1900	53	25	200	1520
Paraquat + Metribuzin	0.84 + 0.4	100	0	0	1990	78	16	140	1440
Paraquat + Metribuzin	1.12 + 0.1	95	1	5	1870	73	16	50	1360
Paraquat + Metribuzin	1.12 + 0.2	98	0	1	2060	78	13	90	1300
Paraquat + Metribuzin	1.12 + 0.4	100	0	0	1680	83	3	10	1250
LSD (0.05)		7	27	105	650	18	45	240	720

[a] Abbreviations: DAT, days after treatment; NIS, nonionic surfactant.
[b] All paraquat treatments included NIS at 0.5% v/v.

Table 3.4 Control of 15-cm horseweed, plant density, and horseweed biomass 28 DAT, and soybean yields[a].

		2007				2008			
Treatment	Rate	Control 28 DAT	Density 28 DAT	Biomass 28 DAT	Yield	Control 28 DAT	Density 28 DAT	Biomass 28 DAT	Yield
	kg ai/ha	—%—	plants/m^2	g/m^2	kg/ha	—%—	plants/m^2	g/m^2	kg/ha
Nontreated		0	44	1260	160	0	59	450	1140
Paraquat[b]	0.56	60	23	110	1230	53	46	420	1520
Paraquat	0.84	73	17	130	990	70	35	240	1580
Paraquat	1.12	90	9	70	1860	70	37	240	1410
Paraquat + Metribuzin	0.56 + 0.1	85	16	50	1680	78	19	80	1560
Paraquat + Metribuzin	0.56 + 0.2	93	3	10	1960	85	10	80	1620
Paraquat + Metribuzin	0.56 + 0.4	88	17	80	1710	80	24	150	1610
Paraquat + Metribuzin	0.84 + 0.1	90	8	30	2240	93	14	110	1470
Paraquat + Metribuzin	0.84 + 0.2	95	4	10	2270	95	7	120	1610
Paraquat + Metribuzin	0.84 + 0.4	100	0	0	2340	95	2	10	1580
Paraquat + Metribuzin	1.12 + 0.1	98	1	30	2410	95	8	50	1550
Paraquat + Metribuzin	1.12 + 0.2	100	0	0	2460	98	6	80	1630
Paraquat + Metribuzin	1.12 + 0.4	98	5	10	1940	98	1	10	1750
LSD (0.05)		9	21	240	660	14	32	240	720

[a] Abbreviations: DAT, days after treatment; NIS, nonionic surfactant.
[b] All paraquat treatments included NIS at 0.5% v/v.

CHAPTER IV

SAFLUFENACIL EFFICACY ON HORSEWEED AND ITS INFLUENCE ON THE ABSORPTION AND TRANSLOCATION OF GLYPHOSATE

Abstract

Field studies were conducted in 2008 to evaluate the effect of adjuvants on the control of horseweed with saflufenacil. The highest level of horseweed control with 0.025 kg ai/ha saflufenacil was 14 days after treatment (DAT) with 1 and 2% methylated seed oil (MSO), giving 91 and 93% control, respectively. Horseweed control with the addition of nonionic surfactant (NIS) was no better than saflufenacil alone 14 DAT. The addition of 1 and 2% crop oil concentrate (COC) to saflufenacil improved control of horseweed to 85 and 86%, respectively, over the addition of NIS, but was not comparable to either rate of MSO 14 DAT. Greenhouse studies were conducted in 2009 to evaluate the addition of glyphosate to saflufenacil on the control of glyphosate-resistant (MDOT) and glyphosate-susceptible (S102) horseweed. All levels of saflufenacil controlled both S102 and MDOT at least 93% and 100% at 14 and 21 DAT, respectively; control of horseweed with the combination of saflufenacil + glyphosate was additive. Studies were conducted in 2009 to determine saflufenacil effects on absorption and translocation of glyphosate in MDOT and S102. Overall, S102 absorbed 11 and 13% more ^{14}C-glyphosate than MDOT at 24 and 48 hours after treatment (HAT), respectively. The addition of saflufenacil did not affect absorption in S102; however, absorption increased in MDOT from 36 to 44% at 48 HAT compared to glyphosate alone treatments. Overall, the addition of saflufenacil reduced ^{14}C-glyphosate translocation in horseweed at least 6% across trials; however, due to the exceptional efficacy of saflufenacil on horseweed these reductions did not reduce control.

Nomenclature: Glyphosate, saflufenacil; horseweed, *Conyza canadensis* (L.) Cronq.

Key words: ^{14}C-glyphosate, adjuvants, antagonism, burndown.

Introduction

Glyphosate is a non-selective, systemic, postemergence herbicide that has been used extensively for controlling many troublesome weeds. The effectiveness of glyphosate as a herbicide and the utility of glyphosate-resistant (GR) crops have allowed many producers to adopt minimum-tillage and no-tillage practices (Givens et al. 2009a; Halford et al. 2001). Consequently, this widespread adoption has led to an increase in the number of glyphosate applications made during the growing season (Givens et al 2009b; Young 2006). The increased use of glyphosate has created tremendous selection pressure resulting in the development of GR weeds. To date, five weed species in Mississippi have been reported to be resistant to glyphosate, including horseweed [*Conyza canadensis* (L.) Cronq.] (Koger et al. 2004), Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] (Nandula et al. 2007a), Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Heap 2010), common waterhemp (*Amaranthus rudis* Sauer) and johnsongrass [*Sorghum halepense* (L.) Pers] (Vijay Nandula, Miss. State Univ., both personal communications).

Horseweed is typically considered to be a winter annual (Bhowmik and Bekech 1993; Buhler and Owen 1997), but also emerges in the spring and summer as well, exhibiting growth habits of a summer annual (Davis and Johnson 2008; Eubank et al. 2006). GR horseweed has become particularly problematic across much of the southeastern United States. Much of the current research in weed science has been primarily focused on investigating glyphosate resistance and alternative means of control for these developing biotypes/populations. Glufosinate controls horseweed well (Eubank et al. 2008; Steckel et al. 2006), but control may be reduced with colder air temperatures at the time of application (Owen et al. 2009; Steckel et al. 2006). Horseweed control with acetolactate synthase (ALS)-inhibiting herbicides has been effective (Owen et al. 2009); however, widespread development of ALS-resistant horseweed is of concern (Davis et al. 2010; Kruger et al. 2009). Possible control options of GR weeds may include tank-mixing herbicides with glyphosate. The addition of 0.84 kg ae/ha 2,4-D to 0.86 kg ae/ha glyphosate improved horseweed control greater than 95% compared to glyphosate alone at 65% (Eubank et al. 2008), whereas Owen et al. (2009) reported the addition of 0.28 kg ae/ha dicamba to glyphosate controlled horseweed 89%. However, there are limitations as to when phenoxy-type products can be applied prior to planting soybean and cotton due to plant-back restrictions and possible crop injury. Additionally, there have been concerns raised over the recent lack of control with spring-applied applications of 2,4-D and dicamba on horseweed (personal observation; Larry Steckel, Univ. of Tenn., personal communication). This raises concerns about the possible development of phenoxy-resistant horseweed. Kruger et al. (2008) reported that some horseweed populations in Indiana exhibited a three-fold tolerance to 2,4-D in a recent greenhouse study. Alternative control options for the postemergence control of GR horseweed are needed.

Saflufenacil is a new herbicide currently being developed by BASF Corporation and has shown potential as an alternative means for the control of GR horseweed (Bowe et al. 2009). The mode-of-action of saflufenacil is through the inhibition of protoporphyrinogen oxidase (PPO) activity with a peroxidative mode-of-action (Grossman 2010). PPO herbicides inhibit protoporphyrinogen IX oxidase, which ultimately leads to the accumulation of protoporphyrin IX (Proto) (Duke et al. 1991). Proto is a strong photosensitizer which generates high levels of singlet oxygen in the presence of oxygen and light (Duke et al. 1991). These singlet oxygen products lead to the production of hydrogen peroxide, leading to rapid necrosis and wilting of leaf tissues (Grossman et al. 2010). Herbicides which exhibit rapid necrosis of plant tissue may cause the disruption of cell membranes, which may in turn inhibit the uptake and translocation of other herbicides when applied in combination. Reduced glyphosate absorption associated with mixing contact herbicides with glyphosate has been well documented (Hydrick and Shaw 1994; Norris et al. 2001; Starke and Oliver 1998). This poses the question as to whether tank-mixing saflufenacil with a product such as glyphosate may influence the absorption and translocation of glyphosate. In preliminary field studies, a tank mixture of 0.025 kg ai/ha saflufenacil and 0.84 kg/ha glyphosate controlled GR horseweed 97% (unpublished data). The additive effects that saflufenacil has on glyphosate is unclear. Further research investigating uptake and translocation of a systemic herbicide such as glyphosate as influenced by saflufenacil is needed.

The use of adjuvants to increase herbicide efficacy has been well documented (Hatzios and Penner 1985; McWhorter and Jordan. 1976; Nandula et al. 2007b; Penner 1989; Wanamarta et al. 1989). Preliminary field studies have shown that 0.025 kg/ha saflufenacil alone gave 75% of GR horseweed, while the addition of crop oil concentrate (COC) improved GR horseweed control to 97% (unpublished data). There is little data available on the influence adjuvants have on the efficacy of saflufenacil. Further research is needed on the use of the adjuvants with saflufenacil.

The objective of this study was to determine the most efficacious adjuvant system for the control of horseweed with saflufenacil, to investigate interactions between saflufenacil and glyphosate mixtures on the control of horseweed, to determine patterns of uptake and translocation of glyphosate applied alone and in combination with saflufenacil.

Materials and Methods

Effect of Adjuvants

Field studies were conducted in 2008 near the Delta Research and Extension Center, Stoneville, MS (33^{o} 25’09.16” N and 90^{o} 53’09.37” W) to evaluate the effect of adjuvants on the control of horseweed with saflufenacil. The soil type was a Dundee very fine sandy loam (fine-silty, mixed, active, thermic Typic Endoaqualfs) with a pH of 6.1 and organic matter content of 1.2%. Experiments were established following several years of no-tillage GR soybean and were naturally infested with GR horseweed. Treatments were initiated when horseweed reached a growth stage of 10- to 15-cm in height. Treatments were applied on April 21, 2008, and May 5, 2008. The herbicide rate evaluated was 0.025 kg/ha saflufenacil. Adjuvant systems included: no ammonium sulfate (AMS) and 2% w/v AMS; no adjuvant, 0.25 and 0.5% v/v nonionic surfactant (NIS); 1 and 2% v/v COC; and 1 and 2% v/v MSO. A nontreated control was included for comparison. Plot size was 3 x 12 m, and treatments were applied with a tractor-mounted compressed-air sprayer calibrated to deliver 140 L/ha using flat fan nozzles[8] at a pressure of 207 kPa.

Visual control ratings for horseweed control were determined using a 0 to 100 scale (0, no control; 100, complete control) and were collected at 14 and 28 days after treatment (DAT) as percent weed control.

The experimental design was a factorial arrangement of treatments with one factor being AMS and the second factor being adjuvant system. Treatments had four replications and were repeated in time. All data were subjected to ANOVA with experiment being used as a random-effect parameter (SAS 2003). Experiment, replications (nested within experiment), and all interactions containing these effects were considered random effects; herbicide treatment was considered a fixed effect. Considering experiment an environmental or random effect permits inferences about treatments to be made over a range of environments (Carmer et al. 1989). Least square means were calculated and mean separation ($p \leq 0.05$) was produced using PDMIX800 in SAS, which is a macro for converting mean separation output to letter groupings (Saxton 1998).

Saflufenacil Interactions with Glyphosate

Greenhouse studies were conducted in 2009 to evaluate the effect of addition of glyphosate to saflufenacil on the control of horseweed. Mature seed from a GR horseweed population (MDOT) were collected from Washington County, MS (33^{o} 25'09.16" N and 90^{o} 53'09.37" W). Cropping history for the MDOT population was preceded by at least 5 years of no-till GR soybean. A glyphosate-susceptible horseweed population (S102), from Coahoma County, MS (34^{o} 12'07.18" N and 90^{o} 32'09.70" W) was selected for comparison. Horseweed seeds (MDOT and S102) were surface planted into separate 25 x 25 x 6 cm trays containing Jiffy mix potting media.[9] Trays were subirrigated with distilled water and placed in a growth chamber at

[8] TeeJet XR11002VS flat-fan nozzle, Spraying Systems Co., North Ave., Wheaton, IL 60189.
[9] Jiffy Products of America Inc. Batavia, IL. 60510.

24/18 C day/night temperatures with supplemental lighting set to a 14 h photoperiod. When emerged horseweed plants attained at least 3 true leaves in growth individual horseweed plants were transplanted to 10 cm pots. Pots were then transferred to a greenhouse with natural light supplemented with sodium vapor lamps set to a 14 h photoperiod. Plants were grown at 25/15 C (± 3 C) day/night temperature. Plants were sub-irrigated as needed. Herbicide treatments were initiated when plants uniformly reached 10- to 15-cm in diameter which corresponded to approximately 35 to 40 leaves per plant.

Herbicide treatments consisted of 0.5X, 1X, and 2X rates of glyphosate and saflufenacil applied alone and in tank mixture with one another. Treatments were glyphosate at 0, 0.42, 0.84, and 1.68 kg/ha; saflufenacil at 0, 0.0125, 0.025 and 0.05 kg/ha. A nontreated control was also included for comparison. All treatments, including the nontreated, included an adjuvant system of 2% (w/v) AMS and 1% (v/v) COC. Treatments were applied using an indoor spray chamber equipped with an air-pressurized flat-fan nozzle[10] calibrated to deliver a spray volume of 140 L/ha at a pressure of 220 kPa. After treatment, plants were returned to the greenhouse, separated so as to prevent cross contamination or influence from neighboring plants and watered as needed without wetting foliage. Visual control ratings for horseweed control were determined using a 0 to 100 scale (0, no control; 100, complete control) and were collected at 7, 14, and 21 days after treatment (DAT) as percent weed control. Horseweed biomass was collected at 21 DAT by harvesting the uppermost portion of the plant at the soil line and recorded as fresh weights. Shoot fresh weights were expressed as a percent of the nontreated control for each population.

The experimental design was a randomized complete block with a factorial arrangement of treatments. Factors were horseweed population and herbicide treatment. Each treatment had four replications. The method described by Colby et al. (1965) was used to calculate the expected response for herbicide combinations. To determine the potential for interaction, expected and observed values were compared at the 0.05 level of significance using Fisher's Protected LSD calculated for the observed data (Koger et al. 2007; Wehtje and Walker 1997). If the observed

[10] TeeJet XR8002EVS flat-fan nozzle, Spraying Systems Co., North Ave., Wheaton, IL 60189.

response of a herbicide combination was either significantly lower or greater than the expected value, the combination was declared antagonistic or synergistic, respectively. Combinations were considered to be additive when the observed and expected responses were similar. All data were subjected to ANOVA using the general linear model and means separated using Fisher's Protected LSD at the alpha = 0.05 level of significance (SAS 2003).

^{14}C-Glyphosate Absorption and Translocation

Studies were conducted in 2009 to determine saflufenacil effects on absorption and translocation of glyphosate in horseweed. Two horseweed populations, a GR (MDOT) and glyphosate-susceptible (S102) were propagated in the same manner as described previously. When plants attained a diameter of 4- to 6-cm randomly selected individual plants from both populations were treated with 0.11 kg ae/ha glyphosate to confirm uniformity of resistance and/or susceptibility to glyphosate.

Trial treatments were initiated when horseweed plants uniformly reached 10- to 15-cm in diameter, which corresponded to approximately 35 to 40 leaves per plant. Prior to overspray the youngest, fully expanded leaf was covered with an 8 x 8 cm piece of aluminum foil to prevent contamination. A factorial arrangement of treatments was utilized with one factor being glyphosate at 0.4 kg ae/ha and the second factor being saflufenacil at 0 and 0.0125 kg ai/ha; and COC at 0 and 1% (v/v). Additionally, four solutions containing ^{14}C-labeled glyphosate (specific activity 2.00 GBq/mmol, 99% purity in an aqueous stock solution of 7.4 MBq/ml as glyphosate acid were prepared in a commercial formulation of glyphosate to give a final concentration of 0.4 kg in 140 L of water (Reddy 2000). First, a solution containing glyphosate at a final concentration of 0.4 kg/ha in 140 L/ha was made using ^{14}C-glyphosate, a commercial formulation of glyphosate, and distilled water. Second, a solution with a final concentration of glyphosate at 0.4 kg/ha + COC at 1% (v/v) was made using ^{14}C -glyphosate, a commercial formulation of glyphosate, a commercial formulation of COC and distilled water. Third, a solution with a final concentration of glyphosate at 0.4 kg/ha + saflufenacil at 0.0125 kg/ha was made using ^{14}C -glyphosate, a commercial formulation of glyphosate, a commercial formulation of saflufenacil and distilled water. Fourth, a solution with a final concentration of glyphosate at 0.4 kg/ha + saflufenacil at 0.0125 kg/ha was made using ^{14}C -glyphosate, a commercial formulation of glyphosate, a commercial formulation of saflufenacil, a commercial formulation of COC and distilled water. All treatments included 2% (w/v) AMS. Herbicide rates were 1/2X of normal field use rates so as to minimize the rapid deleterious effects of glyphosate and saflufenacil on the susceptible population.

Plants were oversprayed with their corresponding treatment to fully evaluate the deleterious effects saflufenacil had on whole plants. Moreover, similar absorption and translocation trends with radiolabeled spotting of herbicides have been reported with non-overspray (Gillespie 1994) and overspray (Camacho and Moshier 1991) treatments of plants. Overspray treatments were applied using an indoor spray chamber equipped with an air-pressurized flat-fan nozzle[11] calibrated to deliver a spray volume of 140 L/ha at a pressure of 220 kPa. Within 30 min after application ten µL of the respective ^{14}C-glyphosate solution, containing 5KBq was distributed in the form of 10 droplets on the adaxial surface of the previously foil-covered leaf. Plants were returned to the growth chamber, separated so as to prevent cross contamination or influence from neighboring plants.

Treated plants were harvested at 24, 48 and 72 hours after treatment (HAT). Treated leaf, including petiole, was excised and immersed in 10 ml methanol/water and shaken for 20 s to remove any ^{14}C-glyphosate remaining on the leaf surface. Leaf wash procedure was repeated using a second vial of 10 ml methanol/water. Two 1-ml aliquots from each leaf wash were mixed with 10 ml scintillation cocktail (EcoLume[12]). Plants were further sectioned into all other leaves, crown and roots. Plant sections were wrapped in Kimwipes[13] tissue paper and dried at 45 C for 48 h. The oven-dried plant samples were weighed and combusted in a biological oxidizer[14] and

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[11] TeeJet XR8002EVS flat-fan nozzle, Spraying Systems Co., North Ave., Wheaton, IL 60189.
[12] EcoLume, ICN, Costa Mesa, CA 92626.
[13] Kimwipes EX-L. Kimberly-Clark Corp, Roswell, GA 30076.
[14] Packard Oxidizer 306, Packard Instruments Co. Downers Grove, IL 60515.

evolved $^{14}CO_2$ was trapped in 10 ml CarboSorb E[15] and 10 ml Permaflour E^+.[14] Radioactivities from oxidations and leaf wash were quantified using liquid scintillation spectrometry.[16] The total amount of radioactivity present in leaf washes and all plant sections was considered as total ^{14}C-glyphosate recovered. Recovery of ^{14}C-glyphosate was 98% of total applied. Sum of radioactivity present in all plant sections was considered as absorbed and was expressed as a percentage of ^{14}C-glyphosate recovered. Translocation was assumed to be the sum of all radioactivity, except the treated leaf, in all other leaves, crown and roots and expressed as a percentage of the ^{14}C-glyphosate absorbed.

Treatments were arranged in a randomized complete block design. Each treatment was replicated four times, and the experiment repeated. All data were subjected to ANOVA using the general linear model in SAS. Means were separated using Fisher's Protected LSD test at alpha $\leq$ 0.05.

Results and Discussion

Effect of Adjuvants

There were no differences in horseweed control due to the addition of AMS (data not presented). At 14 DAT, control of horseweed with saflufenacil alone was visually rated at 78% control (Table 4.2). The highest level of horseweed control was 14 DAT with 1 and 2% MSO giving 91 and 93% control, respectively. Horseweed control from the addition of NIS was no better than saflufenacil alone 14 DAT. The addition of 1 and 2% COC to saflufenacil improved control of horseweed to 85 and 86%, respectively, over the addition of NIS, but was not comparable to either rate of MSO 14 DAT.

At 28 DAT, horseweed control with saflufenacil alone had dropped to 71%, and was not different from the addition of either rate of NIS or 1% COC. Control of horseweed improved to

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[15] CarboSorb I and Permafluor E^+, Packard Instruments Co. Meridian, CT 06450.
[16] Minaxiβ Tri-carb 4000 series liquid scintillation counter, Packard Instruments Co, Downers Grove, IL 60515.

81% with 2% COC and was comparable to 1 and 2% MSO at 83 and 89% control, respectively. These studies suggest that the addition of 1 or 2% MSO or 2% COC gives the most consistent level of control of 10- to 15-cm horseweed. Knezevic et al. (2009) reported similar findings where the addition of MSO or COC to saflufenacil improved the control of prickly lettuce (*Lactuca serriola* L.), field bindweed (*Convolvulus arvensis* L.), dandelion (*Taraxacum officinale* Weber), and shephard's-purse (*Capsella bursa-pastoris* (L.) Medik.) over NIS; however, efficacy of these treatments on the control of *C. canadensis* was not evaluated.

Saflufenacil Interactions with Glyphosate

There were differences between horseweed populations, thus data are presented separately by population. At 14 DAT, the 0.42 kg/ha glyphosate alone gave 80% control of S102, but improved to 90% control 21 DAT (Table 4.3). There were no differences between 0.84 and 1.68 kg/ha glyphosate on the control of S102 with control being at least 96% at 21 DAT. Control of MDOT with 0.42 kg/ha glyphosate alone was less than 43% 21 DAT and was not different than 0.84 kg/ha glyphosate; however, horseweed control improved to 60% at the 1.68 kg/ha glyphosate rate, but was considered less than acceptable levels of control. All levels of saflufenacil alone controlled S102 95% at 14 DAT and horseweed control was 100% 21 DAT. Similarly, MDOT was controlled at least 93% 14 DAT while complete control occurred 21 DAT, regardless of saflufenacil rate. When comparing expected levels of control to the observed there was some evidence of antagonism among the S102 population with the addition of 0.012 kg/ha saflufenacil to the 0.42 and 1.68 kg/ha glyphosate 14 DAT at 93% and 92%, respectively. At 21 DAT there were no differences between any herbicide combinations; all combinations of saflufenacil + glyphosate controlled 100% across both horseweed populations. The control of horseweed with the combination of saflufenacil + glyphosate was additive. Control of GR horseweed can be problematic because of the lack of postemergence options for its control. Saflufenacil holds great potential as an alternative means of control for GR horseweed and a valuable tool in the management of resistant weeds. Saflufenacil has also been listed as a possible alternative control for 2,4-D-resistant prickly lettuce (Burke et al. 2009).

^{14}C-Glyphosate Absorption and Translocation

There were no differences in ^{14}C-glyphosate absorption due to trial, so results were combined. There were no differences in ^{14}C-glyphosate absorption due to the addition of COC (data not presented). This may have been due to the ideal environmental conditions of the growth chamber environment. There were differences in absorption of ^{14}C-glyphosate by horseweed population and harvest timing for glyphosate alone (Figure 4.1). S102 absorbed 12% more ^{14}C-glyphosate than MDOT. Glyphosate continued to accumulate over time in both populations; however, absorption slowed significantly in S102 at 72 HAT. Treated leaves of S102 were severely chlorotic by 72 HAT and this apparently limited further uptake of ^{14}C-glyphosate. Other research has suggested that maximum movement of ^{14}C-glyphosate occurred by 48 HAT (Koger and Reddy 2005; Nandula et al. 2008). Conversely, ^{14}C-glyphosate absorption continued to increase in the glyphosate-resistant population as plant growth was unaffected by glyphosate alone in that there was a gradual increase in ^{14}C-glyphosate absorption from 32 to 44% for MDOT. Similarly, this trend in absorption has been reported in horseweed (Feng et al. 2004) and GR soybean (Pline et al. 1999). Overall, the addition of saflufenacil increased ^{14}C-glyphosate absorption in MDOT from 36 to 44% at 48 HAT compared to glyphosate alone (Figure 4.2). It is unclear why an increase in ^{14}C-glyphosate absorption was observed when combined with saflufenacil. Absorption of glyphosate in GR soybean was reduced when applied in combination with pelargonic acid, which causes rapid desiccation of plant tissues (Pline et al. 1999). Steele et al. (2008) reported that the addition of diuron reduced glyphosate absorption from 75% down to 38% in sharppod morningglory (*Ipomoea cordatotriloba* Dennst). In general, the addition of saflufenacil did not affect ^{14}C-glyphosate absorption in S102 when compared to glyphosate alone. S102 absorbed 17 and 13% more ^{14}C-glyphosate than MDOT at 24 and 72 HAT, respectively. There were no differences in ^{14}C-glyphosate absorption 48 HAT between the two populations.

Saflufenacil-treated leaves from both populations were near complete senescence by 72 HAT; however, MDOT seemed more susceptible to the application of saflufenacil and further absorption was limited due to severe desiccation of treated leaves.

There were differences in ^{14}C-glyphosate translocation by trial, thus data are presented separately. Differences may have been due to plants being slightly smaller in trial 2 compared to trial 1. There were no differences in ^{14}C-glyphosate translocation by HAT for either trial (data not presented). In trial 1, glyphosate alone translocated at least 7% more ^{14}C-glyphosate out of the treated leaf than treatments containing saflufenacil (Table 4.4). Similarly, the addition of COC to glyphosate reduced ^{14}C-glyphosate translocation by at least 3%. There were no differences in overall translocation of ^{14}C-glyphosate by horseweed population within trial 1; however, there were differences in trial 2 in that S102 translocated 2% more ^{14}C-glyphosate out of the treated leaf than did MDOT (Table 4.5). For S102 the addition of saflufenacil reduced translocation of glyphosate from 17% to at least 8%, conversely, there were no differences between MDOT and herbicide treatment. Overall the addition of saflufenacil to glyphosate reduced ^{14}C-glyphosate translocation by at least 6% compared to glyphosate alone across trials. Similarly, the addition of COC to glyphosate alone reduced translocation by 4%. This decrease in translocation was likely due to the deleterious effects of saflufenacil on plant processes limiting ^{14}C-glyphosate movement within the plant. There were no differences in translocation of ^{14}C-glyphosate across saflufenacil treated plants regardless of population or HAT in trial 2 (Table 4.5).

Differences in ^{14}C-glyphosate distribution within the treated leaf were observed between herbicide treatments in that 6% more ^{14}C-glyphosate remained in the treated leaf in saflufenacil-treated plants than in glyphosate alone (Table 4.4). The addition of COC reduced translocation of ^{14}C-glyphosate by 3% compared to glyphosate alone. For trial 2 there were observed differences in 14C-glyphosate distribution within the treated leaf across all factors (Table 4.5). In general, there was no difference in ^{14}C-glyphosate in treated leaf across MDOT and were similar to S102 treated with saflufenacil. Roughly 6% less ^{14}C-glyphosate moved out of S102 when glyphosate was applied with COC and another 4% with addition of saflufenacil + COC. These data highlight the negative effects saflufenacil and COC have on the translocation of ^{14}C-glyphosate in susceptible horseweed. These findings are similar to those of Steele et al. (2008) where diuron reduced translocation of ^{14}C-glyphosate in sharppod morningglory.

Approximately, 6% more ^{14}C-glyphosate moved into the crown of S102 with glyphosate alone compared to MDOT within trial 1 (Table 4.4). The addition of COC reduced the movement of ^{14}C-glyphosate into the crown of S102 by nearly 4% compared to glyphosate alone, whereas the addition of saflufenacil reduced levels by another 7%. There were no differences in the accumulation of ^{14}C-glyphosate within the crown of MDOT plants, regardless of treatment, and were comparable to saflufenacil treated S102. Likewise for trial 2, there were no differences in ^{14}C-glyphosate levels across MDOT for any herbicide treatment (Table 4.5). Roughly 9% more ^{14}C-glyphosate moved into the crown of S102 for glyphosate alone compared to MDOT. Again, the addition of COC to glyphosate reduced translocation of ^{14}C-glyphosate 6% in S102 compared to glyphosate alone. Within S102, the addition of saflufenacil further reduced ^{14}C-glyphosate translocation by an additional 4% compared to glyphosate + COC.

There were differences in translocation of glyphosate among all other leaves by horseweed population in that S102 translocated 1.3% of ^{14}C-glyphosate absorbed into other leaves compared to MDOT at 0.8% in trial 1 (Table 4.4). These findings were nearly identical of those for trial 2 where S102 translocated 0.7% more ^{14}C-glyphosate compared to MDOT (Table 4.5). These findings are similar to those of Feng et al. (2004), where more glyphosate was moved to other leaves within the susceptible than the resistant population.

Distribution of ^{14}C-glyphosate in the roots was not affected by herbicide treatment within either trial; however, there were differences in horseweed population in that MDOT translocated 2% more ^{14}C-glyphosate to the roots than did S102 (Tables 4.4 and 4.5). These data conflict with the findings of Koger and Reddy (2005) where more ^{14}C-glyphosate was translocated to roots in the susceptible horseweed population than the resistant. A possible explanation for these differences may have been a rapid sequestration mechanism, moving glyphosate away from the site-of-action within meristematic tissues of the crown and into vacuoles (Ge et al. 2010).

To remove variability due to plant tissue weights, the radioactivity distribution data were expressed as concentration (ng of ^{14}C-glyphosate per g plant tissue) to normalize the data for plant weights (Tables 4.6 and 4.7). The pattern of ^{14}C-glyphosate concentration in plant parts was nearly identical to the ^{14}C-glyphosate accumulation expressed as percent of absorbed. These findings were similar to those of Koger and Reddy (2005). Overall, radioactivity was distributed throughout MDOT with ^{14}C accumulation decreasing in the order, treated leaf > crown > roots > other leaves. For S102 ^{14}C accumulation decreasing in the order, treated leaf > crown > other leaves > roots. More radioactivity was retained in the treated leaf, crown, and other leaves of S102 compared to MDOT. This differs from the findings of Koger and Reddy (2005) in that the resistant retained more ^{14}C-glyphosate than the susceptible. Possible differences may have been that Koger and Reddy (2005) utilized a single harvest interval of 48 HAT, whereas in our study the movement of ^{14}C over 72 HAT may have shunted more ^{14}C-glyphosate into other plant parts.

Overall, the addition of saflufenacil reduced ^{14}C-glyphosate translocation in horseweed at least 6% across experiments; however, due to the exceptional efficacy of saflufenacil on horseweed it is not believed that this would have no negative effects on horseweed control in the field. Additionally, this research suggests that the addition of COC to glyphosate reduces translocation of glyphosate in horseweed into other plant parts. Nandula et al. (2007b) advised against adding COC to glyphosate spray mixtures due to possible antagonism. Current labeling for saflufenacil recommends the addition of 1% (v/v) COC or methylated seed oil (MSO). Tank mixtures of saflufenacil and glyphosate will likely be utilized by producers to improve control of many broadleaf weed species. Additional research may be needed to determine if saflufenacil will have similar reductions in translocation of glyphosate on other broadleaf species. Saflufenacil holds great potential as an alternative control option for GR horseweed and a valuable tool in the management of resistant weeds.

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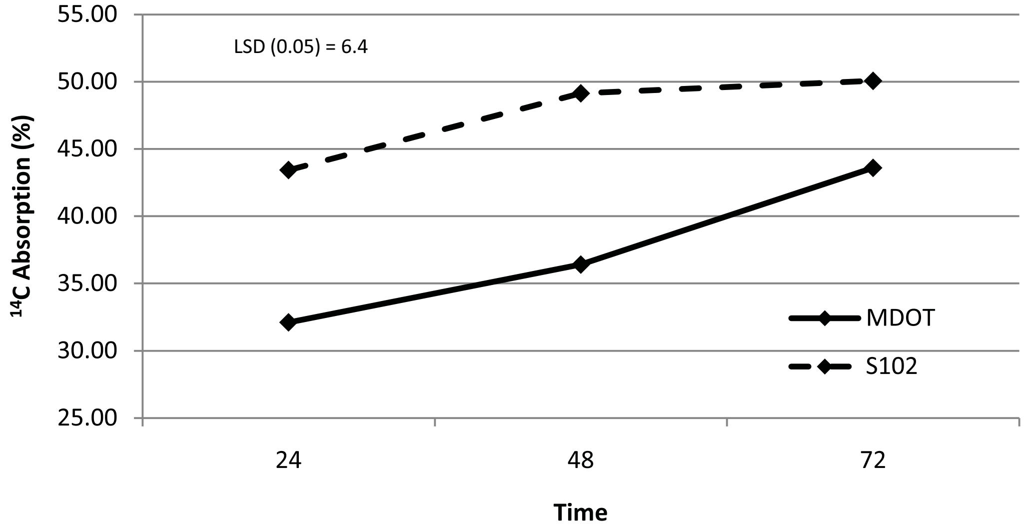

Figure 4.1 Absorption of ^{14}C-glyphosate over time in glyphosate-resistant (MDOT) and glyphosate-susceptible (S102) horseweed treated with 0.4 kg ae/ha glyphosate alone.

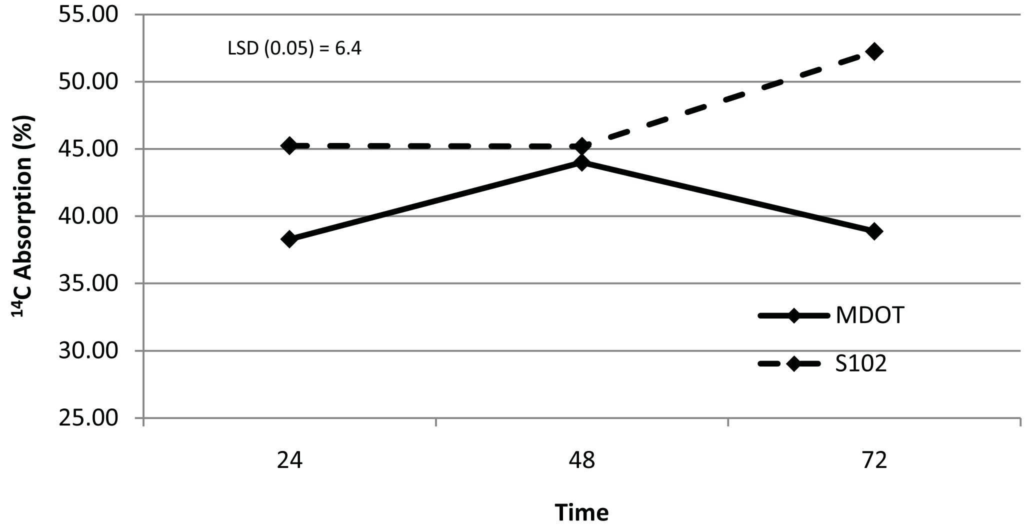

Figure 4.2 Absorption of ^{14}C-glyphosate over time in glyphosate-resistant (MDOT) and glyphosate-susceptible (S102) horseweed treated with 0.4 kg ae/ha glyphosate + 0.0125 kg ai/ha saflufenacil.

Table 4.1 Control of 10- to 15-cm horseweed 14 and 28 DAT with saflufenacil[a,c].

Treatment	Rate v/v	Control 14 DAT	Control 28 DAT
	—%—	—%—	—%—
No Adjuvant[b]		78 c	71 c
NIS	0.25	78 c	72 c
NIS	0.50	79 c	74 c
COC	1.00	85 b	74 c
COC	2.00	86 b	81 b
MSO	1.00	91 a	83 ab
MSO	2.00	93 a	89 a

[a] Abbreviations: DAT, days after treatment; NIS, nonionic surfactant; COC, crop oil concentrate; MSO, methylated seed oil.
[b] All treatments included 0.025 kg/ha saflufenacil.
[c] Means followed by same letter for each evaluation are not significantly different at $p \leq 0.05$.

Table 4.2 Control of 15-cm horseweed with postemergence applications of glyphosate alone and in combination with saflufenacil on glyphosate-resistant and -susceptible horseweed[a]

		S102 Control[a]		MDOT Control[a]	
Treatment[b]	Rate	14 DAT	21 DAT	14 DAT	21 DAT
	kg/ha[c]	%		%	
Glyph[d]	0.42	80	90	40	43
Glyph	0.84	87	96	37	50
Glyph	1.68	90	100	57	60
Safl	0.012	95	100	95	100
Safl	0.025	95	100	93	100
Safl	0.050	95	100	95	100
Glyph + Safl	0.42 + 0.012	93 (99)	100 (100)	93 (97)	100 (100)
Glyph + Safl	0.42 + 0.025	95 (99)	100 (100)	90 (96)	100 (100)
Glyph + Safl	0.42 + 0.050	95 (99)	100 (100)	95 (97)	100 (100)
Glyph + Safl	0.84 + 0.012	95 (99)	100 (100)	95 (97)	100 (100)
Glyph + Safl	0.84 + 0.025	95 (99)	100 (100)	95 (96)	100 (100)
Glyph + Safl	0.84 + 0.050	95 (99)	100 (100)	95 (97)	100 (100)
Glyph + Safl	1.68 + 0.012	92 (100)	100 (100)	95 (98)	100 (100)
Glyph + Safl	1.68 + 0.025	95 (100)	100 (100)	95 (97)	100 (100)
Glyph + Safl	1.68 + 0.050	95 (100)	100 (100)	95 (98)	100 (100)
LSD (0.05)		5	5	7	7

[a] Expected values are calculated as described by Colby (1967) and are enclosed in parenthesis. Interactions were considered significant if differences between observed and expected control exceeded the appropriate LSD value.
[b] Abbreviations: DAT, days after treatment; MDOT, glyphosate-resistant; S102, glyphosate-susceptible; Glyph, glyphosate; Safl, saflufenacil.
[c] Glyphosate herbicide rates are in kg ae/ha while saflufenacil rates are in kg ai/ha. All treatments included 1% v/v COC and 1% w/v AMS.

Table 4.3 [14]C-glyphosate translocation and distribution in glyphosate-resistant and glyphosate-susceptible horseweed as influenced by saflufenacil (Trial 1)

			^{14}C-glyphosate distribution[d]			
Population[a]	Treatment[b]	Trans-location[c]	Treated Leaf	Crown	Other leaves	Roots
		— % of absorbed —				
MDOT	A	10	90	4.8	1.2	4.1
	B	8	92	4.6	0.7	3.3
	C	5	95	3.3	0.4	1.3
	D	5	95	2.7	0.8	2.6
S102	A	13	87	10.3	2.3	0.6
	B	8	92	6.5	1.2	0.3
	C	4	96	2.8	0.8	0.2
	D	5	95	3.6	0.9	0.5
LSD (0.05)		ns	ns	2.1	ns	ns
		— % of absorbed —				
MDOT	---	7	93	3.8	0.8	2.8
S102	---	8	92	5.8	1.3	0.4
LSD (0.05)		ns	ns	1.1	0.4	0.9
		— % of absorbed —				
---	A	12	89	7.5	1.7	2.3
---	B	8	92	5.5	0.9	1.8
---	C	5	95	3.0	0.6	0.8
---	D	5	95	3.1	0.9	1.5
LSD (0.05)		2	2	1.5	0.5	ns

[a] Abbreviations: HAT, hours after treatment; MDOT, glyphosate-resistant; S102, glyphosate-susceptible; COC, crop oil concentrate.
[b] Treatments: A, 0.42 kg ae/ha glyphosate alone; B, 0.42 kg ae/ha glyphosate + 1% v/v COC; C, 0.42 kg ae/ha glyphosate + 0.125 kg ai/ha saflufenacil; D, 0.42 kg ae/ha glyphosate + 0.125 kg ai/ha saflufenacil + 1% v/v COC.
[c] ^{14}C-glyphosate outside of treated leaf (other leaves, crown, and roots) is considered as translocation from trial 1.
[d] ^{14}C-glyphosate distribution throughout the plant is based on percent of ^{14}C-glyphosate absorbed.

Table 4.4 ^{14}C-glyphosate translocation and distribution in glyphosate-resistant and glyphosate-susceptible horseweed as influenced by saflufenacil (Trial 2)

			^{14}C-glyphosate distribution[d]			
Population[a]	Treatment[b]	Trans-location[c]	Treated Leaf	Crown	Other leaves	Roots
		% of absorbed				
MDOT	A	9	91	5.7	1.0	2.7
	B	9	91	5.4	1.3	2.3
	C	9	91	5.2	1.4	2.3
	D	7	93	3.8	0.8	2.5
S102	A	17	83	14.6	2.0	0.6
	B	11	89	8.6	1.7	0.3
	C	8	92	6.0	1.7	0.7
	D	7	93	4.9	1.8	0.8
LSD (0.05)		3	3	2.4	ns	ns
		% of absorbed				
MDOT	---	9	91	5.0	1.1	2.4
S102	---	11	89	8.5	1.8	0.6
LSD (0.05)		2	2	1.2	0.4	0.6
		% of absorbed				
---	A	14	87	10.1	1.5	1.6
---	B	10	90	7.0	1.5	1.3
---	C	8	92	5.6	1.5	1.5
---	D	7	93	4.3	1.3	1.7
LSD (0.05)		2	2	1.7	ns	ns

[a] Abbreviations: HAT, hours after treatment; MDOT, glyphosate-resistant; S102, glyphosate-susceptible; COC, crop oil concentrate.
[b] Treatments: A, 0.42 kg ae/ha glyphosate alone; B, 0.42 kg ae/ha glyphosate + 1% v/v COC; C, 0.42 kg ae/ha glyphosate + 0.125 kg ai/ha saflufenacil; D, 0.42 kg ae/ha glyphosate + 0.125 kg ai/ha saflufenacil + 1% v/v COC.
[c] ^{14}C-glyphosate outside of treated leaf (other leaves, crown, and roots) is considered as translocation from trial 2.
[d] ^{14}C-glyphosate distribution throughout the plant is based on percent of ^{14}C-glyphosate absorbed.

Table 4.5 ^{14}C-glyphosate concentration in treated leaf, other leaves, crown, and roots of glyphosate-resistant and glyphosate-susceptible horseweed (Trial 1)

		Plant portion[c]			
Population[a]	Treatment[b]	Treated leaf	Crown	Other leaves	Roots
		ng ^{14}C-glyphosate/g tissue dry weight			
MDOT	A	655	26	4	20
	B	656	27	2	15
	C	907	25	4	8
	D	747	19	3	5
S102	A	765	66	12	5
	B	989	48	6	4
	C	853	19	4	3
	D	950	27	5	4
LSD (0.05)		116	13	3	5
		ng ^{14}C-glyphosate/g tissue dry weight			
MDOT	---	741	24	3	12
S102	---	889	40	7	4
LSD (0.05)		58	7	2	3
		ng ^{14}C-glyphosate/g tissue dry weight			
---	A	710	46	8	12
---	B	823	37	4	9
---	C	880	22	4	6
---	D	849	23	4	5
LSD (0.05)		82	9	2	4

[a] Abbreviations: MDOT, glyphosate-resistant; S102, glyphosate-susceptible; COC, crop oil concentrate.
[b] Treatments: A, 0.42 kg ae/ha glyphosate alone; B, 0.42 kg ae/ha glyphosate + 1% v/v COC; C, 0.42 kg ae/ha glyphosate + 0.125 kg ai/ha saflufenacil; D, 0.42 kg ae/ha glyphosate + 0.125 kg ai/ha saflufenacil + 1% v/v COC.
[c] ^{14}C-glyphosate distributions throughout plant is based on percent of ^{14}C-glyphosate absorbed from trial 1.

Table 4.6 ^{14}C-glyphosate concentration in treated leaf, other leaves, crown, and roots of glyphosate-resistant and glyphosate-susceptible horseweed (Trial 2)

		Plant portion[c]			
Population[a]	Treatment[b]	Treated leaf	Crown	Other leaves	Roots
		ng ^{14}C-glyphosate/g tissue dry weight			
MDOT	A	717	34	4	14
	B	635	30	5	11
	C	608	29	5	10
	D	682	23	3	10
S102	A	667	92	9	5
	B	808	61	8	3
	C	779	39	7	5
	D	787	31	6	5
LSD (0.05)		ns	15	ns	ns
		ng ^{14}C-glyphosate/g tissue dry weight			
MDOT	---	661	29	4	11
S102	---	760	56	8	4
LSD (0.05)		73	8	1	3
		ng ^{14}C-glyphosate/g tissue dry weight			
---	A	692	63	7	9
---	B	722	46	6	7
---	C	693	34	6	7
---	D	735	27	5	8
LSD (0.05)		ns	11	ns	ns

[a] Abbreviations: MDOT, glyphosate-resistant; S102, glyphosate-susceptible; COC, crop oil concentrate.
[b] Treatments: A, 0.42 kg ae/ha glyphosate alone; B, 0.42 kg ae/ha glyphosate + 1% v/v COC; C, 0.42 kg ae/ha glyphosate + 0.125 kg ai/ha saflufenacil; D, 0.42 kg ae/ha glyphosate + 0.125 kg ai/ha saflufenacil + 1% v/v COC.
[c] ^{14}C-glyphosate distributions throughout plant is based on percent of ^{14}C-glyphosate absorbed from trial 2.