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## **Control of Glyphosate Resistant Horseweed (*Conyza canadensis*) with Saflufenacil and Tank-Mixture Partners.**

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To the Graduate Council:

I am submitting herewith a thesis written by Brock Steven Waggoner entitled "Control of Glyphosate Resistant Horseweed (*Conyza canadensis*) with Saflufenacil and Tank-Mixture Partners.." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

Lawrence E. Steckel, Major Professor

We have read this thesis and recommend its acceptance:

Thomas C. Mueller, Gregory R. Armel, Christopher L. Main

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

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**Control of Glyphosate Resistant Horseweed (*Conyza canadensis*)**

**with Saflufenacil and Tank-Mixture Partners.**

A thesis

Presented for the

Master of Science Degree.

The University of Tennessee,

Knoxville

**Brock Steven Waggoner**

**December 2010**

## Abstract

Field and laboratory studies were conducted to determine the efficacy of saflufenacil alone and with mixture partners for burndown. Field studies were conducted in 2009 and 2010 to evaluate saflufenacil in mixtures with glyphosate, glufosinate, or paraquat for control of glyphosate-resistant (GR) horseweed prior to planting cotton. Saflufenacil and saflufenacil mixtures were applied 7 days before planting (DBP). Saflufenacil at 25 and 50 g ai ha<sup>-1</sup> in mixture with all three non-selective herbicides provided similar GR horseweed control when compared to the current standard of glyphosate plus dicamba. Control of GR horseweed was also not different at the 25 and 50 g ai ha<sup>-1</sup> of saflufenacil across all mixtures from the standard of glyphosate plus dicamba.

Laboratory studies were initiated to determine the uptake and translocation of saflufenacil alone and when mixed with glyphosate and paraquat. It was found that glyphosate plus saflufenacil had a greater absorption of saflufenacil at 2 and 8 HAT. By 24 HAT there were not any differences between the amount of saflufenacil absorbed into GR horseweed between treatments. Translocation data also confirmed that the majority of saflufenacil stayed in the treated leaf at 72 HAT.

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## **Thesis Introduction**

Horseweed is an annual plant that is part of the Asteraceae family and is classified as a winter or summer annual weed (Uva et al. 1997). Horseweed has thrived in reduced or no-tillage systems (Sauer and Struik 1964). Regeher and Bazzaz (1979) reported that horseweed germinated in the spring months and Main et al. (2006) found that it can germinate 10 months out of the year in Tennessee. This long potential window of germination has made horseweed difficult to manage in summer annual crops, particularly in a reduced-till environment (Steckel and Culpepper 2006).

Since horseweed (*Conyza Canadensis*) was first confirmed to be glyphosate resistant (GR) in the state of Delaware (Van Gessel 2001), it has become an increasing problem for no-till cotton producers (Koger et al 2004, Main et al. 2004). Horseweed control prior to the development of glyphosate resistant biotypes consisted of an application of glyphosate prior to or just after planting. Bruce and Kells (1990) reported that 840 g ae ha<sup>-1</sup> of glyphosate applied pre-plant provided 98 to 100% horseweed control. Brown and Whitwell (1988) stated that glyphosate at 1.4 kg ha<sup>-1</sup> provided complete horseweed control.

In 2001, horseweed was reported to be resistant to glyphosate in Tennessee (Main et al. 2004). It can now be found in most row crop counties throughout the mid-south (Heap 2008). Spring tillage has been an option for control of GR horseweed and helps in preparing the seed bed for the crop to be planted (Kapusta 1979). Indeed some Tennessee growers have moved to more tillage since the advent of GR horseweed. From 2003 to 2005, conservation tillage hectares of cotton in Tennessee were reduced 15%. Conversely, conservation tillage hectares of cotton have risen 20% from 2005 to 2009 (USDA 2010). Cotton growers have been able to go

back to no-till by utilizing 2,4-D, dicamba and glufosinate for GR horseweed control prior to planting (Scott et al. 2009, Owen et al. 2009, Steckel et al. 2006). Targeting GR horseweed with these herbicides has provided control similar to tillage but is not always consistent. In field situations where there are dense GR horseweed populations and inadequate soil moisture, control has been inconsistent (Steckel and Culpepper 2006). This inconsistent GR horseweed control is illustrated most recently by research concluding that dicamba and 2,4-D provided inconsistent control of GR horseweed (Steckel et al. 2006). Glufosinate can also provide erratic control of GR horseweed with most researchers concluding that GR horseweed control with glufosinate was temperature dependent, with reduced control at lower temperatures (Anderson et al. 1993; Steckel et al. 2006; Wild et al. 1987). Steckel et al. (2006) found that control 14 days after application (DAA) was better with mixes of glufosinate and 2,4-D, flumioxazin, or dicamba than with glufosinate alone. Also, glufosinate mixed with high rates of dicamba and 2,4-D controlled GR horseweed 30 DAA, suggesting that some residual control was obtained from the dicamba and 2,4-D. Finally, GR horseweed in the southern United States can germinate and emerge 10 months out of the year. Even with successful burndown, subsequent germinations are often a problem if no residual herbicide are used (Main et al. 2006). Therefore new herbicide technologies could improve control of GR horseweed control prior to planting no-till cotton.

Saflufenacil is a new herbicide for pre-plant burndown and/or preemergence (PRE) weed control in corn (*Zea mays* L.), soybean [*Glycine max* (L.) Merr], and cotton (*Gossypium hirsutum*) (Anonymous 2008). Saflufenacil is an inhibitor of protoporphyrinogen oxidase (PPO), and exhibits foliar and residual herbicide activity on broadleaf weed species including horseweed (Anonymous 2008). Injury symptoms from applications to susceptible species normally appear within a few hours, and susceptible plants usually die in 1 to 3 days (Liebl et al. 2008).

Saflufenacil is translocated mainly in the xylem and has limited mobility in the phloem (Liebl et al. 2008). Field research found that rates as low as 25 g ai ha<sup>-1</sup> provided control of horseweed while causing no cotton injury (Owen et al. 2010). This is in contrast to other research that found PPO herbicides like fomesafen applied PRE can injure cotton (Troxler et al. 2002). The current saflufenacil label restricts cotton planting to 42 days after application due to cotton injury concerns (Anonymous 2010). Though saflufenacil has provided good control of GR horseweed it has not controlled other winter annual weed species such as henbit (Anonymous 2010). Growers often want to apply a tank-mixture of herbicides that provide complete weed control to start a cotton crop weed free with one burndown application.

Though saflufenacil has provided good control of GR horseweed it has not provided control of other winter annual weed species (unpublished data). Growers often want to use mixtures of herbicides that provide complete weed control. Glyphosate and paraquat are selected as they are often used in burn-down applications (Steckel et. al. 2010).

Glyphosate is the most widely used herbicide since the advent of glyphosate-tolerant crops (Young 2006). Glyphosate is a weak acid herbicide that has four different pKa values between the pH ranges of 5 to 9 (Sprankle et. al. 1975). Glyphosate is phloem mobile allowing it to move to sensitive meristematic regions while it inhibits the enzyme 5-enol-pyruvylshikimate-3phosphate synthase (EPSPS). As glyphosate use has increased, the number of GR weed species have also increased. A question of this research was how does including glyphosate in mixture with saflufenacil, when applied to GR horseweed, affect the uptake of saflufenacil? Feng et. al. (2004) found that resistance in GR horseweed is due to reduced translocation. A question addressed by this research is, does this resistance mechanism of reduced translocation affect translocation of a tank-mix partner like saflufenacil?

Paraquat is a non-selective herbicide that does not move in the xylem or phloem tissues. Instead Soar et. al. (2003) concluded that paraquat moves in the apoplastic water within the leaf that is in the transpiration stream. Efficacy of paraquat is dependent on being able to get it through the lipid layer of the targeted tissue and into individual cells.

Following application, herbicides are not immediately taken into the plant. Different herbicides take various amounts of time to move through the lipid layers of a plant (Sterling et. al. 2004). This movement is highly dependent on the specific charge of an herbicide and what surfactants are applied in mixture with those herbicides. The amount of time after application that herbicide needs to be absorbed into a weed at a high enough level to control it is defined as a herbicides rainfast period.

## **Chapter 1**

**Control of glyphosate-resistant horseweed (*Conyza canadensis*) with  
saflufenacil tank-mixtures in no-till Cotton (*Gossypium hirsutum*)**

## **Abstract**

Glyphosate-resistant (GR) horseweed management continues to be a challenge in no-till cotton systems in Tennessee and Mississippi. Field studies were conducted in 2009 and 2010 to evaluate saflufenacil in mixtures with glyphosate, glufosinate, or paraquat for control of glyphosate-resistant (GR) horseweed prior to planting cotton. Saflufenacil and saflufenacil mixtures were applied 7 days before planting (DBP). The saflufenacil rates were mixed with the three non-selective herbicides were 0, 6.25, 12.5, 25, and 50 g ai ha<sup>-1</sup>. Dicamba plus glyphosate and flumioxazin plus glyphosate are the most widely used mixtures in Tennessee and Mississippi for control of GR horseweed prior to planting cotton and were included as the grower standards. Saflufenacil at 25 and 50 g ai ha<sup>-1</sup> in mixture with all three non-selective herbicides provided similar GR horseweed control when compared to the current standard of glyphosate plus dicamba. Control of GR horseweed was also not different at the 25 and 50 g ai ha<sup>-1</sup> of saflufenacil across all mixtures from the standard of glyphosate plus dicamba. Moreover, saflufenacil, on silt loam soil evaluated in this study, showed no more cotton injury than glyphosate applied 7 or more days before planting. Saflufenacil at 25 g ai ha<sup>-1</sup> alone provided lower control of GR horseweed than the standard which translated to lower lint yield compared to the glyphosate plus dicamba treatment or saflufenacil with each mixture partner. The 12.5 g ha<sup>-1</sup> rate of saflufenacil mixed with either paraquat or glufosinate provided less GR horseweed control (<85%) than higher rates of saflufenacil(>95%). Across all saflufenacil rates, lint cotton yields were similar among the glyphosate, glufosinate, and paraquat tank-mixtures. Based on GR horseweed control and cotton lint yield, this research suggests that saflufenacil at 25 g ai ha<sup>-1</sup> is the most optimal rate for tank-mixtures with glyphosate, glufosinate or paraquat. It also reaffirms

earlier research that the 25 g ai ha<sup>-1</sup> saflufenacil rate can safely be applied inside of the currently labeled 42 day waiting period between a saflufenacil application and cotton planting.

## **Introduction**

Horseweed is an annual plant that is part of the Asteraceae family and is classified as a winter or summer annual weed (Uva et al. 1997). Horseweed has thrived in reduced or no-tillage systems (Sauer and Struik 1964). Regeher and Bazzaz (1979) reported that horseweed germinated in the spring months and Main et al. (2006) found that it can germinate 10 months out of the year in Tennessee. This long potential window of germination has made horseweed difficult to manage in summer annual crops, particularly in a reduced-till environment (Steckel and Culpepper 2006).

Since horseweed (*Conyza Canadensis*) was first confirmed to be glyphosate resistant (GR) in the state of Delaware (Van Gessel 2001), it has become an increasing problem for no-till cotton producers (Koger et al 2004, Main et al. 2004). Horseweed control prior to the development of glyphosate resistant biotypes consisted of an application of glyphosate prior to or just after planting. Bruce and Kells (1990) reported that 840 g ae ha<sup>-1</sup> of glyphosate applied pre-plant provided 98 to 100% horseweed control. Brown and Whitwell (1988) stated that glyphosate at 1.4 kg ha<sup>-1</sup> provided complete horseweed control.

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hectares of cotton in Tennessee were reduced 15%. Conversely, conservation tillage hectares of cotton have risen 20% from 2005 to 2009 (USDA 2010). Cotton growers have been able to go back to no-till by utilizing 2,4-D, dicamba and glufosinate for GR horseweed control prior to planting (Scott et al. 2009, Owen et al. 2009, Steckel et al. 2006). Targeting GR horseweed with these herbicides has provided control similar to tillage but is not always consistent. In field situations where there are dense GR horseweed populations and inadequate soil moisture, control has been inconsistent (Steckel and Culpepper 2006). This inconsistent GR horseweed control is illustrated most recently by research concluding that dicamba and 2,4-D provided inconsistent control of GR horseweed (Steckel et al. 2006). Glufosinate can also provide erratic control of GR horseweed with most researchers concluding that GR horseweed control with glufosinate was temperature dependent, with reduced control at lower temperatures (Anderson et al. 1993; Steckel et al. 2006; Wild et al. 1987). Steckel et al. (2006) found that control 14 days after application (DAA) was better with mixes of glufosinate and 2,4-D, flumioxazin, or dicamba than with glufosinate alone. Also, glufosinate mixed with high rates of dicamba and 2,4-D controlled GR horseweed 30 DAA, suggesting that some residual control was obtained from the dicamba and 2,4-D. Finally, GR horseweed in the southern United States can germinate and emerge 10 months out of the year. Even with successful burndown, subsequent germinations are often a problem if no residual herbicide are used (Main et al. 2006). Therefore new herbicide technologies could improve control of GR horseweed control prior to planting no-till cotton.

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## **Materials and Methods**

Field experiments were conducted in 2009 and 2010, at the West Tennessee Research and Education Center in Jackson Tennessee and in 2009 at the Delta Research and Extension Center in Stoneville Mississippi. Soil at the Jackson location is a Lexington silt loam (fine-silty, mixed, thermic, Typic Paleudalfs) with organic matter of 1.5% and a pH of 6.6. Plots consisted of two 97 cm spaced rows x 9.1 m long planted using no-tillage practices into cotton stubble from the previous year. Cotton variety Phytogen 375 Widestrike Round-up Ready Flex (WRF) was planted at a rate of 116,000 seeds ha<sup>-1</sup>. Cotton plots were planted using a John Deere vacuum planter into a natural GR horseweed population. Treatments were applied 7 days before planting

(DBP) using a CO<sub>2</sub> pressurized backpack sprayer calibrated to 93 L ha<sup>-1</sup>. Nitrogen in the form of liquid urea ammonium nitrate (UAN) was applied as a side dress application at 90 kg ha<sup>-1</sup> of nitrogen. Mepiquat was applied in two applications at 590 mL ha<sup>-1</sup> per application to manage cotton development. Two applications of glyphosate were applied in season for control of other weeds. All other agronomic practices such as insect control and harvest aides followed current University of Tennessee recommendations.

Soil at the Stoneville location is 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%. Plot size was four 102 cm spaced rows x 12.2 m long planted using no-tillage practices into cotton stubble from the previous year. Cotton variety PhytoGen 375 Widestrike Round-up Ready Flex (WRF) was planted at a rate of 110,000 seeds ha<sup>-1</sup>. Plots were planted into a natural GR horseweed population. Treatments were applied 7 DBP using a tractor-mounted sprayer calibrated to 140 L ha<sup>-1</sup>. Nitrogen in the form of liquid UAN was applied as a side dress application at 135 kg ha<sup>-1</sup> of Nitrogen. Two applications of glyphosate were applied in season as blanket treatments for control of other weeds. All other agronomic practices such as insect control and harvest aides followed current Mississippi State University recommendations.

Three common herbicides that are recommended for burndown in both Tennessee and Mississippi were used in this study for mixing with saflufenacil. The herbicides were glyphosate at 1060 g ai ha<sup>-1</sup>, glufosinate at 450 g ai ha<sup>-1</sup>, and paraquat 702 g ai ha<sup>-1</sup>. Saflufenacil rates included 6.25, 12.5, 25, and 50 g ai ha<sup>-1</sup>. These rates were chosen to represent one-fourth, one-half, one, and two times the proposed labeled rate of 25 g ai ha<sup>-1</sup>. Glyphosate at 1060 g ai ha<sup>-1</sup> plus dicamba at 280 g ai ha<sup>-1</sup> was included as a comparison standard. Also glyphosate at 1060 g ai ha<sup>-1</sup> plus flumioxazin at 71 g ai ha<sup>-1</sup> was included to compare efficacy of another PPO

herbicide. One non-treated treatment was included at each location. The non-treated check did not receive any burndown treatments but received all other agronomic treatments during the growing season specified for each location. Superb® HC (83% petroleum oil plus 17% surfactant emulsifier) surfactant at 0.5% v v<sup>-1</sup> was used at the Jackson location with paraquat plus saflufenacil and saflufenacil alone as neither herbicide formulation contains a surfactant. Agri-Dex® (99% paraffinic oil and polyol fatty acid esters) was included at 0.25 % v v<sup>-1</sup> with paraquat plus saflufenacil and saflufenacil alone at Stoneville. Cotton was mechanically harvested with a spindle picker and cotton seed yield recorded. Treatments each year at each location had samples taken and mixed together to create a composite sample that was used for determining gin turnout, lint yield, and also for classing.

Control of GR horseweed was visually estimated 7 and 30 DAA. Cotton injury was visually estimated 30 DAA. All visual evaluations were made on a scale of 0 to 100% scale (0= no control, 100= complete control). Emerged GR horseweed was counted 20 and 30 DAA within a 1m<sup>2</sup> area in each plot.

Data was analyzed using Proc Mixed in SAS 9.2 (2010). The experimental design was a randomized complete block design with four replications. Each year and location was considered a different environment that was sampled at random (Carmer et al. 1989). Assigning environments as random effects will determine if treatment means are different over a collection of environments. Environments, blocks (nested within environments), and effects associated with these factors were considered random in the model. Herbicide treatments were selected as fixed effects. Fisher's protected LSD was used to detect treatment differences at the P > 0.05 level. In the model, environments did not differ so data were pooled. The data for all parameters measured was normally distributed. Single degree of freedom contrast statements were

constructed in order to compare each mixture partner across saflufenacil rates and each saflufenacil rate over mixture partner.

## **RESULTS AND DISCUSSION**

### **Glyphosate resistant horseweed control**

GR horseweed control was evaluated 7 and 30 DAA and was found to be significant with P value of  $<0.0001$ . Therefore, those data were averaged across three environments and presented in Table 1. The grower standard of dicamba plus glyphosate (Steckel et. al 2010) applied 7 DBP provided 75% control at planting (7 DAA) which was less than all mixture treatments containing saflufenacil. However, by 30 DAA the dicamba plus glyphosate mixture provided excellent horseweed control (99%). The GR horseweed density taken 20 and 30 DAA mirrored these results. This would be in contrast to some Tennessee growers who have reported inconsistent control with dicamba plus glyphosate (Steckel 2006). At 7 and 30DAA, glyphosate and glyphosate plus flumioxazin provided the lowest GR horseweed control ( $< 50\%$ ). Horseweed densities were 32 and 45 plant  $m^2$  at the 30 DAA evaluations in glyphosate and glyphosate plus flumioxazin plots, which supported the visual estimates of those two treatments providing the poorest control.

At 7 DAA, all glyphosate plus saflufenacil treatments controlled GR horseweed  $>90\%$  (Table 1). Likewise, GR horseweed densities with treatments containing saflufenacil were less than glyphosate alone or glyphosate plus flumioxazin 20 DAA. However, by 30 DAA the mixtures of glyphosate plus saflufenacil at 6.25 and 12.5 g ai  $ha^{-1}$  provided 62 and 82% control, respectively, which was 13 to 37% less than control from the higher rates of saflufenacil mixed with glyphosate as well as the dicamba plus glyphosate standard.

Results from the glufosinate plus saflufenacil mixtures were similar to the glyphosate plus saflufenacil mixtures. Control across all rates of saflufenacil mixed with glufosinate were >91% by 7 DAA. A notable difference between the glyphosate and glufosinate based treatments was that glufosinate alone provided 93% GR horseweed control 7 DAA, whereby glyphosate only obtained 27% control of GR horseweed. Another notable difference between the glyphosate and glufosinate based mixtures was at the 30 DAA evaluation, only glufosinate plus saflufenacil at 25 g ai ha<sup>-1</sup> showed differences in visual control or horseweed density at 30 DAA among saflufenacil rates mixed with glufosinate. Moreover, the addition of saflufenacil at a rate of 25 and 50 g ai ha<sup>-1</sup> provided better GR horseweed control than saflufenacil alone although these mixtures were not as good as the dicamba plus glyphosate standard.

Results from the paraquat plus saflufenacil mixtures were similar to the glyphosate plus saflufenacil and glufosinate plus saflufenacil mixtures (Table 1). GR horseweed was controlled > 95% by 7 DAA with the 25 and 50 g ai ha<sup>-1</sup> rates of saflufenacil when mixed with paraquat. A notable difference between the glyphosate and paraquat based treatments, was that paraquat alone provided 84% GR horseweed control at 7 DAA while glyphosate alone was 27%. Horseweed density at 20 DAA did not show any differences between paraquat and glufosinate alone but each had a significantly lower population than glyphosate alone. Another notable result was that the addition of saflufenacil at a rate of 25 and 50 g ai ha<sup>-1</sup> to paraquat provided better horseweed control at 30 DAA than saflufenacil alone although this treatment was not better than the dicamba + glyphosate standard.

Another objective of this research was to determine what the optimum saflufenacil rate is for control of GR horseweed. Single degree of freedom contrasts were conducted to compare the main effect of saflufenacil rate averaged across all mixture partners (Table 4.). The 50 and 25 g

ai ha<sup>-1</sup> rates provided 94 and 93% GR horseweed control, respectively, and were not different across mixture partners (> 0.1664). This would suggest that the 25 g ai ha<sup>-1</sup> rate would be the best choice when factoring in both horseweed efficacy and cost when applying saflufenacil.

### **Visual Cotton Injury and Lint Cotton Yield**

Cotton injury varied from 1 to 15% by 30 DAA in the flumioxazin treatments, though no differences were detected when data was pooled (data not shown). This differs from Owen et. al. (2009) who found that flumioxazin PRE injured cotton 33% and reduced cotton final stand by 64%. No injury was found in saflufenacil treatments. These results are consistent with Owen et al. (2009) who found that saflufenacil at 25 and 50 g ai ha<sup>-1</sup> applied up to 7 DBP did not injure cotton. Final cotton stand was also recorded and showed that treatments containing flumioxazin reduced cotton stand (>20%), whereas all other treatments had no impact on final cotton stand (data not shown).

The effect of herbicide treatments on lint cotton yield was found to be significant >0.0001. Lint cotton yield following the glyphosate plus dicamba standard was 1270 kg ha<sup>-1</sup> (Table 1). This result reaffirms previous research findings that glyphosate plus dicamba is one of the best burndown options for controlling GR horseweed (Owen et. al. 2010; Steckel et. al. 2006) in no-till cotton. The addition of dicamba increased yield over glyphosate alone which yielded 840 kg ha<sup>-1</sup>. There was no yield difference between the glyphosate alone treatment vs. the non-treated check. This is consistent with previous findings of Main et. al. (2004) and Koger et. al. (2004) who both found that glyphosate no longer provided an effective control for managing GR horseweed in Tennessee and Mississippi. The glyphosate plus flumioxazin treatment also yielded lower than the standard and all glyphosate plus saflufenacil tank-mixtures except the lowest rate

of saflufenacil tank-mixed with glyphosate. This agrees with Owen (2009) and Steckel and Gwathmey (2009) that found GR horseweed can be competitive to cotton. However, it would differ from Bruce and Kells (1990) who found that glyphosate provided good control of horseweed.

Tank-mixing saflufenacil with glyphosate at 50, 25, or 12.5 g ai ha<sup>-1</sup> rates produced yields consistent with the standard glyphosate plus dicamba. The 12.5 g ai ha<sup>-1</sup> rate yielded the same as the two higher rates is notable since control at 30 DAA was less than that with the 50 or 25 g ai ha<sup>-1</sup> rate. This would suggest that though some re-growth occurred with the 12.5 g ai ha<sup>-1</sup> rate, GR horseweed was injured enough to not drastically impact yield. The low 6.25 g ai ha<sup>-1</sup> rate of saflufenacil yielded less than the 50 and 25 g ai ha<sup>-1</sup> tank-mixtures and also the standard of glyphosate plus dicamba.

The glufosinate alone treatment had yields that were the same for all the glufosinate plus saflufenacil tank-mixtures and the standard of glyphosate plus dicamba. These results are consistent with research conducted by Steckel et. al. (2006) where glufosinate applied before planting provided good GR horseweed control and cotton yield. As with the glufosinate based treatments, there were no differences in lint cotton yield between paraquat alone and all paraquat plus saflufenacil treatments or the standard. This again is notable since GR horseweed control was less with the low saflufenacil rate in these tank-mixtures.

The saflufenacil alone treatment yielded less than the standard of glyphosate plus dicamba. It also yielded less than all tank-mix treatments that included saflufenacil at 50 g ai ha<sup>-1</sup>. These results are inconsistent with the current saflufenacil label that prohibits using more than 25 g ai ha<sup>-1</sup> applied sooner than 42 days before planting cotton (Anonymous). The reason for the

current directions found on the label is concern over cotton injury (personal communication with BASF research biologists). Results of the current work would suggest that the 25 g ai ha<sup>-1</sup> rate can be applied 7 DBP and still have good crop safety to cotton. These results are consistent with findings of Owen et. al. (2010) who showed that the 25 g ai ha<sup>-1</sup> rate showed good crop safety to cotton when applied 7 DBP.

One objective of this research was to determine if adding a broad-spectrum herbicide in with saflufenacil would have any effect on GR horseweed control. A single degree of freedom contrast was constructed to compare the tank-mix partners across saflufenacil rates 30 DAA. All tank-mix partners improved GR horseweed control (>0.0001) over saflufenacil alone. Moreover, all tank-mixtures increased lint cotton yield over saflufenacil alone (Table 2). In addition, each non-selective herbicide used in this study provided similar control when tested against each other when tank-mixed with saflufenacil. This would suggest that glyphosate, glufosinate, and paraquat can all be effective tank-mix partners with saflufenacil. Cotton growers can then tailor the saflufenacil partner to address other winter annual weeds in their fields without sacrificing GR horseweed control.

Another goal was to determine what the optimum saflufenacil rate was in a tank-mix. A single degree of freedom contrast statement was constructed that compared the main effects of saflufenacil rate averaged across all tank-mixtures (Table 3). This contrast showed that across all 4 rates only the 6.25 and 12.5 g ai ha<sup>-1</sup> rates were different in their control of GR horseweed from the 50 g ai ha<sup>-1</sup>. No differences were observed when comparing the 25 g ai ha<sup>-1</sup> against all other treatments. This data suggests that one of the lower two rates may be the best in a tank-mixture. However, in looking at the control and GR horseweed density data coupled with the fact that the 25 g ai ha<sup>-1</sup> rate yielded as well as the 50 g ai ha<sup>-1</sup> rate the authors would suggest that

the optimal saflufenacil rate is 25 g ai ha<sup>-1</sup> when used in a tank-mixture. This would agree with the current saflufenacil labeled us rate in cotton (Anonymous 2010).

This research clearly showed that glyphosate, glufosinate and paraquat can be good tank-mix partners with saflufenacil for management of GR horseweed prior to planting cotton. Those tank-mix partners can increase the control of GR horseweed compared to saflufenacil alone. It also showed that growers could use lower rates than the label rate of 25 g ai ha<sup>-1</sup> tank-mixed with a non-selective herbicide prior to cotton planting and obtain yield comparable to the 25 g ai ha<sup>-1</sup> rate. However, when looking closely at the reduced horseweed control in this study with the lower than 25 g ai ha<sup>-1</sup> rate, this could be a risky strategy. Moreover, recent experience by the authors walking grower fields where saflufenacil provided inconsistent control in the spring of 2010 would suggest that growers should use the labeled rate. Grower applications are often made in a fashion where coverage is not as thorough as the applications in this research and lower than optimum herbicide rates in this environment often produce poor weed control.

This research also reaffirms that saflufenacil can be a safe herbicide to cotton at rates up to 50 g ai ha<sup>-1</sup>. Currently, the label for saflufenacil states that it can be applied up to 42 days before cotton planting (Anonymous 2010). Our data would suggest that cotton may be safely planted up to 7 DBP. Saflufenacil, at least on the silt loam soil types evaluated in this study which are common soil types for many mid-south cotton hectares, appears to be a good option in cotton for controlling GR horseweed much closer to cotton planting than 42 DBP. This current label has greatly discouraged cotton growers from using saflufenacil before planting. With only having to wait 7 DAA, burndown applications could be more flexible to help cotton growers to better time herbicide applications particularly if earlier burndown applications are unsuccessful.

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Table 1. Glyphosate-resistant (GR) horseweed control 7 and 30 days after application (DAA), GR horseweed density at 20 and 30 DAA, cotton injury 30 DAA, and lint yield taken at harvest.

Data averaged across three environments (TN 2009, 2010; MS 2009).

Herbicide		GR Horseweed					
Treatment	Saflufenacil rate g ai ha <sup>-1</sup>	7 DAA	30 DAA	20 DAA	30 DAA	Injury	Lint Yield
		% Control		Density 1 m <sup>2</sup>		30 DAA	kg ha <sup>-1</sup>
glyphosate + dicamba	---	75	99	28	5	4	1270
glyphosate+ flumioxazin	---	32	37	26	45	5	940
glyphosate	---	27	46	38	32	9	840
glyphosate+ saflufenacil	50	96	95	20	3	5	1280
glyphosate+ saflufenacil	25	97	96	12	2	4	1210
glyphosate+ saflufenacil	12.5	95	82	10	12	5	1190
glyphosate+ saflufenacil	6.25	91	62	7	15	13	1010
glufosinate	---	93	77	17	5	13	1210
glufosinate+ saflufenacil	50	98	84	12	2	15	1290
glufosinate+ saflufenacil	25	96	83	15	5	11	1320
glufosinate+ saflufenacil	12.5	97	87	10	8	1	1170
glufosinate+ saflufenacil	6.25	91	73	13	13	10	1220
paraquat	---	84	70	13	18	12	1190
paraquat+ saflufenacil	50	97	84	12	7	14	1230
paraquat+ saflufenacil	25	95	81	13	8	11	1270
paraquat+ saflufenacil	12.5	86	84	15	7	1	1260
paraquat+ saflufenacil	6.25	81	81	8	8	10	1220
saflufenacil	25	96	65	10	17	15	1040
non-treated check	---	0	0	32	52	1	670
LSD <sub>0.05</sub>	---	7	12	6	6	22	190

Table 2. Single degree of freedom contrasts comparing main effect of tank-mix partner averaged across saflufenacil rate on visual GR horseweed control 30 days after application.

Contrast	mixture Partner			
	Tank-mix partner			
g ai ha <sup>-1</sup> (% control)	glyphosate (78)	glufosinate (86)	paraquat (89)	saflufenacil (59)
glyphosate (78)	---	0.1536	0.0682	<0.0001
glufosinate (86)	---	---	0.6769	<0.0001
paraquat (89)	---	---	---	<0.0001
saflufenacil (59)	---	---	---	---

Table 3. Single degree of freedom contrasts comparing the main effect of saflufenacil rate averaged across tank-mix partners on visual GR horseweed control 30 days after application

Contrast	Saflufenacil rate (g ai ha <sup>-1</sup> )			
Saflufenacil rate				
g ai ha <sup>-1</sup> (% control)	6.25 (81)	12.5 (88)	25 (93)	50 (94)
6.25 (81)	---	0.1664	0.2320	0.0103
12.5 (88)	---	---	0.3747	0.0237
25 (93)	---	---	---	0.1664
50 (94)	---	---	---	---

Table 4. Single degree of freedom contrasts comparing the main effect of tank-mixture partners on lint cotton yield averaged across saflufenacil rates.

Contrast	Cotton lint yield			
	Tank-mix partner			(kg ha <sup>-1</sup> )
Tank-mix partner (kg ha <sup>-1</sup> )	glyphosate (1320)	glufosinate (1390)	paraquat (1390)	saflufenacil (1150)
glyphosate (1320)	---	0.1819	0.1937	0.0065
glufosinate (1390)	---	---	0.9715	<0.0001
paraquat (1390)	---	---	---	<0.0001
saflufenacil (1150)	---	---	---	---

## **Chapter 2**

**Uptake and translocation of saflufenacil with mixture partners glyphosate and paraquat on glyphosate resistant horseweed (*Conyza canadensis*)**

## **Abstract**

Glyphosate resistant (GR) horseweed, has caused producers to change to control of vegetation prior to planting from a glyphosate only herbicide applications. Saflufenacil is a new herbicide for pre-plant burndown and/or preemergence (PRE) weed control in corn (*Zea mays* L.), soybean [*Glycine max* (L.) Merr], and cotton (*Gossypium hirsutum*). Studies were initiated to determine the uptake and translocation of saflufenacil alone and when mixed with glyphosate and paraquat. It was found that glyphosate plus saflufenacil had a greater absorption of saflufenacil at 2 and 8 HAT. By 24 HAT there were not any differences between the amount of saflufenacil absorbed into GR horseweed. Translocation data also confirmed that the majority of saflufenacil stayed in the treated leaf by 72 HAT.

## **Introduction**

Most of the cotton hectares in Tennessee are in some sort of conservation tillage program (USDA 2010). Therefore, the weight of weed control is carried by herbicides. Many of the top yielding cotton varieties are genetically modified with glyphosate tolerant technology. For this reason, many hectares are planted with this technology and receive numerous glyphosate applications (Young 2006). Acceptable weed control from glyphosate application may be obtained when managing glyphosate susceptible (GS) weeds (Culpepper and York 1999). However, now that glyphosate resistant (GR) horseweed (*Conyza canadensis*) (Heap 2008), has become so widespread, producers have struggled to control this weed pest with glyphosate alone.

No-till growers have been utilizing 2,4-D, dicamba, and glufosinate for GR horseweed control prior to planting (Scott et al. 2009, Owen et al. 2009, Steckel et al. 2006). Targeting GR horseweed with these herbicides has provided control on par with tillage but is not always consistent. In field situations where there are dense GR horseweed populations and inadequate soil moisture, control has been inconsistent (Steckel and Culpepper 2006). Several researchers have shown that GR horseweed control with glufosinate was temperature dependent, with less control at lower temperatures (Anderson et al. 1993; Steckel et al. 2006; Wild et al. 1987). Steckel et al. (2006) found that control 14 days after application (DAA) was better with tank mixes of glufosinate and 2,4-D, flumioxazin, or dicamba than with glufosinate alone. Also, glufosinate tank-mixed with high rates of dicamba and 2,4-D controlled GR horseweed 30 DAA. These results suggested that some residual control was obtained from the dicamba and 2,4-D. Finally, GR horseweed in the southern United States can germinate and emerge 10 months out of the year. Even with successful burndown, subsequent germinations are often a problem if no residual herbicide is used (Main et al. 2006). New herbicide technologies could improve control of GR horseweed control prior to planting no-till cotton.

Saflufenacil (N-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)-pyrimidinyl)-benzoyl]-N-isopropyl-N-methylsulfamide) is a new herbicide for pre-plant burndown and/or preemergence (PRE) weed control in corn (*Zea mays* L.), soybean [*Glycine max* (L.) Merr], and cotton (*Gossypium hirsutum*) (Anonymous 2008). Saflufenacil (N-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)-pyrimidinyl)-benzoyl]-N-isopropyl-N-methylsulfamide) is a protoporphyrinogen oxidase (PPO) inhibitor herbicide in the pyrimidinedione chemical family. It has foliar and soil residual activity on selected weed species used for control of winter annual weeds between planting in cotton

(*Gossypium hirsutum*), soybean (*Glycine max*), sorghum (*Sorghum bicolor*), and corn (*Zea mays*) (Anonymous 2010). Saflufenacil is a weak acid with a pKa of 4.41, like many other herbicides (Sterling 1994), which allows it to be absorbed through the hydrophobic lipid structures of plant tissues. Saflufenacil as a weak acid is readily water soluble and allows movement in the xylem with some phloem movement (Liebl et. al 2008).

Though saflufenacil has provided good control of GR horseweed it has not provided control of other winter annual weed species (unpublished data). Growers often want to use mixtures of herbicides that provide complete weed control. Glyphosate and paraquat are selected as they are often used in burn-down applications (Steckel et. al. 2010).

Glyphosate is the most widely used herbicide since the advent of glyphosate-tolerant crops (Young 2006). Glyphosate is a weak acid herbicide that has four different pKa values between the pH ranges of 5 to 9 (Sprankle et. al. 1975). Glyphosate is phloem mobile allowing it to move to sensitive meristematic regions while it inhibits the enzyme 5-enol-pyruvylshikimate-3phosphate synthase (EPSPS). As glyphosate use has increased, the number of GR weed species have also increased. A question of this research was how does including glyphosate in mixture with saflufenacil, when applied to GR horseweed, affect the uptake of saflufenacil? Feng et. al. (2004) found that resistance in GR horseweed is due to reduced translocation. A question addressed by this research is, does this resistance mechanism of reduced translocation affect translocation of a tank-mix partner like saflufenacil?

Paraquat is a non-selective herbicide that does not move in the xylem or phloem tissues. Instead Soar et. al. (2003) concluded that paraquat moves in the apoplastic water within the leaf

that is in the transpiration stream. Efficacy of paraquat is dependent on being able to get it through the lipid layer of the targeted tissue and into individual cells.

Following application, herbicides are not immediately taken into the plant. Different herbicides take various amounts of time to move through the lipid layers of a plant (Sterling et. al. 2004). This movement is highly dependent on the specific charge of an herbicide and what surfactants are applied in mixture with those herbicides. The amount of time after application that herbicide needs to be absorbed into a weed at a high enough level to control it is defined as a herbicides rainfast period.

The objectives of this research were to 1) determine glyphosate and paraquat impact the uptake and translocation of saflufenacil in GR horseweed. 2) Evaluate the rainfastness of saflufenacil when applied alone or in mixture with glyphosate.

## **Materials and Methods**

**Plant Materials:** Laboratory experiments were conducted in 2010 to examine how mixtures of saflufenacil with glyphosate and paraquat impacted foliar uptake and translocation of <sup>14</sup>C- saflufenacil on GR horseweed. Horseweed was removed from a field in April of 2010 that had a known high population of glyphosate resistant biotype. The field was located at the West Tennessee Research and Education Center at Jackson, TN. Plants that were already bolted to 10 to 15 cm in height were selected, as those sized plants best represent what is found in a growers field (Authors Personal Experience). Horseweed was removed with a 10.2 cm diameter core extractor with a depth 10.2 cm. Horseweed plants were placed into 10.2 x 10.2 x 10.2 cm pots,

with each plant/ pot being classified as an individual experimental unit. Peat moss growing medium was used to fill in the remaining space of each pot. Samples were transported the day following transplanting to Knoxville, TN. in an enclosed vehicle. Plants were placed under an outside shade cloth structure that provided 25% shading. Transplanted plants were watered daily and fertilized as needed using Miracle-Gro<sup>1</sup> 24-8-16 mix. Plants were grown for 21 days after transplanting to allow for acclimation of plants prior to treatment. Three days before treating plants they were moved into the laboratory where treatments would be administered to allow time for acclimation in the lab environment. A 16 hour light and 8 hours of darkness photoperiod was initiated in the lab with a constant temperature of 21° C being present in the laboratory. Plants were kept watered by placing them in a basin with water filled to 4 cm and maintained at that depth throughout the laboratory phase of the experiment. Plants were divided by height into 2 runs. Plants 25 to 30 cm tall were selected as run one while plants that were 18 to 25 cm tall were placed in run two. Each run had three replications of plants/ pots that were treated as an individual experimental unit.

**Absorption and Translocation:** A treated leaf was chosen slightly below the whorl and marked with a black marker\* for identification when treating and harvesting. Plants were then moved outside in order to overspray a cold treatment of glyphosate (Touchdown Hi-Tech®)<sup>2</sup> + saflufenacil (Sharpen SG®)<sup>3</sup>, paraquat (Gramoxone Inteon®)<sup>4</sup> + saflufenacil, and saflufenacil alone (Table 5). Cold treatments were applied using a hand held boom calibrated to 140 L ha<sup>-1</sup>. Each solution of paraquat + saflufenacil and saflufenacil alone had Superb® HC (83% petroleum oil + 17% surfactant emulsifier) surfactant at 0.5% v v<sup>-1</sup>. Plants were then moved back into the lab area and were dosed one hour later with <sup>14</sup>C- saflufenacil dissolved in a solution of 0.6mL acetonitrile, 0.6 mL deionized water, and 0.012 mL of Superb® surfactant which had a total of

0.362 kBq  $\mu\text{L}^{-1}$  used for dosing the plants. 6  $\mu\text{L}$  drops, or 2.172 kBq, of  $^{14}\text{C}$ - saflufenacil (specific activity, 4.65 MBq  $\text{mg}^{-1}$ ; radiochemical purity 99.3%) were placed on the previously marked leaf of the horseweed. Samples were collected at 1, 2, 4, 8, 24, 48, and 72 hours after treatment (HAT). Non-absorbed  $^{14}\text{C}$ - saflufenacil was quantified by washing the treated leaf with a 5 mL solution of 90:10 deionized water: acetonitrile. This rinse solution was collected and 10mL of Ecoscint H. Biodegradable Scintillation Solution was added. This mixture was analyzed using a Tri-Carb Liquid Scintillation Analyzer<sup>5</sup>, utilizing liquid scintillation spectrometry (LSS) that was performed for one minute per sample. Immediately after washing of the treated leaf each treated plant was harvested into sections; treated leaf, all plant tissue above the treated leaf, plant tissue below the treated leaf, and the roots. Each section of plant tissue was placed into an individual whirl pack bag and stored at  $-20^{\circ}\text{C}$  until further analysis on plants could be completed. Plant tissue was placed into tin weigh boats and dried using a forced air drier set at  $40^{\circ}\text{C}$  for a minimum of twelve hours. Samples were then homogenized by crushing them together and samples were weighed to insure uniformity when oxidizing. All plant parts were then placed into a Biological Oxidizer OX700-2T<sup>6</sup> and burned for 3 minutes per sample.  $^{14}\text{CO}_2$  was trapped in a scintillation cocktail from R.J. Harvey Instrument Company. Samples were then quantified using the aforementioned LSS procedure.

**Rainfast Study:** Further investigations into how glyphosate affected the uptake of saflufenacil were conducted. Horseweed plants were sourced and treated the same as in the adsorption and translocation study. Plants were divided into plants that had a rain simulation and non-rain simulated treated plants. Plants were further divided into plants that were to be treated with glyphosate plus saflufenacil, saflufenacil alone, and a non-treated check (Table 5) with two replications and three plants per replication. Treatments were applied using a  $\text{CO}_2$  pressurized

backpack sprayer calibrated to 140 L ha<sup>-1</sup> with each replication being treated one day apart from each other. Plants that were selected to have a simulated rain event were allowed to dry for 15 minutes after herbicide treatments. Plants were placed into an Devries Manufacturing Spray Booth<sup>8</sup> calibrated to deliver 1 inch of rain in 5 minutes. Plants were allowed to dry and then were moved back to the shade structure. Ratings were taken 7 DAT and plants were harvested. Plants were dried down and dry weights were taken.

Data was analyzed using Proc Mixed in SAS 9.2 (2010). The experimental design was a randomized complete block design with three replications. Each run was considered a separate environment and was sampled at random (Carmer et al. 1989). Assigning runs as random effects will determine if treatment means are different over a collection of runs. Runs, plants (nested within runs), and effects associated with these factors were considered random in the model. Herbicide treatments were selected as fixed effects. Fisher's protected LSD was used to detect treatment differences at the  $P > 0.05$  level. In the model, runs did not differ, so data were pooled. The data for all parameters measured was normally distributed.

## **Results and Discussion**

**Absorption:** Each hour was treated as a separate rating period and comparisons on each herbicide mixture was conducted. A significant difference in absorption of mixtures into horseweed was not found at 1, 4, 24, 48, and 72 HAT. Differences between herbicide mixtures were found at 2 HAT ( $p > 0.0049$ ) and 8 HAT ( $p > 0.0411$ ). Horseweed at 2 HAT treated with glyphosate plus saflufenacil absorbed 47% of the applied <sup>14</sup>C saflufenacil (Table 6). This is different from saflufenacil alone (21%) and paraquat plus saflufenacil (22%) which both absorbed less <sup>14</sup>C saflufenacil than glyphosate plus saflufenacil. Saflufenacil alone and paraquat

plus saflufenacil were not different from the other. These data suggests that glyphosate helps in absorption of saflufenacil when mixed together.

There were no differences between treatments at 4 HAT though another difference occurs at 8 HAT. Horseweed absorbs  $^{14}\text{C}$  saflufenacil with no significant differences between mixtures of glyphosate plus saflufenacil (60%) and saflufenacil alone (48%). There is also not a difference in absorption between saflufenacil alone and paraquat and saflufenacil (36%) but there is between mixtures containing glyphosate and paraquat. Glyphosate plus saflufenacil absorbed more  $^{14}\text{C}$  saflufenacil than did mixtures containing paraquat. At 24, 48, 72 HAT there was no difference in absorption of  $^{14}\text{C}$  saflufenacil.

**Translocation:** Analysis was conducted measuring the interaction between plant part and herbicide mixture and a p-value of  $> 0.0001$  was found at 72 HAT sampling period. Greater than  $>96\%$  of the  $^{14}\text{C}$  saflufenacil stayed in the tissue of the TL (Table 7). There was not a significant amount found throughout the rest of the plant though. There was no difference ( $p > 0.1205$ ) in how mixture partners affected translocation of  $^{14}\text{C}$  saflufenacil in the horseweed. These data would suggest that in GR horseweed saflufenacil is not translocating within the plant and is staying in the TL tissue (Table 7). These results are in contrast to Ashigh and Hall (2010) who reported that translocation of saflufenacil occurred and that the addition of glyphosate reduced translocation in cabbage, buckwheat, and glyphosate susceptible canola.

**Rainfast Study:** Findings from the leaf wash data on GR horseweed showed that glyphosate mixed with saflufenacil was absorbing saflufenacil into the plant faster than saflufenacil alone (Table 7). To further confirm these findings a small rainfast study was

initiated. Saflufenacil 3 DAT (50%) (data not shown) had a lower level of visual control than did glyphosate plus saflufenacil (58%). Though when comparing treatment by washes as there was no significant differences this interaction had a P-value of  $>0.0835$ . 7 DAT there were no differences between saflufenacil (95%) and saflufenacil plus glyphosate (97%). When comparing the treatments that received the simulated rain event vs. those that did not, there was not a difference in control. These data suggests that within 15 minutes after application that saflufenacil is rainfast and that the addition of glyphosate is not significant in increasing control by 7 DAT.

### **Source of Materials**

<sup>1</sup>Miracle-Gro, Scotts Help Center, 14111 Scottslawn Rd., Marysville, OH 43041

<sup>2</sup>Syngenta Crop Protection, INC., 410 Swing Rd., Greensboro, NC 27409

<sup>3</sup> BASF Corporation, 100 Campus Drive, Florham Park, New Jersey 07932

<sup>4</sup>Syngenta Crop Protection, INC., 410 Swing Rd., Greensboro, NC 27409

<sup>5</sup>AgriSolutions Inc. 31832 Delhi Road, Brighton, IL 62012

<sup>6</sup>PerkinElmer, Inc., 940 Winter St., Waltham, MA 02451

<sup>7</sup>Harvey Biological Oxidizer, 11 Jane Street, Tappan, NY 10983

<sup>8</sup>Devires Manufacturing, 28081 870<sup>th</sup> AVE., Hollandale, MN 56045

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Table 5. Herbicides formulations, formulated products, and application rates.

Herbicide Formulation	Product name	Rates in g ai ha <sup>-1</sup>
glyphosate + saflufenacil	Roundup Weathermax® + Sharpen™	1061 + 25
paraquat + saflufenacil	Gramoxone Inteon® + Sharpen™	702 + 25
saflufenacil	Sharpen™	25

Table 6. Percent <sup>14</sup>C saflufenacil recovered from leaf washes

treatment	% recovered <sup>14</sup> C saflufenacil from leaf washes by hour						
	1 hour	2 hours	4 hours	8 hours	24 hours	48 hours	72 hours
glyphosate plus saflufenacil	70	53	64	36	22	24	37
saflufenacil	83	79	62	48	35	27	31
paraquat plus saflufenacil	73	78	51	60	30	35	26
LSD <sub>0.05</sub>	N/S	10	N/S	12	N/S	N/S	N/S

Table 7. Percent <sup>14</sup>C saflufenacil recovered by each treatment at 72 HAT by plant section. p > 0.1205 and all treatments pooled by plant section p > 0.0001

treatments	Means of % recovered <sup>14</sup> C saflufenacil by plant part			
	TL	ATL	BTL	R
saflufenacil	97	1	1	1
glyphosate plus saflufenacil	93	4	1	2
paraquat plus saflufenacil	97	1	1	1
LSD <sub>0.05</sub>	N/S	N/S	N/S	N/S
all three treatments	96	2	1	1
LSD <sub>0.05</sub>	2	2	2	2

## **Thesis Conclusion**

Using saflufenacil at 6.25, 12.5, 25, and 50 g ai ha<sup>-1</sup> in mixture with glyphosate, glufosinate, gramoxone is safe to use 7 DBP in cotton. Glyphosate plus saflufenacil at 25 and 50 g ai ha<sup>-1</sup> provides comparable control to glyphosate plus dicamba.

Glyphosate when mixed with saflufenacil provides an increase in uptake of saflufenacil at 4 and 8 HAT but by 24 HAT there are no differences between any of the mixtures used. Saflufenacil does not translocate in GR horseweed and stays in the TL tissue.

## **APPENDICES**

**APENDIX A**  
**SAS Codes**

## Chapter One SAS Codes

```
data one;
```

```
input trt block loc year locyr rateone earlyct in ratetwo ratethree count;
```

```
datalines;
```

```
proc mixed ;
```

```
class locyr block trt;
```

```
model earlyct= TRT/ddfm=satterth;;
```

```
random locyr Block(locyr) TRT*BLOCK(locyr);
```

```
lsmeans TRT/pdiff;
```

```
run;
```

```
proc mixed ;
```

```
class locyr block trt;
```

```
model count= TRT/ddfm=satterth;;
```

```
random locyr Block(locyr) TRT*BLOCK(locyr);
```

```
lsmeans TRT/pdiff;
```

```
run;
```

```
proc mixed ;
```

```
class locyr block trt;
```

```
model in= TRT/ddfm=satterth;;  
  
random locyr Block(locyr) TRT*BLOCK(locyr);  
  
lsmeans TRT/pdiff;  
  
run;
```

```
proc mixed ;
```

```
class locyr block trt;  
  
model rateone= TRT/ddfm=satterth;;  
  
random locyr Block(locyr) TRT*BLOCK(locyr);  
  
lsmeans TRT/pdiff;  
  
run;
```

```
proc mixed ;
```

```
class locyr block trt;  
  
model ratethree= TRT/ddfm=satterth;;  
  
random locyr Block(locyr) TRT*BLOCK(locyr);  
  
lsmeans TRT/pdiff;  
  
run;
```

```
proc mixed ;
```

```
class block ratethree partner locyear;
```

```
model ratethree= partner/ddfm=satterth;;  
  
random locyear partner*BLOCK(locyear);  
  
lsmeans partner/pdiff;  
  
contrast "partner" partner 0 1 -1;  
  
run;
```

```
proc print;
```

```
run;
```

```
proc mixed ;
```

```
class block ratethree safrate partner locyear;
```

```
model ratethree= safrate/ddfm=satterth;;
```

```
random locyear safrate*BLOCK(locyear);
```

```
lsmeans safrate/pdiff;
```

```
contrast "safrate" safrate 0 1 -1;
```

```
run;
```

## Chapter 2 SAS Codes

```
data one;

input trt      hr      run      plt      per;

if hr=2 then delete;

if hr=4 then delete;

if hr=8 then delete;

if hr=24 then delete;

if hr=48 then delete;

if hr=72 then delete;

datalines;
```

```
data one;

input trt      hr      run      plt      per;

if hr=1 then delete;

if hr=4 then delete;

if hr=8 then delete;

if hr=24 then delete;

if hr=48 then delete;

if hr=72 then delete;

datalines;
```

```
data one;

input trt      hr      run      plt      per;
```

```
if hr=1 then delete;
if hr=2 then delete;
if hr=8 then delete;
if hr=24 then delete;
if hr=48 then delete;
if hr=72 then delete;
datalines;
```

```
data one;
input trt      hr      run      plt      per;
if hr=1 then delete;
if hr=2 then delete;
if hr=4 then delete;
if hr=24 then delete;
if hr=48 then delete;
if hr=72 then delete;
datalines;
```

```
data one;
input trt      hr      run      plt      per;
if hr=1 then delete;
if hr=2 then delete;
if hr=4 then delete;
```

```
if hr=8 then delete;  
if hr=48 then delete;  
if hr=72 then delete;  
datalines;
```

```
data one;
```

```
input trt      hr      run      plt      per;
```

```
if hr=1 then delete;  
if hr=2 then delete;  
if hr=4 then delete;  
if hr=8 then delete;  
if hr=24 then delete;  
if hr=72 then delete;  
datalines;
```

```
data one;
```

```
input herb$ run hour rep part$ per;
```

```
if hour = 1 then delete;  
if hour = 2 then delete;  
if hour = 4 then delete;  
if hour = 8 then delete;  
if hour = 24 then delete;
```

```
if hour = 48 then delete;
```

```
datalines;
```

```
proc mixed ;
```

```
class trt hr run plt    per;
```

```
model per= trt/ddfm=satterth;;
```

```
random plt run(plt) trt*run(plt);
```

```
lsmeans trt/pdiff;
```

```
run;
```

```
data one;
```

```
input herb$ run hour rep part$ per;
```

```
if hour = 1 then delete;
```

```
if hour = 2 then delete;
```

```
if hour = 4 then delete;
```

```
if hour = 8 then delete;
```

```
if hour = 24 then delete;
```

```
if hour = 48 then delete;
```

```
datalines;
```

```
proc mixed;
```

```
class herb run rep part per;
```

```
model per= part herb part*herb/ddfm=satterth;;
```

```
random run rep(run)
herb*rep(run)
part*rep(run)
part*herb*rep(run);
lsmeans part|herb/pdiff;
run;
```

```
data one;
input wash$ trt$ day run rep con;
If day= 7 then delete;
if day= 1 then delete;
datalines;
```

```
data one;
input wash$ trt$ day run rep con;
If day= 3 then delete;
if day= 1 then delete;
datalines;
```

```
proc mixed;
class wash trt run rep con;
model con= wash trt wash*trt/ddfm=satterth;;
```

```
random run rep(run)
```

```
trt*rep(run)
```

```
wash*rep(run)
```

```
trt*wash*rep(run);
```

```
lsmeans wash|trt/pdiff;
```

```
run;
```

## VITA

Brock Steven Waggoner

On 21 May 1984 I was born and subsequently raised on a farm in Southern Illinois. I graduated from Salem Community High School in Salem, IL. During high school I was active in The Boy Scouts of America and earned the rank of Eagle. Continuing after my education after high school I enrolled at Rend Lake Community College where I focused my studies on diesel mechanics while earning an associate's degree. After this time period I took two years to serve a mission for The Church of Jesus Christ of Latter Day Saints. After two years I returned to school at Southern Illinois University at Carbondale and earned a Bachelor's of Science in Plant and Soil Science in December 2008. While attending SIU-C I worked for Dr. Bryan Young, leading weed scientist in the U.S., who introduced me to the idea of graduate level studies. Because of Dr. Young's influence I pursued further study to earn a Master's of Science Degree in Plant Science from the University of Tennessee under the direction of Larry Steckel, another leading weed scientist in the U.S. Upon completion a master's degree in December 2010 I will further my studies at the University of Missouri as a Ph.D. student.