MISSISSIPPI SOYBEAN PROMOTION BOARD
PROJECT NO. 44-2014 (YEAR 3)
FINAL REPORT

TITLE: APPLICATION TIME OF DAY (TOD) EFFECT ON GLUFOSINATE WHEN TANK-MIXED WITH CLETHODIM ON BARNYARDGRASS EFFICACY

PI: DAN REYNOLDS

EXECUTIVE SUMMARY

Recent trends of increasing farm size and reliance on non-selective POST herbicides for weed control have forced pesticide applicators to make POST applications during a wider interval of each day, including nighttime hours. However, glufosinate has been reported to be sensitive to application time of day (TOD).

Field studies were conducted to evaluate the effect of clethodim (Select) and glufosinate (Liberty) tank mixtures applied at differing TOD's on barnyardgrass control.

Clethodim at 76 g ai ha^{-1}, pooled over glufosinate rates, provided 91 to 97% barnyardgrass control regardless of spraying TOD. At 21 and 28 DAT, glufosinate controlled barnyardgrass 88 to 89% compared to 95% control by clethodim applied alone. By 56 DAT, clethodim was more efficacious than glufosinate and glufosinate plus clethodim.

Barnyardgrass control as affected by glufosinate rate and application TOD was significant at all ratings. Barnyardgrass control with glufosinate at 594 g ai ha^{-1} differed significantly at the differing times of application.

Glufosinate applications at midnight showed reduced barnyardgrass control compared to applications made at noon and 6 P.M. Early morning applications at 6 A.M. also showed reduced barnyardgrass efficacy compared to applications at 6 P.M. Environmental factors such as temperature and light conditions at the time of application are likely responsible for the time of day effects observed in this study. Although relative humidity (RH) and presence of dew may have had an effect on glufosinate efficacy, they may have been overshadowed by application TOD.

These data indicate that tank mixtures of glufosinate and clethodim should be applied at noon or early evening to avoid potential TOD effects that reduce barnyardgrass efficacy over the long term following application.

Introduction

The widespread infestation of glyphosate-resistant (GR) Palmer amaranth (*Amaranthus palmeri* S. Wats.) in soybean (*Glycine max* Merr.) has been a major contributor to the greater adoption of LibertyLink® (LL) soybean in the Midsouthern United States (Riar et al. 2013a; Riar et al.

2013b). The LL system utilizes the genetically-modified (GM) crop resistance to the herbicide glufosinate (Liberty). Glufosinate is a non-selective, non-residual POST herbicide used in GM crops including canola (*Brassica napus* L.), corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.) and soybean (Anonymous 2011 and Senesman 2007). Phosphinothricin [homoalanin-4-yl-(methyl)phosphicic acid], the active portion of the glufosinate molecule, inhibits glutamine synthetase, the enzyme that converts ammonia and glutamate to glutamine (Coetzer and Al-Khatib 2001; Devine et al. 1993; Hess 2000). The accumulation of ammonia and stomatal closure in the plant directly inhibits photosystem I and II reactions by reducing the pH gradient across cell membranes which uncouples photophosphorylation, leading to cell destruction and eventually plant death (Coetzer and Al-Khatib 2001;Senesman 2007).

Glufosinate has the ability to control weeds that are difficult to control with glyphosate, and include *Ipomoea* spp. and hemp sesbania [*Sesbania herbacea* (P. Mill.) McVaugh], as well as GR weeds such as horseweed [*Conyza canadensis* (L.) Cronq.], and *Amaranthus* spp (Culpepper et al. 2000; Green and Owen 2011; Whitaker et al. 2011). However, compared to glyphosate, glufosinate applied alone may provide less than adequate summer annual grass control; thus, producers may choose to tank-mix glufosinate with other herbicides to broaden the weed control spectrum and reduce application cost by combining applications (Beyers et al. 2002; Corkern et al.1998; Culpepper et al. 2000; Green 1989; Kim et al. 2005; Vidrine 1989; Vidrine et al. 1995; Zhang et al. 1995). In order to improve grass weed control performance in the LL cropping system, producers can combine applications of glufosinate and clethodim.

Clethodim, a graminicide herbicide, is utilized for POST annual and perennial grass control without causing injury to dicotyledonous weeds and crops (Anonymous 2010; Devine et al. 1993; Senesman 2007). The primary mode of action of clethodim is the inhibition of the de novo fatty acid biosynthesis at the acetyl-CoA (ACCase) enzyme, located in the stroma of plastids (Anderson 1996; Devine et al. 1993; Senseman 2007). Plant growth ceases soon after application, initially affecting the actively growing tissues, followed by destruction of the intercalary meristem within in 1 to 3 wks after application (Anderson 1996; Senseman 2007). Older leaves often turn orange, purple or red before turning necrotic (Anderson 1996; Senseman 2007).

Recent trends in increasing farm size and reliance on non-selective POST herbicides for weed control have forced pesticide applicators to make POST applications over a wider application interval (Anonymous 2014; Sellers et al. 2004). Additionally, wind velocity is oftentimes lower near sunrise and sunset, thus reducing the potential for herbicide drift to non-target plants. The reduced efficacy of herbicides when applied near or after sunset is referred to as the time-of-day (TOD) effect (Sellers et al. 2004). Several POST herbicides, including glufosinate, have been reported to be sensitive to application time of day (Anderson et al. 1993a; Anderson et al. 1993b; Martinson, et al. 2005; Sellers et al. 2003; Sellers et al. 2004; Stewart et al. 2009; Stopps et al. 2013; Waltz et al. 2004).

Several environmental factors contribute to the influence of herbicide efficacy throughout the day. For instance, POST herbicide applications may be impacted by high wind speed, increasing the potential of spray drift onto non-target crops (Duke 2005). Therefore, producers will apply herbicides in the morning or late evening hours when wind speeds are lowest (Waltz et al. 2004).

However, herbicide efficacy may be reduced at these times of day. The presence of dew on leaf surfaces at these times may interfere with herbicide absorption and may increase herbicide loss through runoff (Anderson 1996; Fausey and Renner 2001; Kogan and Zungia 2001; Waltz et al. 2004). To avoid the interaction with dew presence, POST herbicide applications can be applied during the day after the dew has evaporated from the leaf surface.

Increased relative humidity (RH) and air temperature during this time frame may also improve POST herbicide efficacy (Anderson 1996; Anderson et al. 1993a; Coetzer et al. 2001; Fausey and Renner 2001; Friesen and Wall 1991; Kumaratilake and Preston 2005; Martinson et al. 2005; Peterson and Hurle 2001; Stopps et al. 2013; Waltz et al. 2004). Control of giant foxtail was less than 50% when fluazifop-P plus bromoxynil were applied in temperatures less than 25°C regardless of application time of day (Friesen and Wall 1991). Uptake and translocation of radiolabeled glyphosate was significantly greater in Florida baggarweed [*Desmodium tortuosum* (Sw.) DC.] at 95% RH than at 45 and 70% RH (Sharma and Singh 2001). Differential herbicide response due to diurnal leaf movement can also contribute to the time of day effect. However, diurnal leaf movement is not known to exist in grass species; therefore, reduced grass efficacy following a herbicide application due to diurnal fluctuations can be eliminated as a contributing factor to the TOD effect.

Previous research has shown that glufosinate is sensitive to many factors that can contribute to the TOD effect. Glufosinate applications made at 95% RH provided greater control of giant foxtail compared to applications made at 40% RH (Anderson et al. 1993a). Kumaratilake and Preston (2005) reported that absorption of glufosinate was not affected by temperature; however, translocation of glufosinate to the meristematic regions of wild radish (*Raphanus raphanistrum* L.) increased in warm temperatures compared to translocation of glufosinate in cold temperatures. For a herbicide like glufosinate that has very minimal translocation, it can be expected that increased translocation to meristematic regions will result in increased efficacy. Anderson et al. (1993b) reported greater ammonia accumulation and visual injury of giant foxtail when glufosinate was applied at the end of the photoperiod, rather than at the beginning. Sellers et al. (2004) reported that radiolabeled glufosinate applied at 10 P.M. had greater translocation than when applied at 2 P.M.; however, less glutamine synthetase inhibition and ammonia accumulation occurred at the 10 P.M. application. The difference in glutamine synthetase inhibition and ammonia accumulation between the application times may explain the differences in the TOD effect with glufosinate.

Barnyardgrass is one of the most troublesome grasses in soybean production systems in the Midsouthern United States. As one of the top 10 most common and troublesome weeds in the southern U. S., it can produce up to 39,000 seed per plant (Bagavathiannan et al. 2012; Webster 2013). Barnyardgrass exhibits prolific seed dormancy, prolonged emergence period, and ability to grow rapidly and flower in a range of photoperiod and environmental conditions (Bagavathiannan et al. 2011; Maun and Barrett 1986; Mitch 1990; Potvin 1986). Barnyardgrass densities of 0 to 500 per m of row reduced soybean yield 0 to 78%, with an average yield reduction of 0.25% for each barnyardgrass plant per m of soybean row (Vail and Oliver 1993).

Information pertaining to TOD effects on barnyardgrass control when clethodim is applied with glufosinate may be valuable to producers who want to optimize barnyardgrass control in LL soybeans. Therefore, it is important to better define times during the day when environmental and physiological conditions exist to achieve optimum barnyardgrass control and prevent resupplying the soil seedbank. The objective of this research was to determine the optimal TOD to apply tank mixtures of glufosinate with clethodim to maximize barnyardgrass control.

Materials and Methods

Field studies were conducted in 2013 and 2014 to evaluate the effect of clethodim and glufosinate tank-mixtures applied at differing TOD's on barnyardgrass control. Experiments were conducted at the Black Belt Branch Research Station in Brooksville, MS on an Okolona silty clay (Fine, smectitic, thermic Oxyaquic Hapluderts) with 8% sand, 51% silt, 41% clay, 2% organic matter, and pH of 6.8. Experiments were conducted in a field that was naturally infested with populations of predominantly barnyardgrass. The experiments in both 2013 and 2014 were conducted in a fallowed field with an average barnyardgrass density of 1,205 plants m^{-2}.

Herbicide treatments consisted of clethodim (Select Max ®, Valent USA. Co., PO Box 8025, Walnut Creek, CA 94596) at 0 or 76 g ai ha^{-1} applied separately or tank-mixed with glufosinate (Liberty® 280 SL, 280 g ai l^{-1}, Bayer CropScience LP, PO Box 12014, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709) at 0 or 594 g ai ha^{-1}. Herbicide treatments were applied at 12 AM (midnight) and 6 AM and 12 PM (noon) and 6 PM. (Table 4.1). Crop oil concentrate (Agri-dex**®**, Helena Chemical Company, 225 Schilling Blvd., Suite 300, Collierville, TN 38017) at 1.0% v v^{-1} was included with all clethodim treatments. No adjuvant was included when glufosinate was applied alone.

Herbicide treatments were applied with a CO_2-pressurized backpack sprayer calibrated to deliver 140 l ha^{-1} spray volume and treatments were applied when barnyardgrass plants had fully expanded three to four true leaves. Visual estimates of barnyardgrass control were recorded at 7, 14, 21, 28, and 56 days after treatment (DAT) using a scale of 0 to 100%, where 0 = no control and 100 = complete control. Chlorosis, height reductions, and regrowth were considered when making visual estimations. During the 2014 growing season, significant rainfall events provided excellent growing conditions for barnyardgrass, and due to the lack of residual activity of clethodim and glufosinate, barnyardgrass control 56 DAT was not evaluated.

The experimental design was a randomized complete block with a factorial arrangement of treatments. Factor A was clethodim rate, Factor B was glufosinate rate, and Factor C was application TOD. Four replications for each treatment were used in each experiment. Data were pooled across years because experimental replication was considered a random variable. Untransformed and square root transformed data were subjected to analysis of variance, but interpretations were similar to untransformed data. Therefore, untransformed data were used for analysis. Data were subjected to ANOVA and means were separated using Fischer's protected LSD test at P = 0.05.

Results and Discussion

Pooled over glufosinate rate, the interaction of clethodim rate by TOD was not significant (data not shown). Clethodim at 0 g ai ha^{-1} provided 42 to 47% barnyardgrass control compared to 91 to 97% control from clethodim at 76 g ai ha^{-1} regardless of TOD when pooled over glufosinate rates. Similar to these results, fluazifop-P controlled wild oat (*Avena fatua* L.) 92 to 100% regardless of the application TOD (Friesen and Wall 1991). Conversely, green foxtail control with fluazifop-P was greatest when applied between 3 and 11 PM (Friesen and Wall 1991). Stopps et al. (2013) reported fomasafen plus quizalofop-P control of common ragweed (*Ambosia artemisiifolia* L.), common lambsquarters (*Chenopodium albmum* L.), pigweed and velvetleaf was not affected by application TOD.

The interaction of glufosinate rate by clethodim rate was significant when pooled over application TOD at all data evaluations. Barnyardgrass control at 7 DAT was greatest when glufosinate was applied with clethodim (Table 4.2). Glufosinate plus clethodim controlled barnyardgrass 96%, compared to 89% and 94% control by clethodim and glufosinate applied alone, respectively. However, at 14 DAT, clethodim applied alone and tank-mixed with glufosinate provided similar control at 96%. At 21 and 28 DAT, glufosinate applied alone controlled barnyardgrass 88 to 89% compared to 95% control by clethodim applied alone. By 56 DAT, clethodim was slightly more efficacious (97%) than glufosinate (90%) and glufosinate plus clethodim (92%). These results are consistent with previous research where clethodim controlled annual grasses more effectively than clethodim plus glufosinate (Burke et al. 2005; Gardner et al. 2006). Burke et al. (2005) reported clethodim provided greater goosegrass control than glufosinate regardless of growth stage at the time of application.

When pooled across clethodim rates, barnyardgrass control as affected by the interaction of glufosinate rate and application TOD was significant at all rating times (Table 4.3). Averaged over clethodim, barnyardgrass control did not differ with application TOD with glufosinate applied at 0 g ai ha^{-1}, with control ranging from 44 to 49%, indicating that clethodim efficacy was unaffected by TOD (Table 4.3). However, barnyardgrass control with glufosinate at 594 g ai ha^{-1} varied with the times of application. Throughout the entire experiment, glufosinate at 594 g ai ha^{-1} applied at 12 midnight had slightly but significantly less barnyardgrass control compared to glufosinate at 594 g ai ha^{-1} applied at 12 noon and 6 PM. At 14 DAT, glufosinate at 594 g ai ha^{-1} applied at 6 AM and at 12 noon and 6 PM had 95% barnyardgrass control. At 28 DAT, glufosinate applied at 6 AM had a reduction in barnyardgrass control and was not different from that of the 12 AM application time (Table 4.3). Glufosinate applied at 6 PM had better barnyardgrass control than the 6 AM and 12 AM applications.

Glufosinate has been reported to be effected by RH, ambient air temperature, and light conditions at the time of application (Anderson et al. 1993a; Anderson et al. 1993b; Kumaratilake and Preston 2005; Sellers et al. 2004). High RH may help increase foliar absorption of herbicides by delaying the evaporation of spray droplets and reducing water-deficit stress in plants (Anderson 1996). Anderson et al. (1993a) reported that green foxtail and barley had greater tolerance to glufosinate when the RH was at 40% compared to 95%. Similarly, Coetzer et al. (2001) reported at 90% RH, redroot pigweed (*Amarantus retroflexus* L.), Palmer amaranth, and common waterhemp (*Amaranthus rudis* Sauer) control ranged from 81 to 90%. However, at 35% RH, control of Palmer amaranth, common waterhemp, and redroot pigweed decreased, ranging from 70 to 72% (Coetzer et al. 2001). Martinson et al. (2005) reported that RH ranging from 41% to 96% was not a significant factor in relation to glufosinate efficacy. In our experiments, relative humidity varied but was consistently greater at the 12 AM application, ranging from 86 to 94%. However, barnyardgrass control was reduced at the 12 A.M application compared to the 12 noon and 6 P.M. applications when RH was lowest.

Although results from previous research indicate that RH may play a role in the TOD effect, our data are indifferent for the effect of RH on barnyardgrass control. However, the effect of RH may be overshadowed by the time of application effect.

Ambient air temperature may alter foliar herbicide absorption by altering the nature of the cuticle by either improving or hindering its permeability to herbicides (Anderson 1996). Kumaratilake and Preston (2005) reported that glufosinate was rapidly absorbed into leaves of wild radish regardless of the temperature at which the plants were grown. Likewise, Coetzer et al. (2001) reported that glufosinate absorption was not altered by temperature when averaged over amaranth species. However, herbicide translocation in the phloem tends to increase at higher temperatures due to increased metabolic processes and enzyme activities (Anderson 1996). Glufosinate has very little translocation following application due to rapid phytotoxicity (Coetzer et al. 2001; Devine et al 1993). Kumaratilake and Preston (2005) reported increased glufosinate translocation to the shoot meristem and the untreated leaves at warm temperatures, whereas at cold temperatures the majority of glufosinate was translocated to tip of the treated leaf. When plants were moved from a cold temperature to a warm temperature after glufosinate was applied, glufosinate efficacy increased compared to plants kept under cold conditions throughout the entire study (Kumaratilake and Preston 2005). Anderson et al. (1993a) reported greater green foxtail injury when glufosinatee was applied in warmer temperature compared to glufosinate applications in cooler temperatures. However, cooler temperatures didn’t remove the phytotoxic injury, but merely reduced the rate of injury development (Anderson et al. 1993a). In our experiments, the highest temperatures were recorded at the 12 and 6 PM compared to the 12 and 6 AM applications at 28 DAT. Our data suggest that ambient air temperature at the time of glufosinate application may also play a role in the TOD effect on barnyardgrass efficacy.

Glufosinate inhibits glutamine synthetase, an enzyme that combines ammonium with glutamate to form glutamine, and requires ATP, a product of the light reactions of photosynthesis (Devine et al. 1993; Hess 2000; Tiaz and Zeiger 2006). Glutamine synthetase activity has been found to be altered by light and carbohydrate levels (Tiaz and Zeiger 2006). Glutamine synthetase is catalytically active in the chloroplast of plant cells during periods of light, and is a sink for glufosinate (Sellers et al. 2004; Tiaz and Zeiger 2006). Sellers et al. (2004) reported when glufosinate was applied at 2 PM, glutamine synthetase inhibition was irreversible and was not reversed by the onset of a dark period. However, glufosinate applied at 10 PM merely minimized glutamine synthetase activity, and upon illumination, glutamine synthetase activity was greater when compared to glufosinate applied at 2 PM (Sellers et al. 2004). Sellers et al. (2004) also reported that glufosinate was translocated 2 times greater when applied at 10 PM compared to the 2 PM application. Yet, in dark period, glutamine synthetase is no longer a sink for glufosinate and it is thought that glufosinate is sequestered into the vacuole, making glufosinate molecules unavailable for glutamine synthetase inhibition (Sellers et al. 2004). These data may help explain why the 12 AM application of glufosinate had reduced efficacy on barnyardgrass compared to the 12 and 6 PM applications.

The University of Georgia (2014) reported 13%, 56%, 88%, and 98% control of Palmer amaranth when glufosinate was applied at sunrise, and at 0.5 hr, 1 hr, and 2 hr after sunrise. Applications of glufosinate applied 2 hr after sunrise were not different from applications made at 4 to 6 hr after sunrise (UGA 2014). These data may also explain why the 6 AM application had slightly reduced control compared to the 12 and 6 PM application timings, yet was slightly greater than the 12 AM application. In contrast to our results, Stewart et al. (2009) reported that barnyardgrass efficacy was not affected by glufosinate at 400 g ai ha^{-1} applied at differing TOD's. However, Stewart et al. (2009) used a spray volume of 200 l ha^{-1}, whereas our treatments were applied at a spray volume of 140 l ha^{-1}, which may explain the difference in TOD response in regard to barnyardgrass control.

The presence of heavy dew on the leaves of target plants may result in poor weed control with applications before 6 AM or after 9 PM from increased herbicide dilution and or runoff of applied solution (Anonymous 2011; Doran and Anderson 1976). In contrast, the presence of dew on leaf surfaces may aid in herbicide absorption by allowing the herbicide to remain in solution for a longer period of time before drying (Kogan and Zuniga 2001). Martinson et al. (2005) reported glyphosate efficacy was affected when dew formation occurred after application; however, glufosinate efficacy was not affected by presence or absence of dew. Stewart et al. (2009) reported that although dew was present at 12 and 6 AM, it did not contribute to reduced glufosinate efficacy as much as other TOD effect factors. In our experiments, dew was present at the 12 and 6 AM applications. Glufosinate at 594 g ai ha^{-1} applied at 12 AM provided the least amount of control among the application times; however, barnyardgrass control following the 6 AM application was variable. Dew may have influenced barnyardgrass control but did not have as significant of an effect compared to other TOD effect factors.

Our experiment showed that clethodim applied alone provided greater season-long barnyardgrass control compared to glufosinate plus clethodim and glufosinate alone. Clethodim was not effected by application TOD. Glufosinate applications at midnight reduced resulted in less barnyardgrass control compared to applications made at noon and 6 PM. Early morning applications at 6 AM also showed reduced barnyardgrass efficacy compared to applications at 6 PM. Environmental factors such as temperature and light conditions at the time of application are likely responsible for the TOD effects observed in this study. Although RH and presence of dew may have an effect on glufosinate efficacy, they did not appear to affect barnyardgrass control in our experiments. These data suggest that in order to optimize barnyardgrass efficacy with tank mixtures of glufosinate and clethodim, applications should be made at noon or earlyevening to avoid potential TOD effects.

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Table 4.1 Environmental data at the time of the glufosinate application.

Application date	Sunrise/ sunset[a]	Application time	Relative humidity	Air temperature	Dew[b]
			%	°C	
August 20, 2013	5:21 A.M.	6:00 A.M.	94	24	Y
	.	12:00 P.M.	71	30	N
	6:34 P.M	6:00 P.M.	66	27	N
		12:00 A.M.	94	24	Y
June 13, 2014	4:45 A.M.	6:00 A.M.	66	18	Y
		12:00 P.M.	73	28	N
	7:05 P.M.	6:00 P.M.	58	30	N
		12:00 A.M.	86	22	Y

[a] Times from Astronomical Applications Department, U. S. Naval Observatory, Washington, DC 20392.
[b] Abbreviations: Y, yes; N, no.

Table 4.2 Barnyardgrass control as affected by clethodim and glufosinate interaction pooled over TOD.

Clethodim rate[a]	Glufosinate rate	Barnyardgrass control				
		7 DAT	14 DAT	21 DAT	28 DAT	56 DAT
------------- g ai ha^{-1} -------------		-- % --				
0	0	0D[b]	0C	0D	0C	0C
0	594	94B	93B	89C	88B	90B
76	0	89C	96A	95A	95A	97A
76	594	96A	96A	92B	92A	92B
LSD (0.05)		1	2	2	3	4

[a] Crop oil concentrate at 1% v v^{-1} was included with clethodim.

[b] Means within a column followed by a common letter are not different according to Fisher's Protected LSD P=0.05. A numerical LSD is given for each column.

95

Table 4.3 Barnyardgrass control as affected by glufosinate and TOD interaction pooled over clethodim rates.

Glufosinate rate	Application TOD	Barnyardgrass control				
		7 DAT	14 DAT	21 DAT	28 DAT	56 DAT
g ai ha^{-1}		%				
0	6:00 A.M.	44C[a]	48C	48D	47C	49D
0	12:00 P.M.	45C	48C	47D	47C	49D
0	6:00 P.M.	44C	48C	47D	48C	49D
0	12:00 A.M.	45C	48C	47D	47C	48D
594	6:00 A.M.	95AB	95A	90B	87B	90BC
594	12:00 P.M.	96A	95A	92AB	91A	92AB
594	6:00 P.M.	96A	95A	94A	94A	94A
594	12:00 A.M.	94B	93B	87C	87B	89C
LSD (0.05)		1	2	2	3	3

[a] Means within a column followed by a common letter are not different according to Fisher's Protected LSD P=0.05. A numerical LSD is given for each column.