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Development of a High throughput Surfactant Screening Procedure using Shikimic Acid Analysis

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By

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Development of a high throughput surfactant screening procedure using shikimic acid analysis

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In field efficacy trials most glyphosate/surfactant combinations tested control barnyardgrass as well as pre-formulated glyphosate products 21 days after treatment (DAT). Pre-formulated glyphosate products controlled barnyardgrass quicker than combination treatments, most likely due to improved glyphosate formulations with improved surfactant systems. In greenhouse trials, pre-formulated glyphosate products exhibited greater fresh weight reductions across all species tested, including barnyardgrass, broadleaf signalgrass, hemp sesbania, johnsongrass, large crabgrass, pitted morningglory, prickly sida, sicklepod, yellow foxtail and Palmer amaranth. Nonionic tallow amine treatments exhibited variable control among species.

A shikimate analysis was developed using non-glyphosate-tolerant soybean to estimate efficacy of surfactants; data were then correlated to the visual efficacy data on barnyardgrass in the field. However, there was not sufficient variability in barnyardgrass control to use the shikimate analysis as a predictor.

DEDICATION

I would like to dedicate this work to my mom, Brenda Friesen. Her support and encouragement through this chapter of my life has been essential. As my frustrations grew I could always count on her positive words to guide me through the rough times. The move to Mississippi to continue my education was a tough decision, she reminded me; no matter where I move, I am only a phone call away and I BETTER call every Sunday.

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CHAPTER I

INTRODUCTION

The ability of a herbicide to be an effective resource for weed management depends on many factors, none more important for postemergence herbicides than retention and absorption of herbicide molecules in target weed species. Modification of these elements of herbicidal activity can be achieved through the use of an adjuvant, such as a surfactant. An adjuvant is defined as: any substance in a herbicide formulation or added to the spray tank to modify herbicidal activity or application characteristics (WSSA Herbicide Handbook 2007). Adjuvants can be broken down into two main categories: (1) those that modify physical characteristics of a spray mixture, and (2) those that enhance the biological efficacy of the crop production (Hazen 2000; Kirkwood 1994). Surfactants fall into the latter of these two categories. All surfactants are adjuvants, but many adjuvants are not surfactants (Van Valkenburg 1982). Surfactants are the most common types of spray adjuvants (McElroy et. al. 2001, Wang and Liu 2007). The term surfactant is derived as an acronym for surface-active agent. Surfactants are compounds that reduce surface tension of water or increase its wettability (Van Valkenburg 1982). More specifically, a surfactant is a material that improves the emulsifying, dispersing, spreading, wetting or other properties of a liquid by modifying its surface characteristics (WSSA Herbicide Handbook 2007). When applied alone, surfactants have no activity on plants, but when added to a herbicide, surfactants can influence activity by increasing spray retention, herbicide penetration into the plant cuticle, or translocation within the plant; as a result, increasing the amount of herbicide reaching its site of action (Streibig and Kudsk 1993).

Surfactants are generally classified as spray modifiers or sorption activators (Kirkwood 1994; McMullan 2000). Spray modifiers adjust the water-based spray solution to enhance wetting, sticking, and spreading properties on plant surfaces (Hess and Foy 2000). Sorption activators are accelerator or enhancer surfactants that are needed to increase the ability of herbicides to partition in and across the plant cuticle and for transportation through the cuticle (Hess and Foy 2000; Hazen 2000). These two categories can be further divided in four groups by charge, dependent on their ability to ionize in aqueous solutions: nonionic, anionic, cationic and amphoteric (Hazen 2000; Penner 2000).

Nonionic surfactants are the most widely utilized type of surfactant in the herbicide industry. Surfactants of this type contain no ionizable polar end groups, but the molecule is comprised of hydrophilic and lipophilic segments (Van Valkenburg 1982). Anionic surfactants usually contain a carboxylate, sulfonate, or phosphate group, while cationic surfactants have a water-soluble ester group which does not readily donate or accept protons. Amphoteric surfactants are a mixture of the previous three groups and are commonly called zwitterions. The functional groups on amphoteric surfactants have both positive and negative charges and can be anionic or cationic, depending on the pH of the solution (Hazen 2000). Surfactant type is critical to avoid compatibility issues with ionic herbicides (Hazen 2000; DeRuiter et al. 1996).

In recent years the cost of developing new herbicides has greatly increased and more attention is now being focused on products for which patents are about to expire; therefore, companies are competing to improve formulations rather than create new active ingredients (Kudsk and Streibig 1993). Glyphosate could be the best example, where new patents are issued monthly and new formulations yearly (Green and Beestman 2005). Since generic glyphosate products have become available, the market has become very competitive and many different surfactant systems accompany these products to the marketplace.

Several methods are generally utilized to evaluate surfactants: $^{14}C^{-}$labeled herbicide absorption and translocation (Buick et al. 1993; Feng et al. 2000; Sharma and Singh 2007), whole-plant experiments in the field (Riechers et al. 1995) or greenhouse (Reichers et al. 1994; Reichers et al. 1995; Molin and Hirase 2005), and through the study of specific processes such as isolated cuticle studies (Schönherr 1993). Physicochemical properties of surfactants provide a firm foundation for the development and understanding of surfactant systems (Stock and Briggs 2000). Other research focuses on measurements such as dynamic surface tension (Stevens 1993) and hydrophile/lipophile balance (Harusawa et al. 1982). This, combined with other published literature about surfactant behavior, can help determine basic behavioral profiles of surfactants (Stocks and Briggs 2000). These properties help define the ability of surfactants to aid in retention and absorption of herbicides on leaf surfaces. Understanding these factors are important considering that even glyphosate, the worlds most used herbicide, efficacy is directly related to the ability of the herbicide to penetrate the leaf surface (Feng et al. 1999). The traditional research process is costly and extremely time consuming (Mercer 2007). An efficient high-throughput surfactant screening procedure would save valuable time and resources.

Penner (2000) suggests that a high-priority research topic is to match generic glyphosate formulations with the most effective surfactant. Glyphosate is highly successful at inhibiting the 5-enolypyruvyl-shikimate-3-phoshpate synthase (EPSPS) pathway (Duke and Powles 2008). Blockage of EPSPS increases levels of shikimic acid, blocks aromatic amino acid synthesis, reduces protein synthesis, and decreases growth leading to early cellular death (Duke 1988; Lydon and Duke 1988). Harring et al. (1998) suggest glyphosate/surfactant concentration and type can significantly affect shikimic acid concentration. Utilizing shikimic acid analysis to evaluate surfactant effectiveness would allow for a fast inexpensive sorting method prior to field trial establishment.

The objectives of the research reported in the following chapters were to evaluate the effect of 18 surfactant/glyphosate combinations on common Mississippi weed species, as well as develop a high-throughput surfactant screening procedure utilizing shikimic acid analysis.

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CHAPTER II

EFFECTS OF DIFFERENT GLYPHOSATE/SURFACTANT COMBINATIONS ON TEN COMMON MISSISSIPPI WEED SPECIES

Abstract

Field and greenhouse trials were conducted to compare various glyphosate/surfactant combinations to pre-formulated glyphosate products on several common Mississippi weed species. All treatments were applied at 0.88 kg ae ha^{-1}. In field efficacy trials most glyphosate/surfactant combinations tested controlled barnyardgrass as well as pre-formulated glyphosate products 21 days after treatment (DAT). Pre-formulated glyphosate products controlled barnyardgrass quicker than combination treatments, most likely due to improved glyphosate formulations with improved surfactant systems. In greenhouse trials biomass reductions were taken 21 days after application on barnyardgrass, broadleaf signalgrass, hemp sesbania, johnsongrass, large crabgrass, pitted morningglory, prickly sida, sicklepod, yellow foxtail and Palmer amaranth. Pre-formulated glyphosate products exhibited greater fresh weight reductions across all species. Increased surfactant concentration increased efficacy on five of ten weed species. Nonionic tallow amine treatments exhibited variability among species, due to differences in specific chemical composition of surfactants which are unknown.

Nomenclature: glyphosate; surfactant; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv.; broadleaf signalgrass, *Urochloa platyphylla* (Munro ex C, Wright) R.D. Webster;

hemp sesbania, *Sesbania herbacea* (Mill.) McVaugh; johnsongrass, *Sorghum halepense* (L.) Pers.; large crabgrass, *Digitaria sanguinalis* (L.) Scop.; Palmer amaranth, *Amaranthus palmeri* (S.) Watts.; pitted morningglory, *Ipomea lacunose* L.; prickly sida, *Sida spinosa* L.; sicklepod, *Senna obtusifolia* (L.) H.S. Irwin & Barneby; yellow foxtail, *Setaria pumila* (Poir.) Roem. & Schult.

Introduction

The ability of a foliar herbicide to be an effective resource for weed management depends on many factors, none more important than retention and absorption of herbicide molecules in target weed species. The initial barrier of all foliar-applied herbicides is the leaf surface. Several leaf surface characteristics can cause limited penetration of herbicides: cuticle, age and stage of development of the leaf, leaf angle and position, and number of stomata and trichomes (Hull et al. 1982). Additionally, surface contact spreading of herbicides can be prevented by trichomes, hairs, and water vesicles (Sanyal et al 2006a). Herbicides gain entry into all plants through two main pathways: the cuticle or through the stomatal pores (Buick et al. 1993). The most effective barrier to leaf penetration is the cuticle (McWhorter 1985). The cuticle is a thin lipophilic layer, 0.1 to 1 μm thick, on the leaf surface which reduces water evaporation from leaf surfaces (Hess and Foy 2000; DiTomaso 1999) and provides a barrier between the environment and plants internal cells.

The cuticle is comprised of epidermal wax, cutin, pectin, and cellulose material (Hull et al. 1982). Each of these cuticle components varies between plant species (Baysinger 2000; Ramsey et al. 2005). Within the cuticle, the epidermal waxes have the greatest impact on herbicide penetration (Kirkwood 1999). Research has shown variation in the amount (Sanyal 2006b; Schulke et al. 1995) and type (Post-Beittenmiller 1996; Baker 1982) of wax between plant species. Chachalis et al. (2001) reported an inverse relationship between leaf wax amount and droplet spread on leaf surfaces. Additional research suggests leaf wax amount is inversely related to leaf surface area (McWhorter 1993). Environmental conditions (temperature, light and humidity etc.) can affect the thickness of the cuticle and the amount of epicuticular wax covering the leaf (Price 1982; Reed 1982); well hydrated cuticles spread the wax deposits on the leaf further apart, increasing herbicide diffusion (Kogan and Bayer 1996).

Surfactants are generally classified as spray modifiers or sorption activators (Kirkwood 1994; McMullan 2000). Activators influence the physical and chemical properties of the spray solution, including surface tension, density, volatility and solubility. These properties will modify the spreading, wetting, retention and penetration of the spray solution. Sorption activators are accelerator or enhancer surfactants that are needed to increase the ability of herbicides to partition in and across the plant cuticle and for transportation through the cuticle (Hess and Foy 2000; Hazen 2000). Increased cuticle penetration can be achieved through the use of a nonionic surfactant (Kirkwood 1993).

One of the most important properties of a nonionic surfactant is the number of ethylene oxide (EO) units it possesses. For nonionic surfactants penetration into and across the cuticle is dependent on the EO content. Surfactants with higher EO contents are poor spreaders (Stock and Holloway 1993), but are more efficient at promoting the uptake of highly water-soluble herbicides (Wang and Liu 2007).

Nonionic surfactants are the most common in herbicide formulations; they contain both hydrophilic and lipophilic portions (McWhorter 1985). The surface active molecules of surfactants congregate at the surface of the liquid, either between the droplet and air or between the droplet and plant surface (McWhorter 1982; Buick et al. 1993). This relationship between the hydrophilic and lipophilic portion is the hydrophile/lipophile balance (HLB). A surfactants effectiveness depends largely on it HLB (Myers 2006). Nonionic surfactants have HLB values between 1 and 20. High HLB surfactants are more effective at increasing absorption of herbicides with high water solubility (Hess and Foy 2000), such as glyphosate (de Ruiter et al. 1996).

Surfactants are generally only soluble in water at low concentrations; however, current weed control practices require increased use rates to be successful (Hess 1999). Surfactants molecules overcome this barrier by aggregating the hydrophilic segments outward toward the water and lipophilic segment inward away from water, creating clusters called micelles (Nassetta 1991; Van Valkenberg 1982). The concentration of surfactant clusters is known as the “critical micelle concentration” (CMC). Micelles can soften lipophilic material and potentially alter herbicides solutions and plant cuticles (Hess 1999). Concentrations over the CMC can cause changes in the surface tension and biological activity of the herbicide formulation (Buick et al. 1993). Much fewer micelles are formed with organosilicone surfactants compared to other traditional organic surfactants (Buick et at. 1993), whereas nonionic surfactants generally have a lower CMC and a greater amount of surface tension reduction (Muherei and Junin 2009).

Another equally important factor in surfactant selection is surface tension reduction. The use of wetter-sticker surfactants can lower contact angles on leaf surfaces; this reduction increases spreading allowing the same amount of active ingredient to cover a greater area (Hazen 2000). Buick et al. (1993) demonstrated that very low concentrations of organosilicone surfactants can achieve significant reductions in surface tension. Consequently, the concentration of active ingredient and surfactant are reduced. The ability of the surfactant to break up the cuticle is lost when its concentration per unit area is reduced (Hazen 2000).

In recent years the cost of developing new herbicides has greatly increased and more attention is now being focused on products with patents about to expire; therefore, companies are competing to improve formulations rather than create new active ingredients (Kudsk and Streibig 1993). Since generic glyphosate products have become available, the market has become very competitive and many different surfactant systems accompany these products to the marketplace. When choosing a suitable surfactant several key factors must be kept in mind: physiochemical properties of the herbicide, contact and control of grasses vs. broadleaf weeds (Hazen 2000), growth stage of target species, environment and species variation. Hess and Foy (2000) summarize this best; "different surfactants interact differently with different agrochemicals on different target species."

Previous research has provided widely varying results in weed control with different surfactant systems added to glyphosate. A portion of the same research shows variation both between species and within the same species. The objectives of this research were to evaluate industry standards (Roundup Weathermax®, Touchdown® and Helosate Plus®) verses different types of surfactants added to glyphosate on ten common Mississippi weed species.

Materials and Methods

Field Efficacy

Field experiments were conducted in 2008 and 2009 at the R.R. Foil Plant Science Research Center, Mississippi State University, Starkville, MS. Glyphosate-resistant soybean [*Glycine max* (L.) Merr.] was planted at 23500 seeds ha^{-1} on August 8, 2008; May 29, 2009; and July 28, 2009 into 1.9 by 6.1 m plots. Each experiment was conducted in a randomized complete block, with four replications.

Treatments consisted of 19 glyphosate/surfactant combinations. Pre-formulated glyphosate products (Roundup Weathermax®, Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, MO, 63167; Touchdown®, Syngenta Crop Protection, Inc, P.O. Box 18300, Greensboro, NC 27419; Helosate Plus®, Helm Agro US, INC., 8295 Tournament Drive, Suite 310, Memphis, TN 38125) were added as industry standards and crop oil concentrate (COC) and an untreated check were added for comparison. Pre-formulated products were chosen to represent differences in formulation: Roundup Weathermax®, potassium salt; Touchdown®, diammonium salt, and Helosate Plus®, isopropylamine salt. All glyphosate/surfactant combination treatments and COC were mixed with an isopropylamine formulation without the addition of a formulated surfactant.

A listing of treatments is presented in Table 2.1. Glyphosate/surfactant combination treatments were mixed in the lab on a w/w basis according to the manufacturer's recommendation. The isopropylamine salt of glyphosate was utilized to mix all glyphosate/surfactant combination treatments. A 200 g stock solution of each combination was mixed before each spaying session. Applications occurred at the V7 stage on September 7, 2008; V6 toV7 stage on June 25 and V7 toV8 stage July 28, 2009, respectively. All treatments were applied at 0.88 kg ae ha^{-1} with a CO_2 backpack sprayer equipped with a handboom and 8002VS nozzles.

Barnyardgrass was at the four to six leaf stage, and ranged from 38 to 56 cm in height, upon application of herbicide treatments. Visual weed control and crop injury ratings were determined 7, 14 and 21 days after treatment (DAT) and data were reported utilizing a scale of 0 to 100. A value of 0 equals no weed control or crop injury and 100 equals complete plant death. Data were pooled over the three experiments and subjected to analysis of variance. Treatment means were compared using Fisher's protected LSD at the 0.05 level of significance.

Greenhouse Study

Lack of weed species variation in the field prompted a multi-species weed screen conducted in 2009 at the research farm greenhouses. Seeds of barnyardgrass, broadleaf signalgrass, hemp sesbania, johnsongrass, large crabgrass, pitted morningglory, prickly sida, sicklepod and yellow foxtail were planted in 9 cm^2 pots which contained formulated growing medium (Metro-Mix 300®, vermiculite, bark, Canadian sphagnum peat moss, course perlite, starter nutrient charge, dolomitic limestone, and wetting agent, Sun Gro Horticulture, 15831 N.E. 8th Street, Suite 100 Bellevue, WA 98008). Plants were surface irrigated daily and thinned to one plant per pot as emergence occurred.

Seeds of Palmer amaranth were planted 1 cm deep in 50 cm by 20 cm plastic trays of growing medium. Trays were sub irrigated with 4 L of 0.001% mefonaxam (RimomilGold® SL, 479.35 g ai L^{-1}, Syngenta Crop Protection, Inc., P.O. Box 18300, Greensboro, NC 27419) plus 0.001% azoxystrobin (Quadris® flowable fungicide, 249.26

g ai L^{-1}, Syngenta Crop Protection, Inc., P.O. Box 18300, Greensboro, NC 27419) solution at planting. Seeds were germinated and individually transplanted into 9 cm^2 pots at two leaf stage. Palmer amaranth was subirrigated throughout the trial.

The greenhouse was maintained at 35/30 C day/night temperature and the photoperiod was 16 h, supplemented by sodium vapor lamps. The treatment list is presented in Table 2.2. All plants were sprayed in a compressed air spray chamber equipped with an XR110015E flat fan nozzle at 0.88 kg ae ha^{-1}.

Plants were treated at larger than optimum size due to increased sensitivity under greenhouse conditions. Treatment height/length of each species at application time is presented in Table 2.3. Size at spraying was determined by letting each plant get slightly larger than label recommendations for each weed species. Fresh weights 21 DAT were taken for growth reduction calculations. Weights were calculated by weighing all above ground biomass. Calculations are presented as percent of untreated check.

The experiment was repeated, and conducted as a randomized complete block with three replications. Using PROC GLM procedure in SAS (statistical software package, Version 9.2, SAS Institute, Inc., SAS Campus Dr., Cary, NC 27513) data were combined over experiments and subjected to an analysis of variance with means separated by Fisher's protected LSD at the 0.05 level of probability.

Results and Discussion

Field Efficacy

No crop injury was observed among any treatment across all experiments. Due to the proprietary nature of the surfactant industry, little information was available from surfactant suppliers to explain differences. Therefore, surfactant type, surfactant concentration or glyphosate formulation are the only factors that differences between treatments can be compared.

Seven DAT all pre-formulated products reached at least 90% control of barnyardgrass (Table 2.4). Surfactants HAI1022-1 and HAI 1022-2 are the same coco amine based surfactant but had different batch/lot numbers; therefore they were treated as two separate surfactants. Glyphosate with surfactant HAI1022-1 controlled barnyardgrass as well as Touchdown® and Roundup Weathermax®, whereas glyphosate/surfactant combination HAI1022-2 controlled barnyardgrass less than Touchdown® and Roundup Weathermax®. Slight differences in composition between batch/lot numbers could create the differences. Manufacturers often change the composition without public knowledge for patent protection purposes (Green and Foy 2000).

Glyphosate/surfactant treatments HAI1024, HAI1026 and HAI1027 performed as well as Helosate Plus® but provided less control than Touchdown® and Roundup Weathermax® 7 DAT. The glyphosate formulation in Helosate Plus® is the same isopropylamine form all surfactant combination treatments were mixed with. Furthermore, the surfactants in these combinations are the same type and chemical classification as many other glyphosate/surfactant combinations that performed as well as Touchdown® and Roundup Weathermax®. Therefore, more information is needed on surfactant chemical composition to justify differences.

Touchdown®, Roundup Weathermax® and HAI1020 exhibited better control than HAI1026, HAI1025 and HAI1024 14 DAT (Table 2.4). These glyphosate/surfactant combination products are all nonionic tallow amine surfactants; therefore, further information is needed to explain differences. Furthermore, Touchdown® also controlled barnyardgrass better than combination treatments HAI1022-1and HAI1022-2, but only glyphosate/surfactant treatment HAI 1022-1 was as effective as Roundup Weathermax® 14 DAT.

All treatments reached a minimum of 93% control of barnyardgrass 21 DAT (Table 2.4). Glyphosate/surfactant treatment HAI1022-2 controlled barnyardgrass less than seven other combination treatments and two pre-formulated glyphosate products 21 DAT, but as previously shown 7 and 14 DAT the same surfactant treatment, HAI1022-1 performed as well as pre-formulated products. Glyphosate/surfactant HAI1022-1 controlled barnyardgrass as well as any other treatment 21 DAT.

The results of this study indicate the majority of glyphosate/surfactant combinations tested can control barnyardgrass equal to pre-formulated products 21 days post treatment. However, glyphosate/surfactant combinations tested exhibit much slower visual response of barnyardgrass compared to pre-formulated products. Therefore, pre-formulated products have improved glyphosate formulations; allowing quicker, but no more effective control with superior surfactant systems. Differences in speed of performance are most likely the surfactant system rather than the glyphosate salt formulation, as significant differences in performance aren't expected when utilizing equivalent rates. Parker et al. (2005) reported no difference in the control of barnyardgrass with different formulations of glyphosate. These findings also agree with Gaskin and Holloway (1992), which suggests formulation, and specifically the adjuvant system, can influence the effectiveness of glyphosate uptake and translocation within the plant.

Greenhouse Study

All three pre-formulated glyphosate products (Roundup Weathermax®, Touchdown® and Helosate Plus®) reduced growth by at least 77%. No surfactant treatment performed as well as these pre-formulated products on all species. Several performed better on either grasses or broadleaf species, but most only controlled a few species as well as pre-formulated glyphosate products.

Johnsongrass fresh weight reductions ranged from 32 to 90%. Johnsongrass was fairly large at treatment (61cm) to accentuate differences; nevertheless, the pre-formulated products Roundup Weathermax®, Touchdown® and Helosate Plus® reduced 21 DAT fresh weights substantially; 90%, 83% and 83%, respectively compared to the untreated control (Table 2.5). Glyphosate plus HAI1027, a nonionic tallow amine surfactant, reduced fresh weights of johnsongrass as well as pre-formulated glyphosate products. Glyphosate/surfactant HAI1027 was superior to ten other nonionic tallow amine surfactant treatments: HAI1020, HAI1033, HAI1023-2, HAI1025, HAI1021, HAI1028, HAI1030, HAI1026, HAI1031 and HAI1024. The specific chemical composition of these surfactants was not provided for proprietary reasons. Several factors could cause the observed differences, but the most likely is the EO content. Gaskin and Holloway (1992) demonstrated that surfactant EO content can affect glyphosate efficacy. HAI1027, COC and pre-formulated glyphosate products also reduced johnsongrass fresh weight more than all cationic treatments: HAI1018, HAI1019 and HAI1029. Surfactant HAI1023, a nonionic tallow amine, was mixed at two rates 15 (HAI1023-1) and 7 (HAI1023-2) percent. The increase in rate also increased efficacy form 64 to 78 percent; enough to reduce fresh weight of johnsongrass equal to Touchdown® and Helosate Plus®, both 83%. COC was superior to all but two nonionic tallow amine surfactants, HAI1023-1 mixed at 15% and HAI1027 which reduced johnsongrass fresh weights as well as any pre-formulated glyphosate product. Only four glyphosate/surfactant combination treatments were equivalent to pre-formulated products or crop oil concentrate: three nonionic tallow amine surfactants (HAI1023-1, HAI1027 and HAI1032) and the only nonionic coco amine surfactant.

Barnyardgrass fresh weight reduction ranged from 57 to 94% (Table 2.5). Similar to johnsongrass results, HAI1027 decreased fresh weights of barnyardgrass more than cationic treatments (HAI1018, HAI1019 and HAI1029), coco amine based surfactant HAI1022-2 and eight nonionic tallow amine surfactants. HAI1023-1 reduced the fresh weigh of barnyardgrass less than all other treatments. One of the better surfactants on johnsongrass only reduced barnyardgrass fresh weights 57%. The increased rate of HAI1023-1(15%) could cause damage at the entry point resulting in poor translocation. Kirkwood et al. (2000) showed tallow amine surfactant concentration can influence glyphosate efficacy on barnyardgrass.

Large crabgrass fresh weight reduction ranged from 31 to 82% (Table 2.5). All pre-formulated products reached a minimum of 77% reduction in fresh weight. Nonionic tallow amine glyphosate/surfactant combinations only showed marginal fresh weight reductions of large crabgrass. Only 8 of 13 products tested reduced fresh weights as much as pre-formulated products or crop oil concentrate. Like johnsongrass and barnyardgrass, cationic glyphosate/surfactant combinationss reduced weights less than all pre-formulated products. Cationic surfactant treatment HAI1018 reduced fresh weights of large crabgrass the least, 31%.

Yellow foxtail fresh weight reduction ranged from 34 to 87% (Table 2.5). The potassium salt formulation of Roundup Weathermax® reduced fresh weights of yellow foxtail more than the diammonium salt formulation of Touchdown®. Like other grass species, cationic surfactants exhibited less growth reduction than Roundup Weathermax® and Helosate Plus®. HAI1028 was the only nonionic tallow amine treatment equal to Roundup Weathermax®. Nonionic coco amine surfactant HAI1022-2 reduced fresh weight the least.

Fresh weight reductions of broadleaf signalgrass ranged from 43 to 88% (Table 2.5).Pre-formulated glyphosate products reached a minimum of 82% fresh weight reduction of broadleaf signalgrass. Cationic surfactant treatments reduced fresh weights of broadleaf signalgrass more than any other grass species. Like johnsongrass, increase in concentration of nonionic tallow amine surfactant HAI1023 caused an increase in fresh weight reduction. Nonionic tallow amine surfactant combination HAI1026 reduced fresh weights of broadleaf signalgrass less than all other treatments.

Prickly sida fresh weight reductions ranged from 55 to 93% (Table 2.5). Nonionic tallow amine surfactant treatments reduced fresh weights of prickly sida more than any other broadleaf species. Glyphosate/surfactant combinations HAI1021, HAI23-1, HAI1023-2, HAI1028, HAI1030, HAI1032 and HAI1033 all reduced fresh weights of prickly sida as much as pre-formulated products Touchdown® and Helosate Plus®, both 83%. Previous research shows excellent biomass reduction of prickly sida with cationic tallow amine surfactants (Norsworthy et al. 2001); however, no cationic tallow amine surfactant achieved over 70% reduction of prickly sida, which can only be attributed to differences in surfactant composition. Added to glyphosate nonionic tallow amine surfactants HAI1032 and HAI1033 also performed well on prickly sida. Increased glyphosate absorption could result from higher surfactant concentration since many nonionic tallow amine surfactants reduced biomass of prickly sida poorly and the same type of surfactant combination, HAI1032 (15%), performed as well or better than pre-formulated glyphosate treatments.

Fresh weight reductions of pitted morningglory ranged from 41 to 87% (Table 2.5). Previous research reports variable control of pitted morningglory with glyphosate (Norsworthy et al. 2001; Shaw and Arnold 2002; Koger et al. 2007). Variability of control has been reported to be caused by plant size (Chachalis et al. 2001) and limited absorption (Norsworthy et al. 2001). No glyphosate/surfactant combination treatment reduced fresh weights of pitted morningglory as much as Roundup Weathermax®. Only two surfactant combinations performed as well as a pre-formulated product: HAI1023-1 and HAI1029. Combination HAI1023 exhibited growth reductions equal to Touchdown® and Helosate Plus at 15% but not at 7%. Increased surfactant concentration could assist in overcoming barriers in the plant cuticle or help translocation from the treated leaf.

Hemp sesbania fresh weight reductions ranged from 36 to 87% (Table 2.5). Natural tolerance to glyphosate has previously been reported (Jordan et al. 1997; Lich et al. 1997; Oliver et al. 1996). Only nonionic tallow amine glyphosate/surfactant HAI1021 reduced fresh weights of hemp sesbania as much as pre-formulated glyphosate products. Additionally, treatment HAI1030 exhibited equal control to Helosate Plus®. Control of hemp sesbania like many other species, with glyphosate and cationic surfactants provided poor to marginal fresh weight reduction. Overall, glyphosate plus a nonionic tallow amine surfactant exhibited marginal growth reductions of hemp sesbania.

Fresh weight reductions of Palmer amaranth showed the most variability between treatments, 26 to 87% (Table 2.5). No treatment displayed as much growth reduction as pre-formulated glyphosate treatments: Roundup Weathermax® (87%), Touchdown® and Helosate Plus® (both 80%). Compared to the more difficult species to control in this trial; pitted morningglory and hemp sesbania, Palmer amaranth would be considered intermediate in sensitivity to glyphosate (Norsworthy et al. 2001). No glyphosate/surfactant treatment, regardless of ion type or chemical classification, reduced fresh weights of Palmer amaranth more than 60%. All surfactant treatments reduced Palmer amaranth fresh weights more than glyphosate and the addition of coco amine surfactant HAI1022-2. Advanced formulations with superior surfactant systems gave pre-formulated glyphosate products an advantage over glyphosate/surfactant combination treatments tested.

A range of 51 percentage points separated sicklepod fresh weight reductions; Roundup Weathermax® (90%) and HAI1022-2 (39%) (Table 2.5). HAI1028 was the only glyphosate/surfactant treatment equivalent to pre-formulated glyphosate products. Like several other weeds, glyphosate and coco amine based surfactant HAI1022-2 provided poor reduction of sicklepod fresh weights.

Several studies have shown glyphosate and a tallow amine based surfactant to be effective; however, many of the tallow amine glyphosate/surfactant combinations tested exhibit poor reductions in fresh weight of species tested. Lack of performance could be attributed to specific chemical differences between surfactants or excessive injury preventing glyphosate export. Feng et al. (1998) showed using microscopic studies that tallow amine surfactants are effective at disrupting the plant cuticle.

Pre-formulated products: Roundup Weathermax® (82-92%), Touchdown® (77-88%) and Helosate Plus® (80-94%) all reached a minimum of 77% fresh weight reduction across all species. HAI1032 was the best combination treatment. Glyphosate and HAI1032 controlled four species; barnyardgrass, large crabgrass, broadleaf signalgrass and prickly sida as well as pre-formulated treatments. No other glyphosate/surfactant treatment controlled as many species as HAI1032. Surfactant HAI1032 was mixed at an increased concentration (15%), the increase in surfactant concentration could aid in cuticle disruption and increased translocation. Increasing concentration of nonionic tallow amine surfactant, HAI1023, caused a significant increase in biomass reduction on five species: johnsongrass, broadleaf signalgrass, pitted morningglory, hemp sesbania and sicklepod. Six of 17 glyphosate/surfactant combination treatments (HAI1019, HAI1024, HAI1025, HAI1026, HAI1029, and HAI1031) showed no control of any species equal to pre-formulated products.

As previous research supports, broadleaf species pitted morningglory, hemp sesbania, Palmer amaranth and sicklepod, were the more difficult species to control. With the exception of HAI1021 on hemp sesbania and HAI1028 on sicklepod no treatment displayed control equal to pre-formulated products. Glyphosate and coco amine based surfactant; HAI1022-2 reduced fresh weights of johnsongrass, barnyardgrass, broadleaf signalgrass and prickly sida more than other species, all species known to have sensitivity to glyphosate (Norsworthy et al. 2001)..

Glyphosate/surfactant combination treatments showed greater variation in fresh weight reduction between weed species than pre-formulated products. Variation could be explained by glyphosate formulation: Roundup Weathermax®, potassium salt;

Touchdown®, diammonium salt, and Helosate Plus®, isopropylamine salt. All glyphosate/surfactant combination treatments and COC were mixed with isopropylamine formulation. Roundup Weathermax®, Touchdown® and Helosate Plus® were consistently the better treatments among species tested, still they had varying results. Roggenbuck and Penner (1997) discuss different glyphosate formulations and their varying efficacy on several weed species. Molin and Hirase (2004) report varying results between glyphosate formulations on morningglory and prickly sida. Commonly, a surfactant company will tolerate less control on one type of weed to gain control of another species (Hazen 2000). The best strategy is to try to match the best surfactant to the herbicide, weed species to be controlled, and the environmental conditions (Penner 2000).

Further delineation of surfactant differences could be possible if more information on specific chemical characteristics were disclosed. On the basis of these data, Roundup Weathermax®, Touchdown® and Helosate Plus® were consistently better treatments on a wide variety of weed species. Improved formulations with superior surfactant system allow these pre-formulated glyphosate products to control a variety of weed species more efficiently than glyphosate/surfactant combinations tested. HAI1032 was the best glyphosate/surfactant combination tested, but still only controlled four of the ten species as well as pre-formulated glyphosate products. It is important to remember that surfactant research is about the treatment that overall did the best, not the treatment that was best on any one species.

Table 2.1 Treatment type, class and mixing percentage for field efficacy trial[a].

Treatment	Surfactant Type	Chemical Classification	% surfactant
Untreated Check	-	-	-
Roundup Weathermax®	NA	NA	NA
Touchdown®	NA	NA	NA
Helosate Plus®	NA	NA	NA
COC[b]	Crop oil concentrate	Crop oil concentrate	0.25% v/v[c]
HAI1018[d]	Cationic	Tallow amine ethoxylate	8
HAI1019	Cationic	Amide	8
HAI1020	Nonionic	Tallow amine ethoxylate	8
HAI1021	Nonionic	Tallow amine ethoxylate	8
HAI1022-1	Nonionic	Coco amine ethoxylate	7
HAI1022-2	Nonionic	Coco amine ethoxylate	7
HAI1023-1	Nonionic	Tallow amine ethoxylate	15
HAI1023-2	Nonionic	Tallow amine ethoxylate	7
HAI1024	Nonionic	Tallow amine ethoxylate	7.5
HAI1025	Nonionic	Tallow amine ethoxylate	7.5
HAI1026	Nonionic	Tallow amine ethoxylate	7.5
HAI1027	Nonionic	Tallow amine ethoxylate	7.5
HAI1028	Nonionic	Tallow amine ethoxylate	7.5
HAI1029	Cationic	Tallow amine ethoxylate	7.5
HAI1030	Nonionic	Tallow amine ethoxylate	7.5
HAI1031	Nonionic	Tallow amine ethoxylate	8
HAI1032	Nonionic	Tallow amine ethoxylate	15
HAI1033	Nonionic	Tallow amine ethoxylate	8
HAI1035	Nonionic/amphoteric blend	Amine oxide	5

[a] all surfactants were added on a weight/weight basis unless otherwise noted.
[b]COC, Majestic®, Estes Inc., 2716 Commerce St,Wichita Falls, TX 76301.
[c]v/v: denotes a volume/volume baisis.
[d]HAI codes are proprietary surfactants.

Table 2.2 Treatment type, class and mixing percentage for greenhouse trial[a]

Treatment	Surfactant Type	Chemical Classification	% surfactant
Untreated Check	-	-	-
Roundup Weathermax®	NA	NA	NA
Touchdown®	NA	NA	NA
Helosate Plus®	NA	NA	NA
COC[b]	Crop oil concentrate	Crop oil concentrate	0.25% v/v[c]
HAI1018[d]	Cationic	Tallow amine ethoxylate	8
HAI1019	Cationic	Amide	8
HAI1020	Nonionic	Tallow amine ethoxylate	8
HAI1021	Nonionic	Tallow amine ethoxylate	8
HAI1022-2	Nonionic	Coco amine ethoxylate	7
HAI1023-1	Nonionic	Tallow amine ethoxylate	15
HAI1023-2	Nonionic	Tallow amine ethoxylate	7
HAI1024	Nonionic	Tallow amine ethoxylate	7.5
HAI1025	Nonionic	Tallow amine ethoxylate	7.5
HAI1026	Nonionic	Tallow amine ethoxylate	7.5
HAI1027	Nonionic	Tallow amine ethoxylate	7.5
HAI1028	Nonionic	Tallow amine ethoxylate	7.5
HAI1029	Cationic	Tallow amine ethoxylate	7.5
HAI1030	Nonionic	Tallow amine ethoxylate	7.5
HAI1031	Nonionic	Tallow amine ethoxylate	8
HAI1032	Nonionic	Tallow amine ethoxylate	15
HAI1033	Nonionic	Tallow amine ethoxylate	8

[a] all surfactants were added on a weight/weight basis unless otherwise noted.

[b]COC, Majestic®, Estes Inc., 2716 Commerce St,Wichita Falls, TX 76301.

[c] v/v: denotes a volume/volume basis.

[d]HAI codes are proprietary surfactants.

Table 2.3 Size of weed species in greenhouse trial at application of glyphosate/surfactant treatments[a].

Weed Species	Latin Name	Size (cm)
Barnyardgrass	*Echinochloa crus-galli* (L.) Beauv.	61
Broadleaf signalgrass	*Urochloa platyphylla* (Nash) R.D. Webster	21
Hemp sesbania	*Sesbania herbacea* (P. Mill.) McVaugh	41
Johnsongrass	*Sorghum halepense* (L.) Pers.	56
Large crabgrass	*Digitaria sanguinalis* (L.) Scop.	21
Palmer amaranth	*Amaranthus palmeri* S. Watts	15
Pitted morningglory[b]	*Ipomoea lacunosa* L.	15
Prickly sida	*Sida spinosa* L.	15
Sicklepod	*Senna obtusifolia* (L.) H.S. Irwin & Barneby	56
Yellow foxtail	*Setaria pumila* (Poir.) Roemer & J.A. Schultes	15

[a]weed sizes are in cm of height.
[b]pitted morningglory is in cm of length.

Table 2.4 Visual control of barnyardgrass 7, 14 and 21 days after postemergence treatments of different glyphosate/surfactant combinations.

Treatment	7 DAT[a]	14 DAT	21 DAT
	------------------------------%------------------------------		
Roundup Weathermax®	95	97	98
Touchdown®	96	98	99
Helosate Plus®	90	94	95
COC[b]	90	95	98
HAI1018	89	93	97
HAI1019	88	95	98
HAI1020	90	97	99
HAI1021	92	96	98
HAI1022-1	85	92	95
HAI1022-2	78	90	93
HAI1023-1	84	94	98
HAI1023-2	87	95	98
HAI1024	79	90	96
HAI1025	82	91	95
HAI1026	80	91	95
HAI1027	81	93	96
HAI1028	83	93	98
HAI1029	86	93	97
HAI1030	84	93	97
HAI1031	87	93	96
HAI1032	89	95	98
HAI1033	88	93	97
HAI1035	85	93	95
LSD[c]	13	5	4

[a]Days after treatment (DAT).
[b]COC, Majestic®, Estes Inc., 2716 Commerce St,Wichita Falls, TX 76301.
[c]LSD: Least significant difference separated by Fishers protected LDS at the 0.05 level of significance.

Table 2.5 Fresh weight reduction of common weed species with different glyphosate/surfactant combinations[a].

Treatment	SORHA[b]	ECHCG	DIGSA	SETLU	BRAPP	SIDSP	IPOLA	SEBEX	AMAPA	CASOB
	%									
Roundup Weathermax®	90	92	82	87	88	88	87	85	89	90
Touchdown®	83	82	77	78	82	83	80	82	85	88
Helosate Plus®	83	94	80	83	84	83	80	80	82	85
COC[c]	89	84	76	75	68	68	71	62	69	80
HAI1018[d]	63	68	31	52	87	60	60	66	53	69
HAI1019	71	77	66	55	78	55	66	52	40	57
HAI1020	68	82	69	79	69	65	48	67	51	42
HAI1021	59	93	48	76	82	77	61	77	45	56
HAI1022-2	81	79	62	34	68	73	41	40	26	39
HAI1023-1	78	57	73	76	67	86	77	56	57	66
HAI1023-2	64	86	82	76	52	86	65	36	54	49
HAI1024	32	77	64	54	59	65	50	37	41	56
HAI1025	63	81	44	67	63	59	48	51	41	55
HAI1026	34	75	55	71	43	73	56	55	45	56
HAI1027	84	94	47	71	70	59	57	62	59	47
HAI1028	48	85	82	83	78	78	42	66	47	82
HAI1029	47	74	65	70	77	70	74	42	41	66
HAI1030	42	73	47	75	68	81	64	70	50	60
HAI1031	33	77	46	69	72	70	59	59	50	65
HAI1032	77	91	73	65	85	93	60	46	44	49
HAI1033	67	87	57	75	59	92	59	53	52	63
LSD[e]	11	11	9	8	9	9	8	10	9	9

[a] Fresh weigh reduction was pooled over experiments and reported as percent of control for each species.
[b] Abbreviations: SORHA, johnsongrass; ECHCG, barnyardgrass; DIGSA, large crabgrass; SERLU, yellow foxtail; BRAPP, broadleaf signalgrass; SIDSP, prickly sida; IPOLA, pitted morningglory; SEBEX, hemp sesbania, AMAPA, Palmer amaranth; CASOB, sicklepod.
[c]COC, Majestic®, Estes Inc., 2716 Commerce St,Wichita Falls, TX 76301.
[d]HAI codes are all proprietary surfactants added to glyphosate at manufactures recommended rates.
[e]LSD: Least significant difference separated by Fishers protected LSD at the 0.05 level of significance.

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CHAPTER III

DEVELOPMENT OF A HIGH THROUGHPUT SURFACTANT SCREENING PROCEDURE USING SHIKIMIC ACID ANALYSIS

Abstract

Sampling procedure experiments were conducted in the greenhouse in 2009 to evaluate different techniques and timing on shikimic acid in soybean. Multiple rates, sampling techniques and collection times were evaluated. The conclusion was made that the optimum rate and sampling procedure was: glyphosate delivered at 0.66 kg ae ha and nine tissue samples collected 72 hours after treatment (HAT). Rate and sampling procedure were then used to collect shikimic acid data on soybean in the greenhouse using the treatment list from the 2008 and 2009 field trials. Data was then correlated to visual efficacy of glyphosate/surfactant treatments on barnyardgrass. Low correlations were noted, primarily because of the lack of variation in barnyardgrass control, thus making it impossible to delineate differences between surfactant treatments. Additional experimentation is needed in order to develop this rapid screening procedure.

Nomenclature: glyphosate; surfactant; shikimic acid; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv.; soybean, *Glycine max* (L.) Merr.

Introduction

Weed management strategies became simpler with the introduction of glyphosate in the early 1970's. Glyphosate is the active ingredient in Roundup® (Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, MO, 63167) and now numerous other brands and generic formulations are on the market. For many years now, glyphosate has been applied to more acres than any other herbicide around the World (Baylis 2000). The use of glyphosate as a non-selective herbicide increased in 1996 with the commercialization of genetically modified crops, and has been growing ever since (Dill 2005).

Glyphosate is a post-emergence, non-selective herbicide that controls a wide range of annual and perennial weed species. Glyphosate is the only compound known that is highly successful at inhibiting the 5-enolypyruvyl-shikimate-3-phosphate synthase (EPSPS; EC 2.5.1.19) pathway (Duke and Powles 2008). Inhibition of EPSPS leads to depletion of the aromatic amino acids tryptophan, tyrosine and phenylalanine, which are essential for protein synthesis or advanced pathways leading to developmental growth (WSSA 2007). Blockage of EPSPS increases levels of shikimic acid, a precursor to aromatic amino acid synthesis. It subsequently reduces protein synthesis, decreases growth, and therefore leads to early cellular death (Duke 1988; Lydon and Duke 1988).

Previous research has reported shikimic acid accumulation in soybean [*Glycine max* (L.) Merr] within 24 hours after treatment (HAT) (Singh and Shaner 1998).

Glyphosate efficacy is dependent on the effectiveness of delivery to the leaf surface, leaf infiltration, and translocation to target tissues (Kirkwood and McKay 1994). Glyphosate has relatively rapid entry followed by a longer stage of slower penetration that can be dependent on the type and concentration of the surfactant (Caseley and Coupland 1985). Harring et al. (1998) suggest the type of surfactant and its concentration can significantly affect shikimic acid concentration.

Two different methods have been utilized to measure shikimate levels in glyphosate treated tissue: an HPLC assay and a spectrophotometric assay (Cromartie and Polge 2000). The HPLC assay is labor intensive and expensive (Harring et al. 1998). The spectrophotometric assay is a simple, rapid and reproducible assay capable of measuring shikimate levels in large sample volume (Henry et al. 2005). Further increasing the speed of the assay, Shaner et al. (2005) developed a tissue collection technique using excised leaf discs 4 mm in diameter.

There are multiple methods for screening surfactant effectiveness. Surfactant testing using whole-plant screens in the field and greenhouse are probably the most definitive; however, testing is time consuming and expensive. If an early surfactant screening procedure using the spectrophotometric methods of Cromartie and Polge (2000) and sampling procedures of Shaner et al. (2005) to measure shikimic acid content could be developed; then suitable surfactant candidates could be recognized in a fast, inexpensive manner prior to field trial establishment.

The objectives of this research were 1) to develop proper sampling procedures and optimum glyphosate rate for shikimic acid analysis on soybean; and 2) correlate shikimic acid concentrations of different glyphosate/surfactant combinations to visual field efficacy data.

Materials and Methods

Rate Titration

Greenhouse experiments were conducted at the R.R. Foil Plant Science Research Center, Mississippi State University, in spring 2010. Non-glyphosate-tolerant soybean was planted in 9 cm^2 pots containing growing medium (Metro-Mix 300®, vermiculite, bark, Canadian sphagnum peat moss, course perlite, starter nutrient charge, dolomitic limestone, and wetting agent, Sun Gro Horticulture, 15831 N.E. 8th Street, Suite 100 Bellevue, WA 98008) and grown at 35/30 C day/night temperature. A 16 h photoperiod was maintained with natural light and supplemented with sodium vapor lamps. Plants were thinned to one plant per pot as emergence occurred and surface irrigated daily for adequate moisture. Soybean was used as the indicator species to reduce genetic variation between plants, and thus maximize precision of response. Plants of equal size and growth habit were selected in order to further reduce variation.

Applications of glyphosate (Helosate Plus®, Helm Agro US, INC., 8295 Tournament Drive, Suite 310, Memphis, TN 38125) were applied when the third trifoliolate was fully expanded. Applications were applied in a compressed air spray chamber equipped with an XR110015E flat fan nozzle at 169 L ha^{-1}. Glyphosate was applied at 0.88, 0.66, 0.44, 0.22 and 0.11 kg ae ha^{-1}. These rates represent 100, 75, 50, 25, and 12.5% of the labeled rate.

Tissue was harvested with a modified cork borer equipped with a spring-loaded plunger following collection techniques described by Shaner et al. (2005). Three, six, nine or twelve leaf discs (4-mm diam) were harvested from the third trifoliolate of each plant (Figure 3.1) 24, 48 or 72 h after treatment (HAT). Samples were placed in vials that contained 1 ml of 0.25 M HCL (VWR International LLC. West Chester, PA 19380) and stored at -18 C prior to analysis. Shikimate standards were developed by adding known amounts of shikimate to vials containing leaf discs not exposed to glyphosate, so shikimate levels could be reported as µg shikimate ml^{-1} HCL solution. Each trial contained six untreated control samples and two fortifications (12.5 and 100 µg ml^{-1} shikimate) for each treatment combination. Samples were reanalyzed when recovery of fortified samples fell outside of 90-110% recovery range. Recovery from fortified samples averaged 95 ± 2% and 97 ± 5% at 12.5 and 100 µg ml^{-1} shikimate respectively. Each treatment had three replications and the trial was repeated.

Prior to starting assays, stock solutions were mixed: 0.25 M HCL, 0.25% periodic acid (Sigma-Aldrich Co., 3050 Spruce Street, St. Louis, MO 63103)/ 0.25% meta-periodate (Alfa Aesar, 26 Parkridge Rd Ward Hill, MA 01835) and 0.6 M sodium hydroxide (J.T. Baker Inc., Phillipsburg, NJ 08865)/ 0.22 M sodium sulfite (Sigma-Aldrich Co., 3050 Spruce Street, St. Louis, MO 63103). Shikimate concentrations were determined following the extraction procedures of Cromartie and Polge (2000). Shikimate levels were determined by transferring 25 µl of solution from each vial to a 96 well microtiter plate (Fisher Scientific Inc.,300 Industry Drive, Pittsburgh, PA 15275) containing 100 µl of 0.25% periodic acid/ 0.25% meta-periodate solution. After one hour incubation at 22 C; 100 µl of 0.6-M sodium hydroxide/ 0.22-M sodium sulfite solution was added to each well to stop the reaction. Absorbance was measured at 380 nm using a spectrophotometer (Power Wave XS Microplate Spectrophotometer with Bio-Tek KC4™ software, Bio Tek Instruments, Inc., 100 Tigan St, Winooski, VT 05404) within

15 minutes after stopping the reaction. Background optical density was determined from wells containing control discs and subtracted from glyphosate treatments.

The experimental design was a five by four by three factorial arrangement of treatments with three replications: Factors were glyphosate rate (0.88, 0.66, 0.44, 0.22 and 0.11 kg ae ha^{-1}) by tissue collection method (three, six, nine or twelve leaf discs) by collection timing (24, 48 or 72 HAT). Data were analyzed with Proc GLM procedure in SAS (statistical software package, Version 9.2, SAS Institute, Inc., SAS Campus Dr., Cary, NC 27513) and means were separated using Fisher's protected least significance difference (LSD) at the 0.05 level of probability.

Greenhouse

Non-glyphosate-tolerant soybean was planted and maintained exactly as they were in the rate titration experiment. Plants were treated with either a pre-formulated glyphosate product (Roundup Weathermax®, Touchdown® and Helosate Plus®) or a glyphosate/surfactant combination treatment (see Table 3 for specific treatments). Application rate (0.66 kg ae ha^{-1}), number of tissue samples collected (9) and number of hours after treatment tissue samples were collected (72) were chosen from the rate titration experiment.

All treatments were applied in a compressed-air spray chamber equipped with an XR110015E flat fan nozzle at an application rate of 169 L ha^{-1}. Nine leaf tissue samples (4 mm in diam) were harvested from the third trifoliolate of each plant 72 HAT. Sampling methods and shikimic acid analysis were completed as previously reported in the rate titration experiment. For each sample set (one replication) five untreated control and two fortified (12.5 and 100 μg ml^{-1} shikimate) samples were prepared. Samples were reanalyzed when recovery of fortified samples fell outside of 90-110% recovery range. Recovery from fortified samples averaged 97 ± 4% and 99 ± 6% at 12.5 and 100 μg ml^{-1} shikimate respectively.

Experimental design was a randomized complete block with three replications and was repeated. Shikimic acid concentrations were correlated to visual efficacy of the same treatments from the previous field trials reported in Chapter 2. Field efficacy data and corresponding shikimic acid concentration from the greenhouse are presented in Table 3.1. Data were averaged across three experiments for the field trial and two experiments for greenhouse correlation experiment using PROC MEANS in SAS. Data were then transformed and visual control in the field was correlated to shikimic acid content in the greenhouse using PROC CORR.

Results and Discussion

Rate Titration

Shikimate concentrations were corrected for endogenous levels using the appropriate untreated control sample (Table 3.1). Increasing glyphosate rate increased shikimic acid concentration at all collection times, except 48 HAT when rate increased from 0.44 to 0.66 kg ae ha^{-1}. Increased shikimic acid concentrations as rate increased were previously reported by Harring et al. (1998), Lydon and Duke (1988), Buehring et al. (2007) and Singh and Shaner (1998). Increasing sample number increased shikimic acid concentration at all collection times except 48 HAT when sample number increased from 3 to 6 and 72 HAT when sample number increased from 6 to 9 (Table 3.3).

Shikimic acid concentrations for identical sample number and glyphosate rate, continuously increased as harvest time increased from 24 HAT to 48 HAT and 48 HAT to 72 HAT, which has been observed by Singh and Shaner (1998). Increased variation in shikimic acid concentration was observed between samples as glyphosate rate and sample number increased for all harvest times. Exact cause is unknown, possible explanations could include: increasing glyphosate rate also increases surfactant concentration, allowing greater disruption of the cuticle and therefore increasing variability of glyphosate infiltration and translocation. Also, shikimic acid is not evenly distributed throughout the leaf (Shaner et al. 2005), consequently small amounts of error from smaller sample numbers add up as sample size increases.

Shikimic acid can be influenced by many uncontrollable factors, even in a greenhouse. The effect of light on shikimate accumulation after glyphosate application has been demonstrated by Amrhen et al. (1980) in buckwheat (*Fagopyrum esulentum* Moench.) and Shaner et al. (2005) in soybean. Even though greenhouse plants were as uniform as possible, differences in placement in the greenhouse, amount of water, and temperature could have caused some of the variability in shikimic acid.

The key factors when choosing the sampling procedure was to measure shikimic acid while it was still increasing and not at a maximum or plateau; and choose a treatment that was significantly different than the rest of the treatments. Data shows a separation between treatments at 0.66 kg ae ha^{-1} glyphosate and nine leaf tissue samples to be collected 72 HAT. This sampling procedure shows shikimic acid is still increasing and is one of the first treatments that is significantly different than treatments around it.

Greenhouse

Shikimic acid concentrations were corrected for endogenous levels as in earlier experiments. Little variation in control levels was observed between treatments in earlier field trials for all observation times (Table 3.2). Shikimic acid content of greenhouse grown soybean displayed greater differences between treatments. Very low correlations were observed for 7, 14 and 21 DAT (Figure 3.2).

Barnyardgrass data from field trials shows most pre-formulated glyphosate products and glyphosate/surfactant combination treatments exhibited excellent control. Excellent control of barnyardgrass with glyphosate has been demonstrated previously (Scroggs et al. 2005, Sikkema et al. 2005, Koger et al. 2007). An increase in weed species in the field could create greater separation between treatments to more competently compare surfactant efficacy. To more appropriately separate treatments; weed species with greater variation in glyphosate control are needed.

On the basis of these data, barnyardgrass does not show significant variation in control between treatments to assess reliability of shikimic acid assay for surfactant screening. Further research is needed with a range of control levels in order to truly assess validity of this screening method.

Table 3.1 Average endogenous shikimate content of soybean control samples[a]

Sample number[b]	Hours after treatment (HAT)[c]		
	24	48	72
#	Shikimate μg/ml		
3	5.68 ± 0.44c	6.19 ± 1.05	6.11 ± 0.94
6	6.29 ± 0.88	7.40 ± 1.02	7.50 ± 1.26
9	8.42 ± 1.27	8.66 ± 1.66	8.85 ± 1.72
12	10.10 ± 1.34	10.55 ± 2.02	11.14 ± 2.06

[a]Average endogenous shikimic acid content ± 1 standard deviation of each combination of sample number and time, 12 samples per combination.
[b]Number of leaf discs harvested from the third trifoliolate.
[c]Time in hours after treatment (HAT) leaf tissue samples were harvested.

Table 3.2 Correlation of the effect of surfactants on the efficacy of glyphosate against barnyardgrass 7, 14 and 21 days after treatment in the field to the shikimic acid content of the same treatments on soybean in the greenhouse.

Treatment	7 DAT[a]	14 DAT	21 DAT	Shikimic acid[b]
Roundup Weathermax®	95	97	98	95
Touchdown®	96	98	99	96
Helosate Plus®	90	94	95	90
COC[c]	90	95	98	90
HAI1018	89	93	97	89
HAI1019	88	95	98	88
HAI1020	90	97	99	90
HAI1021	92	96	98	92
HAI1022-1	85	92	95	85
HAI1022-2	78	90	93	78
HAI1023-1	84	94	98	84
HAI1023-2	87	95	98	87
HAI1024	79	90	96	79
HAI1025	82	91	95	82
HAI1026	80	91	95	80
HAI1027	81	93	96	81
HAI1028	83	93	98	83
HAI1029	86	93	97	86
HAI1030	84	93	97	84
HAI1031	87	93	96	87
HAI1032	89	95	98	89
HAI1033	88	93	97	88
HAI1035	85	93	95	85
R^{2}[d]	0.086	0.187	0.112	

[a] Days after treatment (DAT).
[b]Average shikimic acid content of soybean in greenhouse. Average represents two trials containing three replications each.
[c]COC, Majestic®, Estes Inc., 2716 Commerce St,Wichita Falls, TX 76301.
[d]Correlation coefficients reflect how well shikimik acid of soybean in the greenhouse. explain 7, 14 and 21 day after treatment visual control ratings of barnyargrass in the field.

Table 3.3 Recovery of shikimic acid from soybean tissue as a function of time[a], rate[b] and sampling procedure[cd].

Glyphosate rate	24 HAT				48 HAT				72 HAT			
	3	6	9	12	3	6	9	12	3	6	9	12
kg ae ha^{-1}	μg shikimate ml^{-1}HCL solution											
0.11	4.11	5.70	9.73	12.35	6.72	11.01	16.07	18.96	16.00	26.08	24.15	35.13
0.22	5.15	9.35	12.38	16.62	7.30	15.77	20.01	26.62	22.13	29.67	47.44	49.44
0.44	6.57	10.10	13.79	22.32	9.01	18.11	28.77	35.10	23.71	43.68	50.73	78.33
0.66	7.61	10.90	16.12	25.50	14.60	12.98	35.96	51.28	28.98	54.48	89.44	98.92
0.88	10.32	13.10	19.86	31.00	25.10	35.57	48.09	68.25	49.28	67.72	95.41	105.88
LSD[e]	6											

[a]Tissue samples were collected 24, 48 and 72 hours after treatment (HAT) with glyphosate.
[b]Reduced rates were utilized to magnify the degree of sensitivity of soybean to glyphosate.
[c]Sampling procedure included harvesting either 3, 6, 9 or 12 leaf discs 4-mm in diameter from the third trifoliolate of each plant.
[d]Data represent the average of two trials each with three replications.
[e]LSD: Least significant difference separated by Fishers protected LSD at the 0.05 level of significance.

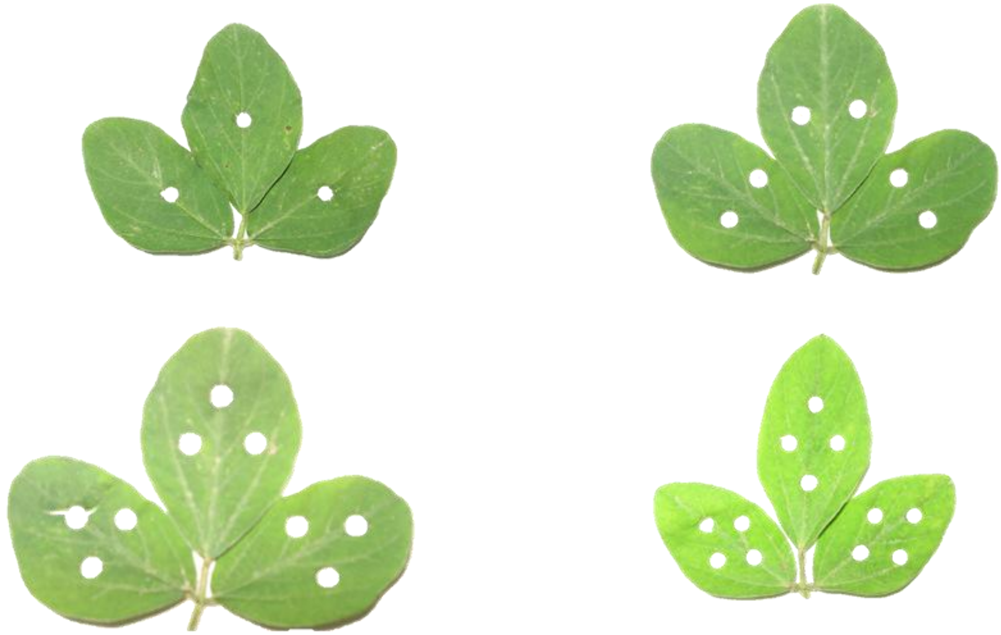

Figure 3.1 Tissue samples harvested from the third trifoliolate of soybean with a modified cork borer for evaluation of shikimic acid content.

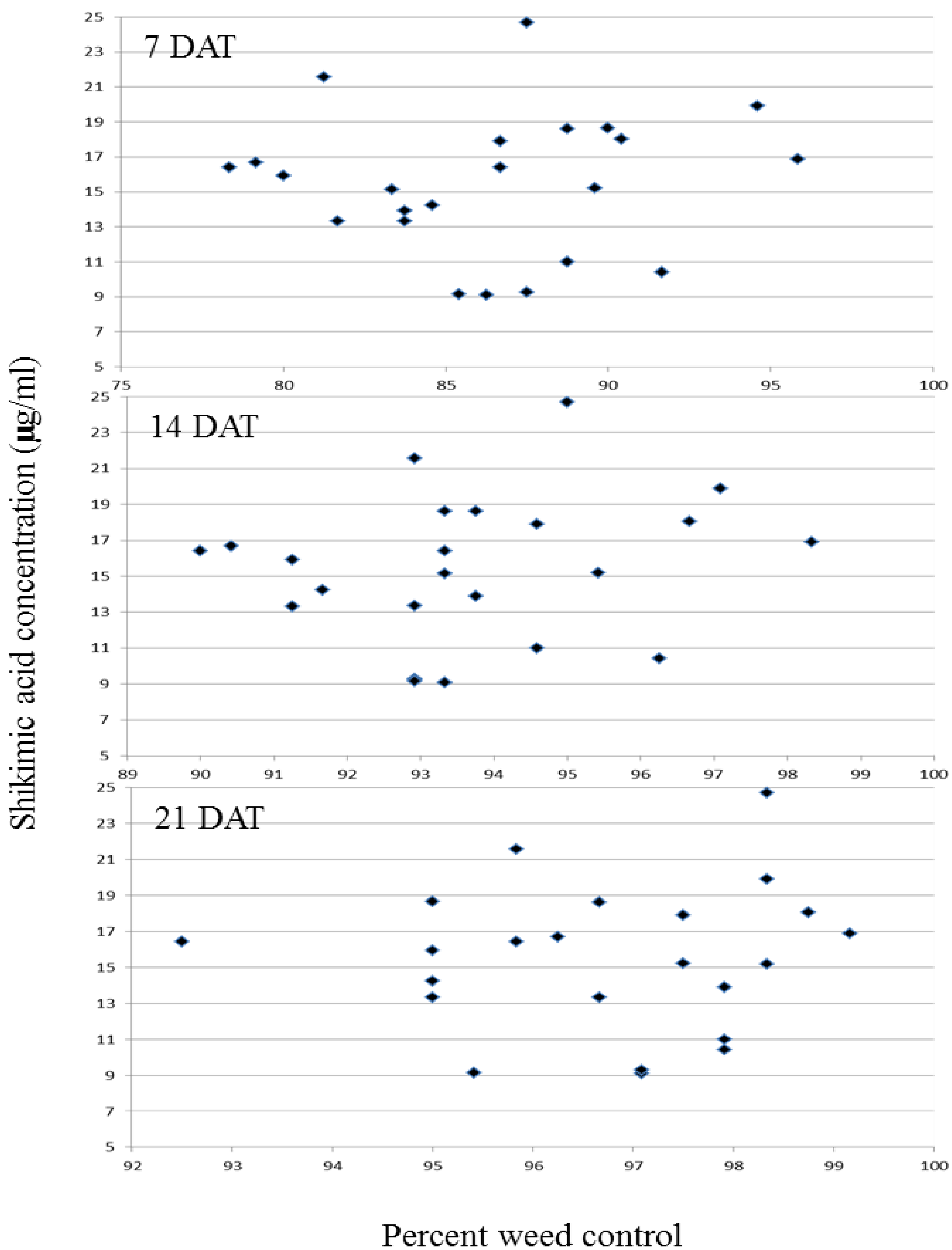

Figure 3.2 Average percent visual control of barnyardgrass in the field 7, 14 and 21 days after glyphosate application compared to average shikimic acid content of greenhouse grown soybean of the same treatment.

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