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The Effect of Anionic Surfactants on Herbicide Mixtures and Solutions

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THE EFFECT OF ANIONIC SURFACTANTS ON HERBICIDE MIXTURES AND SOLUTIONS

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Herbicide mixtures are popular for farmers to delay the evolution of herbicide-resistant biotypes from occurring and control existing herbicide-resistant weeds. Glufosinate is a contact herbicide that has been observed as a mixture partner with many herbicides. In many cases, antagonistic interactions have occurred when using glufosinate in mixture with other herbicides. The antagonistic interactions have resulted in applications with incomplete weed control. Adjuvants have been known to impact an herbicide application by increasing herbicide penetration, spreadability, and efficacy. Adjuvants added to glufosinate mixtures can increase weed control.

The first objective was to investigate the interactions, efficacy, and physical properties of glufosinate, dicamba, or 2,4-D alone or in mixture with one of two different anionic surfactants. The results from the greenhouse study indicated that adding a surfactant to dicamba applied alone or a mixture of dicamba with glufosinate increased biomass reduction to >92 and 96% on common lambsquarters. Results from the field studies showed the highest biomass reduction of Palmer amaranth occurred when dicamba was applied alone (56%). The results from the physical property studies concluded that surfactant two had the lowest surface tension (<35 mN m^{-1}) and the lowest contact angle (41°).

The second objective was to investigate the efficacy, interactions, and physical properties of technical grade glufosinate with no surfactant and glyphosate with a small

formulation of pre-mixed adjuvant applied alone, in mixture, and with one of two different anionic surfactants. The results from the greenhouse experiment indicated that adding a surfactant to glufosinate and glyphosate mixtures applied on common waterhemp resulted in >62% biomass reduction. The results from the field study showed the highest biomass reduction of Palmer amaranth came from a mixture of glufosinate with glyphosate and surfactant two (46%). The results for physical properties concluded that adding a surfactant to glufosinate and glyphosate treatments resulted in an increase in density and viscosity and a decrease in contact angle and surface tension.

The third objective was to evaluate three anionic surfactants at different dose rates added to herbicide mixtures and solutions of glufosinate, dicamba, 2,4-D, and glyphosate. The herbicide by dose effect was significant for both runs. Unformulated glufosinate, Xtendimax, Touchdown Hi-Tech, and mixtures of unformulated glufosinate with Touchdown Hi-Tech or Xtendimax resulted in an increase in biomass reduction when increasing surfactant dose rate.

Dedication

To my mother Dana and to Tony, thank you for always believing in my dreams and aspirations. To my late father, Daniel Anderson, I hope I have made you proud. To my entire family, thank you for all the support.

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CHAPTER 1: LITERATURE REVIEW

Introduction

Agriculture is evolving at an extremely fast pace. With new technology and challenges coming every day, agriculturists are discovering solutions to ongoing issues. One current issue in agriculture revolves around herbicide resistant weeds. Currently, there are 263 weed species expressing herbicide resistance with 23 of the 26 known herbicide sites of action having resistant weeds [1]. Weeds can impact yield dramatically and farmers continue to it a priority to control them as efficiently as possible. One option for controlling herbicide resistant weeds is the inclusion of multiple herbicide modes of action in mixtures. Mixtures containing multiple modes of action will help reduce the evolution of herbicide resistance. Along with multiple modes of action in a tank solution, adjuvants can play a large role in herbicide effectiveness and the efficacy of an application. Research has been conducted to observe how different adjuvants and herbicides can be beneficial or antagonistic when mixed together. It is possible that glufosinate mixtures with the addition of an adjuvant could have a major impact on controlling resistant weeds.

History and Mode of Action of Glufosinate

The glufosinate parent acid was first discovered as a microbial metabolite of *Streptomyces viridochromogenes* in 1972 and was named phosphinothricin or bialophos [2]. The acid was extracted from bacteria to be researched as a potential herbicide. As it was being tested for herbicidal use, glufosinate had the code name of HOE – 39866 (figure 1). In 1981, the ammonium salt of glufosinate was reported as an herbicide [3] with the CAS number listed as 77182-82-2. A nonselective post emergence (POST) herbicide, glufosinate was originally developed as a "burndown" to control vegetation at planting in no-till or stale seedbeds [4]. In 1997, the first transgenic glufosinate-resistant corn was commercially introduced [5].

Duke and Lydon state that glufosinate is a bioactivated herbicide - that is, it must be partly metabolized by the target plant in order to be toxic; Further, it is readily metabolized to phosphinothricin the phytotoxic part of the molecule [6], which inhibits the glutamine synthetase process in plants. In physical form, glufosinate is an ammonia salt, while in its chemical form is made up of phosphinothricin [h0I110alanin-4-yl-(olethyl)phosphinic acid (phosphinothricin acid) [5]. Glufosinate tolerance is conferred to plants by incorporation of either the pat (phosphinothricin acetyltransferase) gene or the bar (bialaphos resistance) gene, whose protein product inactivates glufosinate by acetylation [7]. Inserting either the pat or bar gene into agronomic crops such as corn, soybeans, and cotton has made the glufosinate technology available for post-crop emergence weed control in agronomic crops after crop emergence.

Glufosinate is a contact herbicide that is absorbed by the plant through its foliage. Steckel et al. observed foliar absorption of glufosinate varied depending on the species, absorption of glufosinate increased over time, and foliar absorption of glufosinate was nearly maximum 24 HAT for giant foxtail, barnyardgrass, velvetleaf, and common lambsquarters [8]. When translocating in the plant, glufosinate has been shown to be more phloem-mobile than xylem-mobile. Glufosinate is not very effect in controlling perennial weeds due to its relatively low translocation in plants [7]. Steckel et al. states glufosinate traveled downward to the meristematic tissues and showed great phloem translocation in grasses and observed very little translocation in broadleaved weed species [8]. With limited translocation it is important to have increased coverage of the target weeds when applying glufosinate to obtain adequate weed control.

Glufosinate is classified as a group 10 herbicide. The glufosinate mode of action (MOA) inhibits glutamine synthetase which eventually causes a rapid accumulation of reactive oxygen species; this build up in reactive oxygen species causes the severe phytotoxicity that is associated with glufosinate injury and also causes lipid peroxidation and membrane degradation [9]. Takano explains the ammonia accumulation from this process is a physiological consequence of glutamine synthetase inhibition and does not cause the death of the target plant [9].

Application of Glufosinate

The efficacy of POST herbicides is influenced by environmental conditions before, during, and after the time of application [10]. Among the many environmental factors that can affect herbicide uptake two of the most important are temperature and humidity. Optimal glufosinate uptake is favored by warm, humid conditions [11], with temperature playing a large role. Temperature can affect herbicide uptake by changing the viscosity of cuticle waxes, the rate of diffusion, and in junction with humidity, cuticle hydration [11]. Higher temperatures cause plants to increase respiration rate, causing them to use and uptake more water-soluble solutes. With lower temperatures, plants do not respirate as quickly, causing less water-soluble solutes to be readily taken in. Humidity is vitally important because the cuticle of the leaf must have moisture to allow the herbicide to be absorbed. Low relative humidity prior to, during, and after treatment may cause the cuticle of a plant to be dehydrated, thus possibly reducing absorption of water-soluble herbicides such as glufosinate [8]. Without humid conditions, droplets can dry up quickly before being absorbed by the plant. Coetzer et al. found that four days after treatment, glufosinate rates at 205, 410, and 820 g ha^{-1} controlled Palmer amaranth, redroot pigweed, and common waterhemp on average greater than 80% when plants were grown at 90% relative humidity (RH), whereas glufosinate at 820 g ha^{-1} injured more than 80% of the plants grown at 35% RH [10]. Higher levels of humidity allow glufosinate droplets to not evaporate as quickly, allowing them to be on the leaf surface longer for plant absorption. Based on the previous literature, we can conclude that warm conditions with high humidity are important environmental factors for effective application of glufosinate.

The maturity of plants and the spray application methods are crucial for weed control as well. Steckel et al. reports that young actively growing plants usually have thinner, more permeable cuticles than older plants; thus, water soluble herbicides such as glufosinate may be more effective in penetrating the cuticle of younger plants, and less effective at later application timings [12]. As plants mature, they develop a much denser cuticle, causing the herbicide to have a more difficult time entering the plant. Steckle et al. also observed erratic control of 15 cm tall giant foxtail, common lambsquarters, common cocklebur, and Pennsylvania smartweed was due primarily to an inadequate coverage of spray solution [12]. Smaller weeds have less surface area than larger weeds, allowing for a better chance to receive full coverage when making an application. The labels for glufosinate applications are specific and should be followed to increase the chance of adequate weed control. The Liberty® (BASF, 100 Park Ave, Florham Park, NJ, USA) label states, for ground application, using a nozzle that creates medium to coarse droplets is best for the product because they can provide adequate coverage on the leaf surface, opposed to smaller fine droplet sizes.

Glufosinate Adjuvants

An adjuvant is any substance in an herbicide formulation or added to the spray tank to modify herbicidal activity or application characteristics [13]. Two main categories of adjuvants consists of in-can adjuvants and tank-mix adjuvants. In-can adjuvants are adjuvants added to an active ingredient for the formulation of an herbicide. Tank-mix adjuvants are adjuvants added by the applicator to the tank solution. Adjuvants can help the application of herbicides by improving herbicidal efficacy, but this is not always the case. There are two types of adjuvants that can be formulated into an herbicide or added by the end user: activator adjuvants and utility adjuvants. Activator adjuvants directly enhance the efficacy of an herbicide once it has been deposited on the target surfaces, where utility adjuvants generally work on the properties of the spray solution or the spray mixture and do not directly affect herbicide efficacy [14]. Activator adjuvants help with the absorption of the herbicide droplets into the plant. Utility adjuvants ensure applicators that the herbicide solution interacts homogenously inside the tank. Both activator and utility adjuvants play critical roles when choosing a product and making an application. It is important to follow the label directions for adjuvants based upon the herbicide being used, the crop in which the application will take place, the target weed species, and the size of the targeted weeds.

Adjuvants are used with POST herbicides to improve spray delivery, increase retention of the spray on weed foliage, and enhance foliar penetration, thus increasing herbicide selectivity and effectiveness [15]. Knowing the target weed species is crucial to choosing an adjuvant. Different weed species have different physiological characteristics that need to be overcome for an herbicide to enter a plant. The cuticle structure and composition vary from species to species, although there appear to be five basic types: smooth, ridged, papillose, glaucous (having an additional covering of microcrystalline wax), and glandular where trichomes are present in high number and comprise the main surface of the leaf [11]. Different leaf surfaces could have different effects on the herbicide being sprayed. Regardless of the leaf surface, adjuvants can assist the plant in absorption of the herbicide into the leaf tissue.

An inert ingredient is an ingredient that is premixed into an herbicide product when bought by the applicator. The Liberty® safety data sheet (SDS) states that there are two different inert ingredients used in the Liberty® formulation: alkylethersulfate (sodium salt) and alkyl polysaccharide. The alkylethersulfate used in Liberty® is a polyethylene glycol mono-C12-14-alkyl ether sulfate sodium salt and has a CAS number of 68891-38-3. It is made up of a C12-C14 carbon chain with two moles ethylene oxides attached. The second inert formulated into Liberty® is an alkyl polysaccharide, or called decyl glucoside, CAS number 68515-73-1. It is created by using a condensation of fatty decyl alcohol and a d-glucose polymer and is a non-ionic cleansing agent.

The Liberty® label recommends that ammonium sulfate (AMS) should be added to the tank solution as an adjuvant. The Liberty® label states that AMS is beneficial in difficult environments or when applying glufosinate with hard water due to neutralization of cations. Jones et al. concluded that glufosinate efficacy has been shown to be enhanced with the addition of ammonium sulfate on certain weed species [16]. It has been reported that the use of 5% AMS (w/v) resulted in a significant increase in glufosinate absorption in green foxtail and sicklepod, with absorption remaining unchanged in common milkweed and horsenettle and resulted in a significant decrease in common lambsquarters absorption at 12 h after treatment [17]. Maschhoff et al. concluded that AMS increased the total translocation of absorbed 14C glufosinate out of the treated leaf in velvetleaf from 1 to 4% and in giant foxtail from 5 to 7% but observed no effect on the translocation of 14C from 14C-glufosinate in common lambsquarters [18].

Pratt et al. reported that 2% AMS and Class Act Next Generation® were the only two adjuvants that consistently enhanced glufosinate efficiency for velvetleaf control [19]. The Class Act Next Generation® (WinField United, 4001 Lexington Ave N, Arden Hills, MN, USA) label states that it is a watering condition agent/non-ionic surfactant blend that is composed of ammonium sulfate, corn syrup, and alkyl polyglucoside.

Basta® (BASF, 100 Park Ave, Florham Park, NJ, USA), a glufosinate formulation manufactured by BASF, does not require adjuvants on the label. The label dose state that using adjuvants or wetting agents on hard-to-wet weeds can provide beneficial results. The Basta® label states that using the adjuvant Nu-Film P® (Miller Chemical and Fertilizer, P.O. Box 333 Hanover, Pennsylvania, USA) or Exit® (Miller Chemical and Fertilizer, P.O. Box 333 Hanover, Pennsylvania, USA) will help with control of pine trees in a forest setting. According to the Nu-Film P® label, it is a sticking-extending adjuvant with non-ionic properties that extends the active ingredients life after application. According to the label, Nu-Film P® “produces a film over the top of the plant that does not allow environmental factors to interfere with the application”. The SDS states that it is composed of terpene polymers, mineral oil, alkyl amine ethoxylate. The adjuvant Exit® is designed to be a deposition agent when mixed with herbicides. It also increases surface activity of the herbicide when applied to the target weed species. This causes an increase in absorption and translocation over time under specific environmental conditions. The SDS states it is composed of methyl esters of fatty acids, N, N-Bis 2-(omega-hydroxypolyoxyethylene) ethyl) alkylamine, and tall oil fatty acids. Further research needs to be conducted to better understand the relationship different adjuvants have on a glufosinate application.

Glufosinate Mixed with Glyphosate

Mixing herbicides has been shown to be more effective in reducing resistance evolution than using herbicides in a rotation [20]. Herbicide active ingredients with different modes of action in mixture should have a common weed control spectrum, similar efficacy and persistence, along with different metabolic pathways to effectively reduce the selection pressure and delay the evolution of herbicide-resistant weeds [21]. It has been demonstrated that herbicides applied in mixture or sequentially may interact and result in synergistic, antagonistic, or additive response [22]. Chuah et al. states that the joint action of herbicides in combination is described as 'antagonistic' if the actual control is less than the predicted control, 'synergistic' if the actual control is greater than the predicted control and 'additive' if the weed control from the mixed combination is equivalent to the predicted control [23]. Understanding the interaction when mixing herbicides is important to get the highest weed control possible.

Mixtures of glufosinate with glyphosate can help control weed species that are expressing resistance to glyphosate by giving applicators two modes of action to help delay resistance of non-herbicide resistant weeds and a second MOA, glufosinate, which can control herbicide resistant weeds that already exist. Glyphosate controls a broad spectrum of grass and broadleaf weeds, has a favorable environmental profile and has low mammalian toxicity [24]. Glyphosate is a part of the group 9 herbicide family and inhibits the enzyme 5-enolypruvyl-shikimate-3-phosphate amino acid synthesis in plants [25]. Glyphosate can be sprayed postemergense in glyphosate resistant crops such as corn, cotton, or soybeans.

Applications of glufosinate with glyphosate in mixture has shown to be inconsistent. In giant foxtail, no early synergism was observed at 7 DAT using glyphosate with glufosinate in mixture but at 28 DAT, antagonism was observed with these mixtures when below labeled rates of glufosinate were applied [20]. Chuah et al. observed antagonism with mixtures of glyphosate with glufosinate and reported all nine mixtures showed antagonism on goosegrass (*Eleusine Indica* (L.) [23]. Besancon et al. states fluorescent measurements have confirmed the rapid action of glufosinate results in the breakdown of the PSII system, therefore reducing the glyphosate translocation resulting in an antagonistic interaction [26]. If the PSII system can remain in function, glyphosate can translocate throughout the plant and allow for antagonism to be mitigated. Besancon et al. reported that reduced translocation of glyphosate is the physiological mechanism responsible for the antagonism observed between glyphosate and glufosinate in giant foxtail, and to a lesser extent, in velvetleaf [26].

Inconsistent results with glufosinate mixed with glyphosate has been reported throughout the literature. More research must be conducted to confirm how the antagonism is occurring.

Glufosinate Mixed with Dicamba or 2,4-D

Dicamba is a POST applied herbicide used to control broadleaf weed species in fallow and dicamba tolerant crops such as cotton, corn, and most recently soybeans. It is listed as a group 4 herbicide and attacks normal cell division causing cells to be disrupted. This leads to the plant having malformed growth, tumors, and eventually plant death.

Merchant et al. observed mixing dicamba with glufosinate generally had an increased control of horseweed, common lambsquarters, and Palmer amaranth [27]. Barnett et al. observed combinations of dicamba mixed with glufosinate resulted in increased giant ragweed control when compared with treatments of dicamba alone [28].

Mixing glufosinate with dicamba can also provide residual herbicide activity compared to glufosinate alone. Dicamba at 0.28 kg ai/ha tank mixed with glufosinate provided some residual control compared to glufosinate alone [29]. As mentioned earlier, glufosinate does not provide residual soil activity due to microbes in the soil breaking it down rapidly.

2,4-Dichlorophenoxy acetic acid (2,4-D) is a foliar applied POST herbicide. Because of the activity on broadleaf weeds, low cost, and low probability of resistance, 2,4-D is an attractive option for summer annual broadleaf weed control [30]. 2,4-D is in group 4 and is classified as part of the phenoxy herbicide family. At low doses, 2,4-D promotes plant growth while at high doses it drives plant overgrowth, including cupping and stunting of leaves, brittleness, stunting and twisting of stems, and general abnormal growth [31]. Currently, 2,4-D is labeled for use in a variety of different plant species such as corn, grain sorghum, rice, sugarcane, turf grass, various small grain and grass forage crops, as well as noncrop uses [32]. With such a wide variety of uses, 2,4-D remains a popular choice for herbicide application.

Craigmyle et al. observed the addition of 2,4-D to multiple rates of glufosinate increased the control of common waterhemp compared to sequential applications of glufosinate alone regardless of application timing. Increasing the 2,4-D rate did not improve the level of grass or broadleaf weed control when applied in combination with glufosinate [32]. Barnett et al. observed 2,4-D applied alone only resulted in 47 and 64% control of giant ragweed and the mixture of glufosinate plus 2,4-D provided greater than 96% control [28].

Conclusions

Further research must be conducted to understand how adjuvants impact glufosinate, especially when mixed with other herbicide formulations. AMS is the only adjuvant that is recommend with glufosinate across all labels. Other adjuvants could be beneficial when working with glufosinate applications. Studies have shown that mixing glufosinate with dicamba or 2,4-D can result in better weed control on certain weed species [27.28,32]. Antagonisms caused by mixing glufosinate with glyphoate has been overcome by adding a higher rate of glufosinate [20]. Both dicamba and 2,4-D resulted in better weed control when mixed with glufosinate as opposed to glufosinate alone. With the correct adjuvants, and mixing multiple modes of action, an increase in weed control can occur and help with the management of herbicide resistance.

Objectives

The objectives of this research were: (1) investigate the interactions, efficacy, and physical properties of glufosinate, dicamba, or 2,4-D alone or in mixture with two anionic surfactants; (2) Observe and evaluate the efficacy, interactions, and physical properties of unformulated glufosinate and unloaded glyphosate alone, in mixture, and with two anionic surfactants; and (3) evaluate three anionic surfactants at different dose rates when added to herbicide mixtures and solutions of glufosinate, dicamba, 2,4-D, and glyphosate.

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CHAPTER 2: EFFECT OF SURFACTANTS ASSOCIATED WITH POST EMERGENT HERBICIDES ON PHYSICAL PROPERTIES AND WEED CONTROL

Introduction

Glufosinate is a nonselective, post-emergent (POST), contact herbicide used on glufosinate tolerant crops, orchards, vineyards, and noncropland sites for control of emerged vegetation [1-3]. Currently, glufosinate is applied as a POST over the top application to glufosinate tolerant crops including soybeans, cotton, canola, corn, and sugar beets. Glufosinate inhibits the glutamine synthetase enzyme [1]. The phytotoxicity caused by glutamine synthetase inhibition is caused by the accumulation of reactive oxygen species causing rapid cell death inside the treated plant tissue [4].

Combining multiple modes of action (MOA) in mixture with glufosinate could help control herbicide resistant weeds by allowing for different metabolic pathways to effectively reduce selection pressure and delay the evolution of herbicide-resistant weeds [5]. Bethke et al. states that mixing herbicides has been shown to be more effective in reducing resistance evolution than using different herbicide MOAs in rotation [6]. Applying mixtures of herbicides can be less labor intensive, save time, and result in better weed control of certain species, compared to single MOAs [6].

Dicamba and 2,4-D are both mix options for glufosinate. Both herbicides are synthetic auxins and are applied over the top of crops as a POST source of weed control. Low doses of synthetic auxin herbicides can have similar hormonal properties to natural auxins; high rates can cause growth abnormalities such as leaf epinasty, cupping of leaves, thickening of stems and roots, chlorosis and necrosis [7]. Auxin herbicides MOA can be divided into three consecutive phases in the plant which include the simulation of abnormal growth and gene expression, inhibition of growth and physiological responses (such as stomatal closure), and finally cell death [8,9].

Glufosinate mixed with dicamba or 2,4-D has resulted in control of specific weed species. Merchant et al. reported mixing dicamba with glufosinate caused an increase in control of horseweed, common lambsquarters, and Palmer amaranth [10]. Craigmyle et at. demonstrated the addition of 2,4-D to any rate of glufosinate enhanced the level of common waterhemp control compared to sequential applications of glufosinate alone, regardless of application timing [11].

Mixing multiple MOA can be beneficial resulting in synergism, while in other situations, antagonism can occur. Antagonism has been observed with many different herbicides mixed together [12-16]. Antagonism is caused by a variety of different parameters such as herbicide rate, plant species, and MOAs being mixed [17].

One hypothesis to explain the antagonism of glufosinate mixtures is that glufosinate may cause rapid injury, decreasing the absorption and translocation of the mixed herbicides [18]. Antagonism is a reoccurring issue with multiple herbicides in mixture and further research is needed to better understand why antagonisms continue to occur [6,12].

Adjuvants are used with POST herbicides to improve spray delivery, increase retention of the solution on weed foliage, and enhance foliar penetration, thus increasing herbicide selectivity and effectiveness [19]. The use of adjuvants, especially surfactants, can significantly accelerate the penetration of herbicides into the cuticle [20]. Costa et al.

observed enhanced glufosinate performance with the addition of two nonionic surfactant blended adjuvants on Palmer amaranth [21]. Pratt et al. tested eight adjuvant solutions with glufosinate, and found the treatments containing the highest levels of ammonium preformed the greatest on velvetleaf control [22]. Currently, ammonium sulfate (AMS) is one of few adjuvants recommended for glufosinate.

Adjuvants have been shown to impact herbicide mixture antagonism by increasing herbicide absorption and preventing the formation of less preferred absorption forms of weakly acidic herbicides [19]. Wanamarta et al. reported that adding a surfactant at a rate of 4.8 L ha^{-1} to a mixture of the sodium salt of bentazone and sethoxydim overcame antagonism when compared to both herbicides mixed without the surfactant [23]. Adding a surfactant to glufosinate mixtures could overcome antagonisms that have been documented in literature.

Multiple MOA in mixture can increase weed control of certain weed species. The interactions and how to efficiently use these chemistries mixed together is still unclear. Antagonism of herbicide mixtures is a reoccurring issue [6,12,14] and further research is needed to better understand the interactions that are occurring. Adjuvants have been proven beneficial when used with glufosinate. There is little research regarding adjuvants added to mixtures of glufosinate with dicamba or 2,4-D. The use of an adjuvant could be beneficial in resolving antagonisms involving herbicide mixtures containing glufosinate.

The objective of this study was to 1)investigate the efficacy and interaction of two anionic surfactants added to unformulated glufosinate, dicamba, or 2,4-D alone or together in mixture on three broadleaved species, 2) evaluate and observe the interactions between glufosinate, dicamba, or 2,4-D mixtures and solutions on control of Palmer amaranth and kochia at two locations in Nebraska, and 3) evaluate the physical properties including density, viscosity, surface tension, and contact angle for glufosinate, dicamba, or 2,4-D mixtures and solutions.

Materials and Methods

Greenhouse Study

Greenhouse studies were conducted in the summer of 2019 at the Pesticide Application Technology Laboratory located at The University of Nebraska-Lincoln West Central Research, Education and Extension Center in North Platte, Nebraska. Five weed species were tested: common waterhemp (*Amaranthus tuberculatus (moq.) J. D. Sauer),* velvetleaf (*Abutilon theophrasti Medik),* common lambsquarters (*Chenopodium album L.),* barnyardgrass *(Echinochloa crus-galli (L.) P. Beauv),* and large crabgrass *(Digitaria sanguinalis (L.) Scop)*. Weed species were grown in individual 656 ml cone-tainers (Stuewe and Sons Inc., Corvallis, OR, USA) using a peat moss potting mix (Pro-Mix. Premier Tech, Quakertown PA, USA). Plants were watered with a 5-1-4 fertilizer blend (Wilbur Ellis, San Francisco, CA, USA) injected into irrigation water at 0.2% v:v. Greenhouse temperature was maintained at 28 C during the day and 18 C at night. Supplemental lighting was provided by Philips GreenPower LED toplighting (USA) to achieve a 16-hour photo period. Treatments were applied when plants reached 15-20 cm tall.

Solutions were prepared using 340 g ae ha^{-1} of technical grade unformulated glufosinate (CRODA Atlas Point, New Castle DE, USA) containing no adjuvant package, 280 g ae ha^{-1} of dicamba (Xtendimax® Bayer CropScience, Research Triangle Park, NC, USA), and 530 g ae ha^{-1} of 2,4-D (Enlist One® Corteva Agriscience, Wilmington, DE, USA) alone or in mixture with two experimental anionic surfactants applied at a 1% v/v: S1 and S2 (CRODA Atlas Point, New Castle DE, USA). Herbicide solutions were identified as treatments containing a single herbicide. Herbicide mixtures were identified as treatments containing multiple herbicides. Unformulated glufosinate was created in a laboratory with phosphinic acid, ammonia, and water. The amount of active ingredient was equivalent to Liberty 280 SL® (BASF, Florham Park, NJ, USA). Technical grade unformulated glufosinate was used to deliver the same amount of active ingredient as formulated glufosinate without a pre-mixed surfactant in its formulation, to better understand the reports of antagonism in literature. Reduced rates, compared to label recommended field rates, were used with herbicides to ensure that complete control did not occur in order to better observe differences among treatments.

Applications were made using a single nozzle spray chamber (Devries Manufacturing, Hollandale, MN) with an AI95015EVS TeeJet nozzle (Teejet Technologies, Spraying Systems Co., Springfield, IL, USA) delivering a carrier volume of 140L ha^{-1} with a pressure of 276 kPa at 2.9 k h^{-1}. The AI95015EVS nozzle was specifically used to ensure the correct rate and fan development for the application, as a single nozzle does not achieve the proper nozzle pattern overlap. At 28 days after treatment (DAT), above ground biomass was harvest and placed in a dryer (65 ˚C) for so many days to obtain consistent moisture content between samples.

The experiment was conducted as a completely randomized design with 16 treatments with four replications across two runs. Factorial treatment structure consisted of 5x2 (herbicide x adjuvant) full factorial with the factors consisting of glufosinate, dicamba, 2,4-D, glufosinate-dicamba mixed, and glufosinate-2,4-D mixed by S1 and S2.

Each Species was analyzed separately. Dry biomass data was measured, and percent biomass reduction was calculated. Data was subjected to ANOVA using SAS v9.4 (SAS, Cary, NC) with Fisher's test of least significance ($\alpha = 0.05$).

Field Study

Field studies were conducted during the summer of 2020. Two site locations were used for this experiment with the first location being at the University of Nebraska West Central Research and Extension Center in North Platte Nebraska (41.5˚N, -100.46˚W) and the second location at the University of Nebraska Panhandle Research and Extension Center in Scottsbluff Nebraska (41.8˚N, -103.6˚W). North Platte soil consisted of a Cozad silt loam, while Scotts Bluff soil consisted of a Tripp very fine sandy loam.

North Platte maintenance included a burndown treatment of Paraquat applied at two pints/acre in May of 2020 to help eradicate existing weeds. Palmer Amaranth was the target weed species with 7,750 plants per m^2. Individual treatment plots were three meters wide by seven and a half meters long.

The Scottsbluff trial area had been in fallow for the previous four year with no tillage, irrigation or crops planted. Kochia was allowed to mature to seed and in late fall was mowed using a rotary mower to distribute seed throughout the field. Individual plots were three meters wide by six meters long. Kochia was targeted at 15 to 20 cm tall with 21 kochia per m^2.

At both locations, treatments were applied when plants reached a height of 15 – 20 cm tall. Treatments and treatment rates were the same as described in the greenhouse experiment. The applications were applied using a six nozzle CO2 backpack sprayer with 50 cm nozzle spacing calibrated to deliver 140L ha^{-1} with a pressure of 276 kPa using a

TTI11002 nozzle. At 28 DAT, ten plants per plot were randomly selected and harvested and placed in a dryer (65˚C) to obtain a constant biomass.

The experiment was set up as a randomized complete block design with four replications per treatment. An untreated check was also included. Factorial treatment structure consisted of a 5x2 with factors consisting of five herbicides which included glufosinate, dicamba, 2,4-D, glufosinate-2,4-D, glufosinate-dicamba and the two experimental surfactants which included S1 and S2. Dry biomass data was recorded, and percent biomass reduction was calculated. Data was then subjected to ANOVA using SAS v9.4 with Fisher's test of least significant ($\alpha = 0.05$).

Field treatments containing multiple herbicides were analyzed by the model proposed by Colby [24] to determine if the interaction was synergistic, antagonistic, or additive:

$$E = 100 - \frac{(100X) * (100 - Y)}{100}$$

Where *E* is the percentage of dry weight expected from the mixture, *X* and *Y* are the percent biomass reduction or the percent of dry weights obtained from herbicides applied alone or with S1 or S2. A table with the estimated data through the Colby model was elaborated and preformed comparing observed data percentage of dry weight. To determine the interaction between herbicides, a t-test was preformed comparing estimated data values from Colby's method with data values observed using Banzato and Kronka's [25] equation:

$$t = \frac{\widehat{m} - A}{s(\widehat{m})}$$

Where $\hat{m}$ is the estimated value, A is the observed value, and $s(\hat{m})$ is the standard error of the mean. From this formula, conclusions were made to determine the interaction of the herbicide mixture. Synergism occurred when the data was higher than the estimated data and the "*t*" value was less than 0.05. Antagonism was observed when the data was lower than the estimated data and when the "*t*" value was less than 0.05. When the "*t*" value was greater than 0.05 the interaction was considered additive.

Physical Properties

Density, viscosity, surface tension, and contact angle of 15 spray solutions and water alone were measured at the Pesticide Application Technology Laboratory located at The University of Nebraska-Lincoln's West Central Research, Education, and Extension Center in North Platte, NE. The treatments used for this part of the experiment were the same at mentioned in the greenhouse and field studies.

Density and viscosity measurements were analyzed at a constant temperature of 25 ˚C using a DMA^{TM} 4500 M density meter (Anton Paar USA Inc., Ashland, VA) along with the microviscomter Lovis 2000 M/ME (Anton Paar USA Inc., Ashland, VA) which was attached to the density meter. Further parameters, information, and methodology involving the density and viscosity measurements can be found in Moraes et al. [26] paper.

Surface tension and contact angle measurements were taken using an OCA 15EC (DataPhysics Instruments GmbH, Filderstadt, Germany) using video-based optical contact angle measuring. The equipment uses a video measuring system with a USB camera. The camera is equipped with a high-performance 6X parfocal zoom lens with integrated continuous fine focus, camera tilt angle, and adjustable observation. SCA software is used to collect, analyze, and evaluate the measured data. Surface tension and contact angle measurements were conducted at 25˚C ± 1˚C and at four different relative humidities which included 20, 40, 60, and 80 ± 1%. Temperature and humidity were held constant by an environmental chamber. The chamber temperature was adjusted by a liquid circulator (Julabo USA Inc, Allentown, PA), while the humidity was produced using a humidity generator control (DataPhysics Instruments GmbH, Filderstadt, Germany). Values for humidity and temperature are displayed on the control panel allowing for the operator to check and adjust the parameters in real time. The environmental chamber is built containing three windows made of glass to directly observe samples as measurements are taken. Further parameters, information, and methodology involving the surface tension and contact angle measurements can be found in Moraes et al. [26] paper.

Density, viscosity, surface tension, and contact angle were analyzed separately. Surface tension and contact angle were analyzed based on the relative humidity of 20, 40, 60, or 80%. Data was subjected to analysis of variance (ANOVA) using a generalized linear mixed model (PROC GLIMMIX) in SAS (Statistical Analysis Software, Version 9.4, Cary, NC). Mean separations occurred at an $\alpha = 0.05$ level using Fisher's protected least significant difference (LSD) test and the Tukey adjustment.

Results and Discussion

Greenhouse Study

There was a significant interaction when observing the herbicide by adjuvant interaction across treatments and species (p-value<0.05) (Table 2.1).

Glufosinate alone resulted in <55% biomass reduction across species. Adding S1 or S2 to the tank solution increased biomass reduction across species to >89% (Table

2.2). Common lambsquarters increased from 8% biomass reduction with glufosinate alone to >95% when adding a surfactant. Both S1 and S2 with glufosinate increased biomass reduction to >95% for both grass species in this experiment.

Dicamba resulted in >88% biomass reduction on velvetleaf and common waterhemp, regardless of if it was alone or with a surfactant (Table 2.2). Large crabgrass and barnyard grass both resulted in <37% biomass reduction with no differences when dicamba was applied alone or with a surfactant. Common lambsquarters resulted in 49% biomass reduction with dicamba applied alone and >92% when applied with a surfactant.

2,4-D resulted in >90% biomass reduction across broadleaves, regardless of if it was alone in the tank or if a surfactant was added (Table 2.2). Barnyardgrass and large crabgrass resulted in <60% biomass reduction with 2,4-D treatments.

Both dicamba and 2,4-D performed well on broadleaf species regardless of if a surfactant was added to the tank. Glufosinate was greatly impacted by both surfactants compared to being applied alone (Table 2.2). This can be attributed to the use of unformulated glufosinate for this experiment, which had no surfactant package. Surfactants can be beneficial when incorporated into a tank solution and can help with control of certain weed species. Johnson et al. tested citric ester surfactants with formulated glufosinate and observed that two of the surfactants increased weed control on common lambsquarters and giant foxtail 14 days after treatment compared to the formulated glufosinate alone [27]. Harbour et al. results showed that adding an experimental surfactant to glyphosate increased control of Russian thistle from 8% with no surfactant to 68% with surfactant [28]. This would help explain why a large increase in biomass reduction occurred when adding the surfactant to the unformulated glufosinate.

The only differences observed on broadleaf biomass reduction was with the glufosinate tank solutions across the three species and the dicamba tank solutions on common lambsquarters (Table 2.2). This would lead to the observation that when targeting specific weed species, dicamba or 2,4-D may not need a surfactant added to the tank solution. Harbour et al. observed an increase in phytotoxicity when using surfactants with 2,4-D on kochia and reported no differences in weed control compared to the 2,4-D treatment applied alone [28]. Creech et al. observed no differences when adding a non-ionic surfactant to dicamba on control of grain amaranth or velvetleaf [29]. Species dependent, high control from 2,4-D or dicamba alone may control weeds appropriately, not needing a surfactant to be added to the tank solution.

>95% biomass reduction of grasses occurred when adding a surfactant to glufosinate (Table 2.2). These findings would agree with Costa et al. who found that adding a surfactant to glufosinate resulted in 75% control of broadleaf signalgrass compared to 43% when glufosinate was applied alone [21]. Adding a surfactant to a glufosinate tank solution could be extremely beneficial in controlling grass species. With glufosinate having low activity of grasses, more research is needed to understand surfactants used with glufosinate for grass control.

Mixing dicamba with glufosinate resulted in <62% biomass reduction for common lambsquarters, barnyardgrass, and large crabgrass (Table 2.2). Adding S1or S2 to a mixture of dicamba with glufosinate increased biomass reduction to >96% for common lambsquarters, barnyardgrass, and large crabgrass. >90% biomass reduction of velvetleaf and common waterhemp was observed regardless of if a surfactant was added to a mixture of dicamba with glufosinate. 2,4-D mixtures resulted in >90% biomass reduction across species and treatments.

Mixtures of glufosinate with dicamba resulted in >95% biomass reduction on velvetleaf and common waterhemp (Table 2.2). These results are similar with Steckel et al., who observed both a low and high rate of dicamba mixed with glufosinate resulted in 97 and 94% control of glyphosate resistant horseweed 14 days after treatment [30]. Barnett et al. reported 91 and 88% control of giant ragweed with mixtures of dicamba with glufosinate 30 days after treatment [31]. Species dependent, using mixtures of glufosinate with dicamba can result in weed control.

Mixing dicamba with glufosinate alone without a surfactant resulted in <62% biomass reduction on grasses in this experiment. (Table 2.2). When a surfactant was added to this mixture, biomass reduction increased to >98%. Both glufosinate and dicamba have low activity when applied to grasses, as seen when applied without the surfactant. When adding a surfactant to the tank solution, an increase biomass reduction occurred. This would provide evidence that adding a surfactant to dicamba mixed with glufosinate could result in greater grass biomass reduction.

Mixtures of glufosinate with 2,4-D resulted in >90% biomass reduction for species in this experiment (Table 2.2). Eubank et al. observed 97% control four weeks after treatment when using 2,4-D mixed with glufosinate on glyphosate resistant horseweed [32]. Chahal and Johnson reported mixing 2,4-D with glufosinate resulted in 100% control of glyphosate resistant horseweed three weeks after application and 84% control of glyphosate resistant common lambsquarters four weeks after application [33]. The results from Chahal and Johnson would agree with the greenhouse study that mixing glufosinate with 2,4-D can result in high weed control. Adding a surfactant to a mixture of glufosinate and 2,4-D resulted in the same biomass reduction as the treatment without a surfactant, which would conclude that a surfactant is not needed when mixing both chemistries.

Field Study at the North Platte Location: Palmer amaranth

In the North Platte location, the only effect that was significant was the herbicide effect at an $\alpha=0.05$ (Table 2.3). The surfactant effect and the herbicide*surfactant interaction were not significant.

When applied to Palmer amaranth, surfactants added to herbicide solutions or mixtures did not influence biomass reduction. The herbicides applied did affect the biomass reduction of Palmer amaranth with the highest coming from dicamba alone treatment (56%) (Table 2.4). Glufosinate alone resulted in 32% biomass reduction. Adding glufosinate to mixtures of dicamba or 2,4-D resulted in <51% biomass reduction with no differences when compared to dicamba or 2,4-D applied alone. Colby's equation resulted in synergism for mixtures except for glufosinate with dicamba which resulted in additivity (Table 2.6).

The low biomass reduction of Palmer amaranth can be attributed to the unformulated glufosinate not having a surfactant package. When adding glufosinate to dicamba or 2,4-D, biomass reduction increased to >38% (Table 2.4). Surfactants can influence weed control based on a variety of different factors such as weed species being targeted and the herbicides being applied [34-36]. In this situation, neither surfactant impacted the biomass reduction of Palmer amaranth when added to an herbicide solution or mixture.

The synergistic interactions derived from the Colby equation were expected. When glufosinate was applied alone, low biomass reduction was observed. When mixed with another active ingredient an increase in biomass reduction occurred because of the pre-mixed adjuvants formulated into the dicamba and 2,4-D. There were no differences from the dicamba or 2,4-D alone treatments, compared to when mixed with glufosinate. These results indicate that when using dicamba or 2,4-D, adding glufosinate may not be needed to control Palmer amaranth.

Field Study at the Scottsbluff Location: kochia

At the Scotts Bluff location, there were no significant effects or interactions when observing treatments on kochia biomass reduction at an α=0.05 (Table 2.3).

The only significant treatment on kochia was adding S1 or S2 to glufosinate. Glufosinate alone resulted in 1% biomass reduction (Table 2.5). Adding S1 or S2 to the tank solution increased biomass reduction to >54%. The greatest biomass reduction came from the tank solution of dicamba and S1, resulting in 63%. No differences were observed amongst treatments containing dicamba or 2,4-D.

These results would agree with Harbour et al. who noticed 2%, 68%, and 18% control of Russian thistle when testing different surfactants with glyphosate [28]. Many surfactants are formulated differently and interact differently with different products when added to an herbicide solution or mixture. This could explain why the only differences observed came from glufosinate with S1 or S2 as the glufosinate alone treatment would have contained no surfactant. This shows the beneficial impact that surfactants can have on an herbicide application.

Mixing glufosinate with either growth regulator herbicide resulted in <60% biomass reduction with no differences observed (Table 2.5). When observing the Colby analysis, results indicated synergism and additivity for herbicide mixtures (Table 2.6). It is interesting to note that the synergistic responses came from the mixtures of glufosinate with dicamba or glufosinate with 2,4-D applied with no surfactant, while the other mixtures with surfactants resulted in additivity. This could be due to inert ingredients formulated into the dicamba and 2,4-D formulated herbicides not cooperating with the surfactants added to the mixture. Another theory could be that these herbicide mixtures are species dependent and did not reduce the biomass of kochia. Further research is needed to better understand how inert ingredients in formulated products interact with surfactants added to an herbicide mixture and how these mixtures control multiple weed species.

Physical Properties

Density and Viscosity

Both density and viscosity were significant when ran in ANOVA with a p-value < 0.05 (Table 2.7). The lowest density recorded came from water followed by unformulated glufosinate, which was expected because both of these treatments had no surfactant or adjuvant package in their formulation (Table 2.9). Treatments containing a surfactant or a formulated herbicide containing an adjuvant package increased the density to >1 g cm^{-3}. Overall, the highest density values came from unformulated glufosinate mixed with 2,4-D and S1 or S2, resulting in 1.0020 g cm^{-3}, which could be because the 2,4-D used in this experiment already has a large adjuvant package built into the formulation of the product. Moraes et al. also saw an increase in density when using formulated products and adjuvants compared to water alone [26]. It is also critical to note that even though differences were observed amongst treatments, the highest density observed was 1.0020 g cm^{-3} while the lowest density was water at 0.9987 g cm^{-3} which is only a 0.0033 g cm^{-3} difference in density value.

Water, glufosinate, and dicamba resulted in the lowest viscosity readings (Table 2.9). Once again, this could be attributed to no adjuvants formulated into the water and unformulated glufosinate. The formulation of dicamba used in this experiment also has a small adjuvant package, which could explain why it was similar to that of unformulated glufosinate and water. Treatments containing 2,4-D or a surfactant increased the viscosity to >1.0163 mPa s.

Surface Tension and Contact Angle

The surface tension by relative humidity and the contact angle by relative humidity interactions were both significant at an $\alpha=0.05$ (Table 2.8). Surface tension at 20% RH resulted in water, unformulated glufosinate, dicamba, and glufosinate mixed with dicamba having the highest surface tension at >73 mN m^{-1} and the lowest surface tension from treatments having S2 in solution (Table 2.9). S1 and S2 dropped surface tension of unformulated glufosinate and dicamba from 74 mN m^{-1} without a surfactant to <34 mN m^{-1} with S1 or S2. Curran et al. states that the purpose of surfactants is to reduce the surface tension of the spray solution for more contact between the spray droplet and the plant surface [37]. Xu et al. evaluated the surface tension of two surfactants with distilled water and received a surface tension of <33.7 dyne cm^{-1} compared to 72.8 dyne cm^{-1} with distilled water alone [38]. Surfactants help with the overall surface to droplet contact, which is why a decrease in surface tension is observed when applied to a surface.

40, 60, and 80% relative humidity saw similar trends with water, glufosinate, dicamba, and glufosinate mixed with dicamba having the highest surface tension and the treatments with S2 having the lowest surface tension.

Contact angles ranged from a high of 77˚ to a low of 21˚ across levels of RH and treatments (Table 2.9). Treatments containing S2 tended to have the lowest contact angle amongst treatments, followed by treatments containing S1. Generally, across all levels of RH, adding a surfactant decreased the contact angle for the treatments. This would agree Calore et al. who looked at the contact angle of glyphosate and paraquat treatments on glass and observed that adding an adjuvant decreased the contact angle compared to the herbicides applied alone [39]. It is also interesting to observe that the different RH levels changed the contact angle of certain treatments. For example, it is observed that at 20, 40, and 60% RH the treatment containing 2,4-D and S1 decreased contact angle from 44˚ and 46˚ down to 24˚ when the RH was at 80%. Humidity can play a large factor in herbicide application [40,41]. The results from this study shows that at a higher humidity, a lower contact angle was received when adding a surfactant allowing for greater surface coverage of the droplets. This could lead to greater biomass reduction when using surfactants with herbicide mixtures and solutions.

Conclusions

Surfactants have been known to positively influence herbicide tank mixtures, depending on the chemistry inside the tank and the weed species being targeted. The results from these experiments show that using formulated products or unformulated glufosinate with surfactants can increase biomass reduction. Overall, S1 and S2 were species dependent. Both surfactants did well when incorporated into herbicide mixtures and solutions. S2 was the best preforming surfactant when observing physical properties because it had the lowest surface tension and smallest contact angle. Mixing multiple herbicides with surfactants can increase biomass reduction and enhance physical properties of spray solution. More research should be conducted to better understand how surfactants interact with the inert ingredients already formulated into commercial herbicides and how these inert ingredients could impact physical properties on a tank solution.

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Tables

Table 2.1: ANOVA for greenhouse research. Species were analyzed separately.

		Mean Square Error				
Factors	DF	Common lambsquarters	Velvetleaf	Common waterhemp	Barnyardgrass	Large crabgrass
Herbicide	4	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
Adjuvant	2	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
Herbicide*Adjuvant	8	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
Error	104	4.0544	3.6452	5.1077	7.8462	5.4618

*: Significance at an $\alpha=0.05$.

Table 2.2: Percent biomass reduction on five weed species using glufosinate, dicamba, and 2,4-D mixtures and solutions with S1 or S2 in a greenhouse environment.

		Biomass Reduction				
Herbicide	Surfactant	Common lambsquarters	Velvetleaf	Common waterhemp	Barnyardgrass	Large crabgrass
	1% v:v	%				
Glufosinate[a]	none	8 D[b]	36 B	55 B	22 D	27 E
Glufosinate	S1	95 AB	89 A	95 A	95 A	96 A
Glufosinate	S2	98 A	97 A	98 A	99 A	98 A
Dicamba	none	49 C	88 A	93 A	32 CD	38 DE
Dicamba	S1	92 AB	92 A	95 A	36 C	33 DE
Dicamba	S2	93 AB	94 A	95 A	37 C	32 DE
2,4-D	none	90 B	96 A	96 A	50 B	52 BC
2,4-D	S1	90 B	96 A	96 A	60 B	45 CD
2,4-D	S2	90 B	96 A	98 A	59 B	38 DE
Glufosinate + Dicamba	none	46 C	93 A	95 A	59 B	62 B
Glufosinate + Dicamba	S1	96 AB	97 A	98 A	98 A	99 A
Glufosinate + Dicamba	S2	97 A	97 A	97 A	98 A	99 A
Glufosinate + 2,4-D	none	96 AB	97 A	97 A	90 A	92 A
Glufosinate + 2,4-D	S1	96 AB	97 A	98 A	94 A	95 A
Glufosinate + 2,4-D	S2	97 A	98 A	97 A	99 A	98 A

a: Unformulated glufosinate

b: Comparisons are made within column. Means those within a column followed by the same letter are considered not significantly different (P > 0.05).

Table 2.3: ANOVA for Palmer amaranth and kochia field studies.

		Mean Square Error	
Effect	DF	Palmer amaranth	Kochia
Herbicide	4	0.0007*	0.6894
Surfactant	2	0.7440	0.0526
Herbicide*Surfactant	8	0.7197	0.6723
Error	45	5.4808	17.7799

*: Significance at an α=0.05.

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Table 2.4: Percent biomass reduction of Palmer amaranth at the North Platte location.

% Biomass Reduction of Palmer Amaranth		
Herbicide	Biomass Reduction	
	——%——	
Glufosinate[a]	32	C[b]
Dicamba	56	A
2,4-D	31	BC
Glufosinate + Dicamba	38	AB
Glufosinate + 2,4-D	51	AB

a: unformulated glufosinate

b: means those within a column followed by the same letter are considered not significantly different ($P > 0.05$).

Table 2.5: Percent biomass reduction of kochia at the Scottsbluff location.

% Biomass Reduction of Kochia			
Herbicide	Surfactant	Biomass Reduction[a]	
		———%———	
Glufosinate[a]	none	1	B[b]
Glufosinate	S1	54	A
Glufosinate	S2	55	A
Dicamba	none	34	AB
Dicamba	S1	63	A
Dicamba	S2	60	A
2,4-D	none	40	AB
2,4-D	S1	55	A
2,4-D	S2	16	AB
Glufosinate + Dicamba	none	41	AB
Glufosinate + Dicamba	S1	60	A
Glufosinate + Dicamba	S2	40	AB
Glufosinate + 2,4-D	none	26	AB
Glufosinate + 2,4-D	S1	50	AB
Glufosinate + 2,4-D	S2	29	AB

a: Unformulated glufosinate

b: Means those within a column followed by the same letter are considered not significantly different ($P > 0.05$).

Table 2.6: Colby analysis results when mixing multiple active ingredients on kochia and Palmer amaranth in a field environment.

Colby Analysis							
Herbicide Mixtures	Surfactant	Palmer amaranth			Kochia		
		Estimated control	Observed control	Interaction	Estimated control	Observed control	Interaction
Glufosinate[a] + Dicamba	None	44	38	Additive	0	38	Synergistic
Glufosinate + Dicamba	S1	41	59	Synergistic	57	60	Additive
Glufosinate + Dicamba	S2	33	58	Synergistic	48	33	Additive
Glufosinate + 2,4-D	None	31	51	Synergistic	0	16	Synergistic
Glufosinate+2,4-D	S1	30	48	Synergistic	41	43	Additive
Glufosinate+2,4-D	S2	28	49	Synergistic	0	0	Additive

a: Unformulated glufosinate

Table 2.7: ANOVA for density and viscosity.

	Mean Square Error		
Factors	DF	Density	Viscosity
Herbicide Solution	15	<.0001*	<.0001*
Error	128	0.000071	0.001099

*: Significance at an α=0.05.

Table 2.8: ANOVA for surface tension and contact angle.

		Mean Square Error	
Factors	DF	Surface Tension	Contact Angle
Herbicide Solution	15	<.0001*	<.0001*
RH	3	<.0001*	0.0125*
Herbicide Solution *RH	45	<.0001*	<.0001*
Error	128	.1027	1.5882

*: Significance at an α=0.05.

Table 2.9: Density, viscosity, surface tension, and contact angles of glufosinate mixtures and solutions.

			Surface Tension				Contact Angle			
			mN m^{-1}				degrees			
Treatments	Density[a]	Viscosity	Relative Humidity[b]							
			%							
	g cm^{-3}	mPa s	20	40	60	80	20	40	60	80
Water	0.9987 J	0.9950 I	73 C	74 B	72 A	71 A	65 BC	72 A	54 CDE	72 A
Glufosinate[c]	0.9996 I	1.0047 H	74 C	75 A	72 A	71 A	77 A	72 A	75 A	73 A
Glufosinate + S1	1.0003 GH	1.0560 A	36 E	38 E	38 D	38 C	49 D	29 F	53 CDEF	48 DEF
Glufosinate + S2	1.0003 GH	1.0237 F	33 H	33 J	33 H	33 G	34 EF	31 F	38 HI	41 FG
Dicamba	1.0000 H	1.0060 H	75 A	75 A	71 B	71 A	73 AB	72 A	74 A	60 BC
Dicamba + S1	1.0008 EF	1.0423 BC	34FG	33 J	34 F	34F	44 D	49 BCD	46 EFGH	44 EFG
Dicamba + S2	1.0008 EF	1.0263 F	30 K	30 L	29 K	30 J	21 G	35 EF	29 JK	24 I
2,4-D	1.0004 FG	1.0163 G	36 E	35 GH	33 G	33 G	44 D	56 B	55 CD	52 CDE
2,4-D + S1	1.0012 CD	1.0337 E	33 GH	35 HI	37 E	35 E	44 D	46 CD	44 FGHI	24 I
2,4-D + S2	1.0013 BC	1.0360 DE	32 J	31 K	31 J	31 I	28 EFG	31 F	22 K	28 8
Glufosinate + Dicamba	1.0009 DE	1.0140 G	74 B	73 C	72 B	71 A	63 C	68 A	65 B	65 AB
Glufosinate + Dicamba + S1	1.0015 BC	1.0453 B	34 F	34 I	33 GH	35 D	35 E	49 BCD	48 DEFG	40 FG
Glufosinate + Dicamba + S2	1.0017 AB	1.0367 CDE	31 J	30 L	31 IJ	31 I	23 G	30 F	37 IJ	38 GH
Glufosinate + 2,4-D	1.0016 B	1.0240 F	40 D	41 D	41 C	42 B	64 BC	53 BC	62 BC	65 BCD
Glufosinate + 2,4-D + S1	1.0020 A	1.0397 BCD	33 H	36 F	33 G	33 G	45 D	41 DE	46 EFGH	44 EFG
Glufosinate + 2,4-D + S2	1.0020 A	1.0430 B	32 I	35 FG	32 I	32 H	26 FG	30 F	40 GHI	30 HI

a: Comparisons are made within columns. Means those within a column followed by the same letter are considered not significantly different (P > 0.05).

b: RH consisted of four different levels including 20, 40, 60, and 80% for both surface tension and contact angle.

c: Unformulated glufosinate

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CHAPTER 3: INFLUENCE OF ADJUVANTS ASSOCIATED WITH GLUFOSINATE AND GLYPHOSATE MIXTURES ON WEED CONTROL

Introduction

Powels and Preston state that glyphosate is the "world's most important herbicide because it is extremely versatile, controls a wide spectrum of annual and perennial weeds, has low mammalian toxicity, and has no soil activity" [1]. Glyphosate was released to the market in 1974 as a post-emergent, non-selective herbicide and has been used on glyphosate-resistant crops since being released in 1996 [2]. The glyphosate mode of action inhibits 5-enolpyruvylshikimate-3- phosphate synthase, a nuclear-encoded, chloroplast-localized enzyme in the shikimic acid pathway of plants; this inhibition in the plant prevents the production of aromatic amino acids such as phenylalanine, tyrosine, and tryptophan [3]. It is a systemic herbicide and falls into the organophosphorus family.

Application of glyphosate has been used for many years in agriculture. In more recent times agriculturalists have reported glyphosate-resistant weeds. The first glyphosate-resistant weed, rigid ryegrass, was reported by Powles et al. in 1996 [4], and since 1996 48 weed species have evolved resistance to glyphosate [5]. As the utility of glyphosate is reduced because of glyphosate resistant weeds, alternative weed control methods are needed.

Mixing multiple modes of action (MOA) together in mixture can control resistant weeds. Mixing multiple MOA broadens the selection pressure by targeting multiple metabolic pathways and delay the evolution of herbicide-resistant weeds [6]. Johnson observed mixtures of quinclorac or dithiopyr with MSMA controlled large crabgrass longer than when either was applied alone at the same rate [7]. Applying glyphosate mixed with dicamba to glyphosate resistant giant ragweed at the male's flower bud stage reduced seed production by 80% compared to the control [8].

Glufosinate is a post emergent broad-spectrum herbicide applied as a burndown application or for weed control in glufosinate tolerant crops such as soybeans, corn, and cotton [9-11]. The glufosinate MOA inhibits glutamine synthetase in the plant, which leads to the production and accumulation of reactive oxygen species causing rapid cell death [12]. Glufosinates translocates apoplastically in the xylem, which depends on the transpiration rate of the plant; because of this, glufosinate molecules tend to accumulate in the older leaves with higher transpiration rates instead of younger leaves or apical meristems [13]. Symptoms of glufosinate include chlorosis and wilting occurring within 3-5 days after application, followed by necrosis for the following weeks, which can be enhanced with by bright sunlight, high humidity, and moist soil [14-16].

Glufosinate has been reported to be a successful mix partner with other herbicide chemistries. Steckel et al. observed mixtures of glufosinate with dicamba resulted in 90% control of glyphosate resistant horseweed 56 days after application compared to 52% control of glufosinate applied alone [17]. Waggoner et al. observed glufosinate mixed with saflufenacil on glyphosate resistant horseweed and at 30 days after treatment received 84% control compared to 77% control when glufosinate was applied alone [18]. Glufosinate can help with weed control when mixed with another mode of action but mixing glufosinate with glyphosate has been reported antagonistic [19-21].

Antagonism can be caused by many different factors when mixing multiple MOAs such as herbicide rate, target plant species, and herbicide formulation [22]. Besançon et al. states the reason for antagonism between mixtures of glyphosate with glufosinate is because the glufosinate MOA reduces the translocation of glyphosate, not allowing for the glyphosate MOA to work in the plant [23]. The antagonism between glyphosate and glufosinate still is not fully understood, and more research is needed to better understand what is occurring.

An adjuvant could help with antagonistic issues occurring between mixtures of glyphosate with glufosinate. Adjuvants can impact herbicide antagonism by increasing the herbicide absorption directly and by preventing the formation of less preferred absorption forms of weakly acidic herbicides [24]. Antagonism observed between sethoxydim or clethodim and bentazon was reduced when substituting BCH 815 for crop oil concentrate on barnyardgrass, broadleaf signal grass, and johnsongrass [25]. An antagonistic interaction could be solved by adding an adjuvant to a mixture of glufosinate with glyphosate.

Surfactants are one type of adjuvant that has shown to be beneficial when used with glyphosate or glufosinate. A surfactant is a material that improves the emulsifying, dispersing, spreading, wetting, or other properties of a liquid by modifying its surface characteristics [16]. Adding Kinetic HV to glyphosate increased control on Johnsongrass 14 days after treatment from 81% with no surfactant to 90% when the surfactant was added [26]. Johnson et al. observed the alkyl chain length and the amount of ethylene oxide on surfactants and observed an increase in efficacy when applying surfactants with glyphosate or glufosinate on common lambsquarters and giant foxtail [27]. Costa et al. observed that adding a surfactant to glufosinate increased control of Palmer amaranth 3 days after application from 64% control to 86% control [28]. Surfactants have been shown to improve efficacy when added to glufosinate or glyphosate. Adding a surfactant to both chemistries when mixed has never been tested.

Therefore, a study was conducted to evaluate the efficacy, interactions, and physical properties of glufosinate and glyphosate mixtures and solutions with two anionic surfactants. The objectives of this study were to: 1) evaluate glufosinate-glyphosate mixtures and solutions with two anionic surfactants on biomass reduction of five weed species in a greenhouse setting, 2) conduct a field study to evaluate glufosinate-glyphosate mixtures and solutions with two anionic surfactants on biomass reduction of Palmer amaranth and kochia at two locations in Nebraska, and 3) evaluate the physical properties including density, viscosity, surface tension and contact angle of glufosinate-glyphosate mixtures and solutions.

Materials and Methods

Greenhouse Study

Greenhouse studies were conducted in the winter of 2020 at the Pesticide Application Technology Laboratory located at the West Central Research and Extension Center in North Platte, Nebraska. Three weed species were tested including common waterhemp (*Amaranthus tuberculatus (moq.) J. D. Sauer),* velvetleaf (*Abutilon theophrasti Medik),* and common lambsquarters (*Chenopodium album L.).* Seeds were sown in individual 10 cm cone-tainers (Stuewe and Sons Inc., Corvallis, OR, USA) using a peat moss potting mix (Ball Horticulture Company, West Chicago, IL, USA). Plants were watered with a fertilizer blend (Wilber Ellis, San Francisco, CA, USA) injected into irrigation water. Greenhouse temperature was maintained at 28 C during the day and 18 C at night with a 16-hour photo period. Supplemental lighting was provided by LED lighting (NeoSolTM DS 300W, Illumitex, Austin, TX, USA). Treatments were applied when plants reached 15-20 cm in height.

Treatments were prepared using 340 g ae ha^{-1} of technical grade unformulated glufosinate (CRODA Atlas Point, New Castle Delaware, USA) with no pre-mixed adjuvant and 630 g ae ha^{-1} of a glyphosate (Touchdown Hi-Tech, Syngenta Crop Protection Inc., Greensboro NC, USA) formulation with a small pre-mixed adjuvant concentration alone and in mixtures with two experimental anionic surfactants applied at a 1% v/v: S1 and S2 (CRODA Atlas Point, New Castle Delaware, USA). Herbicide solutions were identified as treatments containing a single herbicide. Herbicide mixtures were identified as treatments containing multiple herbicides. The technical grade unformulated glufosinate and the glyphosate containing a small pre-mixed adjuvant in its formulation were both used in this study to determine if these products could overcome antagonism mentioned in literature [19-21]. Unformulated glufosinate was developed in a laboratory with phosphinic acid, ammonia, and water. The amount of active ingredient was equivalent to formulated glufosinate (Liberty 280 SL® Bayer CropScience, Research Triangle Park, NC, USA) without the pre-mixed adjuvant that Liberty contains. Reduced rates, compared to label recommended rates, were used with herbicides to ensure that complete control was not achieved in order to observe differences amongst treatments.

Applications were made using a single nozzle spray chamber (Devries Manufacturing, Hollandale, MN) with an AI95015EVS TeeJet nozzle (Teejet Technologies, Spraying Systems Co., Springfield, IL, USA) delivering a carrier volume of 140L ha^{-1} with a pressure of 220kPa at 2.9 kph. Because a single nozzle spray chamber was used in this experiment, an AI95015EVS nozzle was chosen for application to ensure appropriate efficacy and fan development of the spray pattern. 28 days after treatment, above ground biomass was harvest and placed in an oven (65˚C) to obtain constant weight.

The experimental design consisted of a completely randomized design with an untreated check, 10 treatments, and four replications across two runs. The factorial structure consisted of a 3x2 full factorial with the factors consisting of unformulated glufosinate, glyphosate, and a mixture of unformulated glufosinate with glyphosate by S1 and S2. Species were analyzed separately. Dry biomass data was measured and converted to percent biomass reduction. Biomass reduction data was subjected to ANOVA using SAS v9.4 (SAS, Cary, NC) with Fisher's test of least significance at an alpha level of 0.05.

Field Study

Field studies were conducted during the summer of 2020. Two site locations were used for this experiment with the first located at The University of Nebraska West Central Research and Extension Center in North Platte Nebraska (41.5˚N, -100.46˚W) and the second located at The University of Nebraska Panhandle Research and Extension Center in Scottsbluff Nebraska (41.8˚N, -103.6˚W). The Scottsbluff soil profile consisted of a Tripp very find sandy loam, while the North Platte soil profile consisted of a Cozad silt loam.

Maintenance at the North Platte location consisted of a burndown treatment on Paraquat applied at two pints/acre in the Spring of 2020 to help control already emerged weeds. Palmer amaranth was target weed species with 7,750 plants/m^2 in each plot. The population of Palmer amaranth at this location consisted of resistant and non-resistant plants. Plots were three meters wide by seven and a half meters long. Rainfall accumulation for this location from time of application until 28 days after treatment when plants were harvested, totaled 5.8 cm.

The Scottsbluff location had been fallow for the previous four years with no irrigation, tillage, or crops planted. During the fall of 2019, kochia was allowed to mature to seed and in late fall was mowed down using a rotary mower. This was done to help distribute seed throughout the field. Plots were three meters wide by seven and a half meters long. Kochia density averaged 21 plants m^2 at the time of applicaiton. Rainfall accumulation for this location from time of application until 28 days after treatment when plants were harvested, totaled 3 cm.

At both locations, plants were targeted when reaching a height of 15 - 20 cm. Treatments for the field studies were the same as the greenhouse treatments described above. The applications were applied using a CO2 backpack sprayer with 50 cm nozzle spacing calibrated to deliver 140L ha^{-1} with a pressure of 276 kPa using an AIXR11002 nozzle. 28 DAT, ten plants per plot at both locations were selected randomly and harvested. Plants were placed in a dryer (65˚C) until reaching a constant biomass.

The experiments were set up as a completely randomized block design with a factorial structure consisting of 3x2 with the factors unformulated glufosinate, glyphosate, and unformulated glufosinate-glyphosate mixed by S1 and S2. There were four replications per treatment. An untreated check was also included. Dry biomass data was converted to percent biomass reduction. Percent biomass reduction data was subjected to ANOVA using SAS v9.4 with Fisher's test of least significant ($\alpha = 0.05$).

Treatments containing multiple herbicides were analyzed using the model proposed by Colby [29] to determine if the interaction was synergistic, additive, or antagonistic:

$$E = 100 - \frac{(100X) * (100 - Y)}{100}$$

Where *E* is the dry weight percentage expected for the mixtures and *X* and *Y* are the percentages of control, dry weight results of herbicides applied alone, or dry weight results when adding S1 or S2 to the mixture. A table with the estimated data through the Colby model was elaborated and preformed comparing observed data percentage of dry weight. To determine the interaction amongst herbicides, a t-test was preformed comparing the estimated data values from the Colby analysis with data values observed using Banzatto and Kronka's [30] equation:

$$t = \frac{\widehat{m} - A}{s(\widehat{m})}$$

Where the estimated value is represented by $\widehat{m}$, *A* represents the observed value, and $s(\widehat{m})$ represents the standard error of the mean. From this formula, conclusions could be made to determine what kind of interaction was occurring when mixing the herbicides. Synergism occurred when the observed data was greater than the estimated data and the "*t*" value was less than 0.05. Additivity occurred when the "*t*" value was greater than 0.05. Antagonism was observed when data was lower than the estimated data and when the "*t*" value was less than 0.05.

Physical Properties

Density, viscosity, surface tension, and contact angle of water alone and nine spray solutions, glufosinate, glyphosate, glufosinate mixed with glyphosate, glufosinate with S1, glufosinate with S2, glyphosate with S1, glyphosate with S2, glufosinate mixed with glyphosate and S1, and glufosinate mixed with glyphosate and S2, were measured at the Pesticide Application Technology Laboratory located at The University of Nebraska-Lincoln's West Central Research, Education, and Extension Center in North Platte, NE.

Density and viscosity measurements were analyzed using a DMA™ 4500 M density meter (Anton Paar USA Inc., Ashland, VA) and a microviscomter Lovis 2000 M/ME (Anton Paar USA Inc., Ashland, VA) attached to the side of the density meter. A constant temperature of 25˚C was used throughout these measurements. Further methodology involving the density and viscosity can be found in Moraes [31] paper.

Surface tension and contact angle measurements were taken using video-based optical contact angle measuring from an OCA 15EC (DataPhysics Instruments GmbH, Filderstadt, Germany). This equipment uses a USB camera with a video measuring system. A high-performance 6X parfocal zoom lens with integrated continuous fine focus, camera tilt angle, and adjustable observation are built into the camera. SCA software is used to collect, analyze, and evaluate the measured data. Surface tension and contact angle measurements were conducted at four different relative humidities which included 20, 40, 60, and 80 ± 1%. The temperature was held at 25˚C ± 1˚C. An environmental chamber allowed for the temperature and humidity to be held constant throughout the experiments. A liquid circulator (Julabo USA Inc, Allentown, PA) was used to adjust the temperature when needed. A humidity generator (DataPhysics Instruments GmbH, Filderstadt, Germany) was used to allow for proper humidity control. Humidity and temperature parameters are displayed on the control panel allowing for the operator to check and adjust the parameters in real time. Further methodology involving the surface tension and contact angle measurements can be found in Moraes [31] paper.

Results and Discussion

Greenhouse Results

The herbicide by adjuvant interaction was significant for common lambsquarters and velvetleaf at an $\alpha = 0.05$ (Table 3.1). Common waterhemp did not have a significant interaction between adjuvant and herbicide. The adjuvant and herbicide effects were significant.

Applying glufosinate alone resulted in <4% biomass reduction across broadleaved species (Table 3.2). There were no differences observed when adding an anionic surfactant to glufosinate across species. Common waterhemp biomass reduction did increase to 30% when adding S2, but this was not significantly different from the glufosinate alone treatment.

Adding a surfactant to glyphosate increased the biomass reduction of common lambsquarters from 0% when glyphosate was applied alone to >60% when using a surfactant (Table 3.2). Biomass reduction of velvetleaf increased from 41% when glyphosate was applied alone to 72% when glyphosate was applied with S2. Common waterhemp resulted in similar findings resulting in 44% biomass reduction when glyphosate was applied alone and 81% biomass reduction when glyphosate was applied with S2. Adding S1 to glyphosate resulted in no differences in biomass reduction when compared to the glyphosate alone treatment.

Adding surfactants to glyphosate and glufosinate have shown to be beneficial for controlling broadleaved species [28,32-34]. The lack of biomass reduction when adding a surfactant to glufosinate was not expected because the glufosinate used in this experiment contained no pre-mixed adjuvant in its formulation. The increase in biomass reduction for the glyphosate solutions was expected because of the small pre-mixed adjuvant package that is formulated into this product. Anionic surfactants for this experiment worked better with glyphosate than with glufosinate, meaning an anionic surfactant may not be needed for applications of glufosinate.

Glufosinate mixed with glyphosate ranged in between 0 and 57% biomass reduction across species, with common lambsquarters having the lowest and common waterhemp having the highest (Table 3.2). Adding S1 to glufosinate with glyphosate in mixture increased the biomass reduction of common waterhemp. No differences were observed on velvetleaf and common lambsquarters when adding S1 to the mixture compared to the mixture applied alone. Adding S2 to a mixture of glufosinate with glyphosate increased biomass reduction across broadleaved species. The largest biomass reduction when using herbicide mixtures came from S2 added to glufosinate with glyphosate on common waterhemp, resulting in 70%.

It has been documented in literature that glufosinate with glyphosate in mixture has resulted in antagonism [19-21,23]. In the greenhouse experiment, adding an anionic surfactant to a mixture of unformulated glufosinate with a glyphosate formulation containing a small, pre-mixed adjuvant increased the biomass reduction of broadleaved species (>13%). S2 used with glufosinate and glyphosate mixtures and solutions resulted in the largest biomass reduction for species. The formulations of the herbicides being mixed, and the surfactants being added to the tank is critical information needed to be able to understand the relationship and interactions happening in the tank. Jordan observed antagonism can be overcome when using a surfactant with sethoxydim and bentazon [25]. More research should be conducted to better understand how glufosinate and glyphosate interact in mixture, along with the formulations or the products and the surfactants being added to the tank. This could help explain previously reported antagonism [19-21]. The results from this study shows that when using the unformulated and low adjuvant containing products with an anionic surfactant, reduction in biomass for broadleaved weed species can be increased.

Field Study

North Platte Location: Palmer amaranth

At the North Platte location, the herbicide by adjuvant interaction was significant ($\alpha<0.05$) (Table 3.3). There was no difference in biomass reduction with glufosinate with or without a surfactant (Table 3.4). When applying glyphosate, only S1 was significant.

It is important to understand that at the North Platte location, the population of Palmer amaranth was 7,750 plants/m^2. Having such a large volume of Palmer amaranth plants could have resulted in the application being affected by the canopy coverage of the plants. Canopy cover of such a dense population would explain the inadequate droplet to leaf surface contact with the taller plants receiving more herbicide than the shorter, smaller plants. Glufosinate is a contact herbicide that relies on proper droplet to leaf surface contact for it to be effective. This can be a possible explanation for the lack in biomass reduction when using these treatments.

S1 and S2 decreased biomass reduction of Palmer amaranth when added to a tank solution of glyphosate (Table 3.4). This was not expected because surfactants have been shown to improve glyphosate efficacy [26,32,33]. It is important to understand that surfactants can work differently depending on their chemical makeup. For example, Riechers et al. observed control of velvetleaf using glyphosate with one cationic surfactant having two moles of ethylene oxide resulted in 53% visual control 21 DAT compared to another cationic surfactant having 15 moles of ethylene oxide which resulted in 78% visual control 21 DAT [32]. Knoche and Bukovac studied sugar beets and the effect of the oxyethylene (OE) chain length of non-ionic surfactants with glyphosate and noted that at <10 OE chain length resulted in the greatest absorption of glyphosate while 16-30 OE chain lengths resulted in the absorption being like the glyphosate control without a surfactant [33]. Surfactants can fall in the same classification but can be formulated differently. The makeup of the surfactants and how they interacted with glyphosate could be the reason why a decrease in biomass reduction was observed.

Mixing glufosinate with glyphosate resulted in 7% biomass reduction on Palmer amaranth (Table 3.4). Adding a surfactant to the herbicide mixture increased biomass reduction to >34%. When applying the mixtures, adding S2 (46%) resulted in better biomass reduction than S1 (34%). No significant differences were detected amongst mixtures. The Colby analysis resulted in additivity when mixing glufosinate with glyphosate (Table 3.5). Synergistic interactions were observed when adding a surfactant to the glufosinate-glyphosate mixture.

The mixture of glufosinate with glyphosate resulted in poor biomass reduction of Palmer amaranth (Table 3.4). It is important to remember that for this experiment, unformulated glufosinate and a glyphosate formulation containing a low adjuvant concentration were used. Having a smaller adjuvant concentration in the glyphosate and no pre-mixed adjuvants with the glufosinate, this low biomass reduction was expected when the two chemistries were mixed together. It is also important to understand that when mixing these two chemistries additivity was the result. These are different results than what has been observed before when using formulated glufosinate and formulated glyphosate mixed together [19,20]. Further research is needed to better understand how both herbicides interact with each other in the tank, and to better understand the surfactant packages that are in the formulated products that could be causing the antagonism to occur.

Adding a surfactant to a glufosinate with glyphosate mixture resulted in synergism along with larger biomass reduction than the mixture without a surfactant (Table 3.5). These results would agree with Jordan that adding a surfactant to mixed herbicides can help overcome antagonisms in the tank and allow for better weed control [25]. From this experiment, it can be observed that when using unformulated or products containing low adjuvant concentrations, antagonisms can be overcome with some surfactants and result in greater biomass reduction when applied to Palmer amaranth.

Scottsbluff Location: Kochia

At the Scottsbluff location there were no differences in kochia biomass reduction among treatments (Table 3.3).

These results show that regardless of if a surfactant was added to glyphosate, glufosinate, or a mixture of both, the application resulted in the same biomass reduction of kochia. It has been reported in literature that surfactants can impact weed control based on the weed species that is targeted. Sanyal et al. reported that adding a nonionic surfactant to primisulfuron resulted in greater spreadability than primisulfuron alone but observed that the spreadability was greatest on velvetleaf compared to common purslane or common lambsquarters [35]. Different leaf surfaces and leaf structure could explain why a larger biomass reduction was observed when applied to Palmer amaranth, and a lack of biomass reduction was seen with kochia.

Physical Properties

Density and Viscosity

Density and viscosity measurements were both significant when ran in ANOVA (Table 3.6). The two lowest density readings came from water and glufosinate, resulting in <0.9996 g cm^{-3} (Table 3.8). This was expected because these two treatments have no surfactant or adjuvant package incorporated into their formulations. All other treatments recorded >1 g cm^{-3} with unformulated glufosinate mixed with glyphosate and S1 having the highest reading at 1.0059 g cm^{-3}. An increase in the density occurred when adding S1 or S2, regardless of the herbicide or the mixture the surfactant was added to. In a study conducted by Assuncao increases in density occurred when synthetic adjuvants were added to the active ingredient diammonium N-(phosphonate methyl)glycine compared to the active ingredient alone [36].

Viscosity readings resulted in water having the lowest viscosity at 0.9950 mPa s and the highest results coming from unformulated glufosinate with S2 at 1.0560 mPa s (Table 3.8). Treatments besides water resulted in >1 mPa s. Adding a surfactant to unformulated glufosinate and glyphosate alone or mixed together resulted in an increase in viscosity. Assuncao reported similar findings with an increase in dynamic viscosity occurring when adding synthetic adjuvants to the active ingredient diammonium N-(phosphonate methyl)glycine compared to the active ingredient alone [36].

Surface Tension and Contact Angle

The surface tension by relative humidity and contact angle by relative humidity were significant at an α=0.05 (Table 3.7). The highest surface tension observed came from water, unformulated glufosinate, glyphosate, and unformulated glufosinate mixed with glyphosate resulting in >71 mN m^{-1} (Table 3.8). Glyphosate and S1 resulted in the lowest surface tension measuring 29 mN m^{-1} across the four levels of RH. Adding a surfactant to an herbicide mixture or solution greatly decreased the surface tension.

The highest contact angle across RH levels came from water, unformulated glufosinate, glyphosate, and mixtures of glufosinate with glyphosate resulting in >54° angle (Table 3.8). The lowest contact angle consisted of treatments with S1 across the four levels of RH. Adding S1 or S2 to both unformulated glufosinate and glyphosate decreased the surface tension. Mixtures of glufosinate and glyphosate decreased in contact angle when adding a surfactant. S1 provided a lower contact angle compared to S2 when added to a mixture.

Surfactants are surface active agents, and their purpose is to reduce the surface tension of the spray solution for more contact between the spray droplet and the plant surface [37]. From the results above, it can be observed that adding a surfactant to glufosinate and glyphosate treatments decreased the surface tensions and contact angles. Singh observed both decreases in surface tension and contact angle when using organosilicone and non-silicone adjuvants with diuron compared to the diuron treatment alone [38]. With decreases in surface tension and contact angle, a greater leaf to droplet surface contact can occur which could increase weed control of glufosinate mixtures or solutions.

Conclusions

The addition of anionic surfactants to glyphosate and glufosinate applied alone or in mixture can increase the biomass reduction of problematic broadleaved weed species as seen in this research. The anionic surfactants in this experiment also decreased contact angle and surface tension, while raising the density and viscosity of the herbicide mixtures and solutions. Overall, both anionic surfactants performed well across experiments. S2 was the best preforming adjuvant when observing biomass reduction, while S1 performed better when observing physical properties. Overall, the formulation and addition of surfactants to glyphosate with glufosinate mixtures should be researched more in depth to better understand if there is an issue with the formulation of the products or the mode of actions themselves.

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Table 3.1: ANOVA for greenhouse experiment.

		Mean Square Error		
Factors	DF	Common lambsquarters	Velvetleaf	Common waterhemp
Herbicide	2	<.0001*	<.0001*	<.0001*
Adjuvant	2	<.0001*	<.0001*	0.0005*
Herbicide*Adjuvant	4	<.0001*	0.0084*	0.1302
Error	62	3.0984	5.8687	12.7948

*: Significant at an α=0.05.

Table 3.2: Percent biomass reduction of common lambsquarters, velvetleaf, and common waterhemp using glufosinate and glyphosate mixtures and solutions with two anionic surfactants in a greenhouse environment.

		Percent Biomass Reduction		
Herbicide Treatment	Surfactant	Common lambsquarters	Velvetleaf	Common waterhemp
		%	%	%
Glufosinate[a]	None	0 D[c]	0 E	4 E
Glufosinate	S1	0 D	0 E	6 E
Glufosinate	S2	0 D	0 E	30 CDE
Glyphosate[b]	None	0 D	41 B	44 BCD
Glyphosate	S1	60 B	33 BC	32 CDE
Glyphosate	S2	80 A	72 A	81 A
Glufosinate + Glyphosate	None	0 D	22 CD	16 DE
Glufosinate + Glyphosate	S1	4 D	17 D	62 ABC
Glufosinate + Glyphosate	S2	13 C	42 B	70 AB

a: Unformulated glufosinate

b: Touchdown Hi-Tech

c: Means those within a column followed by the same letter are considered not significantly different ($P > 0.05$).

Table 3.3: Field study ANOVA for Palmer amaranth and kochia.

	Mean Square Error		
Factors	DF	Palmer amaranth	Kochia
Herbicide	2	0.0047*	0.4122
Adjuvant	2	0.2214	0.2231
Herbicide*Adjuvant	4	0.0038*	0.8490
Error	27	7.0034	15.6562

*: Significant at an α=0.05.

Table 3.4: Percent biomass reduction of Palmer amaranth and kochia using glufosinate-glyphosate mixtures and solutions with two anionic surfactants.

Percent Biomass Reduction					
Herbicide Treatment	Surfactant	Palmer amaranth		Kochia	
		%			
Glufosinate[a]	None	18	BCDE[c]	19	A
Glufosinate	S1	12	CDE	25	A
Glufosinate	S2	25	BC	24	A
Glyphosate[b]	None	22	BCD	18	A
Glyphosate	S1	0	E	50	A
Glyphosate	S2	3	DE	52	A
Glufosinate + Glyphosate	None	7	CDE	19	A
Glufosinate + Glyphosate	S1	34	AB	25	A
Glufosinate + Glyphosate	S2	46	A	24	A

a: Unformulated glufosinate

b: Touchdown Hi-Tech

c: Comparisons are made within column. Means those within a column followed by the same letter are considered not significantly different ($P > 0.05$).

Table 3.5: Results from the Colby analysis on mixtures of glufosinate and glyphosate on Palmer amaranth and kochia.

Colby Analysis							
Herbicide Mixture	Surfactant	Palmer amaranth			Kochia		
		Estimated control	Observed control	Interaction	Estimated control	Observed control	Interaction
Glufosinate + Glyphosate	None	2	0	Additive	0	0	Additive
Glufosinate + Glyphosate	S1	0	34	Synergistic	29	17	Additive
Glufosinate + Glyphosate	S2	0	46	Synergistic	20	0	Additive

a: Unformulated glufosinate

b: Touchdown Hi-Tech

Table 3.6: ANOVA for density and viscosity.

	Mean Square Error		
Factors	DF	Density	Viscosity
Herbicide Solution	9	<.0001*	<.0001*
Error	80	0.000011	0.001690

*: Significance at an α=0.05.

Table 3.7: ANOVA for surface tension and contact angle.

		Mean Square Error	
Factors	DF	Surface Tension	Contact Angle
Herbicide Solution	9	<.0001*	<.0001*
RH	3	<.0001*	<.0001*
Herbicide Solution *RH	27	<.0001*	<.0001*
Error	80	0.08965	1.1194

*: Significance at an $\alpha=0.05$.

Table 3.8: Physical property measurements of glufosinate and glyphosate mixtures and solutions with two anionic surfactants.

Treatments	Density	Viscosity	Surface Tension mN m^{-1}				Contact Angle degrees			
			Relative Humidity[d] %							
	g cm^{-3}	mPa s	20	40	60	80	20	40	60	80
Water	0.9987 J[c]	0.9950 G	73 C	74 C	72 A	71 B	65 ABC	72 B	54 B	72 A
Glufosinate[a]	0.9996 I	1.0047 F	74 BC	75 B	72 A	71 B	77 A	72 B	75 A	73 A
Glufosinate + S1	1.0019 G	1.0333 CD	30 G	30 G	30 E	29 E	21 DE	23 C	39 C	26 E
Glufosinate + S2	1.0002 H	1.0560 A	36 D	38 E	38 C	38 C	49 ABCD	29 C	53 B	48 BC
Glyphosate[b]	1.0024 F	1.0097 F	74 B	73 D	72 A	72 B	69 AB	77 B	80 A	69 A
Glyphosate + S1	1.0048 B	1.0350 CD	29 H	29 G	29 E	29 E	16 E	19 D	38 C	20 F
Glyphosate + S2	1.0031 E	1.0267 E	32 F	33 F	32 D	31 D	35 DE	41 B	38 C	33 D
Glufosinate + Glyphosate	1.0035 D	1.0190 E	75 A	76 A	71 B	72 A	74 A	78 A	78 A	54 B
Glufosinate + Glyphosate + S1	1.0059 A	1.0483 AB	30 G	30 G	29 E	29 E	39 CDE	36 B	39 C	39 D
Glufosinate + Glyphosate + S2	1.0042 C	1.0400 BC	33 E	33 F	32 D	32 D	42 BCDE	37 B	42 C	47 C

a: Unformulated glufosinate

b: Touchdown Hi-Tech

c: Comparisons made within columns. Means those within a column followed by the same letter are considered not significant ($P > 0.05$)

d: Relative humidity for surface tension and contact angle measurements were at levels of 20, 40, 60, and 80%.

CHAPTER 4: EFFECT OF SURFACTANT DOSE RATE ON HERBICIDE SOLUTIONS AND MIXTURES ON CONTROL OF *Chenopodium album L.*

Introduction

The first agricultural adjuvant was a soap solution [1,2] used to increase the toxicity of arsenical formulations on weeds [3]. Edser reported in 2007 that around 230,000 tonnes of surfactants are used annually in agrochemical products [4], with a formulation typically contained 1-10% of one or multiple surfactants [5]. Adjuvants make up a large portion of the agrochemical market, and it is important to understand their importance when added to an herbicide tank solution.

Many adjuvants are used with POST emergent herbicides to improve spray delivery, to increase retention of the spray on weed foliage, and to enhance foliar penetration, thus increasing herbicide selectivity and effectiveness [6]. With adjuvants having many different benefits to POST emergent herbicide applications, it is known that an increase in weed control can occur when adding an adjuvant to an herbicide tank solution.

One classification of adjuvants that work well with POST emergent herbicides would include surfactants. A surfactant can be defined as a material that improves the emulsifying, dispersing, spreading, wetting, or other properties of a liquid by modifying its surface characteristics [7,3]. Curran et al. states that surfactants are surface active agents and there purpose is to decrease surface tension of spray solutions for more contact between spray droplets and plant surfaces [8]. With better leaf surface to droplet contact, POST herbicides are able to get better contact with the target weed species.

Many POST emergent herbicides rely on surfactants to provide an increase in weed control. Harbour et al observed 40 to 44% fresh weight reduction on kochia when using a surfactant with 2,4-D compared to only 27% fresh weight reduction with 2,4-D alone [9]. Dayan et al used a nonionic surfactant with a POST application of sulfentrazone on velvetleaf and reported 90% phytotoxicity compared to 65% phytotoxicity when sulfentrazone was applied alone [10]. Surfactants can be very beneficial when used with post emergent herbicides.

With surfactants increasing weed control when used with POST herbicides applied alone, they could increase weed control when using mixtures of post emergent herbicides as well. There is very little research in literature observing how beneficial surfactants can be when used with herbicide mixtures. It is also important to understand the threshold at which adequate weed control can be achieved based on the dose of a surfactant. With this in mind the objective of this research was to determine the appropriate dose of three anionic surfactants when used with dicamba, 2,4-D, glufosinate, or glyphosate applied alone or in mixture on the control of common lambsquarters (*Chenopodium album L*).

Materials and Methods

In the fall of 2020 and the winter of 2020, greenhouse studies were conducted at the Pesticide Application Technology Laboratory located at the West Central Research, Extension, and Education Center in North Platte, Nebraska to observe the relationship of different surfactant doses with herbicide tank solutions and mixtures on the control of common lambsquarters (*Chenopoidum album*). Plants were grown in individual 656 ml cone-tainers (Stuewe and Sons Inc., Corvallis, OR, USA) using a peat moss potting mix

(Pro-Mix. Premier Tech, Quakertown PA, USA). Plants were grown until reaching a height of 15 to 25 cm where they were then subjected to application.

Solutions were prepared using distilled water. Solutions consisted of 340 g ae ha^{-1} of technical grade unformulated glufosinate (CRODA Atlas Point, New Castle DE, USA) with no surfactant, 770 g ae ha^{-1} of Roundup PowerMAX® (Bayer CropScience, Research Triangle Park, NC, USA), 340 g ae ha^{-1} of Liberty® (Bayer CropScience, Research Triangle Park, NC, USA), 630 g ae ha^{-1} of Touchdown Hi-Tech® (Syngenta Crop Protection Inc., Greensboro NC, USA), 530 g ae ha^{-1} of Enlist One® (Corteva Agriscience, Wilmington, DE, USA), and 280 g ae ha^{-1} of Xtendimax® (Bayer CropScience, Research Triangle Park, NC, USA) alone and mixtures of 340 g ae ha^{-1} of unformulated glufosinate with 770 g ae ha^{-1} of Roundup PowerMAX®, 340 g ae ha^{-1} of unformulated glufosinate with 630 g ae ha^{-1} of Touchdown Hi-Tech®, 340 g ae ha^{-1} of unformulated glufosinate with 530 g ae ha^{-1} of Enlist One®, and lastly 340 g ae ha^{-1} of unformulated glufosinate with 280 g ae ha^{-1} of Xtendimax®. All solutions and mixtures were applied alone and with the addition of a surfactant. Surfactants included three anionic surfactants (S1, S2, or S3 (CRODA Atlas Point, New Castle DE, USA)) applied at three dose rates of 0.25, 0.50, and 1% v/v. Herbicide solutions were identified as treatments containing a single herbicide. Herbicide mixtures were identified as treatments containing multiple herbicides. Technical grade unformulated glufosinate with no pre-mixed adjuvant was used in this experiment to attempt to overcome antagonistic results when mixing glufosinate with other modes of action as reported in literature [15-18].

Applications were made using a single nozzle spray chamber (Devries Manufacturing, Hollandale, MN) with an AI95015EVS Teejet nozzle (Teejet

Technologies, Spraying Systems Co., Springfield, IL, USA). The AI95015EVS nozzle was chosen for the application to ensure proper fan development occurred during application. The spray chamber was calibrated to deliver 140L ha^{-1} with a pressure of 220kPa at 2.9 kph. 28 days after application, above ground biomass was harvest for each treatment and the untreated check and placed in a dryer (65˚C) until reaching a constant weight.

The experiment was set up as a completely randomized design with 100 treatments and an untreated check. There were four replications per treatment across two runs. The factorial treatment structure consisted of a 10x3x4 factorial with factors consisting of herbicides which included unformulated glufosinate, Liberty®, Xtendimax®, Enlist One®, Roundup PowerMAX®, Touchdown Hi-Tech®, unformulated glufosinate mixed with Xtendimax®, unformulated glufosinate mixed with Enlist One®, unformulated glufosinate mixed with Roundup PowerMAX®, and unformulated glufosinate mixed with Touchdown Hi-Tech® by surfactant which include S1, S2, and S3, followed by doses consisting of 0, 0.25, 0.50, 1.0% v/v.

Dry above ground biomass data was analyzed using ANCOVA in RStudio v3.6 (RStudio, 250 Northern Ave, Boston, MA, USA) at an $\alpha = 0.05$. ANCOVA was used because of the dose factor being considered a covariate. Scatterplots were derived using the sgscatter function in RStudio to determine the linear relationship between control of common lambsquarters and the dose of the surfactant for each herbicide and adjuvant and to assist in verifying statistical assumptions (Figures 1 and 2). The first and second run were analyzed separately as results differed between the two runs.

Results and Discussion

Run One

The dose, herbicide, and adjuvant effects and the dose by herbicide and herbicide by adjuvant interactions were significant at an α=0.05 (Table 4.1). The dose by adjuvant interaction was not significant, indicating all adjuvants behaved similarly regardless of their dose. The three-way interaction of herbicide by dose by adjuvant was not significant.

The dose by herbicide and the herbicide by adjuvant interactions were significant, therefore, the results were separated by herbicide with the dose and adjuvant effect (Table 4.2). There were no differences between surfactants or dose rate of surfactants when added to Liberty or Enlist One (Table 4.2). Liberty and Enlist One both have large pre-mixed adjuvants built into their formulation. This can explain why adjusting the dose of an anionic surfactant did not increase the biomass reduction of common lambsquarters, because the necessary additives are already in the formulation.

Unformulated glufosinate, Touchdown Hi-Tech, and Xtendimax applied alone were not influence by surfactant. An increase in biomass reduction was observed with these herbicides when increasing the dose of the surfactant (Table 4.1). Unformulated glufosinate applied alone with no surfactant resulted in <25% biomass reduction and increased biomass reduction as the dose increased (Figure 4.1). At the 1% surfactant dose, unformulated glufosinate resulted in <50% biomass reduction. Increasing the dose of surfactant to Touchdown Hi-Tech greatly impacted the biomass reduction of common lambsquarters. Touchdown Hi-Tech applied alone resulted in <20% biomass reduction and increased biomass reduction as the surfactant dose increased, resulting in >75%. Xtendimax showed similar trends when increasing the surfactant dose rates for S1 and S2 resulting in 75% biomass reduction. The largest biomass reduction of common lambsquarters came from Xtendimax and S3 at the 0.50% v/v dose rate resulting in 75%. A decrease in biomass reduction was observed when using the 1% v/v dose rate with S3 and Xtendimax.

Biomass reduction of common lambsquarters with Roundup PowerMAX was influenced by the different surfactants and was not influenced by the surfactant dose rate (Table 4.2). S1 provided the largest biomass reduction when added to Roundup PowerMAX resulting in >75% (Figure 4.1). A decrease in biomass reduction was observed when adding S3 to Roundup PowerMAX (75%) compared to when Roundup PowerMAX was applied alone (>75%).

Mixtures of unformulated glufosinate with Touchdown Hi-Tech, Enlist One, or Xtendimax were not influenced by the different surfactants, meaning all surfactants acted in similar ways (Table 4.2). The dose rate of surfactants was significant when added to the herbicide mixtures. Unformulated glufosinate mixed with Touchdown Hi-Tech increased biomass reduction as dose rate increased (Figure 4.1). S1 resulted in the highest biomass reduction at a dose rate of 0.25% v/v and was the same for the 0.50% and 1% v/v rates when used with unformulated glufosinate mixed with Touchdown Hi-Tech. S2 and S3 at the 1% v/v dose resulted in the highest biomass reduction (>50%) compared to the other doses when used with glufosinate mixed with Touchdown Hi-Tech. The unformulated glufosinate with Enlist One mixture increased biomass reduction when increasing the dose for S3. S1 and S2 did not increase control and leveled out when increasing the dose rate. The unformulated glufosinate with Xtendimax mixture increased biomass reduction when increasing dose rates for S1 and S2, resulting in >75% biomass reduction. 75% biomass reduction was observed when S3 was added at the 0.50% v/v dose rate to a mixture of unformulated glufosinate with Xtendimax. When increasing the dose rate of S3 to 1% v/v, <75% biomass reduction was observed.

The results for the mixture of unformulated glufosinate with Roundup PowerMAX showed that the different adjuvants did impact biomass reduction and the dose of the surfactant was not a factor (Table 4.2). S1 and S2 both resulted in similar weed biomass reduction, providing >50% (Figure 4.1). Biomass reduction decreased to <50% when S3 was added to a mixture of unformulated glufosinate with Roundup PowerMAX compared to >50% when the herbicides were mixed together or applied alone.

Run Two

The dose, herbicide, and adjuvant effects and the dose by herbicide interaction was significant in run two at an α=0.05 (Table 4.3). With the dose by herbicide interaction being significant, data was separated by herbicide (Table 4.4).

Liberty and Roundup PowerMAX were not influenced by the dose of surfactant (Table 4.4). The treatments of Unformulated glufosinate, Touchdown Hi-Tech, Enlist One, and Xtendimax were improved by surfactant dose (Table 4.4). Unformulated glufosinate, Touchdown Hi-Tech, Xtendimax, and Enlist One increased in biomass reduction when increasing the surfactant dose rate. Adding S1 and S3 to unformulated glufosinate resulted in >40% biomass reduction of common lambsquarters when the surfactant was applied at a 1% v/v dose. Touchdown Hi-Tech significantly increased biomass reduction when increasing the surfactant dose rate resulting in <25% with no surfactant and increasing to >75% when adding S1 at a 0.50% v/v rate. Enlist One ranged between 60% biomass reduction with no surfactant, up to 75% when a surfactant was added, regardless of the surfactant dose rate. Treatments of Xtendimax with a surfactant increased the biomass reduction as the dose of surfactant increased. Surfactants increased biomass reduction to >75%, with the largest coming from a dose of 1% v/v.

Mixing unformulated glufosinate with Enlist One or Roundup PowerMAX did not result in differences when observing the herbicide by dose interaction. Both mixtures resulted in >50% biomass reduction when adding a surfactant. Treatments of unformulated glufosinate mixed with Xtendimax or Touchdown Hi-Tech were impacted by surfactant dose. As the dose increased, biomass reduction of common lambsquarters increased for both treatments across surfactants. Unformulated glufosinate with Touchdown Hi-Tech resulted in <75% biomass reduction across doses and surfactants. Unformulated glufosinate mixed with Xtendimax resulted in 75% weed biomass reduction when using a dose of 1% v/v across surfactants.

Discussion

Both runs resulted in no differences amongst surfactants with unformulated glufosinate, Touchdown Hi-Tech and Xtendimax, which was not expected. All three surfactants are anionic surfactants are different, having their own chemical makeup and structure. Johnson et al. observed citric ester surfactants and found that five out of 32 surfactants increased the control of common lambsquarters when used with glufosinate and noticed a trend that increasing ethylene oxide (EO) numbers increased the surfactant efficacy[11]. The amount of EO that is built into the anionic surfactants could explain why they all acted similarly when applied with an herbicide for these treatments.

Run one and two results showed an increase in weed biomass reduction when increasing the dose of the surfactant for unformulated glufosinate, Xtendimax, Touchdown Hi-Tech and the mixtures of unformulated glufosinate with Xtendimax or Touchdown Hi-Tech. This was anticipated due to unformulated glufosinate containing no premixed adjuvants and Touchdown Hi-Tech and Xtendimax both containing a small amount of pre-mixed adjuvants in their formulations. Surfactants have been added to post emergent herbicides applications to help with spray delivery, increase retention of the spray on weed foliage, and to enhance foliar penetration, thus increasing herbicide selectivity and effectiveness [6]. These treatments do not contain pre-mixed adjuvants in their formulations, and therefore, the treatment would not have the benefits that surfactants contain [3,7,8], which can explain why a large increase in biomass reduction occurred when adding a surfactant. Increasing the dose rate of adjuvants has been observed to increase weed control. Rimsulfuron activity increased from <10% control to >90% control when increasing surfactant concentration from 0.0008 to 1% [20]. Increasing the dose rate of surfactant with nicosulfuron increased control of common foxtail from <20% with no surfactant to >80% at an adjuvant concentration of 0.3% [21]. Increasing the dose rate of surfactants can increase weed control in specific applications.

Surfactants have been shown to impact weed control when applied with glyphosate. Glyphosate with cationic and nonionic surfactants on fresh shoot weight of common lambsquarters resulted in nonionic surfactants having the same level of control as the control while cationic surfactants decreased fresh shoot weights [12]. Collins and Helling studied the effect of glyphosate formulations with adjuvants on two varieties of cocoa and concluded the best adjuvants used were cationic surfactants and a mixture between a crop oil concentrates and an organosilicone surfactant [13]. The addition of an adjuvant could greatly increase weed control when added to glyphosate.

The decrease in biomass reduction from the glufosinate with glyphosate mixture with S3 when increasing the dose rate was not expected. The surfactants used for this experiment were all anionic surfactants. The reasoning behind the decrease in biomass reduction when using S3 cannot be explained. Antagonistic results have been mentioned in literature between glufosinate and glyphosate mixtures [15-17]. S1 and S2 may have been able to overcome these antagonistic results when added to the mixture, which S3 could not, resulting in the decrease in biomass reduction. Antagonism was overcome when mixing sethoxydim and bentazon with a surfactant [18]. This could help explain why S1 and S2 performed well with mixtures of glufosinate with glyphosate because their chemical structure improved the overall efficacy of the treatment.

The dose interaction with Enlist One for run two was not expected because Enlist One contains a large adjuvant package and the addition of a surfactant may not be needed for application. Run one resulted in no differences when increasing the surfactant dose rate for Enlist One. Barnett et al. witnessed 90% control of 2,4-D applied alone and 93% control when applied with glufosinate 30 days after treatment on glyphosate resistant giant ragweed [19]. The surfactant dose rate for Enlist One did not have a large impact on the biomass reduction of common lambsquarters compared to unformulated glufosinate, Touchdown Hi-Tech, and Xtendimax as it can be observed in Figure 1. Surfactant L-77® applied with 2,4-D increased Brazil pusley control to 100% compared to 2,4-D alone, providing 84% control [14]. Further research is needed to better understand the efficacy of 2,4-D when applied with surfactants.

Conclusions

Across both runs, the greatest effects of the surfactants and dose rates in this experiment resulted from the herbicides with little or no pre-mixed adjuvants built into their formulation. The results from run one show that few treatments were impacted by the herbicide by adjuvant interaction while most treatments were impacted by the herbicide by dose interaction. The results from run two showed that surfactant dose rate is an important factor to consider when adding a surfactant to an herbicide application and can increase biomass reduction based off of the herbicide it is applied with. It is important to understand what adjuvants to use when making an application because they may or may not be needed depending on the herbicides being used and the target weed species. Future research should be conducted to determine the impact of surfactants on herbicides with small pre-mixed adjuvants in their formulation, unformulated herbicides, and herbicide mixtures to better understand the impact on weed control.

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Tables

Table 4.1: Run one ANOVA table.

Effect	Num DF	p-value
Dose	1	<.0001*
Herbicide	9	<.0001*
Adjuvant	2	0.0006*
Dose*Herbicide	9	<.0001*
Dose*Adjuvant	2	0.7650
Herbicide*Adjuvant	18	0.0035*
Dose*Herbicide*Adjuvant	18	0.2290

*: significant at an α=0.05.

Table 4.2: Run one ANOVA table for the herbicide*dose and herbicide*adjuvant interactions.

Herbicide(s)	Effect	NDF	p-value
Unformulated Glufosinate	Dose	1	<0.0001*
Unformulated Glufosinate	Adjuvant	2	0.2170
Liberty	Dose	1	0.3920
Liberty	Adjuvant	2	0.0820
Touchdown Hi-Tech	Dose	1	<.0001*
Touchdown Hi-Tech	Adjuvant	2	0.2830
Roundup PowerMAX	Dose	1	0.8000
Roundup PowerMAX	Adjuvant	2	0.0010*
Enlist One	Dose	1	0.1800
Enlist One	Adjuvant	2	0.3130
Xtendimax	Dose	1	0.0060
Xtendimax	Adjuvant	2	0.3660
Unformulated Glufosinate + Touchdown Hi-Tech	Dose	1	<0.0001*
Unformulated Glufosinate + Touchdown Hi-Tech	Adjuvant	2	0.0870
Unformulated Glufosinate + Roundup PowerMAX	Dose	1	0.2100
Unformulated Glufosinate + Roundup PowerMAX	Adjuvant	2	0.0070*
Unformulated Glufosinate + Enlist One	Dose	1	0.0040*
Unformulated Glufosinate + Enlist One	Adjuvant	2	0.7260
Unformulated Glufosinate + Xtendimax	Dose	1	<0.0001*
Unformulated Glufosinate + Xtendimax	Adjuvant	2	0.2520

*: significant at an α=0.05.

Table 4.3: Run two ANOVA table.

Type II Test		
Effect	NDF	p-value
Dose	1	<.0001*
Herbicide	9	<.0001*
Adjuvant	2	<.0001*
Dose*Herbicide	9	<.0001*
Dose*Adjuvant	2	0.0950
Herbicide*Adjuvant	18	0.3240
Dose*Herbicide*Adjuvant	18	0.9600

*: significant at an α=0.05.

Table 4.4: Run two ANOVA table for the herbicide*dose interaction.

Herbicide(s)	Effect	NDF	p-value
Unformulated Glufosinate	dose	1	<.0001*
Liberty	dose	1	0.3020
Touchdown Hi-Tech	dose	1	<.0001*
Roundup PowerMAX	dose	1	0.3680
Enlist One	dose	1	0.0430*
Xtendimax	dose	1	<.0001*
Unformulated Glufosinate + Touchdown Hi-Tech	dose	1	<.0001*
Unformulated Glufosinate + Roundup PowerMAX	dose	1	0.8300
Unformulated Glufosinate +Enlist One	dose	1	0.3190
Unformulated Glufosinate + Xtendimax	dose	1	<.0001*

*: significant at an $\alpha=0.05$.

Figures

Figure 4.1: Scatterplots for run one displaying the relationship between biomass and dose for herbicides and adjuvants. The X-axis is biomass reduction, and the Y-axis is the dose for each adjuvant. Columns from left to right: Enlist One, Unformulated Glufosinate, Unformulated Glufosinate + Enlist One, Unformulated Glufosinate + Roundup PowerMAX, Unformulated Glufosinate + Touchdown Hi-Tech, Unformulated Glufosinate + Xtendimax, Liberty, Roundup PowerMAX, Touchdown Hi-Tech, Xtendimax. Rows from top to bottom: Adjuvant A, Adjuvant, and Adjuvant C.

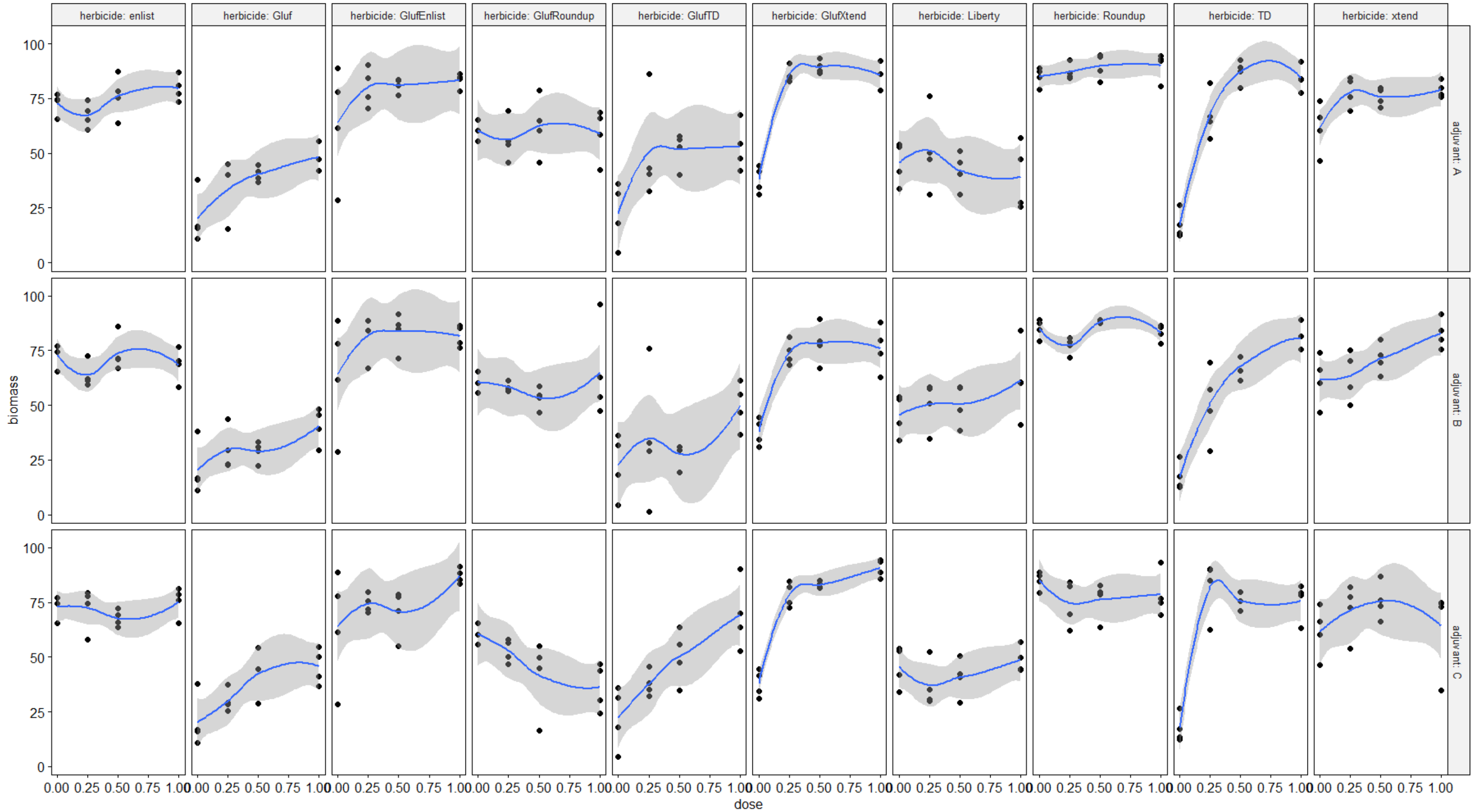

Figure 4.2: Scatterplots for run two displaying the relationship between biomass and dose for herbicides and adjuvants. The X-axis is biomass reduction, and the Y-axis is the dose for each adjuvant. Columns from left to right: Enlist One, Unformulated Glufosinate, Unformulated Glufosinate + Enlist One, Unformulated Glufosinate + Roundup PowerMAX, Unformulated Glufosinate + Touchdown Hi-Tech, Unformulated Glufosinate + Xtendimax, Liberty, Roundup PowerMAX, Touchdown Hi-Tech, Xtendimax. Rows from top to bottom: Adjuvant A, Adjuvant, and Adjuvant C.