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Evaluation of Glyphosate and PPO-Inhibiting Herbicide Tank-Mixtures to Manage Glyphosate Resistance in Soybean

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EVALUATION OF GLYPHOSATE AND PPO-INHIBITING HERBICIDE TANK-MIXTURES TO MANAGE GLYPHOSATE RESISTANCE IN SOYBEAN

by

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Protoporphyrinogen oxidase (PPO)-inhibiting herbicides in combination with glyphosate for postemergence (POST) applications is one of the primary alternatives to manage glyphosate-resistant weeds and the only effective POST chemical option in conventional and glyphosate-tolerant soybean to control glyphosate and ALS-inhibiting resistant weeds. Antagonistic interactions have been reported between many different herbicide modes of action and optimal droplet size may be affected by tank-mixtures of different herbicides. Additionally, the impact of adjuvants on the factors aforementioned as well as on physical properties needs to be thoroughly investigate to maximize herbicide efficacy. Therefore, the objectives of this research were to: 1) conduct greenhouse and field studies to evaluate the impact of glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) applied alone and in tank mixtures on weed control, optimal droplet size, drift potential, and tank mixture interactions, 2) determine the influence of adjuvants on tank mixtures interactions, spray droplet-spectra, drift potential, and physical properties, (3) determine if herbicide efficacy (and thereby, weed control) is correlated to reduced surface tension and contact angle. Overall, applications from the tank mixtures resulted in antagonistic interactions and some of them were overcame

by the addition of adjuvants. Droplet size and percent volume of droplets ≤ 150 μm were highly affected by nozzle type and spray solution. The oil based formulation of lactofen and crop oil concentrates were shattered by TTI nozzles due to its internal turbulence chamber creating smaller droplets and increasing driftable fines. The impact of nozzle selection on weed control was minimal and larger droplets at the rates and carrier volume used in this study could be used without compromising herbicide efficacy reducing drift potential. Adjuvants reduced the surface tension and contact angle of spray solutions; however, herbicide efficacy was only partially explained by the changes in these physical properties. Results emphasized the importance of better understanding the relationship among application variables and weed species. In addition, recommendations should be herbicide- and weed-specific in order to optimize herbicide applications and to maintain herbicide effectiveness.

I dedicate this thesis to my beloved husband Rafael Maschieri Bicudo whose sacrificial care for me and our son made it possible for me to complete this work and to my son Pedro Noah who is indeed a treasure from the Lord. I could not have done it without you. They are my source of inspiration and support.

They are my everything!

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CHAPTER 1

Literature Review

Glyphosate is the most widely used non-selective herbicide worldwide in cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), and soybean [*Glycine max* (L.) Merr.] due to its low toxicity, excellent efficacy, and unique mode of action (inhibits the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase; EPSPS) (Duke and Powles 2008). After its initial introduction, glyphosate was mainly used for preplant burndown applications and for desiccation of certain crops prior harvest. However, the rapid adoption of genetically modified glyphosate-resistant (GR) crops after 1996 has led to a heavy reliance on this broad-spectrum herbicide (Powles and Preston 2006) facilitating its use for postemergence (POST) applications to control several annual and perennial weeds in cropping systems (Corrigan and Harvey 2000; Gonzini et al. 1999). For instance, 56% of the globally used glyphosate has been estimated to occur during POST applications of herbicide-tolerant crops (Benbrook 2016).

A high level of optimism was created about the introduction of GR crops since acetolatate synthase (ALS) resistance was becoming more abundant and no resistant weeds to glyphosate had developed during its first 15 years of use. However, the use of a limited number of herbicide sites of action reduce weed management diversity and increase number of herbicide-resistant weed populations is likely to occur due to a single selection pressure (Knezevic 2007). Therefore, within a few years, three weeds would be confirmed GR including, rigid ryegrass (*Lolium rigidum* Gaudin) in Australia, goosegrass (*Eleusine indica (L.). Gaertn*.) in Malaysia, and horseweed (*Conyza canadensis (L.)*

Cronq) in the United States (Heap 2018). To date, a total of 48 weedy species have been reported to have glyphosate-resistance worldwide (Heap 2018).

To delay the evolution of herbicide resistance, tank mixtures of different herbicide sites of action have been widely recommended. Protoporphyrinogen oxidase (PPO)-inhibiting herbicides in combination with glyphosate for POST applications is a common approach to manage GR weed populations in cotton, corn, and soybean since the latter group injure mostly broadleaf plants. Furthermore, PPO-inhibiting herbicides are the only effective POST chemical option in conventional and GR only soybean to control weeds when resistance to both glyphosate and ALS-inhibiting herbicides is present. The inhibition of the PPO enzyme frequently leads to production of highly reactive singlet oxygen in the presence of light and molecular oxygen, resulting in lipid peroxidation (Duke et al. 1991; Sherman et al. 1991), followed by membrane disruption and plant death. PPO-inhibiting herbicides have many advantages such as low toxicity, low effective rates, quick onset of action, and long residual effect (Hao et al. 2011). In addition, resistance to PPO-inhibiting herbicides has been slow to evolve with only thirteen weed species worldwide and four weed species in the US (Heap 2018).

Antagonistic interactions to specific weed species have been reported in literature when glyphosate and PPO-inhibiting herbicides were applied in combination (Nandula et al. 2012; Starke and Oliver 1998). Glyphosate activity is often antagonized by fast-acting herbicides such as glufosinate and several PPO-inhibiting herbicides (Harre et al. 2018) because these contact herbicides may limit glyphosate translocation (Starke and Oliver 1998). Reduced effectiveness of tank-mixing herbicides to delay the evolution of resistance is likely to occur if mixtures do not show similar efficacy (Beckie and Reboud

2009). The loss of PPO-inhibiting herbicides as an effective chemical class would complicate an already complex agriculture problem since no new herbicide modes of action have been introduced for cotton, corn or soybean in greater than three decades.

Adding other chemicals into the tank-mixture may also affect spray droplet spectra generated from agricultural nozzles (Creech et al. 2015, Bouse et al. 1990). Spray application is a complex process and innumerous factors can affect herbicide efficacy resulting in reduced weed control, economic loss, and environmental contamination. Spray droplet size is recognized as a determining factor for herbicide efficacy (Knoche 1994) since they can affect spray deposition and drift (Taylor et al. 2004). When a spray droplet hits a plant surface, it will be retained, bounce, shatter, or run off. However, the leaf surface type, wettability and orientation, the surface tension and viscosity of the spray solution as well as the droplet size and velocity will influence the outcome (adhesion, bounce, shatter, or run off) of a droplet hitting the target (Zwertvaegher et al. 2014). Spray particle drift is also a concern for pesticide applicators due to the potentially detrimental effects of water contamination and off-target movement; moreover, sublethal glyphosate doses have been reported to confer moderate glyphosate resistance level in a Lolium rigidum Gaudin population (Busi and Powles 2009).

Adjuvants such as surfactants and oil concentrates are tank mixed or pre mixed with foliar-applied herbicides to enhance spray application (Bellinder et al. 2003) or to modify the action of herbicides (Johnson et al. 2006) as well as to increase spray droplet retention on leaf surface and penetration of herbicide active ingredient through the cuticle (Young and Hart 1998). More effective penetration and translocation of the product is likely to occur due to the changes on physical properties such as surface tension (SFT) and contact angle (CA) (Janků et al. 2012). Potential overcome antagonism between two herbicides also have been reported in literature by the addition of adjuvants into the tank-mixture (Campbell and Penner 1982; Young et al. 1996). In addition, adjuvants such as drift control agents have been used to reduce the amount of small spray droplets separating from larger droplets. However, previous research have shown that the performance of adjuvants is dependent on the herbicide with which it is applied, the plant species (and thereby, leaf structure surface), and environmental conditions (Knezevic et al. 2009; Penner 1989).

Objectives

The rapid widespread evolution of herbicide resistance and the lack of new herbicide modes of action highlight the importance of better understanding the relationship among application variables to maximize herbicide efficacy. How glyphosate and PPO-inhibiting herbicides interact when applied in tank mixtures as well as the impact on spray droplet spectra and changes on physical properties influenced by the addition of adjuvants needs to be thoroughly investigated in order to assure effective and sustainable weed management recommendations. The objectives of this research were to: 1) conduct greenhouse and field studies to evaluate the impact of glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) applied alone and in tank mixtures on weed control, optimal droplet size, drift potential, and tank mixture interactions, 2) determine the influence of adjuvants on tank mixtures interactions, spray droplet-spectra, drift potential, and physical properties, (3) determine if herbicide efficacy (and thereby, weed control) is correlated to reduced surface tension and contact angle.

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CHAPTER 2

Nozzle Selection and Adjuvant Impact on the Efficacy of Glyphosate and PPO-Inhibiting Herbicide Tank-Mixtures

Abstract

Antagonistic interactions have been reported when glyphosate and PPO-inhibiting herbicides are applied in tank-mixture and adjuvants may be used to overcome this effect. Herbicide efficacy as well droplet size and drift potential may be impacted by tank mixtures. Therefore, greenhouse experiments were conducted across two years using six nozzles (XR, AIXR, GA, TDXL, ULD, and TTI) and three herbicides (glyphosate, fomesafen, or lactofen) applied alone and in tank mixtures with or without adjuvants (COC, NIS, MSO, or drift retardant) to common lambsquarters, grain sorghum, kochia, and horseweed to better understand droplet-spectra distribution, drift potential, weed control, and tank-mixture interactions. The results of this research indicate that droplet size was not the major contributing factor on herbicide efficacy of PPO-inhibiting herbicides, glyphosate and tank-mixtures of the two, but it is highly affected by nozzle type, herbicide formulation, or the tank mixture. Nozzle type by spray solution interaction were observed for droplet size, but the interactions did not affect the efficacy of the solutions. In order to optimize herbicide applications, herbicide type, adjuvant type, plant species, and environmental conditions should be taken in consideration. Larger droplets could be used effectively without compromising herbicide performance at the majority of treatments reducing the drift potential. Tank-mixtures, applied with or without adjuvants, consistently antagonized common lambsquarters, grains sorghum, and horseweed and the performance of the adjuvant was herbicide- and weed-specific.

Introduction

The development and rapid adoption of glyphosate-resistant (GR) crops has led to a heavy reliance on glyphosate as a chemical option (Powles and Preston 2006) facilitating its use for postemergence (POST) applications in corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] production systems to control several annual and perennial weeds (Corrigan and Harvey 2000; Gonzini et al. 1999). Moreover, the increase of no-till soybeans has modified the weed management practices relying on this broad-spectrum herbicide to control emerged weeds prior planting (Bruce and Kells 1990). Glyphosate is the most widely used non-selective herbicide worldwide in GR crops due to its excellent efficacy, low toxicity, and it is the only molecule that effectively inhibits the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) (Duke and Powles 2008). The repeated use of glyphosate has created a single selection pressure on weed populations (Knezevic 2007) increasing the occurrence of GR weeds. Amongst them, resistant populations of common lambsquarters (*Chenopodium album L*.), horseweed [*Conyza canadensis (L.) Cronq*.], and kochia [*Kochia scoparia (L.) Schrad*.]- placed between the ten most troublesome weeds in broadleaf crops (WSSA 2017) - have been reported in the United States (Heap 2018).

EPSPS-, acetolactate synthase (ALS)-, and protoporphyrinogen oxidase (PPO)-inhibiting herbicides are the only three POST chemical options to manage broadleaf weeds in a GR soybean production system. However, many weeds including the species aforementioned, have also been confirmed to be resistant to ALS-inhibiting herbicides. Therefore, PPO-inhibiting herbicides are the only effective POST chemical option to manage GR and ALS-resistant weeds in a conventional and GR soybean production system. Glyphosate applied in tank mixture with PPO-inhibiting herbicides is a common approach and recommendation to increase weed control spectrum since the latter group injure mostly broadleaf plants. Glyphosate activity is often antagonized by fast-acting herbicides such as glufosinate and several PPO-inhibiting herbicides (Harre et al. 2018) because these contact herbicides may limit glyphosate translocation (Starke and Oliver 1998). The effect of inhibition of plant PPO activity frequently results in the massive production of reactive singlet oxygen, followed by attack of lipid and protein membranes, leading to death of the plant (Duke et al. 1991; Sherman et al. 1991).

Additive and antagonistic herbicide interactions have been reported in literature to occur more often than synergistic interaction when glyphosate is applied in tank mixture with other herbicides (Harre et al. 2018). Furthermore, tank-mixture effectiveness may be reduced when mixtures do not show similar efficacy increasing the potential risk of herbicide resistance evolution (Beckie and Reboud 2009). Adjuvants are used in tank mixtures with herbicides to enhance spray application (Bellinder et al. 2003) or modify the action of herbicides (Johnson et al. 2006) and have been reported to potentially overcome antagonism between two herbicides (Campbell and Penner 1982; Young et al. 1996). However, previous research has shown that the performance of adjuvants is dependent on the herbicide with which it is applied, the plant species targeted, and environmental conditions (Knezevic et al. 2009; Penner 1989).

In addition, spray application factors such as droplet size play a crucial role on spray performance (Butts et al. 2018; Creech et al. 2015; Hanks 1995). Droplet size is highly affected by nozzle type, operation pressure, nozzle orifice size, carrier volume, or adding other chemical into the tank mixture (Creech et al. 2015; Creech et al. 2016;

Etheridge et al. 1999; Bouse et al. 1990). For instance, smaller droplets from XR (extended range) flat fan nozzles have been reported to be more effective than larger droplets when applying POST herbicides at a constant carrier volume (Knoche 1994). In contrast, no differences in control were observed when fomesafen or lactofen were applied to different weed species using XR or air-induction (AI) nozzles (Berger et al. 2014; Sikkema et al. 2008). Although non-air inclusion flat fan nozzles provide more coverage than air inclusion flat fan nozzles and conflicting results can be found in literature, more recent research has shown that herbicide efficacy is not solely affected by droplet size. Herbicide efficacy is highly dependent on nozzle type, nozzle orifice size, spray operation pressure, carrier volume, adjuvants, herbicides, weed size, weed species, and environmental conditions (Brown et al. 2007; Butts et al. 2018; Creech et al. 2015; Creech et al. 2016; Mellendorf et al. 2015; Ramsdale and Messersmith 2001; Sikkema et al. 2008)

Ultimately, spray drift is an important issue for pesticide applicators due to the potentially detrimental effects of water contamination and off-target movement. Moreover, sublethal glyphosate doses have been reported to confer a moderate glyphosate-resistance level in a *Lolium rigidum Gaudin* population (Busi and Powles 2009). Although smaller droplets provide more coverage, spray droplets less than 150 µm have been considered the most prone to drift (Yates et al. 1985). Spray applications are a complex process and studies showing nozzle selection by tank mixture interactions on herbicide efficacy, weed control, and spray-droplet distribution are crucial for understanding and managing herbicide resistance. Therefore, greenhouse studies were conducted across two years using multiple nozzle designs both with and without drift reduction technology (DRT) to provide a wide range of droplet sizes. Moreover, herbicide treatments with or without adjuvants as well as tank mixtures or single applications were included to provide a better understanding in terms of droplet-spectra distribution, drift potential, plants species control, and tank-mixture interactions. The objectives of the greenhouse experiment conducted in 2016 were to: (1) determine the impact of nozzle selection (and thereby, droplet size) on the efficacy of glyphosate and PPO-inhibiting herbicides (lactofen or fomesafen) applied alone and in tank mixtures to four plant species while evaluating their drift potential, and (2) determine the type of interaction when tank mixtures are used. The objectives of the greenhouse experiment conducted in 2017 were to: (1) determine the impact on the efficacy of glyphosate or PPO-inhibiting herbicide (lactofen) applied alone and in tank mixtures by using different adjuvants to four plants while evaluating two extremes in droplet size, (2) evaluate the impact of adjuvants on drift potential, and (3) determine the impact of adjuvants on the type of interaction when tank mixtures are used.

Materials and Methods

Plant Material. Greenhouse experiments were conducted at the Pesticide Application Technology Laboratory (PAT Lab) located at the West Central Research and Extension Center in North Platte, NE, during the years of 2016 and 2017. Seeds from putative glyphosate-susceptible (GS) populations of common lambsquarters and grain sorghum [*Sorghum bicolor (L.) Moench subsp. bicolor*] and GR populations of horseweed (ED_{50} of 639 g ae ha^{-1} based on dry biomass, collected at 40.01°N, W95.44°W) and kochia (ED_{50} of 1607 g ae ha^{-1} based on dry biomass, collected at

41.16°N, W101.99°W) were used in both years. Although grain sorghum is not considered weedy species, it was selected because it is representative of other weed grass species due its similarity in biology and morphology yet much easier to cultivate in the greenhouse.

For the greenhouse experiment conducted in 2016, plants were seeded at different intervals between June and July and grown in D40H cone-tainer cells[1] filled with Berger BM7 Bark Mix[2], which is a growing medium limed to 5.5 to 6.5 pH. Plants were watered with overhead irrigation as needed and fertilized weekly by watering with 1:500 ratio injected 10-4-3 fertilizer[3]. Greenhouse was maintained at a daytime temperature between 25 – 30 C and a nighttime temperature between 16 – 24 C. No supplemental lighting was used. Common lambsquarters and kochia plants were treated with *Bacillus thurigiensis*[4] to avoid *Trichoplusia ni* (Cabbage looper).

For the greenhouse experiment conducted in 2017, seeds were planted at different intervals between May and July and grown in D40H cone-tainer cells[1] filled with Pro-Mix BX[5] general purpose growing medium. Plants were overhead irrigated and fertilized daily with a commercial fertilizer[6] blended with water at 0.2% $v.v^{-1}$. Greenhouse was maintained at a daytime temperature between 26 – 30 C and a nighttime temperature between 18 – 23 C. LED growth lights[8] (520 µmol $s^{-}1$) were used as supplemental lighting during 8-h a day. Plants were treated with *Bacillus thuringiensis*[8]; in addition, common lambsquaters and kochia plants were treated with another *Bacillus thuringiensis*[4] to avoid *Trichoplusia ni* (Cabbage looper).

Herbicide Applications. Greenhouse experiments during 2016 and 2017 were arranged as a randomized complete block design with factorial arrangements of treatments. Each experiment had five replications for each species and two independent experimental runs. Herbicide treatments were applied to 10-15 cm plants height and to 10 cm diameter horseweed rosettes. Spray herbicide applications were made using a three-nozzle research track sprayer[9] with nozzles spaced 50 cm apart and 50 cm above the plants, meeting the manufacturers boom height recommendation to ensure appropriate spray pattern uniformity, delivering 187 L ha^{-1} at 276 kPa at speed of 9.6 kph.

For the greenhouse experiment conducted in 2016, the treatments were arranged in a five by six factorial plus an untreated control consisting of five spray solutions and six nozzle types (Table 1) with the same orifice size. Spray treatments consisted of POST applications using glyphosate[10] at 600 g ae ha^{-1}, fomesafen[11] at 65 g ai ha^{-1}, or lactofen[12] at110 g ai ha^{-1} alone and in tank mixtures. Liquid ammonium sulfate[13] at 2.5% v v^{-1} was added to treatments. Crop oil concentrate[14] (COC) at 1% v v^{-1} was used in treatments except for glyphosate applied alone.

For the greenhouse experiment conducted in 2017, the treatments were arranged in a ten by two factorial plus an untreated control consisting of ten spray solutions and two nozzle types (Table 1) with the same fan angle and orifice size. Spray treatments, which consisted of POST applications of glyphosate[10] at 600 g ae ha^{-1} or lactofen[12] at 110 g ai ha^{-1} alone, lactofen at 110 g ai ha^{-1} with the adjuvants COC at 1% v v^{-1}, NIS[15] at 0.25% v v^{-1}, methylated seed oil[16] (MSO) at 1% v v^{-1}, or drift retardant agent[17] (DRA) at 0.5% v v^{-1}, and herbicides applied in tank-mixture with each of the adjuvants aforementioned. COC was added to the tank-mixture when DRA was used. Liquid ammonium sulfate[13] at 2.5% v v^{-1} was added to treatments.

Data Collection. After plants were sprayed, plants were clipped at the soil surface at 28 days after treatment (DAT) and placed in a dryer for seven days at 65 C until plants reached a constant mass. Dry biomass was recorded and converted into percent biomass reduction as (Equation 1):

$$100 - \left(\frac{X*100}{Y}\right) \qquad [1]$$

where X is the biomass of an individual experimental unit after being treated and Y is the mean biomass of the untreated control replicates. Hereafter, percent biomass reduction will be referred as percent of control.

Analysis of Spray Droplet Size. The spray-droplet distribution for each treatment used in the greenhouse experiments conducted in 2016 and 2017 (water alone was included as treatment for comparison in the greenhouse experiment conducted in 2017) was evaluated using a low-speed wind tunnel at the PAT Laboratory. Each nozzle was tested at 276 kPa and a laminar wind speed velocity of 6.7 m s^{-1} (Fritz et al. 2014). Droplet size measurements were made using a Sympatec HELOS-VARIO/KR laser diffraction instrument with an R7 lens[18] (Sympatech Inc., Clausthal, Germany). This lens is capable of detecting droplets in a range from 18 to 3,500 µm. The spray plume was oriented perpendicular on the laser beam and traversed through the laser beam by means of mechanical linear actuator. The actuator moves the nozzle at a constant speed of 0.2 m s^{-1} such that the entire spray plume would pass through the laser beam. During application, nozzle was traversed through the laser beam 3 times, with each pass serving as one repetition for statistical analysis. The distance from the nozzle tip to the laser was 30 cm. Henry et al. (2014) and Creech et al. (2015) provide detailed information regarding the low-speed wind tunnel and its operation at the PAT Lab. Treatments were compared using the $D_{v0.1}$, $D_{v0.5}$, and $D_{v0.9}$ volumetric droplet size spectra parameters, which represent the droplet size such that 10, 50, and 90% of the spray volume is contained in droplets of smaller diameters, respectively. The percentage of the spray volume contained with droplet diameters less than 150 µm (driftable fines) and the relative span (RS) were measured. RS was calculated to indicate the uniformity of the spray droplet distribution as (Equation 2):

$$RS = \frac{Dv0.9 - Dv0.1}{Dv0.5} \qquad [2]$$

The spray classification category was assigned based on reference curves created from reference nozzle data at the PAT Lab as described by ASABE S572.1 (ASABE 2009) allowing the results to be compared with data derived from other laboratories (Fritz et al. 2014).

Statistical Analyses. Data were subjected to ANOVA using a generalized linear mixed model (PROC GLIMMIX) in SAS (Statistical Analysis Software, version 9.4, Cary, North Carolina, USA) with mean separations made at $\alpha = 0.05$ level using Fisher's protected LSD test and the Tukey adjustment. For the greenhouse experiment conducted either in 2016 or 2017, each species was analyzed separately. To meet the model assumptions, percent of control was analyzed using beta distribution as the data were bound between 0 and 1 (Stroup 2013; Butts et al. 2017). Significant run by treatment interaction was not observed for each plant species within a year; therefore, data were pooled over experimental runs and spray solution and nozzle selection were analyzed as fixed effects while replication as a random effect. For the spray droplet spectra study, to meet the model assumptions, gamma distribution was used to analyze $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ as the data were bound between 0 and infinity whereas Gaussian distribution was used to analyze the percent volume of droplets ≤ 150 µm and relative span (Butts et al., 2017; Stroup, 2013). When beta and gamma distributions were used results were back-transformed for discussion.

Expected responses of the tank mixtures were calculate using Colby´s equation (Colby, 1967). If E is the expected growth reduction as a percent of control using two herbicides in tank-mixture (A + B), and X and Y are the observed growth reduction as a percent of control when herbicide (A or B) was applied alone, then, according to Colby (1967) (Equation 3):

$$E_1 = \frac{X_1 Y_1}{100} \qquad [3]$$

where $E_1 = 100 - E$; $X_1 = 100 - X$; and $Y_1 = 100 - Y$. T tests ($\alpha = 0.05$) in SAS were used to determine the statistical significance of the differences between observed and expected responses as percent of control. Therefore, when the observed control from the tank mixture was less than, equivalent to, or greater than the expected control, the response was considered antagonistic, additive, or synergistic, respectively (Colby, 1967; Lich et al., 1997).

Results and Discussion

Greenhouse Experiment Conducted in 2016. Nozzle selection by spray solution interaction was not significant regardless of the plant species. Therefore, data of each species were combined across nozzles. At a constant carrier volume, main effect of spray solution was significant for the four plant species. In contrast, nozzle selection as main effect was not significant for any of the four plant species. The results from this experiment are consistent with other findings where no differences in glyphosate and fomesafen efficacy were observed using either the XR or AI nozzles on common ragweed (*Ambrosia artemisiifolia L.*), velvetleaf (*Abutilon theophrasti Medik.*), and common lambsquarters control (Sikkema et al. 2008). Likewise, no differences in control of common lambsquarters and shattercane [*Sorghum bicolor* (*L.*) *Moench ssp. verticilliflorum* (*Steud.*) *de Wet ex Wiersema & J. Dahlb.*] using same nozzles were reported from saflufenacil applications (Creech et al. 2016). Similarly, droplet size increases were negatively correlated with lactofen performance on the control of Palmer amaranth (Berger et al., 2014). Conflicting results can be found in literature regarding to droplet size impacting weed control illustrating the complexity of herbicide applications. For instance, Creech et al. (2016) observed increased control of common lambsquarters from applications using a Fine spray when cloransulam-methyl and glufosinate were applied. Similarly, Butts et al. (2018) reported increased weed control as droplet decreased using dicamba and glufosinate; however, increased carrier volume (187 l ha^{-1}) buffered this effect. Therefore, the impact of nozzle selection (and thereby, droplet size) for achieving satisfactory weed control varies among herbicides, carrier volumes and targeted plant species (Butts et al. 2018; Creech et al. 2015, 2016; Sikkema et al. 2008).

The $Dv_{0.5}$ ranged from 240 to 787 µm which represents a change from Fine to Ultra Coarse on the spray droplet classification category (Table 2). Additionally, the $Dv_{0.1}$, $Dv_{0.5}$, $Dv_{0.9}$ values ranked from smallest to largest, using the XR nozzle, followed by the AIXR, GA, TDXL, ULD, and TTI nozzles, regardless of the spray solution (Table 2). The results indicate that nozzle selection is more important than tank solution in determining droplet size, which is also confirmed by Creech et al. (2015) and Henry et al. (2016). In contrast, the percent of driftable fines was affected by the interaction of nozzle type and spray solution. Irrespective of the spray solution, the percentage of driftable fines followed a trend of largest to smallest for applications using the XR, ULD, and TTI nozzles, respectively (Table 2). Additionally, applications using the TTI nozzle reduced the percentage of fines by greater than 94% when compared to applications from the XR nozzle, regardless of the spray solution (Table 2). Either PPO herbicide applied alone produced larger values of $Dv_{0.5}$ when compared to single applications of glyphosate except for the TTI nozzle or the ULD nozzle combined with fomesafen (Table 2). An interaction by the combination of TTI nozzle and oil based products on droplet size and percentage of fines produced has been observed before (data not published), and therefore was not surprising. The TTI nozzle incorporates an internal turbulence chamber inside of the nozzle body increasing droplet size, reducing fine droplets and improving the spray pattern uniformity (Klein and Kruger 2011). It is hypothesized that the emulsion formed by emulsifiable concencetrate (EC) products such as lactofen as well crop oil concentrate adjuvants is shattered when passing through the turbulence chamber creating smaller droplets (smaller $Dv_{0.5}$ values) and increasing the percentage of fines compared to other product formulations. Different from the nozzles used in this study, the ULD nozzle incorporates two pre-orifice openings. This feature as part of the DRT may have interacted with fomesafen a soluble liquid product applied alone decreasing the $Dv_{0.5}$ value compared to glyphosate alone, however, the mechanism is not completely known or understood. Results from this study show that besides nozzle type, herbicide formulation (or the interaction of both) may affect drift potential (Stainier et al. 2006).

The observed percent of control values obtained from applications using glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) alone and in tank mixtures and the expected responses from the tank mixtures as calculated by Colby´s equation are summarized in Table 3. Applications of glyphosate alone resulted in the highest control of common lambsquarters and grain sorghum with 91 and 96%, respectively, and the lowest control of kochia and horseweed with 27 and 28%, respectively. However, when applied in tank mixture with either PPO herbicide, no differences in control were observed for common lambsquarters or grain sorghum compared to glyphosate applied alone. The applications of lactofen alone resulted in better control of kochia and horseweed compared to fomesafen applied alone; furthermore, lactofen alone provided the highest control of both species with 89 and 54%, respectively. However, same control was observed for kochia when applied in combination with glyphosate. In contrast, no differences in control of grain sorghum were observed when using single applications of fomesafen or lactofen. Similarly, fomesafen applied alone improved the control of common lambsquarters in 11% compared to lactofen applied alone. The addition of fomesafen or lactofen into the tank mixture did not improve the control of grain sorghum and common lambsquarters compared to glyphosate applied alone and antagonistic interactions were observed for both species. Likewise, fomesafen applied in combination with glyphosate reduced the control of kochia and horseweed in 4 and 3%, respectively, compared to fomesafen applied alone. Although differences were not significant, antagonistic interactions were also observed. The most dramatic antagonistic interaction was observed when lactofen and glyphosate were applied in tank mixture to horseweed. The combination of both herbicides reduced the control in 11% compared to lactofen applied alone. These findings were similar to those of Starke and Oliver (1998), who found that the combination of glyphosate and a PPO-inhibitor, such as sulfentrazone or fomesafen, caused reduced efficacy of both herbicides when applied to several weed species. Likewise, flumiclorac antagonized glyphosate when applied to Palmer amaranth (Nandula et al., 2012).

Greenhouse Experiment Conducted in 2017. Nozzle selection by spray solution interaction was not significant regardless of the plant species. Therefore, data of each plant species were combined across nozzles. At a constant carrier volume using half of the labeled rates, main effect of spray solution was significant for the four plant species. In contrast, nozzle selection as main effect was significant only for kochia (Table 2.4.). Percent of kochia control increased by 14% from applications using the XR nozzle compared to the TTI nozzle and differences in control may be affected by the addition of adjuvants. Observations from this experiment have been in consensus with previous research, Zabkiewicz (2000) reported the influence of a specific adjuvant on herbicide efficacy may depend upon the nozzle selection.

The $Dv_{0.5}$ ranged from 248 to 809 µm which represents a change from Fine to Ultra Coarse on the spray droplet classification category (Table 2.5.). Additionally, the XR and TTI nozzles produced smallest and largest the $Dv_{0.1}$, $Dv_{0.5}$, $Dv_{0.9}$ values and largest and lowest percentage of fines, respectively, regardless of the spray solution (Table 2.5.). These results are consistent with literature where nozzles that incorporate DRT´s produce larger droplets than non-air inclusion flat fan nozzles at a given pressure and reduce the percentage of driftable fines (Etheridge et al. 1999). Although spray-droplet distribution was affected by the addition of adjuvants into the tank mixture, the results from this exsperiment confirm the results from the greenhouse experiment conducted in 2016 indicating that nozzle selection is more important than tank solution in determining droplet size and percentage of fine droplets. Irrespective of the spray solution, the percentage of driftable fines was minimized by the use of the TTI nozzle, which reduced this percentage in greater than 90% when compared to applications from the XR nozzle. However, interactions between nozzle type and spray solution was observed in both experiments. For instance, applications of lactofen in combination with COC plus DRA using the XR nozzle did not increase the $Dv_{0.5}$ nor reduced the percentage of driftable fines when compared to lactofen applied alone or in combination with COC, NIS, or MSO. Moreover, the same pattern was observed among tank mixtures. In contrast, an opposite behavior either for the single applications of lactofen or for the lactofen in tank mixtures were observed from applications using the TTI nozzle. Drift retardants normally mitigate drift potential by increasing viscosity and reducing the number of fine droplets (Mcmullan 2000). However, conflict results have been observed in literature. For example, Johnson et al. (2006) reported that any of the three drift retardants evaluated using the XR nozzle and two using the AI nozzle reduced the injury drift distance on sorghum. Similarly, (Creech et al. 2018) observed decrease in $Dv_{0,5}$ values and increase in percentage of fines when using a different drift retardant in combination with the TTI and AITTJ at same pressure used in this experiment but with a different nozzle orifice size (11005). Therefore, it is hypothesized that unexpected results using drift retardants are due to a nozzle design, nozzle orifice size, operation pressure, and product formulation interaction. Likewise, an interaction by the combination of TTI nozzle and EC and oil based adjuvant formulation on droplet size and percentage of fines produced was observed in this experiment confirmed and explained by the experiment conducted in 2016.

The observed percent of control values obtained from applications using lactofen or glyphosate alone, lactofen alone in combination with adjuvants (COC, NIS, MSO, or COC plus DRA), and tank mixtures of lactofen and glyphosate applied in combination with the aforementioned adjuvants as well as the expected responses from the tank mixtures as calculated by Colby´s equation are summarized in Table 2.6. Same population of each species used in the experiment conducted in 2016 was used in this study. Percent of control of the four plant species increased when lactofen was applied in combination with COC, NIS, or MSO compared to lactofen applied alone. Although no significant differences in control were observed when any of the adjuvants were applied in combination with lactofen, regardless of the plant species, the control of kochia decreased by 8 and 15% when NIS was applied with lactofen compared to the addition of COC or MSO, respectively. Likewise, the control of grain sorghum decreased by 8 and 12% when NIS was applied with lactofen compared to the addition of MSO or COC, respectively. Similarly, no significant differences in control were observed when any of the adjuvants were applied in tank mixtures to horseweed, kochia, and grain sorghum. In contrast, significant differences in control were observed when glyphosate and lactofen were applied in tank mixture with COC, NIS, or MSO to common lambsquarters. The addition of NIS into the tank-mixture increased the control of common lambsquarters by 19 and 35% compared to the addition of MSO or COC, respectively. Although, the Cobra® label discourages the use of this herbicide in combination with drift retardants, no significant differences in control were observed when the DRA was applied in combination with lactofen and COC compared to applications of lactofen and COC only, regardless of the plant species. However, the efficacy of a specific adjuvant is dependent on the herbicide with which it is tank mixed, the plant species, and environmental conditions (Penner 1989).

Kochia was the only species where antagonistic interactions were not observed, regardless of the adjuvant added into the tank-mixture. In contrast, observed responses of horseweed were less than the expected by 7, 10, and 14% by the addition of COC, MSO, or NIS into the tank mixture, respectively. Likewise, observed responses of common lambsquarters were less than the expected by 25, 28, and 41% by the addition of MSO, DRA plus COC, or COC into the tank mixture, respectively. Observed responses of grain sorghum were less than the expected by 5 and 7% by the addition of MSO and COC, respectively. Observed and expected differences in control aforementioned were significant resulting in antagonistic interactions. Observations from this experiment are in consensus with the findings from the experiment conducted in 2016 and previous research. For instance, combinations of several PPO-inhibiting herbicides in tank mixtures with glyphosate were antagonized on a number of broadleaved weeds (Creech et al. 2016, Harre et al. 2018, Nandula et al. 2012, Starke and Oliver 1998). In contrast, the addition of NIS into the tank mixture overcame the antagonistic interactions when applied to kochia and grain sorghum. Likewise, antagonistic interactions were observed for horseweed, common lambsquarters, and grain sorghum by the addition of COC into the tank mixture; however, this interaction changed from antagonistic to additive for horseweed and grain sorghum by the addition of the DRA into the tank mixture. These observation are in consensus with (Kammler et al. 2010), who observed that the antagonism of clethodim and sethoxydim by halosulfuron was weed species- and adjuvant-specific.

The results observed in this research indicate that nozzle selection (and thereby, droplet size) was not the major contributing factor on herbicide efficacy. However, herbicides, adjuvants, plant species, and environmental conditions should be taken in consideration in selection of the nozzle type to optimize spray applications. Larger droplets could be used effectively without compromising herbicide performance at the majority of treatments tested in this research reducing the drift potential. Glyphosate and PPO-inhibiting herbicides in tank mixtures applied with or without adjuvants were consistently antagonized to common lambsquarters, grain sorghum, and horseweed. Results emphasize the complexity of application variables and the importance of additional research to identify common trends related to application parameters among tank mixtures and across multiple weed species. Moreover, spray droplet size produced cannot be predictable and may be affected by nozzle type and herbicide formulation interaction. Off-target movement of spray applications as well as antagonistic interactions should be avoided to delay the evolution of herbicide resistance and to maintain herbicide effectiveness.

Source of Materials

[1] Stuewe and Sons Inc., Corvallis, OR 97389

[2] Berger.ca, Saint-Modeste, QC Gol 3W0

[3] Nature’s Source® Professional Plant Food, Ball Food, Ball DPF, LLC Sherman, TX 75090

[4] Thuricide®, Bonide Products, Inc., Oriskany, NY 13424

[5]Premier Tech Horticulture Ltd, Rivière-du-Loup, QC G5R 6C1, Canada

[6] Wilbur-Ellis Agribusiness, 3300 South Parker Road, Suite 500, Aurora, CO 80014

[7] Philips Lighting Holding B.V., Somerset, NJ 08873

[8] DiPel®, Valent, 1600, Riviera Avenue, Suite 200, Walnut Creek, Ca 94596

[9] DeVries Manufacturing, Hollandale, MN 56045

[10] Roundup PowerMax®, Monsanto Company, St Louis, MO 63167

[11] Flexstar®, Syngenta Crop Protection, Greensboro, NC 27419

[12] Cobra®, Valent USA Corporation, Walnut Creek, CA 94596

[13] Bronc®, Wilbur-Ellis Company, Fresno, CA 64596

[14] R.O.C®, Wilbur-Ellis Company, Fresno, CA 64596

[15] R-11®, Wilbur-Ellis Company, Fresno, CA 64596

[16] High Load®, Wilbur-Ellis Company, Fresno, CA 6459

[17] Intact™, Precision Laboratories LLC, Waukegan, IL 60085

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Table 2.1 Nozzle selection used in the experiment conducted in 2016 or 2017 classified by their manufacturer and spray drift reduction technology (DRT) feature.

Experiment Year	Common name	Nozzle type[a]	DRT Feature	Manufacturer
2016 / 2017	Extended Range	XR	None	Teejet Technologies, Spraying Systems Co., Wheaton, IL, 62703
2016	Air-Induction Extended Range	AIXR	Venturi, pre-orifice	Teejet Technologies, Spraying Systems Co., Wheaton, IL, 62703
2016 / 2017	Turbo Teejet Induction	TTI	Venturi, pre-orifice, anvil shaped	Teejet Technologies, Spraying Systems Co., Wheaton, IL, 62703
2016	Guardian Air	GA	Venturi, pre-orifice, off-set angle	Pentair Hypro, New Brighton, MN, 55112
2016	Ultra Lo-Drift	ULD	Venturi, pre-orifice	Pentair Hypro, New Brighton, MN, 55112
2016	TurboDrop® XL	TDXL	Dual cap, Venturi, pre-orifice	Greenleaf Technologies, Covington, LA, 70434

[a] The listed nozzle types were all orifice size "04" with a manufacturer-rated spray plume angle of 110° except for ULD nozzles that were 120.

Table 2.2. The effect of different spray solutions on spray droplet size distribution from six nozzle types using a low-speed wind tunnel at the Pesticide Application Technology (PAT) Laboratory in North Platte, Nebraska. Experiment conducted in 2016.

Nozzle type	Spray solution[b]	Spray-droplet distribution[a]					
		$D_{v0.1}$	$D_{v0.5}$[c]	$D_{v0.9}$	≤ 150 µm	RS	CC[d]
		———	µm	———	%		
XR	Glyphosate	105 t	240 v	406 y	21.30 a	1.25 a	F
XR	Fomesafen + COC	139 u	265 u	413 x	12.14 c	1.03 e	M
XR	Lactofen + COC	140 u	268 t	425 w	12.05 c	1.06 d	M
XR	Glyphosate + Fomesafen + COC	138 u	266 u	430 v	12.37 b	1.09 c	M
XR	Glyphosate + Lactofen + COC	140 u	269 t	416 x	11.96 c	1.03 e	M
GA	Glyphosate	192 t	397 r	594 u	5.35 d	1.01 fghi	C
GA	Fomesafen + COC	234 p	432 p	622 s	2.31 i	0.90 o	VC
GA	Lactofen + COC	237 o	443 o	687 q	2.24 i	1.02 efgh	VC
GA	Glyphosate + Fomesafen + COC	220 q	409 q	598 tu	2.69 h	0.92 n	C
GA	Glyphosate + Lactofen + COC	207 g	393 s	602 t	3.39 f	1.01 ghij	C
AIXR	Glyphosate	220 q	453 n	723 o	2.91 gh	1.02 ef	VC
AIXR	Fomesafen + COC	261 l	473 m	675 r	1.70 j	0.88 p	VC
AIXR	Lactofen + COC	256 mn	481 l	742 o	1.68 j	1.01 fghi	VC
AIXR	Glyphosate + Fomesafen + COC	212 r	444 o	726 o	3.81 e	1.16 b	VC
AIXR	Glyphosate + Lactofen + COC	255 n	471 m	711 p	1.83 j	0.97 k	VC
TDXL	Glyphosate	233 p	505 j	822 j	3.08 g	1.16 b	VC
TDXL	Fomesafen + COC	292 j	527 i	758 m	1.17 l	0.88 op	VC
TDXL	Lactofen + COC	300 i	540 h	810 k	1.04 lm	0.94 lm	XC
TDXL	Glyphosate + Fomesafen + COC	272 k	504 j	743 n	1.24 kl	0.93 mn	VC
TDXL	Glyphosate + Lactofen + COC	258 lm	500 k	797 l	1.4 1k	1.08 d	VC
ULD	Glyphosate	310 h	610 f	933 fg	1.06 lm	1.02 efg	XC
ULD	Fomesafen + COC	325 de	602 g	889 i	0.70 nopq	0.94 lm	XC
ULD	Lactofen + COC	329 d	624 e	938 f	0.71 nopq	0.98 k	XC
ULD	Glyphosate + Fomesafen + COC	323 ef	610 f	906 h	0.85 mn	0.95 l	XC
ULD	Glyphosate + Lactofen + COC	317 g	609 f	927g	0.81 no	1.00 hij	XC

TTI	Glyphosate	399 a	787 a	1136 b	0.52 q	0.94 lm	UC
TTI	Fomesafen + COC	338 c	640 d	973 d	0.6 opq	0.99 j	XC
TTI	Lactofen + COC	341 c	653 c	996 c	0.58 pq	1.00 hij	XC
TTI	Glyphosate + Fomesafen + COC	364 b	754 b	1174 a	0.50 q	1.07 d	UC
TTI	Glyphosate + Lactofen + COC	319 fg	613 f	951 e	0.76 nop	1.03 e	XC

[a] Abbreviations = $D_{v0.1}$, $D_{v0.5}$, and $D_{v0.9}$: Parameters which represent the droplet diameter such that 10, 50, and 90% of the spray volume is contained in droplets of lesser diameters, respectively;
≤ 150 µm = Percent of spray volume with droplet diameters less than 150 µm;
RS: Relative span, a dimensionless parameter that estimates the uniformity of a droplet size distribution.

[b]Abbreviation: COC, crop oil concentrate.

[c] Means within a column followed by the same letter are not different (P > 0.05).

[d] The classification category for this study were made based on reference curves created from reference nozzle data at the Pesticide Application Technology Laboratory as described by ASAE 572.1 where F = Fine, M = Medium, C = Coarse, VC = Very Coarse, XC = Extremely Coarse, and UC = Ultra Coarse

Table 2.3. Observed and expected responses calculated using Colby's equation of four plants species control based on dry biomass using glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) applied alone and in tank mixtures in North Platte, Nebraska. Experiment conducted in 2016.

Spray solution[a]	**Common lambsquarters**			**Grain sorghum**			**Kochia**			**Horseweed**		
	—— % ——		P-value	—— % ——		P-value	—— % ——		P-value	—— % ——		P-value
Glyphosate	91[b] a			96 a			27 c			28 c		
Fomesafen + COC	74 b			48 b			84 b			44 b		
Lactofen + COC	63 c			50 b			89 a			54 a		
Glyphosate + fomesafen + COC	90 a	(98)[c]	<.0001	94 a	(98)	<.0001	80 b	(88)	0.0010	41 b	(59)	<.0001
Glyphosate + lactofen + COC	89 a	(97)	<.0001	95 a	(98)	<.0001	89 a	(92)	0.3374	43 b	(67)	<.0001

[a] Abreviations: COC, crop oil concentrate.

[b] Means within a column followed by the same letter are not different ($P > 0.05$).

[c] The expected values for the tank mixtures as calculated by Colby´s equation are presented in parentheses. Observed responses were separated from expected responses using t-tests ($\alpha = 0.05$) in SAS and the P-values are presented in the table. If observed control from the tank-mixture was less than, equivalent to, or greater than the expected control, the response was considered antagonistic, additive, or synergistic, respectively.

Table 2.4. Percent of control of plants species based on dry weights according to the nozzle type used in the experiment conducted in 2017.

Nozzle type[a]	Common lambsquarters[b]	Grain sorghum	Horseweed	Kochia
XR	63 a	60 a	47 a	66 a
TTI	64 a	60 a	46 a	52 b

[a] Abbreviations = XR, Extend Range; TTI, Turbo Teejet Induction.

[b] Means within a column followed by the same letter are not different ($P > 0.05$).

Table 2.5. The effect of different spray solutions on spray droplet size distribution from two nozzle types using a low-speed wind tunnel at the Pesticide Application Technology (PAT) Laboratory in North Platte, Nebraska. Experiment conducted in 2017.

		Spray-droplet distribution[a]					
Nozzle type	Spray solution[b]	$D_{v0.1}$	$D_{v0.5}$[c]	$D_{v0.9}$	≤ 150 µm	RS	CC[d]
		——— µm ———			%		
XR	Water	109 q	252 o	426 ijk	19.73 a	1.26 b	M
XR	Glyphosate	109 q	248 p	418 kl	20.05 a	1.25 b	F
XR	Lactofen	151 i	287 i	452 h	9.88 i	1.05 gh	M
XR	Lactofen + COC	148 j	284 j	445 h	10.25 h	1.04 gh	M
XR	Lactofen + NIS	130 n	263 m	423 jkl	14.42 d	1.11 c	M
XR	Lactofen + MSO	134 l	270 l	431 ij	13.39 f	1.10 cd	M
XR	Lactofen + COC + DRA	118 o	260 m	449 h	17.72 c	1.27 ab	M
XR	Glyphosate + Lactofen + COC	147 k	280 k	435 i	10.61 g	1.03 h	M
XR	Glyphosate + Lactofen + NIS	132 m	261 m	415 l	14.00 e	1.09 cde	M
XR	Glyphosate + Lactofen + MSO	131 mn	262 m	421 kl	14.22 de	1.10 cd	M
XR	Glyphosate + Lactofen + COC + DRA	117 p	257 n	449 h	18.23 b	1.29 a	M
TTI	Water	356 c	716 d	1092 c	0.61 klmn	1.03 h	UC
TTI	Glyphosate	367 b	765 c	1215 a	0.53 lmn	1.10 cd	UC
TTI	Lactofen	307 h	598 h	922 g	1.01 j	1.03 h	XC
TTI	Lactofen + COC	315 g	615 g	957 f	0.92 jk	1.04 gh	XC
TTI	Lactofen + NIS	328 d	643 e	955 de	0.67 klmn	1.03 h	XC
TTI	Lactofen + MSO	325 e	639 ef	1005 de	0.68 jklm	1.07 efg	XC
TTI	Lactofen + COC + DRA	412 a	791 b	1154 b	0.37 mn	0.94 j	UC
TTI	Glyphosate + Lactofen + COC	314 g	611g	961 f	0.87 jk	1.06 fgh	XC
TTI	Glyphosate + Lactofen + NIS	321 f	638 ef	1013 d	0.76 jkl	1.08 def	XC
TTI	Glyphosate + Lactofen + MSO	322 ef	634 f	988 e	0.77 jkl	1.05 gh	XC
TTI	Glyphosate + Lactofen + COC + DRA	415 a	809 a	1222 a	0.33 n	1.00 i	UC

[a] Abbreviations = $D_{v0.1}$, $D_{v0.5}$, and $D_{v0.9}$: Parameters which represent the droplet diameter such that 10, 50, and 90% of the spray volume is contained in droplets of lesser diameters, respectively;
≤ 150 µm = Percent of spray volume with droplet diameters less than 150 µm;

RS: Relative span, a dimensionless parameter that estimates the uniformity of a droplet size distribution.

[b]Abbreviation: COC, crop oil concentrate; NIS, non-ionic surfactant; MSO, methylated seed oil; DRA, drift retardant agent.

[c] Means within a column followed by the same letter are not different (P > 0.05).

[d] The classification category for this study were made based on reference curves created from reference nozzle data at the Pesticide Application Technology Laboratory as described by ASAE 572.1 where F = Fine, M = Medium, C = Coarse, VC = Very Coarse, XC = Extremely Coarse, and UC = Ultra Coarse

Table 2.6. Observed and expected responses calculated using Colby's equation of four plants species control based on dry biomass using glyphosate or lactofen alone, lactofen in combination with COC, NIS, MSO, or COC plus DRA, and glyphosate and lactofen in tank mixtures using the adjuvants aforementioned in North Platte, Nebraska. Experiment conducted in 2017.

Spray solution[a]	**Horseweed**			**Kochia**			**Common lambsquarters**			**Grain sorghum**		
	—— % ——		P-value	—— % ——		P-value	—— % ——		P-value	—— % ——		P-value
Glyphosate	27[b] e			7 c			82 a			94 a		
Lactofen	36 de			47 b			50 c			8 d		
Lactofen + COC	48 bc			68 ab			56 bc			22 bc		
Lactofen + NIS	43 cd			60 ab			52 c			10 cd		
Lactofen + MSO	48 bc			75 a			53 bc			18 bcd		
Lactofen + COC + DRA	49 abc			68 ab			57 bc			31 b		
Glyphosate + Lactofen + COC	55 ab	(62)[c]	0.0150	72 a	(70)	0.6938	51c	(92)	<.0001	89 a	(96)	0.0007
Glyphosate + Lactofen + NIS	45 bcd	(58)	0.0003	57 ab	(63)	0.1505	86 a	(91)	0.0789	95 a	(95)	0.8004
Glyphosate + Lactofen + MSO	53 abc	(62)	0.0013	77 a	(77)	0.9246	67 b	(92)	<.0001	90 a	(95)	0.0057
Glyphosate + Lactofen + COC + DRA	60 a	(63)	0.5796	75 a	(70)	0.4813	64 bc	(92)	<.0001	91 a	(96)	0.2018

[a] Abreviations: COC, crop oil concentrate; NIS, non-ionic surfactant; MSO, methylated seed oil; DRA, drift retardant agent.

[b] Means within a column followed by the same letter are not different ($P > 0.05$).

[c] The expected values for the tank mixtures as calculated by Colby´s equation are presented in parentheses. Observed responses were separated from expected responses using t-tests ($\alpha = 0{,}05$) in SAS and the P-values are presented in the table. If observed control from the tank-mixture was less than, equivalent to, or greater than the expected control, the response was considered antagonistic, additive, or synergistic, respectively.

CHAPTER 3

Response of Palmer amaranth to Glyphosate and PPO-Inhibiting Herbicide Tank-Mixtures

Abstract

Protoporphyrinogen oxidase (PPO)-inhibiting herbicides in combination with glyphosate for POST applications is a common approach to manage glyphosate resistant weeds and commonly the only effective POST chemical option in glyphosate-resistant only soybean to control glyphosate and ALS-inhibiting resistant weeds. Antagonistic interactions have been reported between many different herbicide modes of action. Additionally, optimal droplet size may be affected by tank-mixtures of different herbicides. Therefore, a field study was conducted across two years at three Nebraska locations to investigate the following objectives: (1) determine the response of Palmer amaranth to glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) applied alone and in combination, (2) investigate the type of interaction when these herbicides were applied in tank mixtures, and (3) determine the impact of nozzle selection (and thereby, droplet size) on weed control when systemic and contact herbicides are used in mixtures. Treatments consisted of POST applications of glyphosate, fomesafen, or lactofen alone and in combination using three nozzle types (XR, AIXR, and TTI). Glyphosate applied in tank-mixture with fomesafen or lactofen did not improve Palmer amaranth control compared to glyphosate applied alone on a glyphosate-susceptible population. Overall, lactofen worked better than fomesafen either applied alone or in tank mixture with glyphosate. Applications from the tank mixtures resulted in antagonistic interactions. Droplet size and percent volume of droplets ≤ 150 µm were highly affected

by nozzle type and spray solution (and thereby, herbicide formulation). The emulsion formulation of lactofen and oil based adjuvants are shattered by TTI nozzles due its internal turbulence chamber creating smaller droplets and increasing driftable fines. Conversely, this trend was not observed with the XR and AIXR nozzles as the emulsion formulations generated larger droplets. The impact of nozzle selection on Palmer amaranth control was minimal and larger droplets at the rates and carrier volume used in this study could be used without compromising herbicide efficacy reducing drift potential. Results emphasized the importance of better understanding the relationship among application variables and weed species. In addition, recommendations should be herbicide- and weed-specific in order to optimize herbicide applications and to maintain herbicide effectiveness.

Introduction

Palmer amaranth (*Amaranthus palmeri* S. Watson), a C_4 summer annual native to the US (Sauer 1957), is one of the most invasive and aggressive species in the pigweed (*Amaranthaceae*) family. Its rapid erect growth and prolific seed production (Culpepper et al., 2006; Ward et al., 2013) combined with an extended the period of seedling emergence make it one of the most troublesome weeds in cotton (*Gossypium hirsutum* L.), corn *(Zea mays* L.), and soybean [*Glycine max* (L.) Merr.] in the US (Webster and Nichols 2012). Due to its ability to compete with crops (Klingaman and Oliver 1994), densities of eight and nine plants m^{-2} can create yield losses up to 91% in corn (Massinga et al. 2001) and up to 79% in soybean (Bensch et al. 2003), respectively. In addition, as a dioecious plant, Palmer amaranth is an obligate outcrosser (Franssen et al. 2001) allowing it to quickly spread herbicide resistant-genes (Steckel 2007) reducing the herbicide options for weed management. For example, glyphosate resistance has been reported to be dispersed through pollen flow across a distance of at least 300 m (Sosnoskie et al. 2012).

Glyphosate is the most widely used non-selective herbicide worldwide in cotton, corn, and soybean due to its excellent efficacy, low toxicity, and unique mode of action (inhibits the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase; EPSPS) (Duke and Powles 2008). The rapid adoption of genetically modified glyphosate-resistant (GR) crops after 1996 has led to a heavy reliance on this broad-spectrum herbicide as a chemical option to manage weed species in cropping systems including pigweed species (Powles and Preston 2006). The use of a limited number of herbicide sites of action reduced weed management diversity and increased the number of herbicide-resistant weed populations due to selection pressure. To date, Palmer amaranth populations have evolved resistance not only to glyphosate but to multiple herbicides that target microtubule assembly, photosystem II, acetolactate synthase (ALS), protoporphyrinogen oxidase (PPO), and 4-hydroxyphenylpyruvate dioxygenase (HPPD) in the US (Heap, 2018) with the majority of the populations being resistant to EPSPS and ALS inhibitors (or both) (Culpepper et al. 2006, Wise et al. 2009).

To delay the evolution of herbicide resistance, tank mixtures of different herbicide sites of action have been widely recommended. Furthermore, PPO-inhibiting herbicides in combination with glyphosate for postemergence (POST) applications is one alternative and common approach to manage GR weed populations in cotton, corn, and soybean. PPO-inhibiting herbicides are the only effective POST chemical option in glyphosate-tolerant only soybean to control weeds when resistance to both glyphosate and ALS-inhibiting herbicides is present in the field. The inhibition of the PPO enzyme frequently leads to production of highly reactive singlet oxygen in the presence of light and molecular oxygen, resulting in lipid peroxidation (Duke et al., 1991; Sherman et al., 1991), followed by membrane disruption and plant death. PPO-inhibiting herbicides have many advantages such as low toxicity, low effective rates, quick onset of action, long residual effect, and activity against both monocotyledon and dicotyledon weeds (Hao et al. 2011). In addition, resistance to PPO-inhibiting herbicides has been slow to evolve with only thirteen weed species worldwide and four weed species in the US (Heap, 2018). Herbicide-resistant pigweed species such as common waterhemp (*Amaranthus tuberculatus var. rudis*) (Shoup et al. 2003) and Palmer amaranth have already been reported in the US due to the overreliance on this group of herbicides after the widespread occurrence of resistance to glyphosate and ALS-inhibiting herbicides (Salas et al. 2016).

Antagonistic interactions to specific weed species have been reported in literature when glyphosate and PPO-inhibiting herbicides were applied in combination (Starke and Oliver 1998; Nandula et al., 2012). Furthermore, reduced effectiveness of tank-mixing herbicides to delay the evolution of resistance is likely to occur if mixtures do not show similar efficacy (Beckie and Reboud 2009). The loss of PPO-inhibiting herbicides as an effective chemical class would complicate an already complex agriculture problem since no new herbicide modes of action have been introduced for cotton, corn or soybean production in greater than three decades. In addition, spray application factors such as droplet size play a crucial role on spray performance (Butts et al. 2018). Droplet size is highly affected by nozzle type (Butler Ellis et al. 2002, Etheridge et al. 1999), nozzle size (Nuyttens et al. 2007), or adding other chemicals into the tank-mixture (Creech et al. 2015, L. F. Bouse et al. 1990).

Better understanding how these herbicides interact in tank mixtures as well as the impact on droplet size produced from these applications needs to be thoroughly investigated in order to assure effective and sustainable weed management recommendations. Therefore, the objectives of our research were to: (1) evaluate the response of Palmer amaranth to glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) applied alone and in combination, (2) investigate the type of interaction when these herbicides were applied in tank mixtures, and (3) determine the impact of nozzle selection (and thereby, droplet size) on weed control when systemic and contact herbicides are used in mixtures.

Materials and Methods

Field Experiments and Data Collection. Nebraska-location, GPS coordinates, application date, weather conditions during application, weed densities, and weed heights can be found in Table 3.1. Field experiments were established in a fallow environment infested with Palmer amaranth, during the summers of 2016 and 2017. The density of Palmer amaranth at these locations varied from 50 to 120 plants m^{-2} and plants had already flowered at third location. Ulysses silt loam was the soil type at first location and Holdrege silt loam was the soil type at both second and third locations. The experiment at each location was arranged in a randomized complete block design with factorial arrangements of treatments with four replications. Treatments were arranged in a five by three factorial plus an untreated control consisting of five spray solutions (glyphosate, fomesafen, or lactofen applied alone and glyphosate plus fomesafen or lactofen applied in tank-mixture), and three nozzle types (Extend Range- XR, Air Induction Extended Range- AIXR, and Turbo Teejet Induction-TTI). Spray treatments consisted of POST applications of glyphosate (Roundup PowerMax®, Monsanto Company, St Louis, MO 63167) at 1200 g ae ha^{-1}, fomesafen (Flexstar®, Syngenta Crop Protection, Greensboro, NC 27419) at 130 g ai ha^{-1}, or lactofen (Cobra®, Valent USA Corporation, Walnut Creek, CA 94596) at 220 g ai ha^{-1} alone and in tank mixtures. Liquid ammonium sulfate (Bronc®, Wilbur-Ellis Company, Fresno, CA 64596) at 2.5% v v^{-1} was added to treatments and crop oil concentrate (R.O.C®, Wilbur-Ellis Company, Fresno, CA 64596) at 1% v v^{-1} was used in treatments except for glyphosate applied alone. Herbicide treatments were applied using a CO_2 sprayer mounted to a Bobcat 3400 UTV equipped with a four-nozzle boom with nozzles spaced 50 cm apart and 50 cm above the plants delivering 187 L ha^{-1} at 276 kPa at speed of 9.6 kph. Non-air inclusion and air-inclusion flat fan tip nozzles (XR, AIXR, and TTI) (Teejet Technologies, Spraying Systems Co., Wheaton, IL 62703) with the same fan angle and orifice size (11004) were chosen to produce a wide range of droplet sizes. After plants were sprayed, Palmer amaranth visual estimations of injury, hereafter referred as percent of Palmer amaranth control, were collected at different evaluation times, approximately 7, 14, 21, and 28 days after treatment (DAT) using a scale of 0 to 100% control, with 0% being no herbicidal damage and 100% being complete death.

Tank-mixture Interactions. Tank-mixture efficacy in terms of percent of weed control may be predicted using the responses of herbicides applied singly (Colby 1967).

Therefore, glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) were sprayed alone and in combination. After applications, observed responses from herbicides applied alone were used to calculate the expected responses of them applied in tank mixtures. Colby's equation was used to obtain the expected percent of Palmer amaranth control responses when herbicides where applied in tank mixtures and to describe the type of interaction. If E is the expected response as a percent of Palmer amaranth control using two herbicides in tank-mixture (A + B), and X and Y are the observed responses as a percent of Palmer amaranth control when herbicide (A or B) was applied alone, then, according to (Colby 1967):

$$E_1 = \frac{X_1 Y_1}{100} \qquad [1]$$

where $E_1 = 100 - E$; $X_1 = 100 - X$; and $Y_1 = 100 - Y$.

Analysis of Spray Droplet Size. The spray-droplet distribution for each treatment was evaluated using a low-speed wind tunnel at the Pesticide Application Technology (PAT) Laboratory in North Platte, NE. Each nozzle was tested at 276 kPa and a laminar velocity of 6.7 m s^{-1} (Fritz et al. 2014). Droplet size measurements were made using a Sympatec HELOS-VARIO/KR laser diffraction instrument with an R7 lens (Sympatech Inc., Clausthal, Germany). Henry et al. (2014) and (Creech et al. 2016) provide detailed information regarding the low-wind speed wind tunnel and its operation at the PAT Laboratory. Treatments were compared using the $D_{v0.1}$, $D_{v0.5}$, and $D_{v0.9}$ volumetric droplet size spectra parameters, which represent the droplet diameter such that 10, 50, and 90% of the spray volume is contained in droplets of smaller diameters than reported, respectively. In addition, the percentage of the spray volume with droplet diameters less than 150 µm (driftable fines) and the relative span (RS) were measured. The RS indicates the uniformity of the spray droplet spectrum and was calculated following equation:

$$RS = \frac{Dv_{0.9} - Dv_{0.1}}{Dv_{0.5}} \qquad [2]$$

The spray classifications for this study shown, in Figure 1, were made based on reference curves created from reference nozzle data at the PAT Laboratory as described by ASABE S572.1 (ASABE, 2009) allowing the results to be compared with data derived from other laboratories (Fritz et al. 2014).

Statistical Analyses. Data were subjected to ANOVA using a generalized linear mixed model (PROC GLIMMIX) in SAS (Statistical Analysis Software, version 9.4, Cary, North Carolina, USA) with mean separations made at $\alpha = 0.05$ level using Fisher's protected LSD test and the Tukey adjustment. Spray solution and nozzle type were analyzed as fixed effects whereas location and block were analyzed as random effects and data were pooled across locations. Visual estimations of percent Palmer amaranth control and percent volume of droplets ≤ 150 µm were analyzed using beta distribution as the data were bound between 0 and 1, while gamma distribution was used to analyze $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ as the data were bound between 0 and infinity, and Gaussian distribution was used to analyze the relative span (Stroup, 2013; Butts et al., 2017). When beta and gamma distributions were used results were back-transformed for discussion.

The paired t-test in SAS was used to determine the statistical significance of the differences between observed and expected responses as percent of Palmer amaranth control. Therefore, when the observed weed control from the tank-mixture was less than, equivalent to, or greater than the expected control, the response was considered antagonistic, additive, or synergistic, respectively (Colby 1967, Lich et al. 1997).

Results

Percent of Palmer amaranth Control. The Palmer amaranth control values are shown in the Table 3.2. using glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) applied alone and in tank mixtures. GR Palmer amaranth has not been reported at the three locations used to conduct the experiments. Therefore, the application of glyphosate alone resulted in the highest control with 89% at 14 DAT; however, when applied in combination with lactofen control was similar (P = 0.8183). Likewise, lactofen applied in tank-mixture provided the highest control with 76% at 28 DAT but not different when compared to glyphosate applied alone (P = 0.9998). Moreover, applications of the PPO-inhibiting herbicides in combination with glyphosate increased the Palmer amaranth control compared to either PPO herbicide applied alone. The tank-mixture of fomesafen and glyphosate increased the control by 39 and 26% at 14 and 28 DAT, respectively, when compared to fomesafen applied alone. Similarly, increased control of 23 and 22% by the addition of glyphosate into the tank-mixture was observed at 14 and 28 DAT, respectively, when compared to lactofen applied alone.

The applications of fomesafen alone resulted in the lowest control regardless of the evaluation time. Palmer amaranth control using lactofen improved in 20 and 14% at 14 and 28 DAT, respectively, when compared to fomesafen (Table 3.2.). Therefore, results suggested that lactofen at 220 g ha^{-1} provides better Palmer amaranth control than fomesafen at 130 g ha^{-1} using 187 L ha^{-1} carrier volume. Additionally, in the tank-mixture greater control was observed when lactofen was applied in combination with glyphosate than fomesafen regardless of the evaluation time.

Tank-Mixture Interactions. The expected responses of Palmer amaranth control determined by Colby's equation were greater and significant ($P < 0.0001$) compared to the observed responses regardless of the evaluation time. Observed responses were less than the expected responses when adding fomesafen into the tank-mixture by 11 and 19% at 14 and 28 DAT, respectively. Similarly, observed responses were less than the expected responses when adding lactofen into the tank-mixture by 9 and 13% at 14 and 28 DAT, respectively. Therefore, fomesafen or lactofen in tank-mixture with glyphosate at the rates and carrier volume used in this study resulted in antagonistic interactions.

Nozzle Type and Spray Droplet Size. Although nozzle type by spray solution interaction was not significant either at 14 DAT ($P = 0.9717$) or at 28 DAT ($P = 0.8853$), main effects of spray solution ($P < 0.0001$) and nozzle type ($P = 0.0309$) were significant at 28 DAT. In contrast, only spray solution was significant ($P < 0.0001$) as main effect at 14 DAT. Irrespective of spray solution, applications using the XR nozzle resulted in greater control than the TTI nozzle at 28 DAT (Table 3.3.). There were no differences between the AIXR and the XR ($P = 0.0685$) or the TTI nozzle ($P = 0.9856$).

The $Dv_{0.5}$ ranged from 230 to 796 µm (Table 3.4.) which represents a change from Fine to Ultra Coarse on the spray droplet classification category (Figure 3.1). Additionally, the XR and TTI nozzles produced the smallest and largest $Dv_{0.5}$ values, respectively, regardless of the spray solution, indicating nozzle selection is more important than tank solution in determining droplet size. Furthermore, the percent volume of droplets ≤ 150 µm decreased as $Dv_{0.5}$ increased (Table 3.4.), showing a strong relationship between nozzle type and drift potential. The TTI nozzle showed the lowest RS variation followed by the AIXR and XR nozzle.

For the XR and AIXR nozzles, lactofen or fomesafen applied alone had greater $Dv_{0.5}$ values compared to applications of tank mixtures with glyphosate. Conversely, an opposite behavior was observed for fomesafen herbicide using TTI nozzle. Among spray solutions, glyphosate applied alone had the smallest $Dv_{0.5}$ values using the XR or AIXR nozzles; however, the greatest value when using the TTI nozzle (Table 3.4.). This pattern was also observed in the percent volume of droplets ≤ 150 µm produced, which increased considerably when glyphosate was applied alone with the XR or AIXR nozzle. In contrast, the lowest percentage of fines was observed using the TTI nozzle with glyphosate alone. The percent volume of fine droplets produced were 0.41, 5.73, and 23.84% when glyphosate was applied alone using the TTI, AIXR, and XR nozzles, respectively. Therefore, these results show the droplet spectra produced from applications is also affected by the interaction of nozzle type and herbicide formulation.

Discussion

Extremely tall Palmer amaranth plants present in the first and second locations and high densities across locations likely caused reduced efficacy of the herbicide applications. For instance, Whitaker et al. (2010) reported 100% control at 30 DAT with glyphosate at 1000 g ha^{-1} when Palmer amaranth was between 10 and 15 cm tall. In contrast, Gower et al. (2003) reported reduced weed control, including pigweed species, from 94 to 79% when plants were 10 and 30 cm tall, respectively, with single glyphosate application at 840 g ha^{-1} at 14 to 21 DAT. Palmer amaranth control using applications of

PPO-inhibiting herbicides is highly dependent on weed height and environmental conditions showing poor control with plants > 10 cm tall (Chahal et al. 2015). Berger et al. (2014) has reported reduced Palmer amaranth control with lactofen at 210 g ha^{-1} as weed height increased. Observations from this study using fomesafen versus lactofen have been consistent with other findings. For example, less control was reported using fomesafen at 280 g ha^{-1} compared to lactofen at 213 g ha^{-1} in four pigweed species across two locations at 21 DAT (Sweat et al. 1998). Despite greater control, applications using lactofen have also been reported to cause higher level of injury on soybean (Patzoldt et al. 2002) and peanut (Sperry et al. 2017) when compared to applications using fomesafen. In addition, increased Palmer amaranth (Patzoldt et al. 2002) and common waterhemp (Hager et al. 2003) control have been reported using higher fomesafen rates. For instance, (Bond et al. 2006) reported 96% Palmer amaranth control based on visual ratings at 21 DAT using fomesafen at 420 g ha^{-1} when plants were 15 cm tall.

Besides weed height, weed developmental stage also plays an important role in weed control and spray performance using single applications of POST herbicides. Spray applications at the third location were made late August to six cm tall Palmer amaranth plants that had already started flowering. Therefore, it is hypothesized that redirection of glyphosate translocation to reproductive and developing seed tissues also may have contributed to reduced herbicidal damage from applications using glyphosate alone. For example, (Duke et al. 2003) first reported the presence of glyphosate in seeds of GR soybeans with later applications of glyphosate.

Applications of fomesafen or lactofen in tank-mixture with glyphosate in this study were antagonistic 100% of the time. Previous research has shown antagonistic interactions using fomesafen and glyphosate rate combinations to goosegrass [*Eleusine indica* (L.) Gaertn.], sicklepod [*Senna obtusifolia* (L.) H.S. Iewin & Barneby], Palmer amaranth, velvetleaf (*Abutilon theophrasti* Medik.), and entireleaf morningglory (*Ipomoea hederacea* var. integriuscula Gray) (Starke and Oliver 1998). Additionally, sulfentrazone in tank-mixture with glyphosate was antagonistic to barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] and Palmer amaranth at all rate combinations and to goosegrass and entireleaf morningglory at three of the four combinations (Starke and Oliver 1998). Likewise, an antagonistic interaction was observed when tank mixtures of flumiclorac and glyphosate on Palmer amaranth control (Nandula et al. 2012). Studies have indicated reduced absorption and translocation of glyphosate in some weed species caused by PPO-inhibiting herbicides (Nandula et al. 2012, Starke and Oliver 1998). The absorption and translocation of glyphosate influenced by either fomesafen or lactofen were not evaluated in this study.

Nozzle selection (and thereby, droplet size) and spray solution interaction was not significant for Palmer amaranth control using glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) applied alone and in tank-mixtures at a constant carrier volume. Smaller droplets from non-air inclusion nozzles for POST herbicides applications at a constant volume have been reported to be more effective than larger droplets (Knoche 1994). In contrast, many studies have reported no difference in weed control regarding droplet size. For example, no differences in lactofen efficacy were observed using either the XR or air-induction (AI) nozzles on Palmer amaranth control (Berger et al. 2014). Likewise, no differences in control of common ragweed (*Ambrosia artemisiifolia* L.), velvetleaf, and common lambsquarters (*Chenopodium album* L.) from XR and AI nozzle applications were reported using fomesafen or glyphosate when applied at the manufacturer's rate (Sikkema et al. 2008). Conflicting results found in the literature shows that herbicide efficacy is not solely dependent on droplet size. Differences in control are related to nozzle type, carrier volume, herbicide and weed species (Ramsdale and Messersmith 2001; Brown et al. 2007; Sikkema et al. 2008; Creech et al. 2016; Butts et al. 2018).

Droplet size was more important at 28 DAT where control increased as droplet size decreased. Spray coverage decrease as droplet size increase allowing faster regrowth of plants from applications using contact herbicides. Although improved control from the XR nozzle, regardless of the spray solution and evaluation time, was observed in this study, differences among nozzles were minimal having no impact in realistic terms at a constant carrier volume of 187 L ha^{-1}. Butts et al. (2018) reported increased weed control as droplet size decreased across herbicides (dicamba and glufosinate) and carrier volumes; however, this droplet size effect was minimized as result of the increased carrier volume (187 L ha^{-1}).

Spray-droplet distribution results were affected by nozzle type. Irrespective to spray solution, $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ values ranked from smallest to largest, using the XR nozzle, followed by the AIXR and TTI nozzles. Moreover, as droplet size increased, the driftable fines decreased (Creech et al. 2015). Conversely to the AIXR and TTI nozzles, the XR nozzle lacks DRT features in its design, producing smaller droplets and increasing drift potential. In addition to nozzle type, spray solution had an impact on spray-droplet distribution. Among spray solutions, applications of glyphosate alone using either the XR or AIXR nozzles generated the smallest and greatest values of $Dv_{0.5}$ and droplets ≤ 150 µm within a given nozzle type, respectively. Conversely, the TTI nozzle produced the largest values for $Dv_{0.5}$ and smallest percentage of fines for applications using glyphosate alone. The results indicated that the combination of nozzle type and herbicide formulation dramatically affected droplet size. Differently from glyphosate, a water-soluble herbicide, lactofen is classified as emulsifiable concentrates (EC). An emulsion (oil in water) is formed when EC products are mixed with water requiring some agitation to keep the emulsion from separating (Goodman 2004). In addition to the air-inclusion and pre-orifice technology used in the AIXR nozzle, the TTI nozzle also incorporates an internal turbulence chamber. This chamber is inside of the nozzle body increasing droplet size, reducing fine droplets and improving the spray pattern uniformity (Klein and Kruger n.d.). Therefore, the authors hypothesized that the emulsion formed by EC products such as lactofen and oil based adjuvants such as COC is shattered when passing through the turbulence chamber creating smaller droplets (smaller $Dv_{0.5}$ values) and increasing the percent volume of droplets ≤ 150 µm produced compared to other product formulations.

Conclusions

The results observed of this study indicated that none of the POST applications using the herbicides alone nor in tank mixtures provided a desired Palmer amaranth control of 90% or more at 28 DAT. Moreover, glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) in tank-mixture interacted in an antagonistic way when assessing Palmer amaranth control. Application timing should be strictly adhered to achieve effective weed control, especially with high weed densities or later applications when older plants at the flowering stage may affect herbicide performance. Furthermore, antagonistic herbicide interactions combined with taller plants as well as high densities may result in low weed control accelerating the evolution of herbicide resistance.

Droplet size was not the major contributing factor on herbicide efficacy but it was highly affected by nozzle type and herbicide formulation interactions. Results in this study indicated that the impact of nozzle type on weed control are herbicide- and weed-specific. Nozzles that produce larger droplets can be used effectively without compromising herbicidal efficacy at rates and carrier volumes used in this study to control Palmer amaranth. In addition, these nozzles will reduce the likelihood for off-target movement working towards drift mitigation.

The rapid widespread evolution of herbicide resistance has highlighted the importance of diversifying weed management strategies, including preemergence and POST herbicide applications combined with non-chemical options. Moreover, better understanding the relationship among application variables and weed species are required to optimize herbicide applications and to maintain herbicide effectiveness.

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Table 3.1. Description of the locations used to evaluate the response of Palmer amaranth to glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) applied alone and in combination.

	Location		
Parameters	**1st**	**2nd**	**3rd**
Year	2016	2017	2017
City	Beaver City	Beaver City	Macon
GPS coordinates	40.16°N, 90.91°W	40.13°N, 99.88°W	40.23°N, 98.95°W
Application date	06/30	07/06	08/22
Temperature (°C)	28	33	21
Relative humidity (%)	50	45	60
Weed density (plants m^{-2})	50-70	60-80	100-120
Weed height (cm)	58	31	15
Soil type	Ulysses silt loam	Holdrege silt loam	Holdrege silt loam

Table 3.2. Observed and expected responses calculated using Colby's equation of Palmer amaranth control based on visual estimations of injury pooled across three locations using glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) applied alone and in tank mixtures in Nebraska.

		14 DAT[a]				28 DAT			
Spray solution	**Rate**	**Observed[b]**	**Expected**	**P-value**	**Interaction[c]**	**Observed**	**Expected**	**P-value**	**Interaction**
	g ae or ai ha^{-1}	——— % ———				——— % ———			
Glyphosate	1200	89 a				75 a			
Fomesafen	130	44 d				40 d			
Lactofen	220	64 c				54 c			
Glyphosate + fomesafen	1200 + 130	83 b	94	< 0.0001	Antagonistic	66 b	85	< 0.0001	Antagonistic
Glyphosate + lactofen	1200 + 220	87 a	96	< 0.0001	Antagonistic	76 a	89	< 0.0001	Antagonistic

[a] DAT: days after treatment.

[b] Means within a column followed by the same letter are not different (P > 0.05).

[c] If observed Palmer amaranth control from the tank-mixture was less than, equivalent to, or greater than the expected control, the response was considered antagonistic, additive, or synergistic, respectively.

Table 3.3. Percent of Palmer amaranth control based on visual estimations of injury using glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) alone and in tank mixtures according to the nozzle type across locations at 14 and 28 days after treatment (DAT).

Nozzle type[a]	Palmer amaranth control (%)	
	14 DAT[b]	28 DAT
XR	78 a	65 a
AIXR	76 a	62 ab
TTI	76 a	61 b

[a] Abbreviations = XR, Extend Range; AIXR, Air Induction Extended Range; TTI, Turbo Teejet Induction.

[b] Means within a column followed by the same letter are not different ($P > 0.05$).

Table 3.4. The effect of different spray solutions on spray droplet size distribution from three nozzle types using a low-speed wind tunnel at the Pesticide Application Technology (PAT) Laboratory in North Platte, Nebraska.

		Spray-droplet distribution[a]					
Nozzle type	**Spray solution**	**$D_{v0.1}$[b]**	**$D_{v0.5}$**	**$D_{v0.9}$**	**≤ 150 µm**	**RS**	**CC[c]**
		µm			%		
XR	Glyphosate	100 o	230 n	400 k	23.84 a	1.30 a	F
	Fomesafen	135 k	262 j	412 j	13.10 e	1.05 e	M
	Lactofen	129 l	251 k	409 j	15.16 d	1.12 d	M
	Glyphosate + fomesafen	124 m	240 l	392 l	17.06 c	1.12 d	F
	Glyphosate + lactofen	121 n	236 m	397 k	18.63 b	1.17 c	F
AIXR	Glyphosate	186 j	403 i	688 f	5.73 f	1.24 b	C
	Fomesafen	234 g	438 g	643 h	2.24 i	0.93 i	VC
	Lactofen	250 f	465 f	679 g	1.87 j	0.92 i	VC
	Glyphosate + fomesafen	224 i	434 h	645 h	2.63 g	0.97 h	VC
	Glyphosate + lactofen	228 h	433 h	631 i	2.43 h	0.93 i	VC
TTI	Glyphosate	392 a	796 a	1200 a	0.41 o	1.01 fg	UC
	Fomesafen	312 e	610 e	956 e	0.87 k	1.05 e	XC
	Lactofen	337 c	648 c	985 c	0.60 m	1.00 g	UC
	Glyphosate + fomesafen	350 b	689 b	1041 b	0.52 n	1.00 fg	UC
	Glyphosate + lactofen	319 d	628 d	964 d	0.74 l	1.03 f	XC

[a] Abbreviations = $D_{v0.1}$, $D_{v0.5}$, and $D_{v0.9}$: Parameters which represent the droplet diameter such that 10, 50, and 90% of the spray volume is contained in droplets of lesser diameters, respectively;
≤ 150 µm = Percent of spray volume with droplet diameters less than 150 µm;
RS: Relative span, a dimensionless parameter that estimates the uniformity of a droplet size distribution.

[b] Means within a column followed by the same letter are not different ($P > 0.05$).

[c] The classification category for this study were made based on reference curves created from reference nozzle data at the Pesticide Application Technology Laboratory as described by ASAE 572.1 where F = Fine, M = Medium, C = Coarse, VC = Very Coarse, XC = Extremely Coarse, and UC = Ultra Coarse

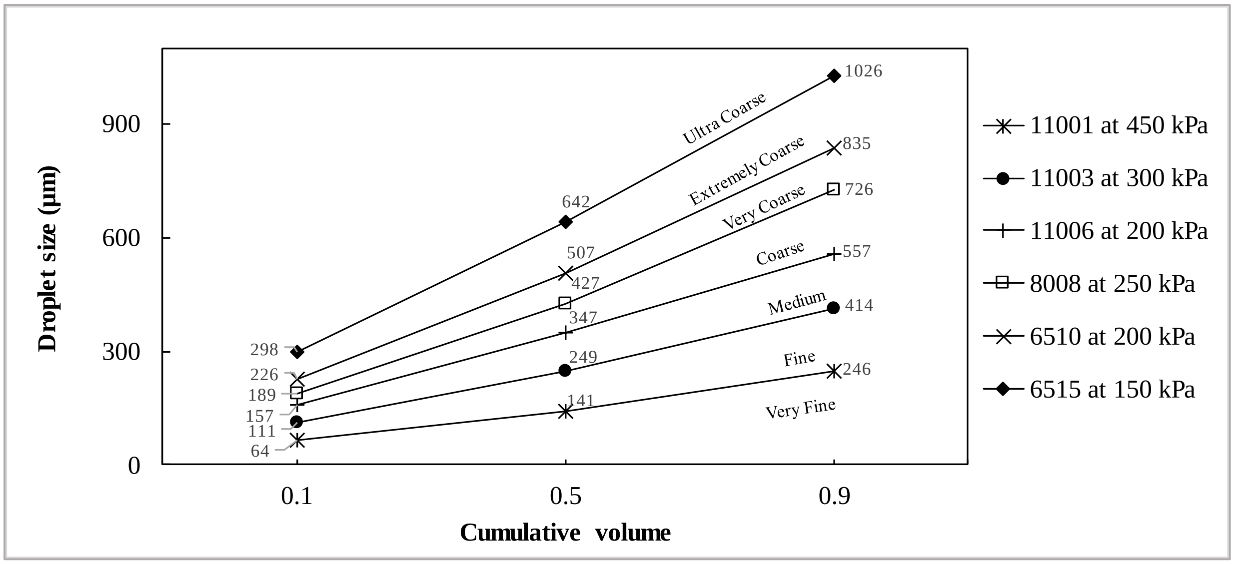

Figure 3.1. Spray category classification based on reference curves generated from reference nozzle data at the Pesticide Application Technology (PAT) Laboratory using ASABE S572.1 guidelines.

CHAPTER 4

Effect of Adjuvants on Physical Properties of Glyphosate and PPO-Inhibiting Herbicide Spray Mixtures

Abstract

Adjuvants can be pre mixed or tank mixed with foliar-applied herbicides to enhance spray droplet retention on leaf surface and penetration of herbicide active ingredient through the cuticle. Additionally, adjuvants are known to cause changes on physical properties such as density, viscosity, surface tension (SFT), and contact angle (CA) increasing leaf wettability. More penetration and translocation of the product is likely to occur due reduced SFT and CA. However, previous research have shown that the performance of adjuvants is dependent on the herbicide with which is applied, the plant species, and environmental conditions. Therefore, a study was conducted at the Pesticide Application Technology Laboratory at North Platte, NE, to: (1) determine the effect of adjuvants (oil concentrates, non-ionic surfactant, and drift retardant) on density, viscosity, SFT, and CA when glyphosate and lactofen are applied alone and in combination, (2) determine the impact of leaf structure surfaces on the CA formed by these spray solutions, and (3) to determine if reduced SFT and CA influence herbicide efficacy. Observations from this study highlighted the importance of adjuvants on reducing the SFT and CA properties of spray solutions; however, herbicide efficacy is only partially explained by the changes on these physical properties. Therefore, other factors that impact herbicide penetration and absorption by the plant should be taken in consideration.

Introduction

The use of different herbicide sites of action in tank-mixtures is a common approach to delay the evolution of herbicide resistance. For instance, glyphosate applied in combination with protoporphyrinogen oxidase (PPO)-inhibiting herbicides has been widely used in glyphosate-resistant (GR) corn, soybean, and cotton cropping systems to manage troublesome weeds resistant to glyphosate. Furthermore, PPO inhibiting herbicides are the only effective postemergence (POST) chemical option to control broadleaves when resistance to glyphosate and acetolactate synthase (ALS)-inhibiting herbicides are present in conventional and GR soybean fields. Spray applications are complex processes and innumerous factors from the time of application until complete herbicide absorption can affect herbicide efficacy resulting in reduced weed control, economic loss, and environmental contamination.

Foliar-applied systemic herbicides must be absorbed in adequate concentration into the shoot tissue and translocated to the site of action to be active (Hess and Falk 1990). However, the leaf surface type, wettability and orientation, the surface tension and viscosity of the spray solution as well as the droplet size and velocity will influence the outcome (adhesion, rebound, or shatter) of a droplet hitting the target (Zwertvaegher et al. 2014). Leaf surface structures play an important role affecting the wetting and penetration of foliar-applied herbicides (Hess 1985; Hull et al. 1982; Wanamarta and Penner 1989; Koch et al. 2008; Kraemer et al. 2009). Characteristics of a leaf surface include the cuticle (epicuticular wax, cutin, and pectin), number of stomata and trichomes, leaf angle and position, and leaf age (Hess 1985; Hull et al. 1982; Wanamarta and Penner 1989). Leaf epicuticular waxes are an effective barrier to herbicide absorption due to their hydrophobic surface characteristic but it is affected by the composition of the wax and plant species (Chachalis et al. 2001). On the other hand, droplets from spray applications may shatter or bounce upon impact of a hairy surfaces and, depending on the density, air pockets beneath the spray droplets are likely to occur due to closely spaced trichomes (Hess et al. 1974).

Adjuvants used in tank-mixtures or pre-mixtures with foliar-applied herbicides enhance spray droplet retention on the leaf surface and penetration of herbicide active ingredient through the cuticle (Young and Hart 1998). More effective penetration and translocation of the product is likely to occur due to the changes in physical properties such as surface tension (SFT) and contact angle (CA) (Janků et al. 2012). The CA formed between the spray droplet and leaf surface has been the main method used to characterize the wettability on plant surfaces; for instance, water droplets tend to spread on wettable surfaces showing a low CA and poor wettability are characterized by spherical water droplets with high CA (Kraemer et al. 2009). In addition, the CA will be affected by the SFT of the liquid, solid surface, and surrounding vapor (Kraemer et al. 2009). Therefore, decreased SFT and CA enhance the spread of spray droplets and thereby, leaf surface coverage (Basu et al. 2002). Previous research has shown that the performance of adjuvants is dependent on the herbicide with which it is applied, the plant species, and environmental conditions (Knezevic et al. 2009, Penner 1989).

Palmer amaranth (*Palmer amaranth S. Watson*), common lambsquarters (*Chenopodium album L*.), horseweed [*Conyza canadensis (L.) Cronq*.], and kochia [*Kochia scoparia (L.) Schrad*.] are among the ten most troublesome weeds in broadleaf crops (WSSA 2017) and resistant populations to glyphosate and ALS-inhibiting herbicides have been reported in the United States (Heap 2018). However, little is known about the changes on physical properties when glyphosate and PPO-inhibiting herbicides are applied to these weed species influenced by the addition of different adjuvants and by their leaf structures. The objectives of this study were to: (1) determine the effect of adjuvants (oil concentrates, non-ionic surfactant, and drift retardant) on density, viscosity, SFT, and CA when glyphosate and lactofen are applied alone and in combination, (2) determine the impact of leaf structure surfaces on the CA formed by these spray solutions, and (3) to determine if reduced SFT and CA influence herbicide efficacy.

Material and Methods

The physical properties - density, viscosity, SFT, and CA of 10 spray solutions and water alone - were measured at the Pesticide Application Technology Laboratory (PAT Lab) located at the University of Nebraska-Lincoln's West Central Research and Extension Center in North Platte, NE. Treatments consisted of 10 spray solutions using half of the labeled rates and a carrier volume of 187 L ha^{-1} of glyphosate (Roundup PowerMax®, Monsanto Company, St Louis, MO 63167) at 600 g ae ha^{-1}, or lactofen (Cobra®, Valent USA Corporation, Walnut Creek, CA 94596) at 110 g ai ha^{-1} alone, lactofen at 110 g ai ha^{-1} with the adjuvants crop oil concentrate-COC (R.O.C®, Wilbur-Ellis Company, Fresno, CA 64596) at 1% v v^{-1}, non-ionic surfactant-NIS (R-11®, Wilbur-Ellis Company, Fresno, CA 64596) at 0.25% v v^{-1}, methylated seed oil-MSO (High Load®, Wilbur-Ellis Company, Fresno, CA 6459) at 1% v v^{-1}, or drift retardant agent-DRA (Intact™, Precision Laboratories LLC, Waukegan, IL 60085) at 0.5% v v^{-1}, and herbicides in combination with each of the adjuvants aforementioned, water alone was included for comparison. COC was added to the spray solution when DRA was used. Liquid ammonium sulfate (Bronc®, Wilbur-Ellis Company, Fresno, CA 64596) at 2.5% v v^{-1} was added to spray solutions.

Density and viscosity analyses were performed at 20 C using a DMA™ 4500 M density meter (Anton Paar USA Inc., Ashland, VA 23005) and the microviscometer Lovis 2000 M/ME (Anton Paar USA Inc., Ashland, VA 23005) attached to the density meter, respectively. Surface tension and contact angle analyses were performed using a video-based optical contact angle measuring instrument - OCA 15EC (DataPhysics Instruments GmbH, Filderstadt, Germany). The instrument is composed by a video measuring system with USB camera of high performance 6x parfocal zoom lens with integrated continuous fine focus, and adjustable observation and camera tilt angle. The SCA software is used to collect, assess and evaluate the measured data. An environmental chamber was used to keep the temperature and the relative humidity at 25 C ± 1 C and at 60% ± 1%, respectively. The temperature is adjusted by a liquid circulator (Julabo USA Inc, Allentown, PA 18109) and the air humidity is provided by a humidity generator and controller - HCG (DataPhysics Instruments GmbH, Filderstadt, Germany); both values are displayed on the control panel of the HCG device allowing to be checked in real time. The chamber has three windows made of special optimal glass to directly observe the sample. For detailed information, the experimental devices are illustrated in the Figure 1 and Figure 2.

Density and Viscosity. A syringe was used to inject the bubble-free liquid sample into the measuring cell placed inside of the density meter where density was calculated using the fade-out method. The liquid passes through the measuring cell followed by the capillary glass tube placed inside of the microviscometer containing a steel rolling-ball which measures the rolling time of the ball through the liquid sample based on Hoeppler´s falling ball principle. Each treatment sample as described earlier was replicated three times. Between each treatment, both measuring cell and capillary glass tube were cleaned with distilled water and isopropyl alcohol (91%) and a new syringe was used.

Surface tension. The SFT was determined by using the pendant drop-method (drop hanging on a needle) and calculated from the shape and size of a pendant drop using the Laplace-Young equation. A 500 µl Hamilton® dosing syringe was prepared and mounted with a 1.65 mm outer needle diameter placed inside of the environmental chamber and used as reference size. A live image from the camera can be seen on the computer screen using the software SCA. Before dispensing the liquid, a record was initialized. The liquid was dispensed at a slow and continuous dosing rate (0.5 µL/s) until forming a drop as big as possible. After achieving a perfect drop the record sequence was stopped and used for measurements. The software detects the needle between two magnification lines and calibrates the magnification of the image based on its reference size. The profile feature was used to detect the drop contour automatically and the Laplace-Young method was used to calculate the surface tension. Each treatment sample, as described earlier, was replicated five times. Between each treatment, both needle and syringe were cleaned five times with distilled water. After the cleaning procedure, the syringe was filled with the next treatment sample five times to assure that both needle and syringe only contained the liquid of the treatment to be analyzed.

Static Contact angle. The static CA was determined by using the sessile drop-method. With a diffuse light source the sessile drop is illuminated from one side and from the other side the contour is observed. The CA is the angle formed by a liquid at three-phase boundary where the liquid, gas, and solid intersect. A 500 µl Hamilton® dosing syringe was prepared and mounted with a 0.52 mm outer needle diameter placed inside of the environmental chamber and used as reference size. A live image from the camera could be seen on the computer screen using the software SCA. The dosing volume of 1 µL (1241 microns) was dispensed with a very fast dosing rate (5 µL/s). The environmental chamber (with the surface sample on) was moved carefully upwards, without touching the needle tip, until the drop was settled down. After achieving a perfect drop the image was snapped and the snapshot was used for measurements to reduce errors from vibration. Magnification lines are used to define the region of interest enabling the software to detect the base line and the drop contour. The Ellipse method was used to calculate the static CA on specific surfaces. The static CA of droplets of the treatment samples described earlier was measured on the adaxial leaf surface of Palmer amaranth, common lambsquarters, kochia, horseweed, and grain sorghum [*Sorghum bicolor (L.) Moench subsp. bicolor*] selected daily at random just before measurement from greenhouse-grown plants that were 10 to 15 cm in height and 10 cm diameter for horseweed rosettes. In addition, mylar plastic cards were also used as surface sample for comparison. Each treatment sample was replicated five times within a surface type. Between each treatment, both needle and syringe were cleaned five times with distilled water. After the cleaning procedure, the syringe was filled with the next treatment sample five times to assure that both needle and syringe only contained the liquid of the treatment to be analyzed.

Statistical Analyses. Density, viscosity, SFT, and CA were analyzed separately and subjected to ANOVA using a generalized linear mixed model (PROC GLIMMIX) in SAS (Statistical Analysis Software, version 9.4, Cary, North Carolina, USA) with mean separations made at $\alpha = 0.05$ level using Fisher's protected LSD test and the Tukey adjustment. Data met the model assumptions and transformations were not needed.

Results and Discussion

Density and Viscosity. The impact by the addition of the adjuvants into the treatment solutions was greater on viscosity than on density values (Table 1). The smallest density was observed for water alone with 0.9982 g cm^{-3} while the largest one was observed when NIS was added into the solution of herbicides in combination with 1.0090 g cm^{-3}. Although a difference was observed, it represented an increase of only 1.07%. No differences in viscosity was observed when comparing water to the treatments of herbicides (glyphosate or lactofen) alone. The viscosity of lactofen alone increased at least 2.45% by the addition of adjuvants; however, the greatest impact was observed by the addition of the COC plus DRA, representing an increase of 33.6%. Likewise, the viscosity of the herbicide solutions in combination were greater than the solutions of lactofen and adjuvants alone; however, these differences were only different for the herbicide solutions in combination with MSO or COC plus DRA. The highest viscosity values were observed by the addition of COC plus DRA into the solution of herbicides in combination with 1.4420 mPa s. Previous research showed that DRA´s alter the viscoelastic properties of the spray solution increasing viscosity and reducing drift potential (Schampheleire et al. 2009) which is similar to what was found in this study.

Surface Tension. The images of the SFT of the treatment solutions measured by using the pendant drop-method are illustrated in Figure 3. Treatment solutions of glyphosate or lactofen alone had a SFT of 38.34 and 33.04 mN m^{-1}, respectively; both values were less than the corresponding values of the water control. Surface tensions of the different treatment solutions varied from 29.22 to 71.15 mN m^{-1}. Adjuvants added to the lactofen solution decreased the SFT when compared to the lactofen alone solution and the greatest impact was observed by the addition of NIS. Likewise, among the solutions, the smallest SFT was observed by the addition of COC but it was not different from NIS. The primary purpose of a surfactant is to reduce the surface tension and increase the contact between the spray droplet and the plant surface (Curran and Lingenfelter, 2009). Likewise, adjuvants that are primarily oil based tend to increase herbicide penetration but also can reduce surface tension; COC incorporates a percentage of surfactant in its composition and MSO reduces properties of silicone surface (Curran and Lingenfelter, 2009). However, previous research has shown the performance of adjuvants is dependent on the herbicide with which is applied, the plant species, and environmental conditions (Knezevic et al. 2009, Penner 1989).

Contact angle. The images of the contact angles of the treatment solutions measured on different surfaces are illustrated in Figure 3. Irrespective of the surface being tested, water alone resulted in the highest contact angles, indicating that water had the least contact with the solid surface, and the values observed were dependent on the leaf structure type. Although all species contain epicuticular wax, the degree of hydrophobicity varies among species (Chachalis et al. 2001, Hess and Falk 1990), the environment that the plant has grown in, and plant developmental stage (Singh et al. 2002). The glyphosate alone solution resulted in the second highest contact angles followed by the solution of lactofen alone, regardless of the surface type. The difference in contact angle between solutions (excluding water alone) was not significant for horseweed. Additionally, the trend for Palmer amaranth was different than other species tested. This may at least partially describe why Palmer amaranth has become one of the most problematic weeds in row crop production in the United States in recent history. Likewise, contact angles decreased by the addition of adjuvants either to the lactofen alone solution or in combination with glyphosate compared to the solutions with either of the two herbicides alone, regardless of the surface type. Contact angles formed on Palmer amaranth leaf surface were similar across treatment solutions of herbicides with or without the addition of adjuvants further illustrating the strong solution by species interactions that exist. Great variation was observed during measurements when using Palmer amaranth leaves due to the presence of deep veins; therefore, it may have affected the CA results differently than other species.

The CA depended on surface types and adjuvants used (Table 1). These results are consistent with other findings showing the CA formed is dependent on adjuvant type and surface utilized; moreover, differences in CA is observed when using same spray solution but different surfaces (Ebeling 1939, Fogg 1947). Overall, the greatest impact of adjuvants on reducing the CA across the different surfaces was for the lactofen solutions with COC plus DRA or NIS. Likewise, NIS was the adjuvant that most impacted the reduction of the CA formed on different surfaces when using glyphosate or glyphosate plus lactofen. Reduced contact angle is likely to occur with reduced surface tension. Although every solution conform to this trend, in an overall perspective, reduction of SFT and decreased CA were observed by the same adjuvants. Mankowich (1953) observed a correlation between SFT and CA for several surfactants; however, this correlation was not applicable to all surfactants tested in that study either.

Effect of SFT and CA on Herbicide Efficacy. The results in terms of herbicide efficacy of the treatment solutions used in this study applied to common lambsquarters, grain sorghum, kochia, and horseweed are described in Chapter 2. Only treatments using COC in herbicide solution were applied to Palmer amaranth as described in Chapter 3. Although the adjuvants decreased the CA formed on common lambsquartes leaf surfaces compared to lactofen alone, an increase in weed control was not observed. The greatest control of common lambsquarters was observed with the glyphosate alone solution and a slightly improvement was observed when using the herbicides in tank-mixture with NIS. However, contact angle was not the major contributing factor on weed control (and thereby, herbicide efficacy) since the CA formed by glyphosate alone was 107 versus 49 degrees by the addition of NIS into the tank-mixture. Likewise, lower contact angles formed by the addition of adjuvants into the tank-mixtures did not improve the control of grain sorghum when compared to glyphosate alone. Contrary to what was observed for grain sorghum, common lambsquarters control was greater when lactofen was applied with adjuvants compared to lactofen alone. Likewise, the addition of adjuvants increased the control of kochia and horseweed when compared to lactofen alone. However, reduction in SFT and CA could not be correlated to this improvement. Lower SFT and CA were not the major factors impacting herbicide efficacy where the greatest control of

Palmer amaranth was observed with glyphosate or glyphosate plus lactofen tank-mixtures with COC (51 versus 35 degrees, respectively).

Although reduced SFT and CA were observed in this study, the herbicide efficacy of Palmer amaranth, common lambsquarters, grain sorghum, kochia, and horseweed were not correlated to the changes in these physical properties. Likewise, reduction in SFT and CA did not always increases weed control (and thereby, herbicide efficacy) (Hess and Falk 1990, Foy and Smith 1965, Singh et al. 2002, Singh and Singh 2006). According to Singh et al. (2002), factors other than SFT and CA have a greater influence on efficacy such as the interaction between herbicide, surfactant, and plant surface. Hess et al. (1974) observed that even when using same active ingredient the herbicide distribution was influenced by the formulation type and leaf structure surface ultimately impacting herbicide efficacy. However, the effect on penetration and herbicide efficacy as influenced by the active ingredient within a spray droplet is not well understood (Hess et al. 1974). Increased leaf wettability is likely to occur with reduced SFT and CA; however, these isolated changes on physical properties do not solely result in better herbicide uptake by the plant and enhanced herbicide efficacy.

Observations from this study highlight the importance of adjuvants on reducing the SFT and CA properties of spray solutions; however, herbicide efficacy is only partially explained by the changes on these physical properties. Therefore, other factors that impact herbicide penetration and absorption by the plant should be taken in consideration. More studies are needed to better understand the factors influencing herbicide uptake and how they are correlated. For instance, leaf angle and position are important factors influencing the contact angle formed by the spray droplet on a leaf surface. Nevertheless, little is known about dynamic contact angle of spray solutions on different leaf surfaces. In addition, other adjuvant properties such as humectancy may increase weed control.

Spray droplets tend to dry faster as a result of a greater spreading. Depending on herbicide formulation the active ingredient absorption by the plant may be negatively affected though. Emulsifiable concentrates such as lactofen tend to separate in the solution, and as the spray droplet dries, the active ingredient may remain as crystals on the leaf surface. The success of a spray application from the time that the droplet leaves the nozzle until it is completely absorption by the plant depends on herbicide formulation and rate, adjuvant type and rate, leaf structure surface, and environmental conditions. Therefore, further investigation is needed to improve recommendations of applications using glyphosate and PPO-inhibiting herbicides alone and in combination in order to maximize herbicide efficacy.

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Table 4.1. Influence of adjuvants on physical properties of treatment solutions containing glyphosate and/or lactofen.

Treatment solution[a]	Density[b]	Viscosity	SFT	CA					
				CARD	CHEAL	SORBI	KCHSC	AMAPA	CNYZC
	g cm^{-3}	mPa s	mN m^{-1}	degrees					
Water	0.9982 k	1.0172 f	71.15 a	71 a	136 a	131 a	125 a	94 a	75 a
Glyphosate	1.0087 b	1.0321 f	38.34 b	49 b	107 b	119 b	100 b	51 bcd	63 b
Lactofen	1.0062 g	1.0302 f	33.04 c	37 c	76 c	78 c	89 c	54 bc	62 b
Lactofen + COC	1.0043 j	1.0750 de	30.64 f	25 de	44 gh	47 g	45 f	56 b	47 c
Lactofen + NIS	1.0064 f	1.0561 e	29. 22 h	21 f	46 g	42 g	37 g	49 bcd	47 c
Lactofen + MSO	1.0054 h	1.0686 de	31.73 d	25 de	54 ef	56 f	68 d	46 cd	45 cd
Lactofen + COC + DRA	1.0052 i	1.3760 b	30.24 fg	19 f	38 h	46 g	37 g	47 cd	39 de
Glyphosate + Lactofen + COC	1.0073 e	1.0775 d	30.09 g	25 de	54 ef	71 d	53 e	35 e	41 cde
Glyphosate + Lactofen + NIS	1.0090 a	1.0684 de	30.47 fg	14 g	49 fg	58 ef	45 f	45 d	39 de
Glyphosate + Lactofen + MSO	1.0079 c	1.1016 c	31.59 de	24 e	60 de	67 d	65 d	52 bcd	37 e
Glyphosate + Lactofen + COC + DRA	1.0077 d	1.4420 a	31.28 e	27 d	63 d	64 de	55 e	47 cd	41 cde

[a] Abreviations: COC, crop oil concentrate; NIS, non-ionic surfactant; MSO, methylated seed oil; DRA, drift retardant agent; SFT, surface tension; CA, contach angle; CARD, maylar plastic card; CHEAL, *Chenopodium album*; SORBI, *Sorghum bicolor*; KCHSC, *Kochia scoparia*; AMAPA, *Amaranthus palmeri*; CNYZC, *Conyza canadensis*.

[b] Means within a column followed by the same letter are not different (P > 0.05).

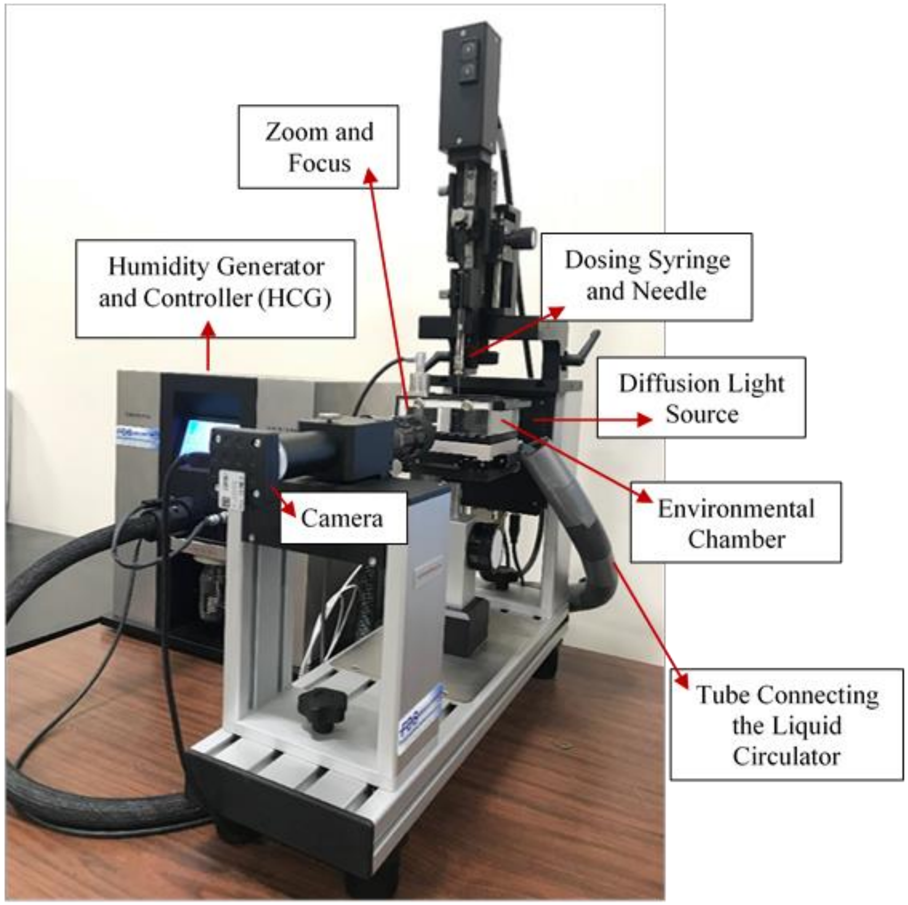

Figure 4.1. Experimental device for Surface Tension and Contact Angle Measurements.

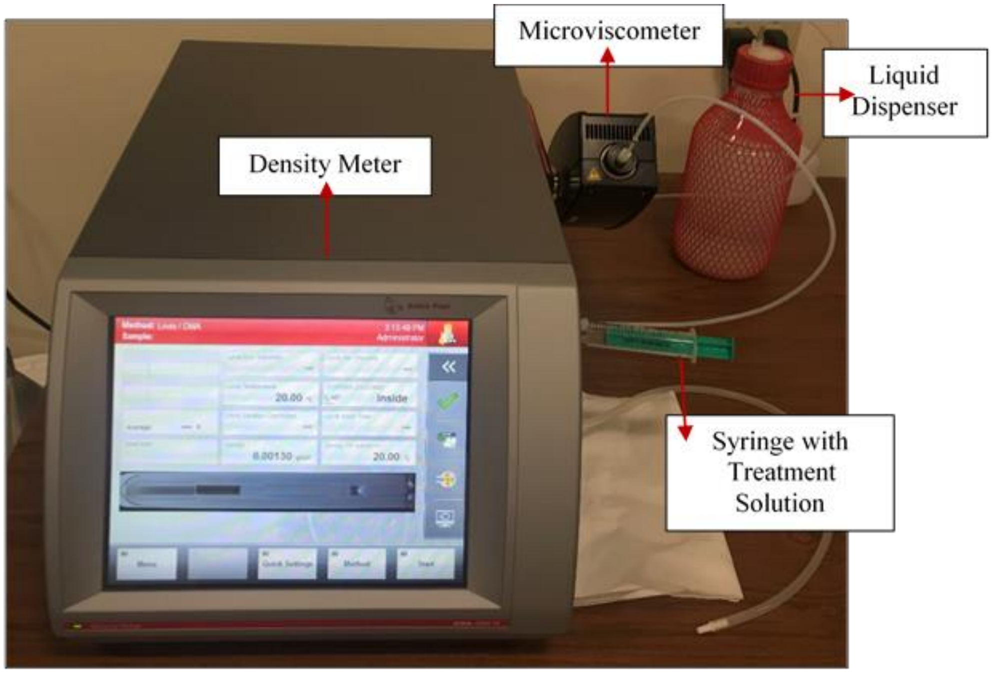

Figure 4.2. Experimental device for Density and Viscosity Measurements.

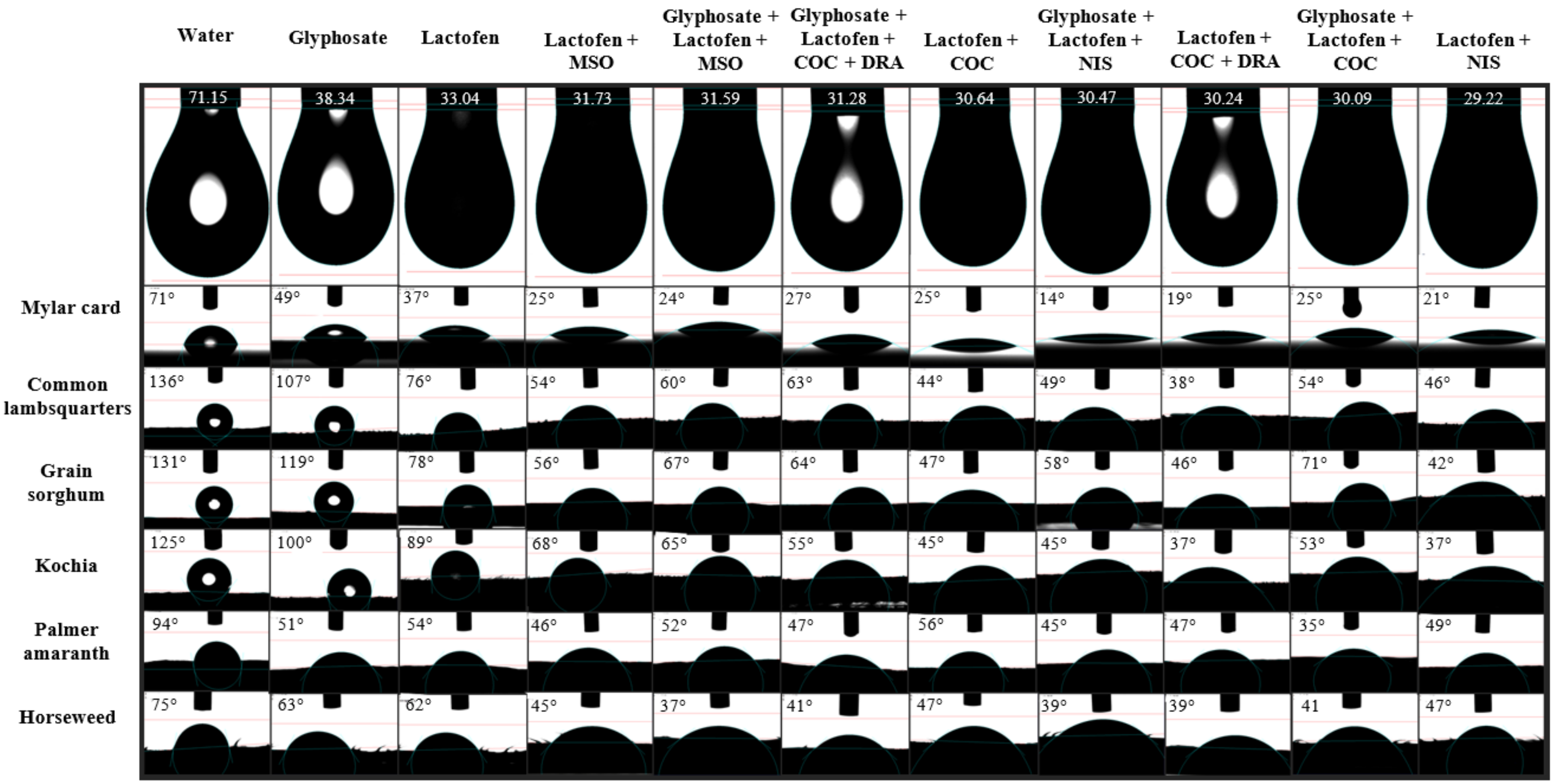

Figure 4.3. Images of the surface tension and static contact angle values using ten spray solutions plus water alone and different surfaces. (Surface tension is expressed as mN m^{-1}).