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

Jesaelen G. Moraes 1,*, Thomas R. Butts 1, Vitor M. Anunciato 2, Joe D. Luck 3, Wesley C. Hoffmann 4, Ulisses R. Antuniassi 5 and Greg R. Kruger 1

1 Department of Agronomy and Horticulture, University of Nebraska-Lincoln, North Platte, NE 69101, USA; tbutts@uada.edu (T.R.B.); grkruger2@icloud.com (G.R.K.)
2 Department of Plant Protection, Sao Paulo State University, Botucatu, SP 18618687, Brazil; vitor.muller@gmail.com
3 Department of Biological System Engineering, University of Nebraska-Lincoln, North Platte, NE 69101, USA; jluck2@unl.edu
4 USDA-ARS Aerial Application Technology Research Unit, College Station, TX 77845, USA; clint.hoffmann@gmail.com
5 Department of Rural Engineering, Sao Paulo State University, Botucatu, SP 18618687, Brazil; ulisses@fca.unesp.br
* Correspondence: jesaelenmoraes@huskers.unl.edu; Tel.: +1-402-219-1674

Abstract: PPO-inhibiting herbicides in combination with glyphosate for postemergence applications is a common approach to manage glyphosate- and ALS-inhibitor-resistant weeds. PPO-inhibitors can reduce glyphosate translocation when applied in tank-mixtures, but adjuvants may be used to overcome this effect. Additionally, optimal droplet size may be affected by tank-mixtures of different herbicides and it can be crucial to herbicide efficacy. Field and greenhouse studies were conducted to investigate the impact of nozzle selection and adjuvants on weed control and interactions when applying PPO-inhibitors (fomesafen or lactofen) alone or in tank-mixture with glyphosate to five weed species using six nozzle types. Ultra-coarse droplets were just as effective as medium droplets regardless of the spray solution, but have a lower likelihood of off-target movement. Tank-mixtures applied were consistently antagonistic to common lambsquarters, horseweed, and Palmer amaranth. Only fomesafen was antagonistic to kochia whereas synergistic interactions were observed when glyphosate plus lactofen were applied in combination with COC, DRA + COC, or NIS. Separate applications are advisable with herbicide- and weed-specific situations to avoid antagonism, which is necessary to achieve optimum weed control and maintain the effectiveness of PPO-inhibitors. Future research should continue to look at these important interactions across a wide range of weed species.

Keywords: air inclusion nozzle; crop oil concentrate; drift reducing adjuvant; methylated seed oil; non-air inclusion nozzle; non-ionic surfactant; soybeans

1. Introduction

Glyphosate is the most widely used non-selective herbicide worldwide due to its excellent efficacy, low toxicity, and unique mode-of-action (inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase; EPSPS) [1]. The repeated use of glyphosate has created a single selection pressure on weed populations [2], increasing the occurrence of glyphosate-resistant (GR) weeds. Amongst them, resistant populations of Palmer amaranth [Amaranthus palmeri S. Watson], kochia [Bassia scoparia (L.) A. J. Scott.], common lambsquarters (Chenopodium album L.), and horseweed [Erigeron canadensis L.] (four of the ten most troublesome weeds in broadleaf crops) [3] have been reported in the United States [4]. EPSPS-, acetolactate synthase (ALS)-, and protoporphyrinogen oxidase (PPO)-inhibiting herbicides are the primary three postemergence (POST) herbicide sites-of-action to manage broadleaf weeds in a glyphosate-tolerant (GT) only soybean production system. Many
weeds, including the aforementioned species, have also been confirmed to be resistant to ALS-inhibiting herbicides [4].

Although the use of different traits which confers soybean tolerance to different herbicides is an option to manage GR weeds in soybean, the use of dicamba or 2,4-D traits for POST applications face an uncertain future as large areas of sensitive vegetation have been injured in the past few years as result of off-target movement [5]. Repeated exposure to sublethal doses of dicamba and 2,4-D has resulted in many weed species with evolved resistance [6–8]. It is important to maximize the effectiveness of PPO-inhibiting herbicides to manage problematic broadleaf weeds in GT only and conventional soybean systems when resistance to both glyphosate and ALS-inhibiting herbicides is present in the field.

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 [9]. 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 [4]. PPO-inhibiting herbicides applied in tank-mixture with glyphosate for POST applications is a common approach to manage GR weed populations and delay the evolution of herbicide resistance. However, antagonistic interactions to specific weed species have been reported in literature when those herbicides were applied in combination [10–12]. Glyphosate activity is often antagonized by fast-acting herbicides such as glufosinate and several PPO-inhibiting herbicides [13] because contact herbicides can limit glyphosate translocation [12].

Additive and antagonistic interactions have been reported in literature to occur more often than synergistic interactions when glyphosate is applied in tank-mixture with other herbicides [13]. Furthermore, tank-mixture effectiveness may be reduced when mixtures do not show similar efficacy [14]. Adjuvants such as surfactants and oil concentrates are used in tank-mixtures or pre-mixtures with foliar-applied herbicides to enhance herbicide activity [15] or modify the action of herbicides [16] as well as to increase spray droplet retention on leaf surface and penetration of herbicide active ingredient through the cuticle [17]. Furthermore, they have been reported to potentially overcome antagonism between two herbicides [17,18]. Previous research has shown that the performance of adjuvants is dependent on the herbicide being applied, the plant species targeted, and environmental conditions [19,20].

In addition, spray application factors such as nozzle selection play a crucial role in spray performance [21–23]. Smaller droplets from XR (extended range) flat fan nozzle have been reported to be more effective than larger droplets when applying POST herbicides at a constant carrier volume [24]. In contrast, no differences in control were observed when fomesafen or lactofen were applied to different weed species using XR or air-inclusion (AI) nozzles [25,26]. Although non-air inclusion flat fan nozzles provide more coverage than air inclusion flat fan nozzles, 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 operating pressure, carrier volume, adjuvants, herbicides, weed size, weed species, and environmental conditions [21,22,26–30].

Spray applications are a complex process and studies showing nozzle selection by tank-mixture interactions on herbicide efficacy and weed control are crucial to assure effective and sustainable weed management recommendations. Field and greenhouse studies were conducted including multiple nozzle designs, herbicide treatments with or without adjuvants on single or herbicide tank-mixtures to investigate the following objectives: (1) determine the impact of nozzle selection on weed control when systemic and contact herbicides are used in tank-mixtures, (2) evaluate the response of several weed species to glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) applied alone or in combination, (3) determine the type of interaction (additive, synergistic, or antagonistic) when these herbicides were applied in tank-mixtures, and (4) determine the impact of adjuvants on the type of interaction when tank-mixtures were used.
2. Materials and Methods

2.1. Experimental Design and Establishment

2.1.1. Field Studies

Study location, GPS coordinates, application date, weather conditions during application, weed densities, and weed heights are summarized in Table 1. Two field experiments were established in a fallow environment infested with Palmer amaranth during the summer of 2016. The experiment at each location was arranged in a randomized complete block design with factorial arrangement of treatments with four replications. Treatments were arranged in a five by three factorial plus a nontreated control consisting of five spray solutions and three nozzle types (Table 2). Spray treatments consisted of POST applications of glyphosate (Roundup PowerMax®, Monsanto Company, St Louis, MO 63167, USA) at 1260 g ae ha\(^{-1}\), fomesafen (Flexstar®, Syngenta Crop Protection, Greensboro, NC 27419, USA) at 130 g ai ha\(^{-1}\), or lactofen (Cobra®, Valent USA Corporation, Walnut Creek, CA 94596, USA) at 220 g ai ha\(^{-1}\) alone and glyphosate in tank-mixture with either fomesafen or lactofen. Liquid ammonium sulfate (Bronc®, Wilbur-Ellis Company, Fresno, CA 64596, USA) at 2.5% v/v was added to all treatments and crop oil concentrate-COC (R.O.C®, Wilbur-Ellis Company, Fresno, CA 64596, USA) at 1% v/v was used in all treatments, except for glyphosate applied alone. Herbicide treatments were applied using a CO\(_2\) sprayer mounted to a Bobcat 3400 UTV (Bobcat Company, West Fargo, ND 58078, USA) 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.

Table 1. Description of the locations used to evaluate the response of Palmer amaranth to glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) applied alone or in tank-mixtures.

| Parameters                      | Field Experiments                              |
|---------------------------------|------------------------------------------------|
| City                            | Beaver City, Nebraska                          |
| GPS coordinates                 | 40.16° N, 99.91° W                            |
| Application date                | 30 June 2016                                   |
| Temperature (°C)                | 28                                             |
| Relative humidity (%)           | 50                                             |
| Weed density (plants m\(^{-2}\))| 50–70                                          |
| Weed height (cm)                | 58                                             |
| Soil type                       | Ulysses silt loam                              |
|                                 | Holdrege silt loam                             |

2.1.2. Greenhouse Studies

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 2016 and 2017. Seeds from putative glyphosate-susceptible (GS) populations of common lambsquarters and sorghum [Sorghum bicolor (L.) Moench ssp. 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) [31] and kochia (ED\(_{50}\) of 1608 g ae ha\(^{-1}\) based on dry biomass, collected at 41.16° N, W101.99° W) [31] were used in both years. Although sorghum is not considered a weedy species, it was selected because it is representative of other grass weed species due to its similarity in biology and morphology and was easier to cultivate in the greenhouse.

For the greenhouse experiment conducted in 2016, plants were seeded between June and July and grown in D40H cone-tainer cells (Stuewe and Sons, Inc., Corvallis, OR 97389, USA) filled with Berger BM7 Bark Mix (Berger.ca, Saint-Modeste, QC Gol 3W0, USA), 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 (Nature’s Source® Professional Plant Food, Ball Food, Ball DPF, LLC Sherman, TX 75090, USA). The 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 thuringiensis* (Thuricide®, Bonide Products, Inc., Oriskany, NY 13424, USA) to avoid *Trichoplusia ni* (Cabbage looper). For the greenhouse experiment conducted in 2017, plants were seeded between May and July and grown in the same D40H cone-tainer cells filled with Pro-Mix BX (Premier Tech Horticulture Ltd., Riviè re-du-Loup, QC G5R 6C1, Canada) general purpose growing medium. Plants were overhead irrigated and fertilized daily with a commercial fertilizer (Wilbur-Ellis Agribusiness, 3300 South Parker Road, Suite 500, Aurora, CO 80014, USA) blended with water at 0.2% v/v. The greenhouse was maintained at a daytime temperature between 26–30 °C and a nighttime temperature between 18–23 °C. LED growth lights (Philips Lighting Holding B.V., Somerset, NJ 08873, USA) at 520 μmol s⁻¹ were used as supplemental lighting 8-h per day. Plants were treated with *Bacillus thuringiensis* (DiPel®, Valent, 1600, Riviera Avenue, Suite 200, Walnut Creek, CA 94596, USA); in addition, common lambsquarters and kochia plants were treated with a second *Bacillus thuringiensis* (Thuricide®) to avoid *Trichoplusia ni* (Cabbage looper).

Both greenhouse experiments 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 (DeVries Manufacturing, Hollandale, MN 56045, USA) 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 a speed of 9.6 kph.

For the greenhouse experiment conducted in 2016, the treatments were arranged in a fivebysix factorial plus a nontreated control consisting of five spray solutions and six nozzle types (Table 2). Spray treatments consisted of POST applications using glyphosate (Roundup PowerMax®) at 630 g ae ha⁻¹, fomesafen (Flexstar®) at 65 g ai ha⁻¹, or lactofen (Cobra®) at 110 g ai ha⁻¹ alone and glyphosate in tank-mixture with either fomesafen or lactofen. Liquid ammonium sulfate (Bronc®) at 2.5% v/v was added to all treatments and COC (R.O.C®) at 1% v/v was used in all treatments, except for glyphosate applied alone. For the greenhouse experiment conducted in 2017, the treatments were arranged in a ten by two factorial plus a nontreated control consisting of ten spray solutions and two nozzle types (Table 2). Spray treatments consisted of POST applications of glyphosate (Roundup PowerMax®) at 630 g ae ha⁻¹ or lactofen (Cobra®) at 110 g ai ha⁻¹ alone, lactofen at 110 g ai ha⁻¹ with the adjuvants COC at 1% v/v, drift retardant adjuvant-DRA (IntactTM, Precision Laboratories LLC, Waukegan, IL 60085, USA) at 0.5% v/v, methylated seed oil-MSO (High Load®, Wilbur-Ellis Company, Fresno, CA 6459, USA) at 1% v/v, or non-ionic surfactant-NIS (R-11®, Wilbur-Ellis Company, Fresno, CA 64596, USA) at 0.25% v/v, and herbicides applied in tank-mixture with each of the adjuvants aforementioned. Liquid ammonium sulfate (Bronc®) at 2.5% v/v was added to all treatments. COC was added to the tank-mixture when DRA was used. Half-labeled rates of herbicides were used for the greenhouse studies to avoid complete plant death to be able to account for differences among treatments.

Table 2. Nozzle selection used in the field and/or greenhouse experiments as classified by their manufacturer and the spray droplet classification category in accordance with ASABE S572.1 guidelines.

| Experiment               | Common Name | Nozzle Type a | Droplet Size Classification b,c | Manufacturer                                      |
|--------------------------|-------------|---------------|---------------------------------|---------------------------------------------------|
| Field; Greenhouse 2016/2017 | Extended Range | XR            | M                               | Teejet Technologies, Spraying Systems Co., Wheaton, IL 62703 |
| Field; Greenhouse 2016   | Air-Induction Extended | AIXR          | XC                              | Teejet Technologies, Spraying Systems Co., Wheaton, IL 62703 |
| Greenhouse 2016          | GuardianAIR | GA            | C                               | Pentair Hypro, New Brighton, MN 55112             |
Table 2. Cont.

| Experiment                  | Common Name          | Nozzle Type  | Droplet Size Classification \(b,c\) | Manufacturer                                      |
|-----------------------------|----------------------|--------------|-------------------------------------|--------------------------------------------------|
| Greenhouse 2016             | TurboDrop® XL        | TDXL         | VC                                  | Greenleaf Technologies, Covington, LA 70434       |
| Greenhouse 2016             | Ultra Lo-Drift ULD   | ULD          | UC                                  | Pentair Hypro, New Brighton, MN 55112             |
| Field; Greenhouse 2016/2017 | Turbo Teejet® Induction | TTI         | UC                                  | Teejet Technologies, Spraying Systems Co., Wheaton, IL 62703 |

\(a\) The listed nozzle types were all orifice size “04” with a manufacturer-rated angle of 110° except for ULD nozzles that were 120. \(b\) Based on water at 40 psi. \(c\) Abbreviations: M = Medium, C = Coarse, VC = Very Coarse, XC = Extremely Coarse, and UC = Ultra Coarse.

2.2. Data Collection

2.2.1. Field Studies

After the plants were treated, percent of Palmer amaranth control evaluations based on visual estimations of injury were collected at 28 days after treatment (DAT) using a scale of 0 to 100%, with 0% being no herbicidal damage and 100% being complete plant death.

2.2.2. Greenhouse Studies

Treated plants were clipped at the soil surface at 28 DAT and placed in a dryer at 65 °C until plants reached a constant mass. Dry biomass was recorded and converted into percent biomass reduction using Equation (1):

\[
100 - \left( \frac{X \times 100}{Y} \right)
\]

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

2.3. Tank-Mixture Interaction

Percent of weed control can be predicted using the responses of herbicides applied singularly [30]. After applications, observed responses based on the biomass reduction at 28 DAT from herbicides applied alone were used to calculate the expected responses of applied in tank-mixtures. Expected responses were only calculated for greenhouse studies since dry biomass was not collected for field studies. Colby’s equation was used to obtain the expected percent of control responses when herbicides were applied in tank-mixtures and to describe the type of interaction [32]. If \(E\) is the expected response as a percent of control using two herbicides in tank-mixture \((A + B)\), and \(X\) and \(Y\) are the observed responses as a percent of control when herbicide \((A\) or \(B)\) was applied alone, then, according to Colby:

\[
E = 100 - \frac{(100 - X) \times (100 - Y)}{100}
\]

2.4. Statistical Analyses

Data from field and greenhouse studies were subjected to ANOVA using R software (R Foundation for Statistical Computing, Wien, Austria) with mean separations made at \(\alpha = 0.05\) level using Fisher’s protected LSD test and the Tukey post-hoc test. For the greenhouse experiments conducted either in 2016 or 2017, each species was analyzed separately. A 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 (block) was considered a random effect. For field experiments, spray solution and nozzle selection were analyzed as fixed effects whereas block was analyzed as a random effect, data were pooled over across locations because of lack of significant run by treatment interaction.
A t-test was performed to compare observed and expected responses to determine the statistical significance of the differences between them using the following equation [33]:

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

where: $\hat{m}$ = the expected value; $A$ = the observed value; and $s(\hat{m})$ = the standard error of the mean.

Using this formula, the level of significance of $t (p)$ could be determined to classify the type of interaction. When the observed percent control from the tank-mixture was less than, equivalent to, or greater than the expected percent control, the response was considered antagonistic, additive, or synergistic, respectively.

3. Results

3.1. Nozzle Selection

No interaction between spray solution and nozzle was observed in the field study for Palmer amaranth control at 28 DAT (Supplementary Materials Table S1) or for sorghum, common lambsquarters, horseweed, and kochia control at 28 DAT from the greenhouse study conducted in 2016 (Supplementary Materials Table S2). Similarly, no interaction between spray solution and nozzle was observed for sorghum, common lambsquarters, horseweed, and kochia control at 28 DAT from the greenhouse study conducted in 2017 (Supplementary Materials Table S3). Although the main effect of nozzle was significant for kochia control from the greenhouse study containing adjuvants conducted in 2017 (Supplementary Materials Table S3), applications using the XR nozzle were 5% greater than the TTI (Supplementary Materials Table S4). Nozzles used in this study produced droplet size classifications ranging from Medium to Ultra Coarse according to their manufacturer catalogs (Table 2).

3.2. Percent of Control by Plant Species

The main effect of spray solution was significant for Palmer amaranth from the field study conducted in 2016 (Supplementary Materials Table S1). Percent of Palmer amaranth control to treatments of glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) applied alone and in tank-mixtures at 28 DAT are shown in Figure 1. These field populations had not been screened for herbicide resistance, but it is likely there was only a low level of glyphosate resistance [34]. Therefore, the application of glyphosate alone resulted in the greatest control (74%). When applied in combination with fomesafen, control decreased to 61% but there was no difference in control when lactofen was used in tank-mixture with glyphosate. Applications of PPO-inhibiting herbicides alone resulted in the lowest control with 33% for fomesafen and 42% for lactofen.

The main effect of the spray solution was significant for sorghum, common lambsquarters, horseweed, and kochia from the greenhouse study conducted in 2016 (Supplementary Materials Table S2; Figure 2). Applications of glyphosate alone resulted in the greatest control of common lambsquarters and sorghum (98 and 93%, respectively) and the lowest control of horseweed and kochia (27 and 19%, respectively) as the latter species were GR populations. When glyphosate was applied in tank-mixture with either PPO herbicide, control of sorghum and common lambsquarters was not different. Lactofen alone provided the greatest control of horseweed (53%) but when applied in tank-mixture with glyphosate, the control was reduced (42%). No differences were observed when fomesafen was applied alone or in combination with glyphosate to horseweed. The greatest kochia control (93%) was observed when lactofen was applied in combination with glyphosate, but it was not different compared to lactofen applied alone (92%). Differences in control of sorghum and kochia were not observed when using applications of fomesafen or lactofen alone. In contrast, fomesafen applied alone to common lambsquarters and horseweed resulted in 10% greater and 14% lower control, respectively, compared to lactofen.
Figure 1. Percent of Palmer amaranth control based on visual estimations of injury at 28 DAT treated with different herbicide tank-mixtures averaged across nozzle types evaluated from a field study conducted in 2016. Bars with same letter do not differ using Tukey post-hoc test at $\alpha = 0.05$.

Figure 2. Percent of (a) sorghum, (b) common lambsquarters, (c) horseweed, and (d) kochia control based on dry biomass at 28 DAT treated with different herbicide tank-mixtures averaged across nozzle types evaluated from a greenhouse study conducted in 2016. Bars with same letter do not differ using Tukey post-hoc test at $\alpha = 0.05$.

The main effect of spray solution was significant for sorghum, common lambsquarters, horseweed, and kochia from the greenhouse study conducted in 2017 (Supplementary Materials Table S3). Percent of control to applications of glyphosate and lactofen applied
alone and in tank-mixtures with or without the adjuvants COC, DRA plus COC, MSO, or NIS at 28 DAT are shown in Figure 3. No differences were observed when glyphosate was applied alone or in tank-mixture with lactofen plus any adjuvant for sorghum control. Lactofen applied in combination with adjuvants did not improve sorghum control compared to lactofen applied alone, except for the spray solution lactofen plus DRA plus COC. In contrast, glyphosate applied in combination with lactofen plus an adjuvant reduced common lambsquarters control by a minimum of 19% compared to glyphosate applied alone, except for the tank-mixture containing NIS. In addition, no differences were observed when lactofen was applied alone or in combination with any of the adjuvants. For horseweed, no differences were observed for glyphosate or lactofen applied alone resulting in the lowest controls with 30% and 36%, respectively. Improved control was observed when lactofen was applied in combination with COC, DRA plus COC, or MSO compared to lactofen alone. In contrast, no differences were observed when applying lactofen alone or in combination with NIS. Greatest horseweed control was observed when glyphosate was applied in tank-mixture with lactofen plus DRA plus COC with 60%, but it was not different from the tank-mixtures (glyphosate plus lactofen) containing either COC only (55%) or MSO (53%). For kochia, the lowest control was observed when glyphosate was applied alone (7%) followed by lactofen applied alone (44%). Spray solutions of lactofen plus an adjuvant or glyphosate in tank mixture with lactofen plus an adjuvant improved kochia control (>78%).

Figure 3. Percent of (a) sorghum, (b) common lambsquarters, (c) horseweed, and (d) kochia control based on dry biomass at 28 DAT treated with different herbicide tank-mixtures as affected by the addition of different adjuvants and averaged over nozzle types evaluated from a greenhouse study conducted in 2017. Bars with same letter do not differ using Tukey post-hoc test at α = 0.05.
3.3. Tank Mixture Interactions

In the field experiments, glyphosate in tank-mixture with either PPO-inhibiting herbicide reduced Palmer amaranth control compared to glyphosate alone. Tank-mixtures containing fomesafen reduced the control from 74% to 61% (Figure 1) compared to glyphosate alone suggesting that glyphosate was antagonized by fomesafen.

Observed and expected responses and the type of interaction calculated for the tankmixtures used in the greenhouse study conducted in 2016 are summarized in Table 3. The addition of fomesafen or lactofen into the tank-mixture did not improve the control of sorghum or common lambsquarters compared to glyphosate applied alone and antagonistic interactions were observed for both species. Differences among responses (observed and expected) were minimal regardless of which PPO-inhibiting herbicide was applied to sorghum. This is likely to occur because of the extremely high observed responses for glyphosate alone (99% sorghum control) and limitations when using Colby’s equation to predict responses. In contrast, differences were present for horseweed as observed responses were less than the expected by 18% and 24% for the tank-mixtures, including fomesafen or lactofen, respectively. An antagonistic interaction was observed for fomesafen applied in tank-mixture with glyphosate for kochia control (11% less control), whereas an additive interaction was observed for lactofen applied in the tank-mixture.

Table 3. Interaction of glyphosate plus fomesafen or lactofen based on dry biomass in glyphosate-susceptible populations of sorghum and common lambsquarters and glyphosate-resistant populations of horseweed and kochia from a greenhouse study conducted in 2016.

| Species          | Herbicide | Observed Response | Expected Response a | t-Value | p b | Interaction   |
|------------------|-----------|-------------------|---------------------|---------|-----|--------------|
| Sorghum          | Fomesafen | 97                | 99                  | 14.82   | <0.0001 | Antagonistic |
|                   | Lactofen  | 97                | 99                  | 15.25   | <0.0001 | Antagonistic |
| Common lambsquarters | Fomesafen | 90                | 98                  | 5.86    | <0.0001 | Antagonistic |
|                   | Lactofen  | 89                | 97                  | 6.23    | <0.0001 | Antagonistic |
| Horseweed        | Fomesafen | 37                | 55                  | 9.57    | <0.0001 | Antagonistic |
|                   | Lactofen  | 42                | 66                  | 9.40    | <0.0001 | Antagonistic |
| Kochia           | Fomesafen | 77                | 88                  | 3.88    | <0.0001 | Antagonistic |
|                   | Lactofen  | 93                | 93                  | 0.99    | <0.0001 | Additive     |

a Expected responses calculated according to Colby model. b Level of significance of p.

Observed and expected responses and the type of interaction calculated for the tankmixtures containing adjuvants used in the greenhouse conducted in 2017 are summarized in Table 4. For sorghum, antagonistic interactions were observed when glyphosate was applied in tank-mixture with lactofen plus COC or MSO whereas additive interactions were observed when glyphosate was applied in tank-mixture with lactofen plus DRA plus COC or NIS. No differences among treatments were observed as explained previously. In contrast, observed responses of common lambsquarters were less than the expected by 29, 29, 30, and 11% when glyphosate was applied in tank-mixture with lactofen plus DRA plus COC, DRA plus COC, MSO, or NIS, respectively, resulting in antagonistic interactions. Likewise, antagonistic interactions were observed regardless of the spray solution applied to horseweed with 7, 4, 11, and 15% reductions when glyphosate was applied in tank-mixture with lactofen plus COC, DRA plus COC, MSO, or NIS, respectively. For kochia, there was no antagonistic interactions observed. Applications of glyphosate in tank-mixture with lactofen plus COC, DRA plus COC, or NIS resulted in synergistic interactions for kochia control, whereas an additive interaction was observed when glyphosate in tank-mixture with lactofen plus MSO was used.
Table 4. Interaction of glyphosate plus lactofen influenced by the addition of different adjuvants based on dry biomass in glyphosate-susceptible populations of sorghum and common lambsquarters and glyphosate-resistant populations of horseweed and kochia from a greenhouse study conducted in 2017.

| Species          | Adjuvant | Observed Response | Expected Response a | t-Value | p b | Interaction          |
|------------------|----------|-------------------|---------------------|---------|-----|----------------------|
| Sorghum          | COC      | 96                | 99                  | 5.03    | <0.0001 | Antagonistic         |
|                  | DRA + COC| 96                | 99                  | 1.39    | 0.173 | Additive             |
|                  | MSO      | 97                | 99                  | 3.30    | 0.002 | Antagonistic         |
|                  | NIS      | 99                | 99                  | 0.12    | 0.898 | Additive             |
| Common lambsquarters | COC   | 63                | 92                  | 13.81   | <0.0001 | Antagonistic         |
|                  | DRA + COC| 65                | 94                  | 15.43   | <0.0001 | Antagonistic         |
|                  | MSO      | 64                | 94                  | 23.44   | <0.0001 | Antagonistic         |
|                  | NIS      | 82                | 93                  | 10.57   | <0.0001 | Antagonistic         |
| Horseweed        | COC      | 55                | 62                  | 4.63    | <0.0001 | Antagonistic         |
|                  | DRA + COC| 60                | 64                  | 2.61    | 0.013  | Antagonistic         |
|                  | MSO      | 53                | 64                  | 4.84    | <0.0001 | Antagonistic         |
|                  | NIS      | 45                | 60                  | 7.05    | <0.0001 | Antagonistic         |
| Kochia           | COC      | 88                | 85                  | 3.27    | 0.002  | Synergistic          |
|                  | DRA + COC| 84                | 73                  | 3.22    | 0.003  | Synergistic          |
|                  | MSO      | 79                | 80                  | 0.18    | 0.859  | Additive             |
|                  | NIS      | 85                | 79                  | 6.94    | <0.0001 | Synergistic          |

a Expected responses calculated according to Colby model. b Level of significance of p.

4. Discussion

Consistent results across field and greenhouse experiments showed that nozzle selection (and thereby, droplet size) was not the major contributing factor on herbicide efficacy, as similar results were observed regardless of the nozzle being used. Smaller droplets from non-air inclusion nozzles for POST herbicide applications at a constant volume have been reported to be more effective than larger droplets [24]. In contrast, many studies have reported no differences in weed control regarding droplet size when PPO-inhibiting herbicides [11,25,26,28,35] were applied to several weed species. 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 [21,22,26–28,30], showing the complexity of optimizing pesticide applications.

In general, less control was observed when using applications of fomesafen alone compared to lactofen alone (except for common lambsquarters). Observations from this research 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 [36]. Higher fomesafen rates have been reported to increase the control of Palmer amaranth [37] and common waterhemp [38]. For instance, Bond et al. [39] reported 96% Palmer amaranth control based on visual ratings at 21 DAT using fomesafen at 420 g ha$^{-1}$ when plants were 15 cm.

Very large Palmer amaranth plants as well as high densities present at both locations where field studies were conducted likely caused reduced efficacy of the herbicide applications. Whitaker et al. [40] 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. [41] reported reduced weed control, including pigweed species, from 94 to 79% when plants were 10 and 30 cm tall, respectively, with a single glyphosate application at 840 g ha$^{-1}$ at 14 to 21 DAT. Palmer amaranth control using applications of PPO-inhibiting herbicides is dependent on weed height and environmental conditions showing poor control with plants >10 cm tall [42]. Berger et al. [25] reported reduced Palmer amaranth control with lactofen at 210 g ha$^{-1}$ as weed height increased.

Tank-mixture interactions were consistent across greenhouse experiments supporting the hypothesis that glyphosate is antagonized by other PPO-inhibiting herbicide when applied to Palmer amaranth plants in the field. Similarly, combinations of several PPO-inhibiting herbicides in tank-mixtures with glyphosate were antagonized on several broadleaf weeds [10,12,13,28]. Interestingly, antagonism was only observed in kochia when
glyphosate was in tank-mixture with fomesafen. Adding COC, DRA plus COC, or NIS to the glyphosate plus lactofen tank-mixture improved the control of kochia resulting in synergistic interactions. Likewise, the addition of NIS to the glyphosate plus lactofen tank-mixture increased common lambsquarters control. Although interaction remained classified as antagonistic, greater than 80% control was observed with no differences when compared to glyphosate applied alone. Additive and antagonistic herbicide interactions have been reported in literature to occur more often than synergistic interactions when glyphosate is applied in tank-mixture with other herbicides [13]. Observations from this research are in consensus with Kammler et al. [43], who observed that the antagonism was weed species- and adjuvant-specific.

Based on these results, it would be advisable to apply glyphosate and fomesafen separately for weed species such as common lambsquarters, horseweed, kochia, and Palmer amaranth. Glyphosate and lactofen should also be applied separately for weed species such as common lambsquarters, horseweed, and Palmer amaranth but for other weed species such as kochia they may be tank-mixed to save an additional application. To effectively address this issue, future research should continue to look at these important interactions across a wide range of weed species and spray application techniques. The herbicide application method using separate booms may avoid glyphosate being antagonized by PPO-inhibitors as it could reduce the antagonism of grass weed control when using acetyl-CoA carboxylase (ACCase)-inhibiting herbicides in combination with glyphosate or auxinic herbicides [44].

5. Conclusions

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, sorghum, common lambsquarters, horseweed, and kochia. Spraying contact herbicides (fomesafen or lactofen) with ultra coarse droplets was just as effective as medium droplets. These larger droplet producing nozzles will reduce the likelihood of off-target movement. None of the POST applications using the herbicides alone or in tank-mixtures provided Palmer amaranth control of 90% or more at 28 DAT. Glyphosate and PPO-inhibiting herbicides (fomesafen or lactofen) in tank-mixture had antagonism on Palmer amaranth control. Glyphosate and PPO-inhibiting herbicides in tank-mixtures applied with or without adjuvants consistently had antagonism on common lambsquarters and horseweed. The potential of adjuvants to overcome antagonistic interactions was dependent on the weed species. Avoiding antagonism of glyphosate and PPO-inhibiting herbicides is necessary to achieve optimum weed control and to maintain the effectiveness of these herbicides.

Supplementary Materials: The following are available at https://www.mdpi.com/article/10.3390/agronomy11040754/s1, Table S1: ANOVA probability values for spray solution, nozzle, and interaction between spray solution and nozzle with respect to visual estimations of injury of Palmer amaranth at 28 DAT following applications of glyphosate, lactofen and fomesafen alone or in tank-mixtures from a field study conducted in 2016, Table S2: ANOVA probability values for spray solution, nozzle, and interaction between spray solution and nozzle with respect to dry biomass reduction of sorghum, common lambsquarters, horseweed and kochia at 28 DAT following applications of glyphosate, lactofen and fomesafen alone or in tank-mixtures from a greenhouse study conducted in 2016, Table S3: ANOVA probability values for spray solution and nozzle with respect to dry biomass reduction of sorghum, common lambsquarters, horseweed and kochia at 28 DAT following applications of glyphosate, lactofen and fomesafen alone or in tank-mixtures from a greenhouse study conducted in 2017, Table S4: Percent of control of plants species based on dry weights according to the nozzle type used from the greenhouse experiment conducted in 2017.
**Author Contributions:** Conceptualization, J.G.M. and G.R.K.; methodology, J.G.M., T.R.B. and G.R.K.; formal analysis, J.G.M., and V.M.A.; investigation, J.G.M.; writing—original draft preparation, J.G.M.; writing—review and editing, J.G.M., T.R.B., G.R.K., J.D.L., W.C.H. and U.R.A.; supervision, G.R.K.; funding acquisition, G.R.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** This project is based on research that was partially supported by the Nebraska Agricultural Experiment Station with funding from the Hatch Multistate Research capacity funding program from the USDA National Institute of Food and Agriculture.

**Institutional Review Board Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Acknowledgments:** The authors would like to thank all the members of the Pesticide Application Technology (PAT) Laboratory for help and support in conducting this research.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Duke, S.O.; Powles, S.B. Glyphosate: A once-in-a-century herbicide. *Pest Manag. Sci.* **2008**, *64*, 319–325. [CrossRef] [PubMed]
2. Knezevic, S.Z. Herbicide tolerant crops: 10 years later. *Maydica* **2007**, *52*, 245–250.
3. [WSSA] Weed Science Society of America. 2019 WSSA Survey Ranks Most Common and Most Troublesome Weeds in Broadleaf Crops, Fruits and Vegetables. Available online: http://wssa.net/wssa/weed/surveys/ (accessed on 18 September 2020).
4. Heap I International Survey of Herbicide-Resistant Weeds. Available online: http://www.weedscience.org (accessed on 18 September 2020).
5. Husted, K. Dicamba Damage Estimate Tops 2.5 Million Acres. Harvest Public Media. Available online: https://www.harvestpublicmedia.org/post/dicamba-damage-estimate-tops-2-5-million-acres (accessed on 18 September 2020).
6. Vieira, B.C.; Luck, J.D.; Amundsen, K.L.; Werle, R.; Gaines, T.A.; Kruger, G.R. Herbicide drift exposure leads to reduced herbicide sensitivity in *Amaranthus* spp. *Sci. Rep.* **2020**, *10*, 1–11. [CrossRef] [PubMed]
7. Ashworth, M.B.; Walsh, M.J.; Flower, K.C.; Powles, S.B. Recurrent selection with reduced 2, 4-D amine doses results in the rapid evolution of 2, 4-D herbicide resistance in wild radish (*Raphanus raphanistrum L.*). *Pest Manag. Sci.* **2016**, *72*, 2091–2098. [CrossRef]
8. Tehranchian, P.; Norsworthy, J.K.; Powles, S.; Bararpour, M.T.; Bagavathiannan, M.V.; Barber, T.; Scott, R.C. Recurrent Sublethal-Dose Selection for Reduced Susceptibility of Palmer Amaranth (*Amaranthus palmeri*) to Dicamba. *Weed Sci.* **2017**, *65*, 206–212. [CrossRef]
9. Hao, G.F.; Zuo, Y.; Yang, S.G.; Yang, G.F. Protoporphyrinogen oxidase inhibitor: An ideal target for herbicide discovery. *Chim. Int. J. Chem.* **2011**, *65*, 961–969. [CrossRef]
10. Nandula, V.K.; Reddy, K.N.; Koger, C.H.; Poston, D.H.; Rimando, A.M.; Duke, S.O.; Bond, J.A.; Ribeiro, D.N. Multiple resistance to glyphosate and pyrithiobac in Palmer amaranth (*Amaranthus palmeri*) from Mississippi and response to flumiclorac. *Weed Sci.* **2012**, *60*, 179–188. [CrossRef]
11. Nandula, V.K.; Molin, W.T.; Bond, J.A. Influence of Water Quality, Formulation, Adjuvant, Rainfastness, and Nozzle type on Efficacy of Fomesafen on Palmer amaranth (*Amaranthus palmeri*) Control. *Am. J. Plant Sci.* **2018**, *9*, 1660. [CrossRef]
12. Starke, R.J.; Oliver, L.R. Interaction of glyphosate with chlorimuron, fomesafen, imazethapyr, and sulflentrazone. *Weed Sci.* **1998**, *46*, 652–660. [CrossRef]
13. Harre, N.T.; Young, J.M.; Young, B.G. Glyphosate-induced antagonism in rapid response giant ragweed (*Ambrosia trifida*). *Weed Technol.* **2018**, *32*, 52–59. [CrossRef]
14. Beckie, H.J.; Reboud, X. Selecting for weed resistance: Herbicide rotation and mixture. *Weed Technol.* **2009**, *23*, 363–370. [CrossRef]
15. Bellinder, R.R.; Arsenovic, M.; Shah, D.A.; Rauch, B.J. Effect of weed growth stage and adjuvant on the efficacy of fomesafen and bentazon. *Weed Sci.* **2003**, *51*, 1016–1021. [CrossRef]
16. Johnson, A.K.; Roeth, F.W.; Martin, A.R.; Klein, R.N. Glyphosate spray drift management with drift-reducing nozzles and adjuvants. *Weed Technol.* **2006**, *20*, 893–897. [CrossRef]
17. Young, B.G.; Hart, S.E.; Wax, L.M. Interactions of sethoxydim and corn (*Zea mays*) postemergence broadleaf herbicides on three annual grasses. *Weed Technol.* **1996**, *10*, 914–922. Available online: www.jstor.org/stable/398793 (accessed on 18 September 2020). [CrossRef]
18. Campbell, J.R.; Penner, D. Compatibility of diclofop and BAS 9052 with bentazon. *Weed Sci.* **1982**, *30*, 458–462. Available online: www.jstor.org/stable/4043741 (accessed on 18 September 2020).
