

Ammonium sulfate improves the efficacy of glyphosate on South African lovegrass (*Eragrostis plana*) under water stress

Research Article

Cite this article: Bastiani MO, Roma-Burgos N, Langaro AC, Salas-Perez RA, Rouse CE, Fipke MV, Lamego FP (2021) Ammonium sulfate improves the efficacy of glyphosate on South African lovegrass (*Eragrostis plana*) under water stress. *Weed Sci.* **69**: 167–176. doi: [10.1017/wsc.2020.97](https://doi.org/10.1017/wsc.2020.97)

Received: 5 August 2020
Revised: 22 October 2020
Accepted: 23 December 2020
First published online: 5 January 2021

Associate Editor:







Vipin Kumar, Kansas State University

Keywords:

Adjuvant; herbicide absorption; invasive species management; rangelands

Author for correspondence:

Fabiane P. Lamego, Embrapa Pecuária Sul, BR 153 Km 632,9, Vila Industrial, Zona Rural, Cx Postal 242, Bagé, RS, 96401-970, Brazil. Email: fabiane.lamego@embrapa.br

Marlon O. Bastiani¹ , Nilda Roma-Burgos² , Ana C. Langaro³ ,
Reiofeli A. Salas-Perez⁴, Christopher E. Rouse⁴ , Marcus V. Fipke¹  and
Fabiane P. Lamego⁵ 

¹PhD Student, Crop Protection Graduate Program (Programa de pós-graduação em Fitossanidade), Universidade Federal de Pelotas, Capão do Leão, RS, Brazil; ²Professor of Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR, USA; ³PhD Student, Department of Crop Protection, Federal University of Viçosa (UFV), Viçosa, MG, Brazil; ⁴PhD Student, Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR, USA and ⁵Research Associate, Embrapa Pecuária Sul, Bagé, RS, Brazil

Abstract

South African lovegrass (*Eragrostis plana* Nees) is the most important weed of native pastures in southern Brazil. Management options are limited under water-stress conditions, and glyphosate has been the main tool for control. This study compared four salts of glyphosate applied at three growth stages and determined the glyphosate tolerance level. In addition, the performance of ammonium sulfate (AMS) under two soil moisture conditions (50% and 100% of water-holding capacity) and the effect of AMS on absorption and translocation of radiolabeled [¹⁴C]glyphosate were evaluated. The potassium salt of glyphosate had the fastest activity across growth stages of *E. plana*, which is more vulnerable to glyphosate at the panicle initiation stage. Isopropylamine salt was the slowest-acting glyphosate formulation. Younger plants were typically more easily controlled than older plants at the full tillering stage. The addition of AMS increased the level of control of drought-stressed *E. plana* compared with glyphosate alone by increasing translocation out of the treated leaf and consequently increasing the concentration of glyphosate in the primary culm. These data can be used to plan an effective management program for *E. plana* that takes into account the developmental stage of desired pasture grass species.

Introduction

South African lovegrass (*Eragrostis plana* Nees), known as “tough lovegrass” (*capim-annoni*) in Brazil, is a C₄ perennial grass native to South Africa that has become increasingly problematic in southern Brazilian grasslands (Barbosa 2016). The management of this species is extremely difficult because of its wide dispersion and invasion of native pasture areas in Brazil. The botanical similarity between *E. plana* and native grass species makes selective control of *E. plana* difficult (Goulart et al. 2012). In recent years, farmers have reported cases of herbicide failures, even in burndown operations with glyphosate, in which plants often regrow after herbicide application. The efficacy of glyphosate on *E. plana* is influenced by growth stage. It is believed that glyphosate does not translocate completely into all tillers, especially the older ones. Instead, minimum amounts are distributed across some tillers, resulting in sublethal doses on a per-tiller basis, allowing several tillers to regrow (Bastiani et al. 2018; Corrêa et al. 2014).

Herbicides can be wiped directly onto the foliage of weeds from the moistened cords of a “wiper applicator.” This technique of wiping on herbicides can eliminate spray drift and allow selective use of nonselective, systemic herbicides (Harrington and Ghanizadeh 2017). A wiper applicator was developed in Brazil exclusively to control *E. plana* in native pasture, where up to 80% weed control can be achieved without causing injury to the native pasture grass, which is shorter than *E. plana* (Perez 2010). Cases of unsatisfactory control of *E. plana* have been reported, whether using the wiper applicator or conventional sprayer. Control failures are due to various factors, including variable plant size, advanced growth stage, herbicide applications under drought periods, and insufficient coverage owing to extremely dense grass canopy (Kim et al. 2011; Ziska 2016). There may be other unknown factors that contribute to the herbicide control failures of *E. plana*. One known factor is the limited translocation of glyphosate to the tillers. Knowledge of the processes endowing tolerance to herbicides is necessary to develop effective and efficient weed management strategies.

In several cases, the differences in the susceptibility of crops or weeds to glyphosate are due to differences in herbicide absorption and/or translocation. This was observed in a perennial ryegrass (*Lolium multiflorum* Lam.) resistant population that had a lower proportion of phloem

vessels than xylem, which reduced the amount of herbicide translocated with photoassimilates across the plant (Galvani et al. 2012). Recently, it was confirmed that impaired absorption and translocation endowed glyphosate resistance in Palmer amaranth (*Amaranthus palmeri* S. Watson), in which a susceptible population had 10% and 20% more herbicide absorbed and translocated, respectively, compared with the resistant population (Palma-Bautista et al. 2019). Shrubby false buttonweed (*Spermacoce verticillata* L.) may also survive glyphosate application due to minimal absorption and translocation (Fadin et al. 2018).

The leaf cuticle and plasma membrane are among the barriers limiting glyphosate entry and mobility in plants (Travlos et al. 2017). In most cases, the various salts of glyphosate cannot cross these barriers efficiently without appropriate adjuvants (Hess and Chester 2000; Satchivi et al. 2000). Sprayable ammonium fertilizer is a popular adjuvant, because it prevents precipitation of glyphosate in the spray tank, decreases surface tension, and increases herbicide spreading and penetration into the leaf (Nalewaja and Matysiak 2000). In particular, the addition of ammonium sulfate (AMS) to the glyphosate spray solution improves glyphosate efficacy (Pline et al. 2000; Salisbury et al. 1991; Soltani et al. 2016). While salts in the water, such as calcium and magnesium, form bonds or complexes with glyphosate, thereby reducing its efficacy (Wills and Mcwhorter 1985), AMS can sequester these salts, allowing optimum glyphosate activity.

The addition of AMS can improve glyphosate activity when weeds are water stressed (Satchivi et al. 2000). Plants growing under drought conditions for a prolonged period develop thicker cuticles or leaf pubescence, which reduces herbicide absorption. Further, reduced photosynthesis and transport of photoassimilates under drought stress contribute to reduced herbicide translocation (De Ruiter and Meinen 1998). Adjuvants like AMS may alleviate this problem in *E. plana* by enhancing absorption and/or translocation of glyphosate under drought stress.

We conducted two main experiments to: (1) determine whether AMS increases glyphosate efficacy on *E. plana* under water stress and (2) evaluate the effect of AMS on the absorption and translocation of glyphosate in *E. plana* compared with a highly sensitive plant, junglerice [*Echinochloa colona* (L.) Link]. To support these experiments, we also performed two preliminary studies to compare the efficacy of various glyphosate salts on *E. plana* at different growth stages and determine the optimum dose of glyphosate for *E. plana* control.

Materials and Methods

The Effect of Formulation and Growth Stage on the Activity of Glyphosate on *Eragrostis plana*

Plants were grown in the greenhouse (environmental conditions not controlled) at Federal University of Pelotas, Capão do Leão, RS, Brazil from January to March 2017. Seeds were collected from a native pasture area (no *E. plana* management implemented in this area) of Federal University of Pelotas, Capão do Leão, RS, Brazil (31.806°S, 52.489°W) in March 2016. In January 2017, seeds were sown in pots (23-cm height; 26-cm diameter) containing 8 kg of sandy loam soil (pH 5.3, 15% clay, and 1.24% organic matter) at various times to attain the following growth stages simultaneously: 5 to 6 leaves, full tillering (20 to 30 tillers per plant), and panicle initiation. Seedlings were thinned to four plants per pot and were kept at field capacity and fertilized as needed. Plants in the

Table 1. Glyphosate formulations tested for *Eragrostis plana* control at the Federal University of Pelotas, Capão do Leão, RS, Brazil, 2017.^a

| Trade name | Glyphosate salt | Concentration | Rate |
|----------------------|-----------------|----------------------|-----------------------|
| | | g ae L ⁻¹ | g ae ha ⁻¹ |
| Control | — | — | — |
| Roundup® Original | Isopropylamine | 360 | 540 |
| Roundup® Original DI | Diammonium | 370 | 540 |
| Roundup® Transorb R | Potassium | 480 | 540 |
| Roundup® WG® | Ammonium | 720 | 540 |

^aAll formulations of glyphosate were from Monsanto, 800 North Lindbergh Boulevard, St Louis, MO 63167.

aforementioned stages were treated with glyphosate (see details in Table 1) at a sublethal dose of 540 g ae ha⁻¹. Four glyphosate formulations were tested (Table 1), along with a nontreated check, with four replications (one replication = four plants per pot). Glyphosate was applied at a constant pressure of 200 kPa using a CO₂-operated backpack sprayer connected to a 2-m spray boom fit with four flat-fan TeeJet® AIXR110015 nozzles (Spraying Systems, North Avenue and Schmale Road, Wheaton, IL 60187) spaced 0.5 m apart and calibrated to deliver a spray volume of 150 L ha⁻¹. We decided to use a sublethal dose of glyphosate to allow better separation of glyphosate salts according to their efficacy. Also, no AMS was added to glyphosate in this experiment.

At 7, 14, 21, 28, and 35 d after application (DAA), the herbicide effect on *E. plana* was evaluated visually on a scale of 0% to 100%, with 0% being the absence of visible symptoms and 100% being plant death. Shoots were collected at 35 DAA and oven-dried at 60 C to determine final shoot dry mass. The data were subjected to ANOVA, and when significant ($P \leq 0.05$), the means were compared using a Tukey's test at 5% probability ($P \leq 0.05$). Data were analyzed separately by growth stage, because the plants did not reach the targeted growth stage at the same time. This experiment was not repeated in time.

Dose Response to Glyphosate

Based on data from the previous experiment, we performed a dose-response assay of glyphosate at full tillering, which was the most tolerant growth stage. The experiment was conducted in the greenhouse (environmental conditions not controlled) at Federal University of Pelotas, Capão do Leão, RS, Brazil from April to June 2017. The climate of the region, according to the climatic classification of Wilhelm Köppen (Köppen and Geiger 1930) is Cfa (C: hot mesothermal climate, with average cold months between 3 and 18 C; f: average monthly rainfall of not less than 60 mm, always humid; and a: temperature of the hottest month above 22 C), with average temperature of 16.7 C during the time when the experiment was conducted.

The seeds used and plant establishment and growth conditions were the same as described in the previous experiment. Glyphosate potassium salt (Roundup® Transorb R, Monsanto, 800 North Lindbergh Boulevard, St Louis, MO 63167) was applied when *E. plana* had 20 to 30 tillers per plant, using a backpack sprayer calibrated as described previously. Glyphosate doses were 0, 22.5, 45, 90, 180, 360, 720, and 1,440 g ae ha⁻¹. No AMS was included in this experiment.

Shoots were collected at 42 DAA and oven-dried at 60 C to determine final shoot dry mass. Data for dry mass were fit with a nonlinear log-logistic regression model, according to Equation 1:

$$y = c + \left\{ (d - c) / \left[1 + \left(\frac{x}{e} \right)^b \right] \right\} \quad [1]$$

where c is the lower limit of y , d the upper limit, e is the x value that reduces the value of y 50% between d and c , and b is the relative slope. The data were analyzed using the DRC package in R Core Team software (Knezevic et al. 2007). This experiment was not repeated in time.

Effect of AMS and Water Stress on Glyphosate Efficacy

This study was conducted in a greenhouse at the Altheimer Laboratory Complex of the Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR, USA, from July to December 2017. Seeds of *E. plana* from the same location as described before (efficacy of glyphosate formulations experiment) were sown in pots containing 8 kg (23-cm height; 26-cm diameter) of field soil (Captina silt loam [fine-silty, siliceous, active, mesic Typic Fragiudults]).

The greenhouse was maintained at $32/25 \pm 3$ C day/night temperature with supplemental light for 14 h. Plants were kept at field capacity until the beginning of the water-deficit treatments at the full tillering stage (20 to 30 tillers per plant). The water-deficit treatment was initiated 4 d before herbicide application. Water-stressed plants were kept at 50% water-holding capacity and compared with plants grown at 100% of the water-holding capacity of the pot (C_w). The C_w was determined using the weight of the soil after water saturation (C_{fm}) and the weight of the soil after drying for 24 h at 105 C (C_{dm}), using the equation $C_w = (C_{fm} - C_{dm}) / C_{fm} \times 100$ (Santos et al. 2015). At the beginning of the water-stress treatment, soil in all pots was saturated, drained, and weighed. The pots were then weighed every day, and the water lost by evapotranspiration was replenished (considering 1 g = 1 ml) to maintain C_w at 50% and 100%. Plants were kept under these conditions for 4 d, after which the herbicide treatments were applied. Drought stress was terminated at 4 d after herbicide application (Figure 1).

The experiment was arranged in a completely randomized design, using a factorial arrangement of treatments with four replications (one replication = four plants per pot) and conducted twice. This experiment consisted of two factors: Factor A was the soil water condition (50% and 100% of water-holding capacity) and Factor B was herbicide by adjuvant treatment [no herbicide; glyphosate alone [312 g ha⁻¹]; and glyphosate [312 g ha⁻¹] + AMS [2,805 g ha⁻¹]]. We used a glyphosate dose of 312 g ha⁻¹ (LD₅₀ determined in the dose-response experiment) to observe the benefit of adding an adjuvant, if any. Glyphosate treatments using potassium salt (Roundup® Power Max II, Monsanto) were applied in the spray chamber at a constant pressure of 276 kPa. The spray boom was fit with two flat-fan TeeJet® 80067 nozzles (Spraying Systems), spaced 0.5 m apart and delivering 187 L ha⁻¹.

Glyphosate efficacy (weed control) was evaluated at 7, 14, 21, 28, and 60 DAA, and shoot dry mass was determined at 60 DAA, as described previously. Weed control data were best described with the nonlinear, sigmoidal, three-parameter Gompertz regression defined by Equation 2:

$$y = a * \exp\{-\exp[-b * (x - c)]\} \quad [2]$$

where y is the weed control (%), a is the asymptote, b is the growth rate, c is the inflection point, and x is the glyphosate dose. The number of days to reach 50% of weed control (IC₅₀), was calculated from the above equation. The a parameter estimates the maximum weed control (M_{WC}).

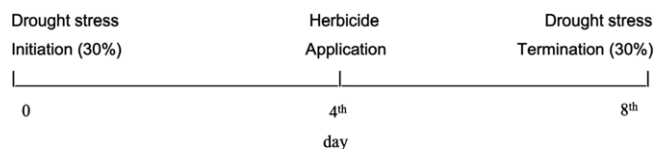


Figure 1. Schematic diagram of the timeline of water-stress duration and herbicide application on *Eragrostis plana* at full tillering (20–30 tillers per plant).

For shoot dry mass, we considered the factors to be runs (Factor A), water-stress treatments (Factor B), and herbicide treatments (Factor C), and then data were subjected to ANOVA. There was no interaction between treatment factors and runs, so the runs were combined. These data were initially tested for normality and homogeneity of variance (there was no need for data to be transformed) and submitted to ANOVA. When significant differences were observed, the water-stress treatments were compared using Student's t -test ($P \leq 0.05$), and herbicide treatment means were compared using Tukey's test ($P \leq 0.05$), with SAS System software v. 9.0 (SAS Institute, 100 SAS Campus Dr, Cary, NC 27513).

AMS Effect on Absorption and Translocation of Glyphosate in *Eragrostis plana*

Eragrostis plana plants were grown in the greenhouse at the Altheimer Laboratory Complex of the Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR, USA, from September 2017 to January 2018. As the pattern of glyphosate absorption and translocation has not been reported for *E. plana*, we used *E. colona* as a highly glyphosate-sensitive reference plant. Seeds of *E. plana* were collected from a native pasture area of the Federal University of Pelotas, Capão do Leão, RS, Brazil, as described earlier. *Echinochloa colona* seeds were collected from a rice field in east Arkansas, USA.

Experimental units were pots with 8 kg of field soil from the Arkansas Main Research Station at Fayetteville, as described previously. Plant material and growth conditions were the same as described in the efficacy experiment, except that only one plant was grown per pot. Experimental units were arranged in a completely randomized design, using a factorial arrangement of treatments with four replications. The experiment was conducted twice. The absorption experiment consisted of two factors: Factor A was the addition of AMS (glyphosate alone and glyphosate + AMS) and Factor B was sampling time [24, 72, and 144 h after herbicide treatment [HAT]]. Four replications of nontreated plants were used to account for background radioactivity. We used 720 g ae ha⁻¹ glyphosate potassium salt (Roundup® PowerMax II, Monsanto) with 2,805 g ae ha⁻¹ AMS as recommended, which controlled *E. plana* 100% (Figure 2). Use of the full dose of glyphosate (720 g ae ha⁻¹) was based on the methodology described by Nandula and Vencill (2015). Sublethal rates may be applicable in certain situations but do not provide the same physiological response as the full rate. *Eragrostis plana* survives a full dose of glyphosate in the field in many cases; hence, a full dose is needed to study glyphosate absorption and translocation in this species. Each treatment was spiked with radioactive [¹⁴C]glyphosate as a tracer. The radiolabeled herbicide was applied to the second and third youngest, fully expanded leaves (Figure 3) using a 50- μ l microsyringe in six 2- μ l droplets containing 12,500 dpm μ l⁻¹. Thus, each plant received a total of 300,000 dpm [¹⁴C]glyphosate. Plants were harvested at 24, 72, and 144 HAT. At each harvest time, plants were removed from the pots, the roots were rinsed,

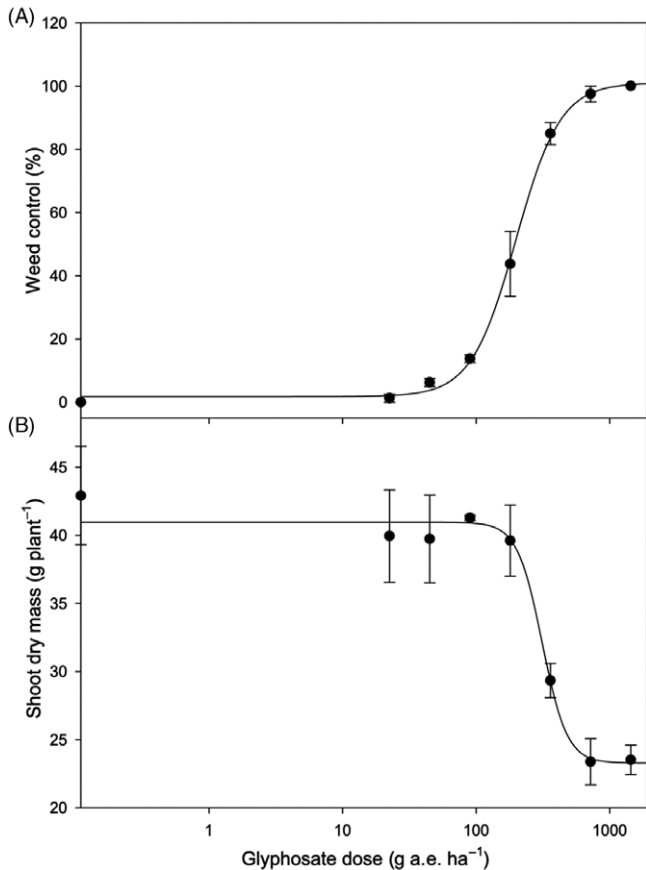


Figure 2. Weed control (A) and shoot dry mass (B) of *Eragrostis plana* at 42 d after glyphosate treatment, Federal University of Pelotas, Capão do Leão, RS, Brazil, 2017. Treatment means ($n = 4$) and standard errors (error bars) are plotted with the regression curve. Data were best described with a nonlinear log-logistic, four-parameter regression: $y = c + \{ (d - c) / [1 + (\frac{x}{e})^b] \}$. Glyphosate as a potassium salt formulation was applied at full tillering growth stage (20–30 tillers per plant) at 150 L ha⁻¹ spray volume.

and the plant was fractionated into six parts (above the treated leaf [ATL], treated leaf [TL], culm of main tiller [CMT], roots of main tiller [RMT], other tillers [OTL], and roots of other tillers [ROT]), as illustrated in Figure 3.

The treated leaves were rinsed with 10 ml of deionized water to remove the unabsorbed glyphosate. The rinsate from the treated leaf of each sample was mixed with 15 ml of scintillation cocktail (Ultima Gold™, PerkinElmer, Waltham, MA, USA) and quantified using a liquid scintillation spectrometer (LSS; Packard Tri-Carb 2100TR liquid scintillation spectrometer, Packard Instrument, Downers Grove, IL, USA). Absorption of glyphosate was calculated as the percentage of total radioactivity recovered (rinsate + radioactivity inside the plant) as described by Equation 3:

$$\text{Absorption (\%)} = \frac{\text{Total of radioactivity in tissues}}{\text{Total of radioactivity in tissues} + \text{radioactivity from leaf rinsate}} \times 100 \quad [3]$$

To evaluate glyphosate translocation, a factorial (two by three by six) arrangement of treatments was used. Factor A was the addition or absence of AMS; Factor B was harvest time (24, 72, and 144 HAT); and Factor C was plant section (Figure 3). Plant sections

were dried at 60 C for 48 h and then oxidized in a biological oxidizer (OX700™, R. J. Harvey Instrument, Tappan, NY, USA). The ¹⁴CO₂ evolved during sample combustion was trapped in a scintillation vial containing 15 ml of scintillation cocktail (Carbon-14 Cocktail, R. J. Harvey Instrument) and quantified using an LSS, as previously described. For the roots of the main tiller (RMT), other tillers (OTL), and roots of tillers (ROT), the tissues were ground in a coffee grinder (BCG2110B, KitchenAid, Benton Harbor, MI, USA). Three subsamples (1 g) were oxidized as described earlier. Considering an efficiency of 85.2% for the biological oxidizer and 95.6% for the LSS counter, the total amount recovered in the translocation experiment was the same in both species at 83.67% for *E. plana* and 84.0% for *E. colona*.

Eragrostis plana and *E. colona* were considered separate experiments. The interaction between treatment factors and runs was not significant for each species, so the runs were combined. The interaction among treatment factors of each experiment was analyzed, and when it was significant, Student's *t*-test ($P \leq 0.05$) was used to separate means using the R Core Team software (R Foundation for Statistical Computing, Vienna, Austria, <http://R-project.org>).

Results and Discussion

The Effect of Formulation and Growth Stage on the Activity of Glyphosate on *Eragrostis plana*

The potassium salt of glyphosate had the fastest activity when applied to plants with 5 to 6 tillers, showing the highest control (55%) at 7 DAA (Table 2), while ammonium and isopropylamine salt were the slowest-acting formulations at this growth stage. Potassium salt remained the best treatment (99% control) at 21 DAA, comparable only to diammonium salt of glyphosate (88% control). Isopropylamine salt was the slowest-acting formulation, with less than 50% activity at 28 DAA. Isopropylamine salt was also the least effective among the glyphosate formulations, as it attained only 72% control at 35 DAA when the experiment was terminated. Diammonium and potassium salt formulations controlled *E. plana* 100% up to 35 DAA in plants with 5 to 6 tillers. At full tillering, the glyphosate formulations did not differ in activity up to 21 DAA. At 3 wk after application, *E. plana* control ranged from 30% to 47% across formulations. None of the formulations killed *E. plana* 100% at 35 DAA (salts ranging from 33% to 57% of control), showing a large reduction in efficacy relative to efficacy on younger plants. Regardless of formulation, glyphosate was more effective at panicle initiation than at full tillering, although the ratings cannot be compared statistically across growth stages (because each growth stage was considered to be a separate experiment). At panicle initiation, potassium and isopropylamine salt formulations had the highest activity up to 28 DAA, when these treatments reached maximum activity at 98% to 99% control. All other formulations reached peak activity at 35 DAA, resulting in >90% control across formulations, except for diammonium salt, which showed numerically lower activity (85%).

All formulations of glyphosate reduced the dry shoot biomass equally compared with the nontreated check at all application timings (Table 3). The degree of biomass reduction was greater when smaller plants were treated, with 79% to 88% reduction at 5 to 6 tillers and 43% to 57% reduction at full tillering. At these stages, the plants were actively growing; thus, the nontreated plants accumulated significantly more biomass from the time of herbicide application, while treated plants ceased growing. At panicle initiation, biomass reduction was generally significant across

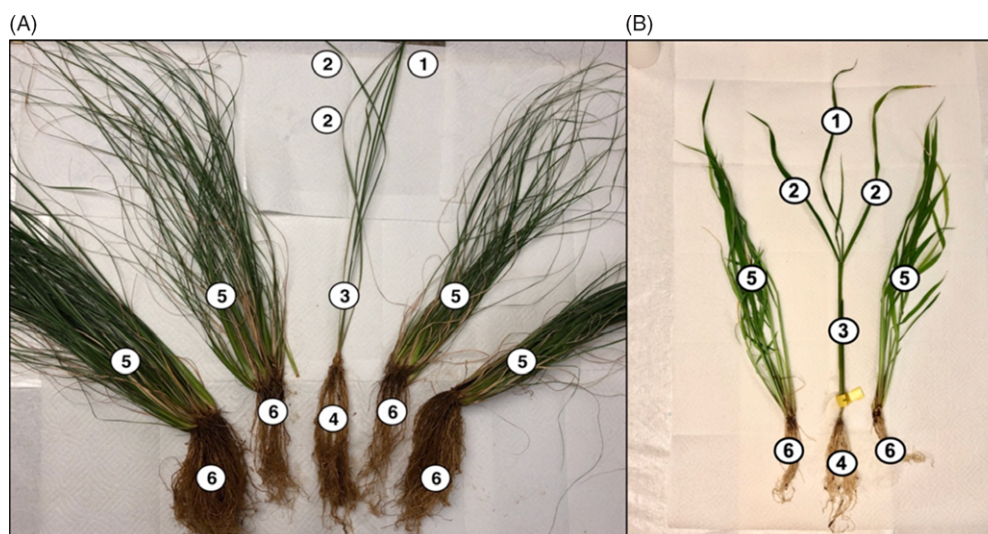


Figure 3. Plant parts of *Eragrostis plana* (A) and *Echinochloa colona* (B) used to evaluate glyphosate translocation. Legend: (1) above treated leaf, (2) treated leaf, (3) culm of main tiller, (4) roots of main tiller, (5) other tillers, (6) roots of other tillers.

formulations, but the magnitude of biomass reduction was lower compared with that of younger plants. This is understandable, as desiccating a fully grown, large bunchgrass like *E. plana* does not eliminate the high amount of biomass already accumulated at the time of herbicide application. The plant at this stage has reached peak growth; therefore, the nontreated check plants would have minimal additional biomass accumulation in the monthlong period after treatment. Overall, glyphosate was least effective on actively tillering *E. plana*.

Dose Response to Glyphosate

The goal of this experiment was to estimate how much glyphosate is needed to achieve a targeted level of control. Because the efficacy of glyphosate varies by growth stage, the amount of glyphosate needed to attain approximately 90% control would differ across growth stages. The log-logistic model with four parameters best fit the dry shoot biomass data. The log-logistic model has been extensively used to describe herbicide effects, mainly to determine the amount of herbicide that would cause 50% growth reduction or 50% control (GR_{50}) in weeds. Complete weed control was attained with 720 g ae ha^{-1} (Figure 2), for which the LD_{50} and LD_{90} were reached at doses of 199 and 461 g ha^{-1} , respectively (Figure 2; Table 4). The GR_{50} for *E. plana* at full tillering was 312 g ha^{-1} of glyphosate (Figure 2; Table 4).

Effect of AMS and Water Stress on Glyphosate Efficacy

The interaction effect between water-stress and herbicide treatments on weed control and shoot dry mass was significant ($P \leq 0.05$). Weed control data across time was fit with regression equations to show the differential progression on *E. plana* control (Figure 4). The regression parameters also provided estimates of the maximum weed control (M_{WC}) and number of days to reach 50% of control (IC_{50}). Glyphosate was less effective on drought-stressed plants than on well-watered ones, regardless of adjuvant treatment, except with glyphosate + AMS, which controlled *E. plana* equally regardless of drought stress at 21 DAA (Table 5). Treatments with glyphosate alone or glyphosate + AMS had equivalent levels of *E. plana* control across the two

water-stress conditions at 28 DAA (Figure 4), which resulted in the same shoot dry mass at 60 DAA (Figure 5).

The addition of AMS clearly enhanced glyphosate efficacy (Figure 4), reaching IC_{50} in 13.5 d compared with 33.5 d for glyphosate alone (Table 5). Visually, the addition of AMS to glyphosate increased efficacy compared with glyphosate alone, except at 14 DAA under drought stress.

Although no differences in shoot dry mass were observed between glyphosate alone and glyphosate + AMS at 60 DAA (Figure 5), there was a significant difference in control between glyphosate + AMS (98% to 99%, respectively, when drought stressed or well watered) and glyphosate alone (63% and 76%, respectively, when drought stressed or well watered) (Figure 4).

AMS Effect on Absorption and Translocation of Glyphosate in *Eragrostis plana*

The previous experiment showed a clear advantage of mixing AMS with glyphosate to control *E. plana*. We hypothesized that AMS increased the absorption and translocation of glyphosate in *E. plana*. The beneficial effect of AMS was apparent whether the plants were drought stressed or well watered; thus, we used only well-watered plants to study the effect of AMS on glyphosate absorption and translocation.

AMS did not increase the absorption of [^{14}C]glyphosate in *E. plana* (Table 6). The [^{14}C]glyphosate absorption leveled off at 72 HAT, with the highest absorption attained at 33% at 144 HAT. The main effects of AMS addition and time of harvest on [^{14}C]glyphosate translocation were significant in *E. plana* (Table 7). The interaction effect of these factors was also significant. Without AMS, [^{14}C]glyphosate translocation was higher (19%) early on (24 HAT) than with AMS (7%). With AMS, glyphosate translocation increased significantly with time, resulting in a higher proportion of absorbed glyphosate translocated at the end of the experiment (63%) compared with the glyphosate-alone treatment (53%).

AMS increased glyphosate absorption in *E. colona* by 6% (Table 6) and continued to rise significantly, reaching 44% at the end of the experiment (144 HAT). In *E. colona*, the interaction between AMS treatment and harvest time on the translocation of [^{14}C]glyphosate out of the treated leaf was not significant (Table 8).

Table 2. Comparative efficacy of glyphosate formulations on *Eragrostis plana* control at three growth stages in the greenhouse at the Federal University of Pelotas, Capão do Leão, RS, Brazil, 2017.

| Glyphosate salt ^a | <i>Eragrostis plana</i> control (%) ^b | | | | |
|------------------------------|--|--------|--------|--------|--------|
| | 7 DAA | 14 DAA | 21 DAA | 28 DAA | 35 DAA |
| | 5–6 tillers | | | | |
| Isopropylamine | 15 c | 33 b | 40 d | 45 c | 72 b |
| Diammonium | 37 b | 72 a | 88 b | 99 a | 100 a |
| Potassium | 55 a | 83 a | 99 a | 100 a | 100 a |
| Ammonium | 15 c | 32 b | 62 c | 77 b | 86 ab |
| CV (%) | 10.6 | 14.5 | 5.4 | 7.8 | 11.1 |
| | Full tillering ^c | | | | |
| Isopropylamine | 38 a | 45 a | 30 a | 32 b | 33 b |
| Diammonium | 27 a | 33 a | 33 a | 30 b | 35 b |
| Potassium | 40 a | 48 a | 47 a | 40 ab | 55 a |
| Ammonium | 33 a | 42 a | 42 a | 50 a | 57 a |
| CV (%) | 21.3 | 23.8 | 25.8 | 17.3 | 16.8 |
| | Panicle initiation | | | | |
| Isopropylamine | 77 a | 93 a | 97 a | 98 a | 98 a |
| Diammonium | 43 c | 73 b | 80 b | 83 b | 85 b |
| Potassium | 65 b | 93 a | 97 a | 99 a | 99 a |
| Ammonium | 38 c | 53 c | 75 b | 85 b | 93 ab |
| CV (%) | 9.0 | 5.1 | 4.8 | 3.5 | 7.2 |

^aGlyphosate was applied at rate of 540 g ae ha⁻¹ and 150 L ha⁻¹ of spray volume.

^bDAA, days after glyphosate treatment. Different lowercase letters indicate statistical differences among glyphosate salts by Tukey's test ($P \leq 0.05$), $n = 4$ replicates.

^cGrowth stage where plants had 20–30 tillers per plant.

Table 3. Comparative efficacy of glyphosate formulations on shoot dry mass accumulation in *Eragrostis plana* at three growth stages in the greenhouse, Federal University of Pelotas, Capão do Leão, RS, Brazil, 2017.

| Glyphosate salt ^a | 5–6 tillers | Full tillering ^b | Panicle initiation |
|------------------------------|--|-----------------------------|--------------------|
| | Shoot dry mass ^c g plant ⁻¹ | | |
| Check | 10.5 a | 17.2 a | 36.6 a |
| Isopropylamine | 2.2 b | 7.4 b | 21.9 b |
| Diammonium | 1.5 b | 9.0 b | 26.9 ab |
| Potassium | 1.7 b | 7.4 b | 20.0 b |
| Ammonium | 1.3 b | 9.8 b | 19.9 b |
| CV (%) | 13.4 | 20.00 | 20.6 |

^aAll glyphosate salts were applied at rate of 540 g ae ha⁻¹ and 150 L ha⁻¹ of spray volume.

^bGrowth stage where plants had 20–30 tillers per plant.

^cDifferent lowercase letters indicate statistical differences among glyphosate salts by Tukey's test ($P \leq 0.05$), $n = 4$ replicates.

Averaged across AMS treatments, the highest percentage of [¹⁴C] glyphosate translocation occurred at 72 HAT (44%). Averaged over harvest times, the addition of AMS increased the translocation of glyphosate in *E. colona*. Thus, AMS improved the translocation of glyphosate in both highly tolerant (*E. plana*) and highly sensitive (*E. colona*) species, contributing to increased glyphosate activity.

The distribution of [¹⁴C]glyphosate in *E. plana* tissues at 24 and 72 HAT was as follows: treated leaf (TL) > culm of main tiller (CMT) > roots of main tiller (RMT) ≥ other tillers (OTL) ≥ roots of other tillers (ROT) ≥ above treated leaf (ATL) regardless of AMS treatment (Table 9). At the end of the study (144 HAT), the amount of [¹⁴C]glyphosate remaining in the TL was equivalent to that in the CMT. However, more [¹⁴C]glyphosate was translocated to the CMT when glyphosate was applied with AMS than when applied alone. The addition of AMS increased the quantity of glyphosate that translocated out of the TL, this being concentrated in the CMT (Table 9). At 144 HAT (the end of the study), the [¹⁴C]glyphosate translocated to the CMT was 9% higher when

glyphosate was mixed with AMS than when applied alone. The quantity of glyphosate translocated to the roots was the same across harvest times. No [¹⁴C]glyphosate was detected in the other tillers regardless of the AMS treatment. Likewise, zero to minimal (1% to 2%) amounts of glyphosate were detected in the ROT with or without AMS.

As was seen with *E. plana*, the concentration of [¹⁴C]glyphosate in *E. colona* (Table 10) was highest in the treated leaf (TL), followed by culm of main tiller (CMT) > roots of main tiller (RMT) ≥ other tillers (OTL) ≥ roots of other tillers (ROT) ≥ above treated leaf (ATL) regardless of AMS treatment. At 24 and 72 HAT, when glyphosate was sprayed alone, the [¹⁴C]glyphosate remaining in the TL was higher than when glyphosate was applied with AMS. The addition of AMS favored a higher translocation of [¹⁴C]glyphosate out of the treated leaf, mainly accumulating in the CMT at 24 and 72 HAT. The addition of AMS did not affect the distribution of [¹⁴C]glyphosate at 144 HAT. As observed with *E. plana*, AMS did not change the quantity of glyphosate translocated to the roots of *E. colona* at any time. Traces of [¹⁴C]glyphosate ranging from 1% to 2% were quantified in the tillers and roots of tillers, regardless of the addition of AMS.

Practical Implications

This research demonstrated that for a difficult-to-control species like *E. plana*, glyphosate formulation and growth stage matters. In this case, potassium salt is best, and the greatest advantage with potassium salt occurs when glyphosate is applied during the early tillering stage. Like all other plant species, the most vulnerable stage is at the onset of the reproductive stage; in grasses that would be at panicle initiation. The majority of related studies had shown no differences between glyphosate formulations. This is expected when the target species are more susceptible to glyphosate than *E. plana*. For example, glyphosate diammonium or glyphosate-isopropylamine salts applied POST at three rates did not differ in control of common ragweed (*Ambrosia artemisiifolia* L.), ivyleaf morningglory (*Ipomoea hederacea* Jacq.), large crabgrass [*Digitaria sanguinalis* (L.) Scop.], or pitted morningglory (*Ipomoea lacunosa* L.) (Richardson et al. 2003). In addition, the absorption and translocation of [¹⁴C]glyphosate formulated as isopropylamine or trimethylsulfonium salts were similar in velvetleaf (*Abutilon theophrasti* Medik.) and giant foxtail (*Setaria faberi* Herrm.). In this case, the absence of differences between these formulations may be because both formulations, upon ionization, yield the same active acid glyphosate. Also, the efficacy of isopropylamine, diammonium, and potassium salts of glyphosate does not differ on yellow nutsedge (*Cyperus esculentus* L.), *I. lacunosa*, broadleaf signalgrass [*Brachiaria platyphylla* (Munro ex C. Wright) Nash], and *A. palmeri*, even when some salts affected the pattern of absorption and translocation (Mueller et al. 2006). Differences in absorption and translocation could be attributed to differences in leaf anatomy (Oliveira et al. 2015) but do not always equate to differences in efficacy of control. The potassium and ammonium salt formulations resulted in higher leaf wetting when applied to hairy beggarticks (*Bidens pilosa* L.), while the isopropylamine salt formulation resulted in lower wettability of southern sandbur (*Cenchrus echinatus* L.) leaves (Oliveira et al. 2015). In waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer], the initial absorption of glyphosate was higher with the isopropylamine salt formulation compared with the diammonium salt formulation at 2 HAT (Li et al. 2005). However, the initial observable differences in absorption and translocation did not result in differentiation of

Table 4. Log-logistic parameters from the dose–response regression fit to the weed control and shoot dry mass data of *Eragrostis plana*.^a

| Variables | Slope | Lower limit | Upper limit | GR ₅₀ or LD ₅₀ | LD ₉₀ |
|----------------|------------|-------------|-------------|--------------------------------------|------------------|
| | <i>b</i> | <i>c</i> | <i>d</i> | g ha ⁻¹ | |
| Weed control | -2.6 ± 0.4 | 1.8 ± 2.4 | 101.0 ± 3.3 | 198.8 ± 12.6 | 461.4 ± 71.4 |
| Shoot dry mass | 4.6 ± 3.1 | 23.3 ± 1.8 | 41.0 ± 1.8 | 312.3 ± 47.5 | — |

^aAbbreviations: GR₅₀, glyphosate dose that caused 50% of shoot dry mass reduction; LD₅₀, glyphosate dose that caused 50% of weed control; LD₉₀, glyphosate dose that caused 90% of weed control. ±, standard error. n = 4 replicates.

Table 5. Maximum weed control (M_{WC}) and number of days to reach 50% weed control (IC₅₀) of *Eragrostis plana*, estimated from the regression equations fit to the dose–response data on weed control from 7 to 60 d after treatment, University of Arkansas, Fayetteville, AR, USA, 2018.^a

| Herbicide treatment | Well watered | | Drought ^b | |
|--|--------------------------|------------------|----------------------|------------------|
| | M _{WC} | IC ₅₀ | M _{WC} | IC ₅₀ |
| | —%— | —d— | —%— | —d— |
| Glyphosate alone | 95.5 ± 12.4 ^b | 32.49 ± 9.7 | 75.8 ± 14.9 | 23.4 ± 6.7 |
| Glyphosate + AMS (2,805 g ha ⁻¹) | 98.0 ± 5.6 | 13.5 ± 2.1 | 98.0 ± 4.5 | 18.8 ± 2.1 |

^aM_{WC} and IC₅₀ were best described with the nonlinear, sigmoidal, three-parameter Gompertz regression function: $y = a \cdot \exp\{-\exp[-b \cdot (x - c)]\}$, n = 8 replicates. ±, standard error.

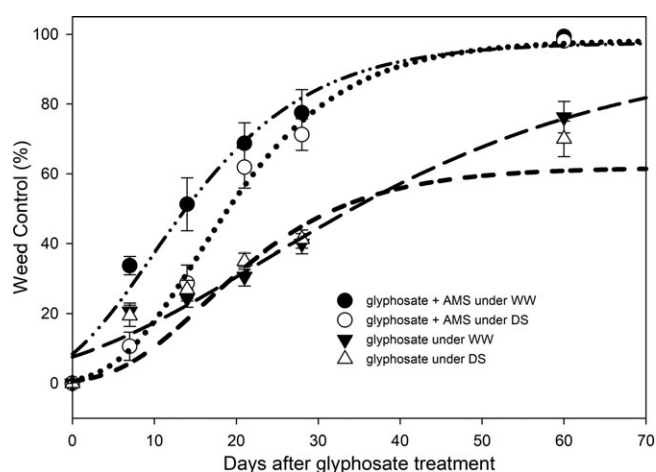


Figure 4. Weed control of *Eragrostis plana* with time after glyphosate treatment under well-watered (WW) and drought-stressed (DS) conditions, University of Arkansas, Fayetteville, AR, USA, 2018. AMS, ammonium sulfate. Treatment means (n = 8) and standard errors (error bars) are plotted with the regression curve. Data were best described with the nonlinear, sigmoidal, three-parameter Gompertz regression function: $y = a \cdot \exp\{-\exp[-b \cdot (x - c)]\}$. Glyphosate was applied at full tillering growth stage (20–30 tillers per plant) at a 150 L ha⁻¹ spray volume.

efficacy between formulations at 72 HAT. Thus, the interaction among glyphosate formulations and leaf surfaces can help predict the efficacy of formulations (Travlos et al. 2017).

Anecdotal evidence suggests that plants are most sensitive to herbicides when young (i.e., seedlings), and herbicide efficacy declines with plant age. Controlling weeds when small also brings an economic advantage, because it is a stage when weeds have minimal or no effect on crop yield. It is also common knowledge that plants are most sensitive to stress (i.e., drought, heat, herbicides) at the onset of the reproductive stage. Thus, herbicide application in the crop season is mostly terminated before the reproductive phase. For a difficult grass weed in a pasture ecosystem (such as *E. plana*), it appeared that the best timing for glyphosate application is during the onset of reproduction. At the reproductive stage,

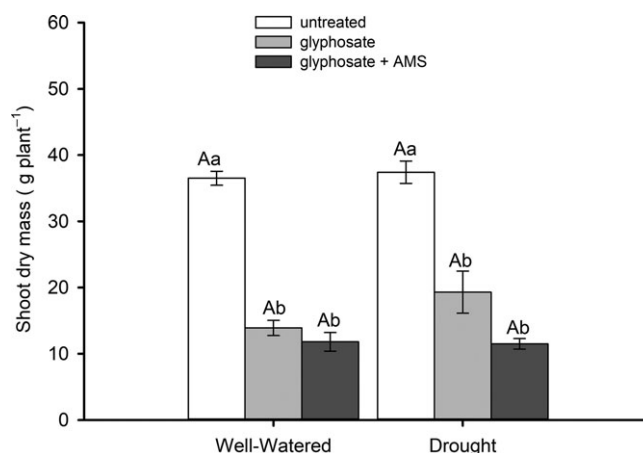


Figure 5. Shoot dry mass of *Eragrostis plana* at 60 d after glyphosate treatment, University of Arkansas, Fayetteville, AR, USA, 2018. Treatment means (n = 8) with standard errors (error bars) are shown. Glyphosate was applied to the plants at full tillering growth stage (20–30 tillers per plant) using a 150 L ha⁻¹ spray volume. Different lower-case letters indicate statistical differences among herbicides treatments by Tukey's test (P ≤ 0.05), and uppercase letters indicate differences between water conditions by Student's *t*-test (P ≤ 0.05). AMS, ammonium sulfate.

plants tend to translocate sugar to roots and reproductive organs, thus also transporting most of the glyphosate molecules. If more glyphosate is translocated basipetally, then more will reach the meristematic tissues and the tillers, resulting in better control. Our previous study showed that *E. plana* sprayed at panicle initiation translocated more [¹⁴C]glyphosate out of the treated leaf (mainly to the main culm) compared with the tillering stage (Bastiani et al. 2018). This timing is suitable for pastures if there is height differentiation between the pasture grass and the weed that allows application by a “wipe-on” technique when the weeds are taller. This timing can also be used when one is doing a complete rejuvenation of pasture grass after *E. plana* infestation is reduced to a minimum or completely eradicated.

The current study informs us that one should start chemical management of *E. plana* at the early tillering stage (or maybe

Table 6. The main effects of ammonium sulfate (AMS) and time of harvest on the total absorption of [¹⁴C]glyphosate in *Eragrostis plana* and *Echinochloa colona*, University of Arkansas, Fayetteville, AR, USA, 2018.

| Factors | Factor level | <i>E. plana</i> | <i>E. colona</i> |
|-----------------------|---------------------------------|------------------|------------------|
| Hours after treatment | 24 | 20 b | 19 c |
| | 72 | 28 a | 27 b |
| | 144 | 33 a | 44 a |
| AMS addition | None | 28 ^{ns} | 27* |
| | AMS (2,805 g ha ⁻¹) | 26 | 33 |
| CV (%) | | 24 | 27 |

^a720 g ae ha⁻¹ of potassium salt of glyphosate (cold herbicide) was applied at full tillering growth stage (20–30 tillers per plant in *E. plana* and 8–12 tillers per plant in *E. colona*) at 150 L ha⁻¹ spray volume. Each plant received a total of 300,000 dpm of [¹⁴C]glyphosate. Different lowercase letters or asterisks (*) indicate statistical differences among harvest times or addition of AMS by Student's *t*-test ($P \leq 0.05$) for each species, $n = 8$ replicates.

Table 7. The interaction effect of ammonium sulfate (AMS) addition and harvest time on total translocation of [¹⁴C]glyphosate out of the treated leaf of *Eragrostis plana*, University of Arkansas, Fayetteville, AR, USA, 2018.

| Harvest time | Glyphosate + AMS (2,805 g ha ⁻¹) | |
|---------------------|--|-------------------------------------|
| | Glyphosate | —% of total absorbed ^a — |
| —h after treatment— | | |
| 24 | 19 Ab | 7 Bc |
| 72 | 49 Aa | 45 Ab |
| 144 | 53 Ba | 63 Aa |
| CV (%) | | 11.96 |

^a720 g ae ha⁻¹ potassium salt of glyphosate (cold herbicide) was applied at full tillering growth stage (20–30 tillers per plant) at 150 L ha⁻¹ spray volume. Each plant received a total of 300,000 dpm of [¹⁴C]glyphosate. Different lowercase letters indicate statistical differences among harvest times; uppercase letters indicate differences between AMS treatments by Student's *t*-test ($P \leq 0.05$), $n = 8$ replicates.

Table 8. Effect of ammonium sulfate (AMS) addition on total translocation out of treated leaf of [¹⁴C]glyphosate in *Echinochloa colona*, University of Arkansas, Fayetteville, AR, USA, 2018.

| Factor | Factor level | Translocation out of treated leaf ^a |
|----------------------------------|---------------------------------|--|
| | | —% of total absorbed— |
| Harvest time (h after treatment) | 24 | 22 c |
| | 72 | 44 a |
| | 144 | 30 b |
| AMS | None | 29 b |
| | AMS (2,805 g ha ⁻¹) | 34 a |
| CV (%) | | 27 |

^a720 g ae ha⁻¹ of potassium salt of glyphosate (cold herbicide) was applied at full tillering growth stage (8–12 tillers per plant) at 150 L ha⁻¹ spray volume. Each plant received a total of 300,000 dpm of [¹⁴C]glyphosate. Different lowercase letters indicate statistical differences among harvest times or between AMS treatments by Student's *t*-test ($P \leq 0.05$), $n = 8$ replicates.

before tillering, but we have not tested this timing). This is the second-best timing compared with panicle initiation. At early tillering stage, the best formulation was potassium salt. Differential performance of glyphosate salts could be explained by the interaction between glyphosate formulations and leaf surfaces, as the leaf surfaces change with time. Young plants are typically more easily controlled compared with older plants, because tissues in the former are less developed, allowing greater absorption of herbicides (Degreeff et al. 2018). When plants are small, the concentration of the herbicide per plant would be higher compared with when

plants are large; hence, foliar herbicides are more effective on seedlings than large plants. Further, the translocation pattern of photosynthates (sugar) changes during the life cycle of the plant; hence, herbicides like glyphosate (which moves in a similar pattern as photosynthates) could be translocated to various tissues, resulting in a dilution of glyphosate reaching the target site and reduced efficacy (Fadin et al. 2018). The early timing may be possible if the desired pasture grass is slightly dormant or at a stage when it can recover from herbicide injury.

At full tillering, when *E. plana* is most difficult to control, the GR₅₀ and LD₅₀ for *E. plana* were 312 and 199 g ae ha⁻¹ of glyphosate, respectively. Based on control failures reported by farmers, we expected a GR₅₀ higher than this, as well as a dose greater than 720 g ae ha⁻¹ to achieve 100% control. *Eragrostis plana* is apparently less tolerant to glyphosate when grown in the greenhouse than when growing in pastures. In our collective experience, greenhouse-grown plants are more sensitive to herbicides than field-grown plants. Some species with innate tolerance to glyphosate such as *Ipomoea grandifolia* (Dammer) O'Donnell and Benghal dayflower (*Commelina benghalensis* L.) have a GR₅₀ around 616 and >1,440 g ha⁻¹, respectively in greenhouse experiments (Lacerda and Victoria Filho 2004), almost 2-fold above the values we obtained in this study. On the other hand, velvetbean [*Mucuna pruriens* (L.) DC], a plant also known to have high tolerance to glyphosate, had a GR₅₀ of 404 g ha⁻¹ in a greenhouse experiment (Rojano-Delgado et al. 2012). Thus, the GR₅₀ values obtained from greenhouse-grown plants are usually less than the observed tolerance level of such species to the same herbicide in the field. For *E. plana*, the capacity to regrow after herbicide application and its high tillering capacity are the main factors linked to failures observed in the field (Supplementary Figure S1). Regrowth was observed even at 5 to 6 tillers in a previous experiment (Supplementary Figure S1).

Under drought conditions, the activity of glyphosate on *E. plana* is greatly reduced; thus, herbicide should not be applied in the dry season. It is common knowledge that drought stress reduces herbicide uptake, translocation, metabolism, and efficacy. Plants grown under drought conditions can develop thicker cuticles or leaf pubescence, which inhibits herbicide absorption; drought reduces photosynthesis and transport of photoassimilates; and transport via the xylem or phloem is reduced, thereby also reducing herbicide translocation (Patterson 1995). The addition of AMS may help in this situation. However, research is lacking on the utility of adjuvants to improve the efficacy of herbicides under drought.

The addition of AMS, an inorganic salt, to the spray solution has been reported for years to improve the efficacy of glyphosate (Pline et al. 2000; Soltani et al. 2016). It has been reported also that certain ions in the water (e.g., Ca²⁺, Mg²⁺) could react with the phosphate group in the glyphosate molecule, consequently inactivating some glyphosate molecules and reducing the effectiveness of the herbicide (Wills and Mcwhorter 1985). The water used in our experiments (Supplementary Table S1) did not contain enough salts to be considered hard water, indicating that the beneficial effect of AMS was unlikely related to inactivation of salts in the water. AMS may enhance glyphosate efficacy through mechanisms other than overcoming antagonistic salts. Glyphosate plus AMS applied using deionized water reduced the fresh weight of quackgrass [*Elymus repens* (L.) Gould] in the greenhouse more than glyphosate sprayed alone (De Ruiter and Meinen 1998). Therefore, the role of AMS in enhancing the efficacy of glyphosate is still not understood. Some researchers reported increased

Table 9. Effect of ammonium sulfate (AMS) addition on the distribution of [¹⁴C]glyphosate in *Eragrostis plana*, University of Arkansas, Fayetteville, AR, USA, 2018.^a

| Plant part | 24 HAT | | 72 HAT | | 144 HAT | |
|------------------------|----------------------------------|------------------|------------|------------------|------------|------------------|
| | Glyphosate | Glyphosate + AMS | Glyphosate | Glyphosate + AMS | Glyphosate | Glyphosate + AMS |
| | % of total absorbed ^b | | | | | |
| Above treated leaf | 0 Ad | 0 Ac | 2 Ad | 1 Ad | 3 Ac | 3 Ad |
| Treated leaf | 81 Aa* | 93 Aa | 52 Ba* | 55 Aa | 47 Ca* | 37 Ab |
| Culm of main tiller | 15 Cb* | 6 Cb | 40 Bb | 39 Bb | 44 Aa* | 53 Aa |
| Roots of main tiller | 4 Ac | 1 Bc | 5 Ac | 7 Ac | 7 Ab | 7 Ac |
| Other tillers | 0 Ad | 0 Ac | 0 Ad | 0 Ad | 0 Ac | 0 Ad |
| Roots of other tillers | 0 Ad | 0 Ac | 1 Ad | 0 Ad | 2 Ac | 0 Ad |

^aAMS rate was 2,805 g ha⁻¹. HAT, h after treatment.

^b720 g ae ha⁻¹ of potassium salt of glyphosate (cold herbicide) was applied to the plants at full tillering growth stage (20–30 tillers per plant) at 150 L ha⁻¹ spray volume. Each plant received a total of 300,000 dpm of [¹⁴C]glyphosate. The different lowercase letters indicate statistical differences among plants parts; uppercase letters indicate differences between harvest times; asterisks (*) indicate statistical differences between AMS addition by Student's *t*-test ($P \leq 0.05$), $n = 8$ replicates.

Table 10. Effect of ammonium sulfate (AMS) addition on the distribution of [¹⁴C]glyphosate in *Echinochloa colona*, University of Arkansas, Fayetteville, AR, USA, 2018.^a

| Plant part | 24 HAT | | 72 HAT | | 144 HAT | |
|------------------------|----------------------------------|------------------|------------|------------------|------------|------------------|
| | Glyphosate | Glyphosate + AMS | Glyphosate | Glyphosate + AMS | Glyphosate | Glyphosate + AMS |
| | % of total absorbed ^b | | | | | |
| Above treated leaf | 0 Ac | 1 Ac | 1 Ad | 2 Ad | 2 Ac | 2 Ac |
| Treated leaf | 80* Aa | 74 Aa | 61* Ca | 52 Ca | 71 Ba | 70 Ba |
| Culm of main tiller | 17* Cb | 21 Bb | 32* Ab | 37 Ab | 22 Bb | 22 Bb |
| Roots of main tiller | 2 Ac | 3 Ac | 5 Ac | 6 Ac | 3 Ac | 4 Ac |
| Other tillers | 0 Ac | 1 Ac | 0 Ad | 2 Ad | 1 Ac | 2 Ac |
| Roots of other tillers | 0 Ac | 0 Ac | 0 Ad | 2 Ad | 1 Ac | 1 Ac |

^aAMS rate was 2,805 g ha⁻¹. HAT, h after treatment.

^b720 g ae ha⁻¹ potassium salt of glyphosate (cold herbicide) was applied at full tillering growth stage (8–12 tillers per plant) at 150 L ha⁻¹ spray volume. Each plant received a total of 300,000 dpm of [¹⁴C]glyphosate. Different lowercase letters indicate statistical differences among plants parts; uppercase letters indicate differences between harvest times; and asterisks (*) indicate statistical differences between AMS treatment means by Student's *t*-test ($P \leq 0.05$), $n = 8$ replicates.

absorption and/or translocation of glyphosate with AMS (Satchivi et al. 2000). Satchivi and colleagues reported increased glyphosate absorption in *A. theophrasti* and *S. faberi* upon the addition of 1% AMS to either isopropylamine or trimethylsulfonium salt formulations. Glyphosate activity under drought stress conditions was also improved by the addition of AMS (Satchivi et al. 2000), which was confirmed by our experiments.

While the addition of AMS did not improve glyphosate uptake in *E. plana*, AMS improved glyphosate uptake in *E. colona* in our study, as it also improved glyphosate uptake in *A. theophrasti* and *S. faberi* (Satchivi et al. 2000). The addition of AMS to both glyphosate formulations increased the quantity of glyphosate translocated out of the treated leaf but did not increase the quantity of glyphosate translocated to the roots. In which case, we would expect increased desiccation but no effect on regrowth capacity of grasses, because the meristems are at the base, close to the roots. Glyphosate translocation varies across species, as our data showed on *E. plana* (58%) and *E. colona* (30%). As *E. plana* is considered glyphosate tolerant, we expected low levels of glyphosate translocation compared with the glyphosate-sensitive *E. colona*. The GR₅₀ for *E. colona* was low (48.45 g ha⁻¹) (Supplementary Table S2), confirming high sensitivity to glyphosate. This could explain the low amount of glyphosate translocation. Extremely low tolerance to glyphosate of *E. colona* means that glyphosate kills the cells and tissues fast (rapid and complete desiccation), preventing continued translocation of glyphosate to other plant organs. It takes more glyphosate to kill a tolerant plant like *E. plana*; therefore, the plant can continue to translocate glyphosate for as long as the cells and tissues are alive.

The addition of AMS to glyphosate increased translocation out of the treated leaf and increased concentration of glyphosate in the

culm of the primary tiller of *E. plana*, which did not occur in *E. colona*. The accumulation of glyphosate in the primary culm of *E. plana* may be the main reason for improved performance of glyphosate with AMS. The regrowth of *E. plana* observed in the field (Supplementary Figure S1) and also in this experiment consists of new tillers growing from the base of the primary tiller. Increased concentration of glyphosate in the primary culm would prevent the development of new ones.

Based on the higher percentages of glyphosate absorption and translocation in *E. plana*, relative to *E. colona* (a highly sensitive species), we can deduce that herbicide failures in the field are unlikely to be due to low absorption. Failure to control *E. plana* in the field can be explained by the absence or lack of [¹⁴C]glyphosate translocation to the secondary and younger tillers. These findings confirm the theory first proposed by Corrêa et al. (2014) and later confirmed by Bastiani et al. (2018), wherein plants of *E. plana* exhibit a mechanism of early termination of dependence among tillers. According to this theory, glyphosate does not translocate up the culm of tillers or a large enough amount does not translocate to result in complete control of the weed. To achieve better control of the whole bunch in the field, each tiller must be exposed to a lethal dose of glyphosate. An additive that enhances translocation of glyphosate from leaves to the base of the tillers is necessary to prevent regrowth. Anything that impedes proper coverage and glyphosate translocation will contribute to control failure.

In summary, the potassium salt of glyphosate had the fastest activity across growth stages of *E. plana*, while isopropylamine salt was the slowest-acting formulation, which may be explained by the higher leaf wettability attained with potassium salt compared with isopropylamine salt. Younger plants were typically more easily

controlled than older plants at the full tillering stage. *Eragrostis plana* was most vulnerable to glyphosate at the onset of the reproductive stage (panicle initiation), when the plants would start translocating sugar to perennating organs (meristematic tissues at the base) and reproductive organs. In this manner, more glyphosate could be carried to sensitive tissues, improving the efficacy of glyphosate. Plants are generally vulnerable to herbicide treatment (or other stresses) at the onset of reproduction. AMS improved the efficacy of glyphosate by increasing translocation out of the treated leaf and consequently increasing the concentration of glyphosate in the primary culm, which would limit the development of new tillers. Drought-stressed *E. plana* cannot be controlled with glyphosate, and AMS increased the level of control compared to glyphosate alone. These data can be used to plan an effective management program for *E. plana* in coordination with the developmental stage of desired pasture grass species.

Acknowledgments. The authors are grateful to the Coordination for the Improvement of Higher Education Personnel (CAPES, finance code 001) for a scholarship to MOB and to the National Council for Scientific and Technological Development (CNPq) for a fellowship to FPL (process no. 305816/2016-0). This research was funded by Embrapa Pecuária Sul; the University of Arkansas Agricultural Research Station, Weed Physiology Laboratory, Fayetteville, AR, USA; and partially by the Federal University of Pelotas (UFPEL). No conflicts of interest have been declared.

Supplementary material. To view supplementary material for this article, please visit <https://doi.org/10.1017/wsc.2020.97>

References

- Barbosa FG (2016) The future of invasive African grasses in South America under climate change. *Ecol Inform* 36:114–117
- Bastiani MO, Lamego FP, Langaro AC, Salas-Perez RA, Rouse CE, Burgos NR (2018) Influence of growth stage on efficacy, absorption and translocation of glyphosate in *Eragrostis plana*. Page 267 in Proceedings of the 31st Congresso Brasileiro Da Ciência das Plantas Daninhas. Londrina, Brazil: Sociedade Brasileira das Plantas Daninhas
- Corrêa EB, Silveira MCT, Morais SL, Trentin G, Perez NB, Natividade RS (2014) Characterization of tough lovegrass tillers dynamic related to glyphosate herbicide translocation. Abstract 1050 in Proceedings of the 23rd Congresso de Iniciação Científica. Pelotas, Brazil: Universidade Federal de Pelotas–UFPEL
- Degreeff RD, Varanasi AV, Dille JA, Peterson DE, Jugulam M (2018) Influence of plant growth stage and temperature on glyphosate efficacy in common lambsquarters (*Chenopodium album*). *Weed Technol* 32:448–453
- De Ruyter H, Meinen E (1998) Influence of water stress and surfactant on the efficacy, absorption and translocation of glyphosate. *Weed Sci* 46:289–296
- Fadin DA, Tornisielo VL, Barroso AAM, Ramos S, Dos Reis FC, Monquero PA (2018) Absorption and translocation of glyphosate in *Spermacoce verticillata* and alternative herbicide control. *Weed Res* 58:389–396
- Galvani J, Rizzardi MA, Carneiro CM, Bianchi MA (2012) *Lolium multiflorum* foliar anatomy susceptible and resistant to glyphosate. *Planta Daninha* 30:407–413
- Goulart ICGR, Nunes AL, Kupas V, Merotto Junior A (2012) Interactions between herbicides and safeners to tough lovegrass control in rangelands. *Cienc Rural* 42:1722–1730
- Harrington KC, Ghanizadeh H (2017) Herbicide application using wiper applicators—a review. *Crop Prot* 102:56–62
- Hess FD, Chester LF (2000) Interaction of surfactants with plant cuticles. *Weed Technol* 14:807–813
- Kim DS, Marshall EJP, Brain P, Caseley, JC (2011) Effects of crop canopy structure on herbicide deposition and performance. *Weed Res* 51:310–320
- Knezevic SZ, Streibig JC, Ritz C (2007) Utilizing R software package for dose-response studies: the concept and data analysis. *Weed Technol* 21:840–848
- Köppen W, Geiger R, eds (1930) *Handbuch der Klimatologie*. Volume 5. Berlin: Gerbrüder Borntraeger. 44 p
- Lacerda ALDS, Victoria Filho R (2004) Dose response curves in weeds with glyphosate use. *Bragantia* 63:73–79
- Li J, Smeda RJ, Sellers BA, Johnson WG (2005) Influence of formulation and glyphosate salt on absorption and translocation in three annual weeds. *Weed Sci* 53:153–159
- Mueller TC, Main CL, Thompson MA, Steckel LE (2006) Comparison of glyphosate salts (isopropylamine, diammonium, and potassium) and calcium and magnesium concentrations on the control of various weeds. *Weed Technol* 20:164–171
- Nalewaja JD, Matysiak R (2000) Spray deposits from nicosulfuron with salts that affect efficacy. *Weed Technol* 14:740–749
- Nandula VK, Vencill WK (2015) Herbicide absorption and translocation in plants using radioisotopes. *Weed Sci* 12:140–151
- Oliveira RB, Dario G, Alves KA, Gandolfo MA (2015) Influence of the glyphosate formulations on wettability and evaporation time of droplets on different targets. *Planta Daninha* 33:599–606
- Palma-Bautista C, Torra J, Garcia MJ, Bracamonte E, Rojano-Delgado AM, Alcantara-De La Cruz R, De Prado R (2019) Reduced absorption and improved translocation endows glyphosate resistance in *Amaranthus palmeri* harvested in glyphosate-resistant soybean from Argentina. *J Agric Food Chem* 67:1052–1060
- Patterson DT (1995) Effects of environmental stress on weed/crop interactions. *Weed Sci* 43:483–490
- Perez NB (2010) Weed control in pastures: use of Clean Field technology. Bagé, RS, Brazil: Comunicado Técnico 72 da Embrapa Pecuária Sul. Pp 1–7
- Pline WA, Hatzios KK, Hagoood ES (2000) Weed and herbicide-resistant soybean (*Glycine max*) response to glufosinate and glyphosate plus ammonium sulfate and pelargonic acid. *Weed Technol* 14:667–674
- Richardson RJ, Bailey WA, Armel GR, Whaley CM, Wilson HP, Hines TE (2003) Responses of selected weeds and glyphosate-resistant cotton and soybean to two glyphosate salts. *Weed Technol* 17:560–564
- Rojano-Delgado AM, Cruz-Hipolito H, De Prado R, Luque De Castro MD, Franco AR (2012) Limited uptake, translocation and enhanced metabolic degradation contribute to glyphosate tolerance in *Mucuna pruriens* var. *utilis* plants. *Phytochemistry* 73:34–41
- Salisbury C, Chandler J, Merkle M (1991) Ammonium sulfate enhancement of glyphosate and sc-0224 control of johnsongrass (*Sorghum halepense*). *Weed Technol* 5:18–21
- Santos ABD, Bottcher A, Kiyota E, Mayer JLS, Vicentini R, Brito MDS, Creste S, Landell MGA, Mazzafera P (2015) Water stress alters lignin content and related gene expression in two sugarcane genotypes. *J Agric Food Chem* 63:4708–4720
- Satchivi N, Wax LM, Stoller EW, Briskin DP (2000) Absorption and translocation of glyphosate isopropylamine and trimethylsulfonium salts in *Abutilon theophrasti* and *Setaria faberi*. *Weed Sci* 48:675–679
- Soltani N, Nurse R, Shropshire C, Sikkema P (2016) Benefit of adding ammonium sulfate or additional glyphosate to glyphosate in corn and soybean. *Agric Sci* 7:759–770
- Travlos I, Cheimona N, Bilalis D (2017) Glyphosate efficacy of different salt formulations and adjuvant additives on various weeds. *Agronomy* 7:1–9
- Wills GD, Mcwhorter CG (1985) Effect of inorganic salts on the toxicity and translocation of glyphosate and MSMA in purple nutsedge (*Cyperus rotundus*). *Weed Sci* 33:755–761
- Ziska LH (2016) The role of climate change and increasing atmospheric carbon dioxide on weed management: herbicide efficacy. *Agric Ecosyst Environ* 231:304–309