Comparison of efficacy, absorption and translocation of three glyphosate formulations on velvetleaf (*Abutilon theophrasti*)†

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Abstract: Growth analysis, absorption and translocation studies were conducted to compare a 1-aminomethanamide dihydrogen tetraoxosulfate (GLY-A) formulation of glyphosate with two isopropylamine (GLY-IPA-1, GLY-IPA-2) formulations of glyphosate on velvetleaf. The two isopropylamine formulations differed by the presence of a surfactant in the formulation, GLY-IPA-1 containing surfactant whereas GLY-IPA-2 did not. Four- to six-leaf velvetleaf was treated with GLY-A and GLY-IPA-1 and GLY-IPA-2 (0, 50, 67, 89, 119, 158, 280, 420, 560 and 840 g AE ha^{-1}) with and without ammonium sulfate (AMS; 20 g L^{-1}). GLY-A and GLY-IPA-2 included a non-ionic surfactant (2.5 mL L^{-1}) in the spray solution at all herbicide concentrations. No additional surfactant was added to GLY-IPA-1. The IC$_{50}$ value for GLY-A was 88 g AE ha^{-1} compared with 346 and 376 g AE ha^{-1} for GLY-IPA-1 and GLY-IPA-2 respectively in the absence of AMS. When AMS (20 g L^{-1}) was added to the spray solution, the estimated IC$_{50}$ values were 143, 76 and 60 g AE ha^{-1} for GLY-IPA-1, GLY-IPA-2 and GLY-A respectively. Absorption of ^{14}C-glyphosate into the third leaf of five- to six-leaf velvetleaf was three- to sixfold greater 72 h after treatment (HAT) when applied as GLY-A compared with GLY-IPA-1 and GLY-IPA-2 respectively in the absence of AMS. AMS (20 g L^{-1}) increased absorption of ^{14}C-glyphosate in all glyphosate formulations two- to threefold, but differences among the formulations remained. Approximately three- and sixfold more ^{14}C-glyphosate applied as GLY-A had translocated out of the treated leaf compared with GLY-IPA-1 and GLY-IPA-2 respectively by 72 HAT. Adding AMS (20 g L^{-1}) increased translocation of ^{14}C-glyphosate out of the treated leaf approximately 2.5-fold for all three formulations. The increased efficacy of GLY-A versus GLY-IPA-1 and GLY-IPA-2 on velvetleaf is due to the greater rate of absorption and subsequent translocation of glyphosate out of the treated leaf. AMS increased the efficacy of all three formulations by increasing absorption and translocation of glyphosate in the plant.
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Keywords: glyphosate; AMADS; velvetleaf; absorption; translocation; growth response

1 INTRODUCTION

Glyphosate [*N*-(phosphonomethyl)glycine] is a non-selective herbicide that kills plants by inhibiting 5-enolpyruvylshikimic acid-3-phosphate (EPSP) synthase (EC 2.5.1.19), an enzyme necessary for aromatic amino acid production. With the introduction of glyphosate-resistant crops, growers increasingly rely on glyphosate to manage their weeds. This reliance has led to a shift to those weed species that are more tolerant to glyphosate.[1]

Velvetleaf (*Abutilon theophrasti* L.) is tolerant to glyphosate.[2] Variable control of velvetleaf was observed where glyphosate controlled velvetleaf at three locations in Iowa – from 46 to 81% when applied at 420 g ha^{-1}.[2] Also, velvetleaf is more tolerant than other species, as the addition of ammonium sulfate (AMS) to the glyphosate spray solution was needed to increase translocation in velvetleaf but not needed in common lambsquarters (*Chenopodium album* L.).[3] One mechanism of velvetleaf tolerance to glyphosate is the presence of calcium on the leaf surface.[4] Glyphosate interacts with calcium to produce a salt or complex that is poorly absorbed.[5] The addition of ammonium sulfate (AMS) to the spray solution can overcome calcium chelation by the precipitation of calcium sulfate and the formation of a glyphosate–ammonium complex.[6] Glyphosate efficacy on velvetleaf can be increased more than fourfold when 20 g L^{1} AMS is added to the spray solution. In one study, velvetleaf GR$_{50}$ values decreased from 451 to 92 g ha^{-1} when AMS at 20 g L^{-1} was added.[3]

Numerous studies have compared the efficacy of different glyphosate formulations on velvetleaf.[7–9] Results of these studies have varied from showing

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†Presented in part at the Western Society of Weed Science Meeting, 2004.
Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.
(*Received 10 February 2006; revised version received 11 May 2006; accepted 23 May 2006*)
Published online 18 September 2006; DOI: 10.1002/ps.1296

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no effect of formulation on glyphosate efficacy on velvetleaf[9] to major differences among formulations.[7,8] One of the problems with comparing these studies is that AMS may or may not have been added to the formulations. Feng *et al.*[7] and Li *et al.*[8] did not add AMS to the spray solution and found differences among different formulations. Satchivi *et al.*[9] found that AMS ($10\,g\,L^{-1}$) in the spray solution increased glyphosate absorption and translocation in velvetleaf for two different formulations of glyphosate. However, there were no differences in uptake or translocation between the two formulations.

Molin and Hirase[10] found that a formulation of glyphosate (GLY-A) containing glyphosate acid ($147.4\,g$ AE L^{-1}) dissolved in 1-aminomethanamide dihydrogen tetraoxosulfate (AMADS) was two- to threefold more active than an isopropylamine salt formulation of glyphosate (GLY-IPA-1) on prickly sida *(Sida spinosa* L.), morningglory *(Ipomeae hederacea* var. *integriuscula* Gray), sicklepod (*Senna obtusifolia* L.) and purple nutsedge (*Cyperus rotundus* L.). AMADS can also act as an adjuvant to improve glyphosate activity.[11,12]

Recent research suggests that cuticular disruption may play a vital role in increasing the efficacy of glyphosate. Feng *et al.*[13] found a direct correlation between the amount of tissue damage caused by a surfactant and the speed and amount of glyphosate absorption. Tissue damage was caused by the surfactant since formulations without the surfactant did not cause damage.[13,14] Scanning electron microscopy showed that the surfactant caused well-defined, irregularly shaped pits on the leaf surface. In tissue cross-sections, the upper and lower epidermal cell layers were collapsed and the menchymal layer was necrotic.[15] Molin and Hirase[10] speculated that the increased activity of GLY-A was due to disruption of the cuticle by AMADS.

However, since AMADS is a mixture of sulfuric acid and urea, it is possible that AMADS may be acting like AMS in increasing glyphosate activity by complexing with calcium ions on the leaf surface. The objectives of the present research were: (1) to compare the efficacy of GLY-A with two different isopropylamine formulations of glyphosate – GLY-IPA-1, which contains a proprietary surfactant, and GLY-IPA-2, which contains no surfactant – on velvetleaf; (2) to determine the effect of AMS on the efficacy of these formulations on velvetleaf; and (3) to measure the absorption and translocation of ^{14}C-glyphosate when applied as GLY-A, GLY-IPA-1 or GLY-IPA-2 with and without AMS.

2 MATERIALS AND METHODS

2.1 Growth analysis

Velvetleaf seeds were planted in greenhouse flat inserts ($4 \times 4\,cm$ per cell) in Metro Mix 200 potting media (Sun Gro Horticulture, Bellevue, WA). Approximately five seeds were placed in each cell and covered with soil (2 cm). Emerged plants were thinned to one plant per cell and fertilized with Osmocote (14-14-14) pellets (Scotts-Sierra Horticultural Co., Marysville, OH). Greenhouse conditions were 24:18 °C day:night temperatures and 14:10 h day:night photoperiod under natural light supplemented with halogen lights with an output of $100\,\mu E\,m^{-2}\,s^{-1}$ to obtain a 14 h photoperiod. The plants were grown to the four- to six-leaf stage. Plants were sprayed with a greenhouse track sprayer equipped with TeeJet™ 8002E nozzles (Spraying Systems Co., Wheaton, IL) and calibrated to deliver $100\,L\,ha^{-1}$ at 207 kPa). Commercial formulations of glyphosate-isopropylammonium, Roundup UltraMax (Monsanto, St Louis, MO; GLY-IPA-1) and Rodeo (Monsanto, St Louis, MO; GLY-IPA-2), and of glyphosate-AMADS, Engame (UAP, Greeley, CO; GLY-A), were applied at 0, 50, 67, 89, 119, 158, 280, 420, 560 and $840\,g$ AE ha^{-1} with or without ammonium sulfate (AMS; $20\,g\,L^{-1}$) in the spray solution. A non-ionic surfactant, Activator 90 (Loveland Industries Inc. Greeley, CO), was added at $2.5\,mL\,L^{-1}$ to the spray solutions with GLY-IPA-2 and GLY-A formulations. No additional surfactant was added to the GLY-IPA-1. The water source for the spray solution contained 42 ppm of calcium and less than 1 ppm of magnesium. Fourteen days after treatment, plants were harvested, dried in an oven at 60 °C and weighed. The experiment was conducted 3 times in a randomized complete block design with four replications.

2.2 Absorption and translocation

Application solutions were made from each of the formulations to deliver glyphosate ($280\,g\,ha^{-1}$) when sprayed at $100\,L\,ha^{-1}$ with or without AMS ($20\,g\,L^{-1}$). Each formulation described above (475 μL) was spiked with ^{14}C-glyphosate (specific activity 9487 kBq mg^{-1}) stock solution (25 μL) to achieve a final specific activity of 62 kBq mg^{-1}. Velvetleaf plants were grown as described in Section 2.1 and were sprayed with each formulation ($280\,g\,ha^{-1}$). Ten droplets (0.2 μL) of radiolabeled formulated material were applied to the third leaf immediately after spraying.

Treated leaves were harvested 0, 6, 24 and 72 h after treatment (HAT) and placed in a scintillation vial containing wash solution (methanol + water, 10 + 90 by volume, containing $2.5\,g\,L^{-1}$ non-ionic surfactant; 5 mL). The leaves were agitated for 15 min and removed, and scintillation cocktail (ScintiSafe Plus 50%, 10 mL; Fisher Scientific, Fairlawn, NJ) was added to the wash solution. Samples were analyzed by liquid scintillation spectroscopy (LSS) (Packard 2500TR; PerkinElmer, Shelton, CT). At 0 HAT, 98% of the radioactivity was recovered in the wash solution, indicating the effectiveness of the leaf washing procedure. Treated leaves were put in an envelope and oven dried at 60 °C. The leaves and apical meristem above the treated leaf, the leaves

Pest Manag Sci **62**:1177–1181 (2006)
DOI: 10.1002/ps

and stem below the treated leaf and the roots were harvested and dried at 60 °C. After drying, plant parts were oxidized using a biological oxidizer (OX500; RJ Harvey Instrument Corp., Hillsdale, NJ) and the radioactivity was determined by LSS. There were three replications for each time point and the experiment was conducted twice. Total recovery of radioactivity applied to the plants ranged from 93 to 99%.

2.3 Statistical analysis

Analysis of variance (ANOVA) was used to determine the differences among the herbicide treatments. Experiment by treatment interactions were not significant, so data from all experiments were combined. Regression analysis was used to determine the effect of herbicide treatment and herbicide dose on velvetleaf growth. Velvetleaf dose response residual mean square and predicted sum of squares were examined. A log-logistic nonlinear curve was fitted to the data for each herbicide to estimate the concentration that caused 50% inhibition of dry weight accumulation (IC_{50}).[16] Regression lines were analyzed by the method of Saxton,[17] which is a method for determining mean separations of nonlinear equations at $P = 0.05$.

3 RESULTS AND DISCUSSION

3.1 Growth analysis

GLY-A was approximately fourfold more active that GLY-IPA-1 and GLY-IPA-2 on velvetleaf in the absence of AMS (Table 1). AMS at 20 g L^{-1} increased the activity of all three glyphosate formulations (Table 1). The activity of GLY-IPA-1 and GLY-IPA-2 increased approximately two- to fivefold respectively when 20 g L^{-1} AMS was included in the spray solution, whereas the activity of GLY-A increased by approximately 1.5-fold with the addition of 20 g L^{-1} AMS.

The differences between GLY-IPA-1 and GLY-IPA-2 could be due to the amount of surfactant added to the spray solution. GLY-IPA-1 includes a proprietary surfactant, and no additional surfactant was added as the concentration of glyphosate was reduced. The concentration of surfactant in GLY-IPA-1 may have fallen below a critical level as the herbicide was diluted, and thus there was not as great a response to AMS as with GLY-IPA-2 where the concentration of surfactant remained constant. These results do support previous studies which have shown that AMS increases the activity of glyphosate formulations on velvetleaf.[6,18]

AMS did not increase the activity of GLY-A on velvetleaf as dramatically as it did for the other two formulations, although there was a significant increase in efficacy. Shaner *et al.*[11] found that, in corn, AMADS was better than AMS in overcoming the antagonism of hard water on glyphosate activity, and the addition of AMADS and AMS was not better than AMADS alone. Since the tolerance of velvetleaf to glyphosate is due to the presence of calcium on the leaf surface, and AMS acts by precipitating calcium sulfate on the leaf surface and forming a glyphosate–ammonium complex,[5] the AMADS in the GLY-A formulation may be acting in a similar manner.

Table 1. Effect of glyphosate formulation and AMS on the estimated IC_{50} on velvetleaf

Herbicide	AMS (g L^{-1})	IC_{50}[a] (g ha^{-1})
GLY-IPA-1	0	346a
	20	143b
GLY-IPA-2	0	376a
	20	76b
GLY-A	0	88b
	20	60 c

[a] Values followed by the same letter are not significantly different ($P = 0.05$) according to Saxton.[17]

3.2 Absorption and translocation

The absorption of ^{14}C-glyphosate into velvetleaf was dependent on formulation and the presence of AMS. In the absence of AMS, more ^{14}C-glyphosate was absorbed from the GLY-A formulation compared with the other two formulations (Fig. 1). The addition of 20 g L^{-1} AMS increased the absorption of ^{14}C-glyphosate two- to threefold with all the formulations. The absorption of ^{14}C-glyphosate with the GLY-IPA-1 formulation when AMS was added to the spray solution was equivalent to that with the GLY-A formulation in the absence of AMS (Fig. 1).

The total amount of ^{14}C-glyphosate translocated out of the treated leaf reflected the amount of herbicide absorbed. Approximately threefold more ^{14}C-glyphosate translocated out of the treated leaf when it was applied in the GLY-A formulation compared with the other two formulations in the absence of AMS (Fig. 2). AMS increased the amount of glyphosate that translocated out of the treated leaf approximately threefold for all the formulations (Fig. 2). The amounts of ^{14}C-glyphosate that translocated to the apical meristem and roots were approximately equal (data not shown).

When the translocation out of the treated leaf was expressed as a percentage of the amount of ^{14}C-glyphosate absorbed into the plant, there were no differences among the different treatments. The amount of herbicide translocated out of the treated leaf was approximately 75% of the absorbed material.

These results are consistent with the whole-plant response to GLY-A and GLY-IPA-1, where the addition of AMS also increased the herbicidal activity of the formulations, and support previous research which showed that AMS increases glyphosate activity on velvetleaf by increasing the amount of herbicide absorbed into the plant.[9] However, the results with GLY-IPA-2 are not consistent with the whole-plant response. Although 20 g L^{-1}AMS did increase absorption and translocation of ^{14}C-glyphosate, the

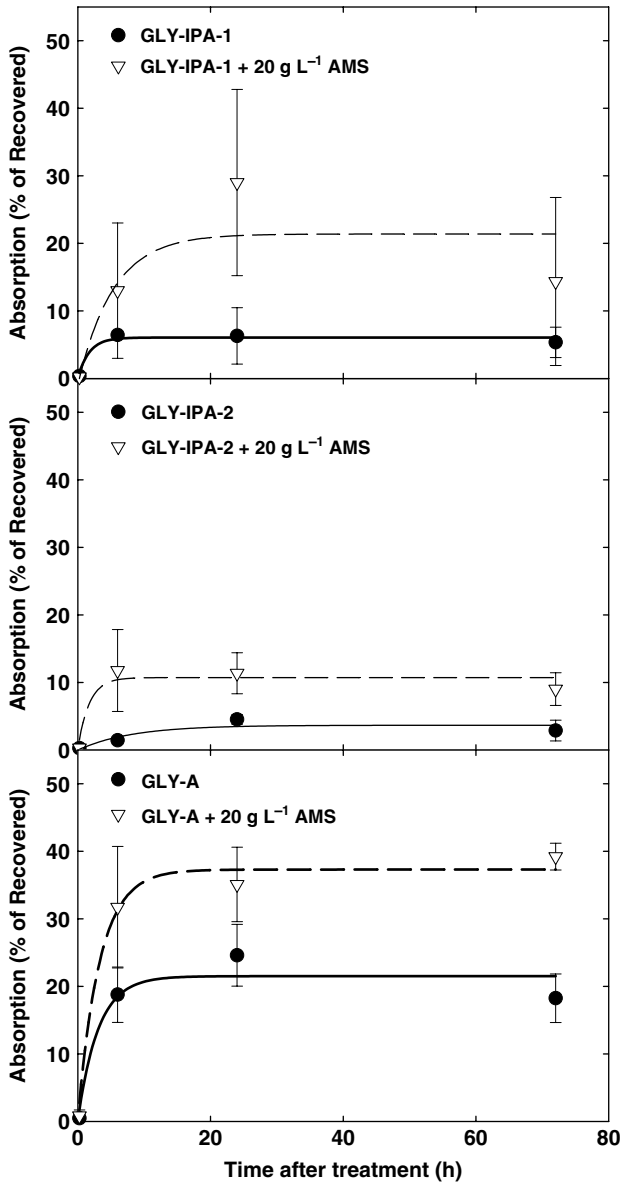

Figure 1. Effect of different formulations of glyphosate with 20 g L^{-1} AMS on the absorption of ^{14}C-glyphosate into the third leaf of velvetleaf.

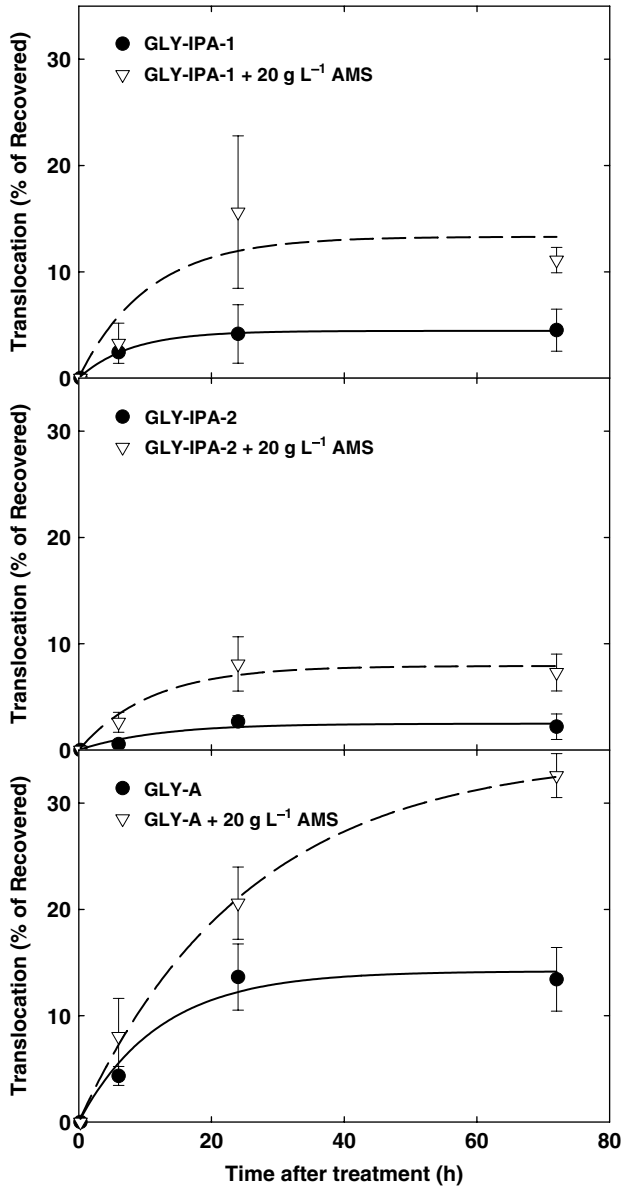

Figure 2. Effect of different formulations of glyphosate with 20 g L^{-1} AMS on the translocation of ^{14}C-glyphosate out of the third leaf of velvetleaf to the rest of the plant.

difference in absorption is not consistent with the increase in activity seen at the whole-plant level. Absorption increased only 2.5-fold with the addition of AMS as opposed to the fivefold increase in activity observed at the whole-plant level. This discrepancy is likely due to the difference in the time of year when the plants were grown and other uncontrolled factors.

4 CONCLUSIONS

These results suggest that, while the AMADS in the GLY-A formulation increases the activity of glyphosate on velvetleaf, this adjuvant is acting differently to AMS. AMS increases the activity of glyphosate on velvetleaf by the precipitation of calcium sulfate on the leaf surface and the formation of a glyphosate–ammonium complex.[4] AMADS, on the other hand, is thought to increase glyphosate activity by disrupting the leaf cuticle.[10] These results suggest that both of these mechanisms may be acting and are additive. If AMADS were acting like AMS, AMS would not be expected to enhance the activity of GLY-A on velvetleaf. However, further research needs to be done to determine the actual mechanism of both of these additives on increasing glyphosate activity on velvetleaf.

REFERENCES

1 Culpepper AS, Flanders JT, York AC and Webster TM, Tropical spiderwort (*Commelina benghalensis*) control in glyphosate-resistant cotton. *Weed Technol* **18**:432–436 (2004).

2 Hartzler RG and Battles BA, Reduced fitness of velvetleaf (*Abutilon theophrasti*) surviving glyphosate. *Weed Technol* **15**:492–496 (2001).

3 Hall GJ, Hart CA and Jones CA, Plants as sources of cations antagonistic to glyphosate efficacy. *Pest Manag Sci* **56**:351–368 (2000).

4 Nalewaja JD and Matysiak R, 2,4-D and salt combinations affect glyphosate phytotoxicity. *Weed Technol* **6**:322–327.
5 Thelen KD, Jackson EP and Penner D, The basis for the hard-water antagonism of glyphosate activity. *Weed Sci* **43**:541–548 (1995).
6 Young BG, Knepp AW, Wax LM and Hart SE, Glyphosate translocation in common lambsquarters (*Chenopidium album*) and velvetleaf (*Abutilon theophrasti*). *Weed Sci* **51**:151–156 (2003).
7 Feng PC, Sandbrink JJ and Sammons SD, Retention, uptake and translocation of ^{14}C-glyphosate from track spray applications and correlation to rain fastness in velvetleaf (*Abutilon theophrasti*). *Weed Technol* **14**:127–132 (2000).
8 Li J, Smeda RJ, Sellers BA and Johnson WG, Influence of formulation and glyphosate salt on absorption and translocation in three annual weeds. *Weed Sci* **53**:153–159 (2005).
9 Satchivi NM, Wax LM, Stoller EW and Briskin DP, Absorption and translocation of glyphosate isopropylamine and trimethylsulfonium salts in *Abutilon theophrasti* and *Setaria faberi*. *Weed Sci* **48**:675–679 (2000).
10 Molin WT and Hirase K, Comparison of commercial glyphosate formulations for control of prickly sida, purple nutsedge, morningglory, and sicklepod. *Weed Biol Manag* **4**:136–141 (2004).
11 Shaner DL, Westra P and Nissen S, AMADS increases the efficacy of glyphosate on corn. *Weed Technol* **20**:179–183 (2006).
12 Penner D, Michael J and Brown WG, A novel water conditioning adjuvant for use with formulated and nonformulated glyphosate. *J ASTM Internat* **2**:1–6 (2005).
13 Feng PC, Ryerse JS and Sammons RD, Correlation of leaf damage with uptake and translocation of glyphosate in velvetleaf (*Abutilon theophrasti*). *Weed Technol* **12**:300–307 (1998).
14 MacIsaac SA, Paul RN and Devine MD, A scanning electron microscope study of glyphosate deposits in relation to foliar uptake. *Pestic Sci* **31**:52–64 (1991).
15 Feng PC, Ryerse JS and Jones CR, Analysis of surfactant leaf damage using microscopy and its relation to glyphosate on deuterium oxide uptake in velvetleaf (*Abutilon theophrasti*). *Pestic Sci* **55**:385–386 (1999).
16 Seefeldt SS, Jensen JE and Fuerst EP, Log-logistic analysis of herbicide dose–response relationships. *Weed Technol* **9**:218–227 (1995).
17 Saxton AM, A macro for converting mean separation output to letter groupings in PROC MIXED. *Proc. 21st SAS® Users Group International Meeting,* Nashville, TN, pp. 1243–1246 (1998).
18 Young BG, Knepp AW, Wax LM and Hart SE, Glyphosate translocation in common lambsquarters (*Chenopodium album*) and velvetleaf (*Abutilon theophrasti*). *Weed Sci* **51**:151–156 (2003).