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GLYPHOSATE–ADJUVANT INTERACTIONS: A REVIEW OF RECENT EXPERIENCES

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ABSTRACT

Glyphosate is the most widely used herbicide in the world. Surfactants are well known to enhance its uptake, translocation and field performance. The interactions between glyphosate formulations and surfactants, however, are not simple and depend on factors which include leaf surface, droplet characteristics, differential wetting of leaves, adjuvant type and the chemical form of the herbicide Understanding the complexity of these interactions is critical in order to reduce the amounts of glyphosate being used world-wide, particularly in environmentally sensitive areas.

In most species, addition of common adjuvants to glyphosate reduces the surface tension of spray solutions and droplet contact angles, leading to increased spray deposition, spray retention and leaf wetting. There are many examples in the published literature where such adjuvant effects translate directly to increased uptake and enhanced glyphosate activity. There are other instances, however, which show that adjuvant effects on glyphosate treatments are more species specific than previously appreciated; increased spray delivery does not always result in increased control of certain weeds. In this paper some examples are discussed, which indicate the complex interactions between glyphosate, adjuvants and plant responses. Reasons for lower effectiveness of glyphosate treatments in the field include the hydrophobic nature of waxes, poor droplet spreading, increased drying time and antagonism in the presence of certain adjuvants.

Crop-based oils and methylated seed oils, which also increase leaf wetting, have increased the effectiveness of glyphosate treatments on many difficult-to-control weed species. Results of recent studies are discussed, indicating that the enhanced activity of glyphosate in the presence of oil-based adjuvants possibly involves humectant action or a solubilization effect on epicuticular waxes on the target leaves.

Keywords: Glyphosate, surfactants, adjuvants,

INTRODUCTION

Glyphosate (N-(phosphonomethyl)glycine is a global herbicide, because of its versatility in economically controlling a broad spectrum of weeds under varied agricultural, industrial, amenity and domestic situations (Bayliss 2000). Once applied to the leaf surface, glyphosate has several barriers to overcome before it becomes lethal to a plant. These are: (a) epicuticular wax on the cuticle; (b) the cuticle itself; (c) walls of epidermal cells, and (d) plasmalemma of cells. The efficacy of glyphosate depends largely on the success of passing through these barriers, before translocation within a plant to its sub-cellular sites of action.

With the aim of overcoming the cuticular barriers, many studies have examined the formulation factors involved- such as glyphosate concentrations, glyphosate-adjuvant mixes, surfactant and/or adjuvant concentration, spray droplet sizes, surface tension reduction in solutions, and leaf surface characteristics. These have revealed the complex nature of the interactions between different surfactants, surfactant rates, herbicide rates and target species, as major factors influencing the uptake of glyphosate (Liu and Zabkiewicz 1997, 2000; Knoche and Bukovac 1993; Kirkwood et al 2000; Leaper and Holloway 2000).

Despite a vast amount of research, the basis of enhancement of foliar uptake of glyphosate caused by adjuvants is still not well understood. Improved glyphosate efficacy with surfactants has been attributed to increased leaf wettability, droplet contact area and penetration, resulting from reduced surface tension and contact angle of droplets on leaf surfaces. Surfactants may also increase glyphosate uptake by influencing spray droplet size, droplet drying time and overall spray retention on leaf surfaces, and may also increase cuticular permeability and/or stomatal penetration (Kirkwood 1993; 1999).

Our research work has focused on using adjuvants to improve the bio-efficacy of glyphosate, so that its rates can be reduced. This review is based on some laboratory and field research, which demonstrate the complexity of the interactions between different weed species and adjuvants on the efficacy of glyphosate.

GLYPHOSATE- SURFACTANT INTERACTIONS

Influence of epicuticular wax

The role of epicuticular wax and the influence of two surfactants on the uptake of glyphosate a.i. were studied using three weed species- teaweed (*Sida spinosa*), velvetleaf (*Abutilon theophrasti*) and sicklepod (*Cassia obtusifolia*) (Sharma and Singh 1999).

These species had different amounts of polar wax (14, 64 and 93%, respectively), as a % of total waxes on adaxial leaf surfaces (25.5, 27.6 and 40.6 $\mu g\ cm^{-2}$), respectively.

Glyphosate (Rodeo™) treatments (0.56 kg a.i. ha^{-1} in 188 L ha^{-1} of water) were given to the three weeds, either alone, or formulated with surfactants X-77 (non-ionic) and Silvet L-77 (organosilicone). During spray treatments, the 3rd fully expanded leaf was kept covered with aluminium foil, and later, after removing the cover, the adaxial surface of this leaf was treated with ^{14}C-glyphosate, applied as 5 x 2 μl droplets (10 μl).

Contact Angles (CA) measured correlated with the amount of polar wax on leaf surfaces, being least on teaweed and highest on sicklepod. Surfactants significantly reduced surface tension (ST) and CA of glyphosate droplets on both Teflon slides and test leaves.

Uptake and translocation of glyphosate without surfactant decreased significantly as the % of polar waxes on adaxial leaf surfaces increased in the species (Table 1). However, incorporation of X-77 or L-77 significantly increased the uptake and translocation of ^{14}C-glyphosate by all species, and this increase was greater with L-77 than with X-77 in all species. The overall effect of increased glyphosate uptake was well correlated with low ST and low CA, increased spreading of droplets in the presence of the surfactants.

Table 1. Effect of surfactants on uptake and translocation of ^{14}C-glyphosate and overall control of test weed species with varying amounts of cuticular polar waxes.

Treatments	Teaweed	Velvetleaf	Sicklepod
		*Uptake as % of applied	
Glyphosate (control)	28.03 e	21.80 f	20.97 f
Glyphosate + X-77	58.27 b	38.60 c	34.23 d
Glyphosate + L-77	66.43 a	56.87 b	56.77 b
		Translocation as % of applied	
Glyphosate (control)	4.48 f	3.78 g	3.4 g
Glyphosate + X-77	9.75 d	5.87 e	6.07 e
Glyphosate + L-77	35.40 a	20.57 b	11.17 c
		Control %	
Glyphosate (control)	45.0 cd	41.3 d	43.8 cd
Glyphosate + X-77	55.0 b	47.5 cd	66.3 a
Glyphosate + L-77	68.8 a	50.0 bc	66.3 a

*In all Tables Uptake = ^{14}C in the whole plant; Translocation = ^{14}C in the whole plant, excluding the treated leaf. Recovery of ^{14}C activity: 95%. Values followed by the same letter are not significantly different (Duncan's New Multiple Range Test).

Greenhouse efficacy evaluation trials consistently showed significantly higher % control of all species achieved by Rodeo + L-77, which is attributed to super spreading properties and increased surface contact promoted by L-77. However, the species-specific nature of the glyphosate-surfactant interaction was also obvious from the study.

The interaction of plant surface waxes and surfactants on uptake of glyphosate was seen in another study (Sharma *et al*., 2001), which used Commelina (*Commelina communis*) and Nightshade (*Solanum nigrum*). The leaf surface properties of these two weeds were significantly different. Nightshade's leaf surface had large amounts of non-polar waxes.

Glyphosate (Rodeo™) treatments given to the weeds were the same as above, either alone, or formulated with Kinetic® (0.25% v/v), which is a blend of polyalkyleneoxide modified poly-dimethyl-siloxane (organosilicone) and non-ionic surfactants. Experimental procedures for ^{14}C-glyphosate uptake studies were also as same as given previously.

Both uptake and translocation of ^{14}C-glyphosate were significantly higher in the presence of the surfactant than without surfactant, in both species. However, between the two species, greater uptake and translocation of ^{14}C-glyphosate was in Nightshade (Table 2).

Table 2. Effect of Kinetic® on ^{14}C-glyphosate uptake and translocation by Commelina and Nightshade.

Harvest time (h)	Uptake (% of applied)				Translocation (% of applied)			
	Commelina		Nightshade		Commelina		Nightshade	
	- S*	+ S	- S	+ S	- S	+ S	- S	+ S
0.25	2	20	6	26	1	2	1	14
1	6	27	9	35	2	3	2	23
6	14	35	24	54	2	5	4	37
24	21	51	51	66	4	6	9	43
48	26	58	59	68	9	16	17	46
LSD ($P \leq 0.05$)	3		2		1		2	

*- S: no surfactant; + S: with surfactant.

The increased entry of glyphosate, with or without surfactant into Nightshade is attributed to its relatively smooth leaf surface, which had less polar wax (or greater amounts of non-polar wax). Uptake and translocation results were well correlated with the % control of these two weeds achieved by glyphosate (± surfactant) (data not presented).

Scanning electron micrographs of treated Commelina leaves showed that the application of glyphosate alone disrupted the epicuticular waxes on the adaxial leaf surface, but the waxes remained intact on the cuticle. Treated with the organosilicone surfactant alone, the waxes appeared dissolved, dried and with cracks. Similar damage to cuticles and epidermal tissues caused by surfactants has been reported (Feng et al 1999). With glyphosate + surfactant, surface waxes were disrupted and dissolved; trichomes were ruptured from the base, and stomata were also disrupted. The surfactant aided disruption of epicuticular wax on Commelina probably allowed increased penetration of glyphosate a.i. into the plant.

In both our studies, glyphosate uptake was greater through non-polar cuticles, and glyphosate was repelled by leaf surfaces having more polar (hydrophilic) components. The presence of surfactants allowed such repellence to be overcome. However, there are other studies, which have produced somewhat different results. For instance, Chachalis *et al*. (2001) reported that lower efficacy of glyphosate was related to the more hydrophobic (non-polar) nature of epicuticular wax of redvine (*Brunnichia ovata*) leaves, compared to those of trumpet creeper (*Campis radicans*), a susceptible species.

The wax mass per unit area (22-37 µg cm^{-2}) was similar in the two species regardless of leaf age. However, trumpet creeper was consistently more susceptible to glyphosate than redvine and the CA of droplets was lower on its leaves. Trumpet creeper leaves have dense trichomes and glands and significant surface micro-roughness compared to redvine, with no trichomes or glands. Therefore, it is possible that leaf surface characteristics played a significant role in increased glyphosate uptake by this susceptible species.

Species-specificity of responses

The species-specific nature of the adjuvant-enhanced uptake of glyphosate was observed in another study with hairy beggarticks (*Bidens frondosa*) and guinea grass (*Panicum maximum*), which differed in leaf surface characteristics (Sharma and Singh 2000).

Plants were treated with glyphosate (Rodeo™ 0.56 kg a.i. ha^{-1} in 188 L ha^{-1} of water), alone, or formulated with adjuvants: (a) non-ionic surfactant Ortho X-77 (0.25% v/v), a mixture of alkylaryl-polyoxyethylene glycols, free fatty acids and isopropanol; (b) organosilicone Silwet L-77 (0.1% v/v), and (c) Methylated Seed Oil, MSO (1% v/v). Measurement of static ST and CA of a range of concentrations of the three adjuvants mixed with glyphosate indicated that L-77 caused the greatest reduction in ST and CA (Figure 1).

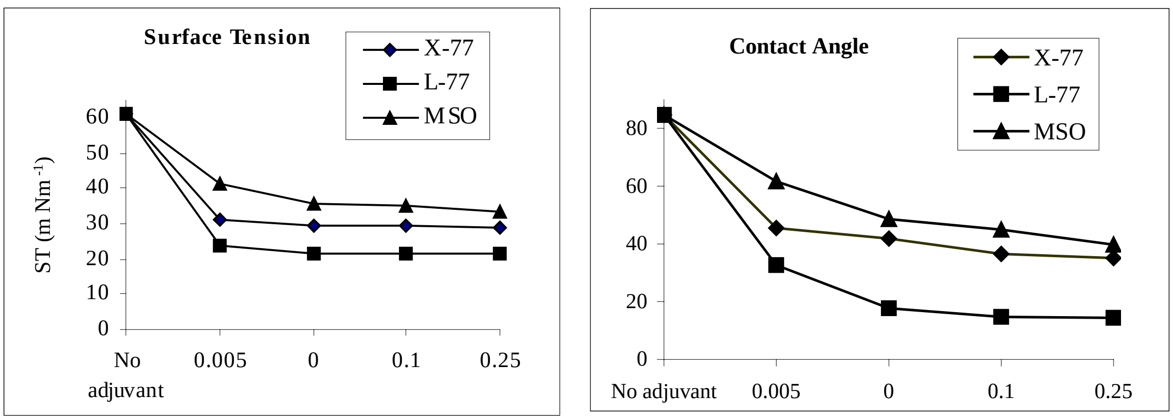

Figure 1. Effect of adjuvant concentrations on (a) Surface tension of glyphosate solutions and (b) Contact angles on a Teflon slide.

In *B. frondosa*, all three adjuvants greatly increased the uptake and translocation of ^{14}C-glyphosate (Table 3). In the presence of L-77 >50% of the applied ^{14}C-glyphosate was taken up by *B. frondosa* within 15 minutes. At 6 h, and thereafter, glyphosate uptake was significantly higher with MSO compared with X-77.

In *P. maximum*, uptake and translocation of ^{14}C-glyphosate increased with both X-77 and MSO, but not with L-77. In fact, compared with the glyphosate alone treatment, L-77 had an antagonistic effect on uptake and translocation of glyphosate in this grass species. The efficacy trial confirmed L-77 induced antagonism of glyphosate uptake by *P. maximum*.

Similar antagonism of glyphosate by L-77 in grasses has been reported (Liu and Zabkiewski 1997; Juying and Dastgheib 2001). The common explanation is that the spread of droplets caused by L-77 in the range of 0.1-0.2% is very large, and hence, the dose/leaf area covered could be too low and not conducive to cuticular penetration through diffusion. It is well known that glyphosate uptake is better from concentrated droplets. This explanation is probably true for our study as well. In addition, the concentration of L-77 (0.1%) may not have been sufficient to permit stomatal infiltration in *P. maximum*.

Both of the above effects could be dependent on the nature of the target species foliage. Usually, grass species are more susceptible to glyphosate than broad-leaved weeds; hence, this antagonism by organosilicone surfactants is significant to note.

Table 3. Effect of surfactant types on ^{14}C-glyphosate uptake and translocation in *Bidens frondosa* and *Panicum maximum*.

Treatments	*B. frondosa*				*P. maximum*			
	Uptake as % of applied							
	0.25 h	6 h	24 h	48 h	0.25 h	6 h	24 h	48 h
Glyphosate (control)	6.9	25	28	28	2.2	14	18	37
Glyphosate + X-77	29.8	45	50.1	49.9	3.5	16	23.5	32.6
Glyphosate + L-77	52.5	77	84.6	83.9	3.6	3.1	5.1	7.5
Glyphosate + MSO	17.2	43	61.5	62.4	2.2	12.5	23.1	36.8
LSD (P = 0.05)	1.2				0.8			
	Translocation as % of applied							
	0.25 h	6 h	24 h	48 h	0.25 h	6 h	24 h	48 h
Glyphosate (control)	2.5	12.3	13.0	14.2	0.9	4.1	15.9	18.1
Glyphosate + X-77	15.1	35.2	40.6	40.6	1.2	5.9	14.4	20.4
Glyphosate + L-77	30.1	65.4	70.4	70.3	1.3	1.9	3.0	5.2
Glyphosate + MSO	7.5	31.1	48.8	48.6	1.0	4.4	13.6	25.2
LSD (P = 0.05)	1.4				0.5			
	% Control							
Glyphosate (control)	36.3 (± 6.3)*				17.5 (± 2.9)			
Glyphosate + X-77	52.5 (± 11.9)				30.0 (± 4.1)			
Glyphosate + L-77	94.0 (± 6.2)				0 (± 0)			
Glyphosate + MSO	77.5 (± 9.6)				58.8 (± 4.8)			

*Numbers in parenthesis are Standard Errors of the Means.

Our study also showed the significantly increased control of both species achieved by glyphosate, assisted by MSO. The increased uptake and translocation of ^{14}C-glyphosate 6 h after treatment is indicative of either a solubilizing effect on the cuticular waxes, or humectant effect on the droplets, or both. These effects have been recorded by others as well (Wyrill and Burnside 1977) and have been reviewed by Kirkwood (1993; 1999).

Glyphosate-surfactant interactions- some field studies

In Australia, several field studies were conducted during 1999-2000, in a watershed catchment to evaluate the efficacy of surfactants on Biactive® glyphosate for the control of large weed infestations. Only Biactive® glyphosate, which does not contain additional surfactants, was registered in Australia for 'over or near water' applications.

In one Trial, the weed infestations were large stands of Lantana (*Lantana camara*) and Groundsel bush (*Baccharis halimifolia*) in the Lake Samsonvale catchment in South-East Queensland. Infestations were 2.0-3.5 m tall shrubs with dense foliage. Both species have great potential for regeneration and regrowth. Field plots were ≈50 m^2 (Lantana) or ≈25 m^2 (Groundsel) and three replicates. Lantana plots were mostly unbroken stands; Groundsel plots were dense clusters of large plants (1-5 plants m^{-2}).

Field treatments were glyphosate Biactive® (a) without surfactant (1.8 or 2.7 Kg a.i. ha^{-1}), (b) + non-ionic surfactant Agral 90™ (0.05 or 0.1% v/v), or (c) + Pulse™ (0.2% v/v), an organosilicone. Plots were treated with either 10 L $plot^{-1}$ (Lantana) or 5 L $plot^{-1}$ (Groundsel) (≈ carrier volume of 2000 L ha^{-1}). The use of slightly higher rates in this field trial was because it was possible that various cations in the lake water sources may antagonize glyphosate. Nalewaja and Matysiak (1991) reported that higher rates could overcome the influence of antagonistic salts in glyphosate spray carriers.

Single applications of Biactive® glyphosate alone were ineffective on the mature stands, as indicated by significant regrowth at 8 WAT. However, the addition of the surfactants to glyphosate significantly increased the control of both species. The increase of glyphosate efficacy caused by both rates of the non-ionic Agral 90 was significantly higher than the enhancement due to the organosilicone surfactant Pulse™.

Table 4. Glyphosate-surfactant interactions on field control of mature woody weed stands of Lantana and Groundsel.

Treatments	Lantana		Groundsel	
	Herbicide Rate (Kg a.i. ha^{-1})			
	1.8	2.7	1.8	2.7
	% Control (4 WAT)*			
Glyphosate (control)	34	56	25	48
Glyphosate + Agral 90 (0.05%)	60	90	55	90
Glyphosate + Agral 90 (0.1%)	65	95	62	95
Glyphosate + Pulse (0.2%)	64	88	38	75
LSD (P = 0.05)	5.4		6.8	
	% Regrowth (8 WAT)*			
Glyphosate (control)	100	65	100	60
Glyphosate + Agral 90 (0.05%)	35	20	25	10
Glyphosate + Agral 90 (0.1%)	20	0	25	10
Glyphosate + Pulse (0.2%)	15	0	60	25
LSD (P = 0.05)	5.0		8.2	

*visual estimation of control (0-100% control)

The organosilicone- Pulse™ increased the efficacy of glyphosate on Lantana, but the influence on Groundsel was much less. The spray containing Pulse™ spread almost immediately on the foliage of both species.

Run-off occurred from leaves of both species with Pulse™ (data not presented), but this run-off from Groundsel leaves was much more marked than from Lantana. This may explain the loss of glyphosate efficacy and the species-specific control response observed.

It is possible that the relative high carrier volume used in the trial, necessitated by the maturity of the stands, may have contributed to loss of activity due to spray run-off from Groundsel. The spread of droplets caused by L-77 may have also caused the glyphosate dose per leaf area to be very low, which would not be conducive to cuticular penetration through diffusion. If anything, these results emphasize the need to be much more mindful of the adjustment required in relation to surfactant concentrations and carrier volume, in using particularly the organosilicones, as has been pointed out (Liu and Zabkiewicz, 1997).

A second adjuvant-glyphosate efficacy Trial was conducted in Botany Wetlands in Sydney, a nutrient-enriched pond system with deeply entrenched infestations of mature, 2-2.5 m tall *Ludwigia peruviana* (Chandrasena et al 1998). The field Trial was conducted in early summer November 1997. Average air temperature was 25^0C and relative humidity 55%.

Treatments were glyphosate Biactive® (a) without adjuvant (1.8, 2.7 or 3.6 Kg a.i. ha^{-1}), (b) + Agral 90™ (0.05% v/v), (c) + Pulse™ (0.2% v/v), or (d) + Synertrol, a vegetable-oil concentrate containing 832 g L^{-1} of emulsifiable Canola oil. Three replicate plots (25 m^2 of 100% *L. peruviana*) per treatment were treated with 5 L $plot^{-1}$ (carrier volume 2000 L ha^{-1}).

The results (Figure 2) indicated the dose-dependent glyphosate control of *L. peruviana* achieved at 4 WAT, and the consistent enhancement of glyphosate efficacy by both Agral and Pulse. Incorporating the vegetable oil- Synertrol also improved the performance of glyphosate on *L. peruviana* significantly. Droplets without adjuvants wetted the leaves poorly and dried on leaf surfaces within 3-5 minutes, whereas the adjuvant-incorporated droplets were moist even after 12-15 minutes (data not presented). Pulse caused some run-off from treated leaves, but run-off was not significant in the other treatments.

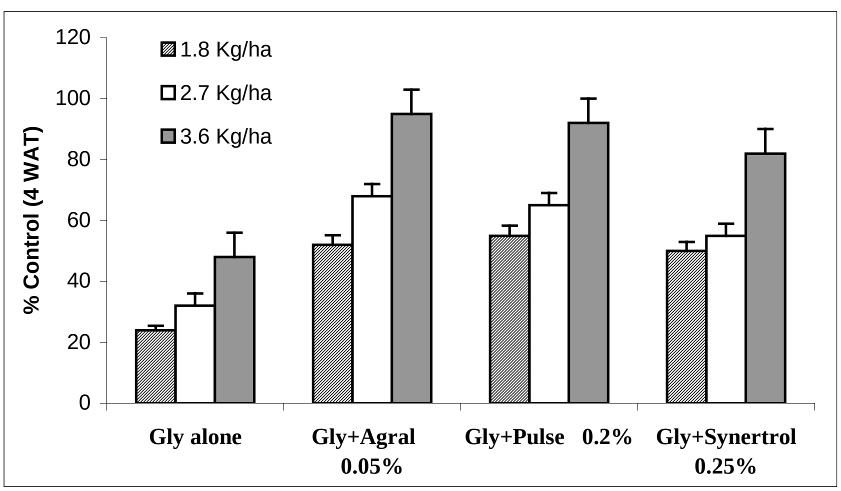

Figure 2. Effect of adjuvants on the efficacy of Biactive® glyphosate on mature stands of *Ludwigia peruviana*.

In both of the field studies reported, the increase of efficacy of Biactive® glyphosate caused by adjuvants was obvious and relatively easily explained by the observed improved wetting of target leaves and increased droplet drying times. There was evidence that Agral 90 was consistently better than others in assisting Biactive® glyphosate uptake, and that carrier volume needs to be lower to benefit fully from incorporating an Organosilicone surfactant.

The increase in glyphosate efficacy caused by the Canola oil-based Synertrol is significant, because this biodegradable adjuvant may be more acceptable to be used with Biactive® glyphosate 'in or near water' under the current conditions in Australia.

CONCLUSIONS

Efficient delivery of a.i. to target sites is a fundamental requirement for herbicide activity. In the case of glyphosate, this can be achieved by influencing the efficiency of spray retention (decreased ST and CA), cuticle penetration and tissue absorption, by using a suitable adjuvant. Selection of a suitable surfactant can reduce the degree of incompatibility between the polar glyphosate and the largely non-polar cuticle.

The herbicide-adjuvant-plant interaction is a complex system. Understanding the different roles of adjuvants in enhancing glyphosate efficacy is essential for optimum utilization of this global herbicide. Glyphosate uptake is very dose dependent, and adjusting the amount of added surfactant to control droplet spread, and hence, applied dosage per leaf, is a simple option for enhancing uptake into any species.

The mechanism of surfactant-induced transfer of glyphosate a.i. across the cuticle is still not well established and could involve one or more of the following processes: (i) change in solubility relationships and partitioning processes that are favorable to transfer, (ii) decrease in the resistance of the cuticle to diffusion, and (iii) activation of specific polar or non-polar routes through the cuticle. Our studies, among others, have established that there is a linear relationship between properties such as ST and CA, and the uptake of glyphosate. However, many glyphosate efficacy studies, including ours, show that greater herbicidal activity can be produced by slightly higher rates of surfactants.

We believe that surfactants do not simply exert their effects on plant surfaces; they could penetrate through the waxy cuticle into the underlying tissues, thereby assisting the uptake of glyphosate. Other adjuvants, such as vegetable oils, may provide for greater uptake of glyphosate by creating and prolonging conditions conducive to cuticular penetration. The interactions between plant-species, glyphosate and surfactant rates are not simple, and need to be considered when planning weed control programs, so that the ultimate aim of reducing the amount of glyphosate used in the world and costs incurred, can be reduced.

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