THE MECHANISM OF ACTION OF SILWET L77^R IN IMPROVING THE PERFORMANCE OF GLYPHOSATE APPLIED TO PERENNIAL RYEGRASS

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Summary. Addition of the surfactant Silwet L77^R at 0.1% (v/v) to formulated glyphosate increased the rate of glyphosate uptake into leaves of perennial ryegrass, *Lolium perenne* L. Silwet L77^R did not alter the fraction of absorbed glyphosate that was translocated and partitioned to plant organs. Silwet L77^R plus formulated glyphosate gave a contact angle with the upper leaf surface of 0°. The increased rate of uptake, as determined by fluorescence, resulted from greater stomatal entry.

INTRODUCTION

One approach to improving the efficacy of herbicides is by changing the formulation to facilitate improved retention, uptake or translocation characteristics. Glyphosate is a highly water soluble herbicide with excellent translocation characteristics but generally limited foliar uptake (4). The addition of surfactants and other adjuvants may improve the performance of formulated glyphosate in several species, although results are frequently variable (2, 6, 10). The explanation of improved herbicide performance following surfactant addition usually relates to improved leaf wetting, cuticle penetration (4) or transfer across the plasmalemma of epidermal cells (10).

The efficacy of glyphosate for the full season control of perennial ryegrass *Lolium perenne* L. is poor and there is a marked period of tolerance in spring which may be overcome by aditional surfactant (2, 8). Triton X 45^R (M. Willocks, pers. comm.), Agral LN^R (8), and Silwet L77^R (2) have proved effective in reducing glyphosate rates for full season control. Silwet L77^R has a number of interesting characteristics and this paper describes experiments which determine its mechanism of action in improving glyphosate performance.

METHODS

All experiments were conducted in a controlled environment chamber maintained at 15°C, a photoperiod of 12 h, with a PPFD of 300 $\mu Em^{-2}\ s^{-1}$ and 75% RH. Plants of perennial ryegrass, *Lolium perenne* L. cv. Grasslands Ruanui, were raised in pots filled with washed river sand and watered twice weekly with a complete nutrient solution. Typically plants were treated when they possessed three fully expanded leaves on the first tiller and 2-8 daughter tillers. All experiments were randomised block designs with at least six replicates. An analysis of variance was carried out to calculate standard errors of the means.

^{14}C-glyphosate solutions were formulated as the isopropylamine salt to be equivalent to 0.72 kg a.i. ha^{-1} glyphosate (as Roundup^R) in 250 L ha^{-1}, $\pm$ the surfactant (Silwet L77^R; Union Carbide Co.) at 0.1-0.5% (v/v). Uptake (Table 1) and translocation (Table 2) experiments involved application of two 1 μl droplets (2,000 and 25,000 d.p.m., respectively) to the mid section of the upper surface of the lamina of the youngest expanded leaf on the main tiller. Uptake was determined by washing the treated leaf with water, while

translocation involved water extraction of glyphosate from plant tissue fractions (treated leaf, other leaves on treated tiller, daughter tillers and roots) that had been previously ground in liquid nitrogen (1). All uptake data are expressed as a percentage of the total activity applied. In the experiment described in Table 2, glyphosate uptake was calculated as described, retention by the treated leaf was expressed as the radioactivity recovered from the treated leaf as a percentage of the total absorbed and relative partitioning to the various plant fractions as a percentage of the total radioactivity exported from the treated leaf.

Contact angle determinations were made by projecting the image from 3 *ul* droplets applied to the leaf surface (3). A minimum of 20 replicates was used in these experiments.

Stomatal penetration of glyphosate solution was visualised by adding 1 mg ml^{-1} sodim fluorescein to solutions of 0.72 kg ha^{-1} glyphosate $\pm$ Silwet L77^R. Single 1 *ul* droplets were applied to the upper lamina surface and washed off after 10 or 60 seconds, to remove surface solution before examination of the leaf surface under a UV microscope at 160-400 fold magnification.

RESULTS AND DISCUSSION

Addition of surfactant to glyphosate (formulated as Roundup^R) reduced surface tension of the solution and increased leaf surface wetting. Complete wetting of the leaf surface occurred in 5-20 seconds with 0.05% Silwet L77^R and in 2-5 seconds at higher surfactant concentrations. The application of formulated glyphosate to the upper leaf surface, at a concentration that gave 0.72 kg ha^{-1} when applied in 250 L ha^{-1}, gave a contact angle of 118°; higher concentrations did not reduce the contact angle. Application of 0.72 kg ha^{-1} glyphosate to the lower leaf surface gave a contact angle of 36° which was not significantly reduced by addition of 0.1% Silwet L77^R.

The addition of 0.1% Silwet L77^R to ^{14}C-glyphosate initiated more rapid uptake into the leaf, with a rise from 18 to 37% after 3 h (Table 1). After this time the rate of uptake from the surfactant treatment declined while the uptake of glyphosate alone continued. By 21 h approximately 40% of applied glyphosate was taken up from both treatments.

Table 1. The effect of applied Silwet L77^R surfactant on the absorption of glyphosate by the upper leaf surface of perennial ryegrass (% of applied ^{14}C-glyphosate)

Silwet L77^R (% v/v)	Time after glyphosate application (h) 0.5	1	2	3	21	45
0	2	5	16	18	39	42
0.1	6	16	28	37	40	50
s.e.m.			4.7			

Uptake of glyphosate from the lower leaf surface was very low, being less than 3% after 3 h, irrespective of whether or not 0.1% Silwet L77^R was added.

Increasing the uptake period to 5 h did not significantly influence the quantity of glyphosate absorbed, although by 48 h 11.5% was absorbed from control treatments and 4.5% from those containing surfactant.

It is clear from the data in Table 1 that addition of 0.1% Silwet L77^R increased the initial rate of uptake of glyphosate from the upper leaf surface. In a shorter term experiment it was shown than 3.3% of applied glyphosate was taken up within 5 minutes and measurable uptake occurred within 10 seconds. Uptake after 3 minutes could be increased to 16.5% by using 0.5% Silwet L77^R.

The speed of uptake suggested entry of solution into protected compartments of the leaf via stomata. The addition of sodium fluorescein to application solutions allowed observation of the movement of the herbicide solution (± surfactant) through the stomata and into adjacent cells. There was no fluorescence by sub-cuticular tissues in the absence of surfactant. Addition of 0.1% Silwet L77^R gave some stomatal infiltration but this was considerably less than that found for 0.5% Silwet L77^R.

With 0.1% Silwet L77^R, translocation of glyphosate from the treated leaf was greater at 27 h than in the control, although there was no change in partitioning to other plant components (Table 2). By 75 h total leaf uptake, translocation from the treated leaf and partitioning of radioactivity between the tillers and roots was similar for the control and 0.1% Silwet L77^R treatments. The higher rate of Silwet L77^R (0.5%) inhibited uptake and translocation of glyphosate but did not influence the pattern of partitioning to plant components.

Table 2. The effect of Silwet L77^R surfactant on the uptake, retention by treated leaf (% applied ^{14}C-glyphosate), and partitioning of glyphosate to other plant components (% of ^{14}C-glyphosate exported from treated leaf).

Time after application (h)	Silwet L77^R (% v/v)	Uptake by treated leaf	Retention by treated leaf	Partitioning of translocated radioactivity		
				Treated tiller	Other tillers	Roots
27	0	37	55	14	34	52
	0.1	37	35	17	28	55
	0.5	25	67	19	26	55
75	0	42	34	12	32	56
	0.1	35	36	21	28	51
	0.5	25	61	13	26	61
s.e.m.		3.2	4.6	2.9	4.3	5.7

When Silwet L77^R was added to commercially formulated glyphosate and applied to the upper leaf surface there was total surface wetting and rapid uptake of ^{14}C-glyphosate (Table 1). The fact that Silwet L77^R failed to increase the total amount of ^{14}C-glyphosate absorbed by, and translocated from, the

treated leaf suggests that leaf permeability, including penetration of the cuticle and/or plasmalemma, was not significantly influenced. One possible explanation involved the role of MON 0818, the surfactant present in all formulated glyphosate solutions. This surfactant has been found to be particularly effective at improving the phytotoxicity of glyphosate compared with other surfactants, despite being shown to be an ineffective wetter, both in the present study and others (10). It is likely that in the present experiments MON 0818 was effective in promoting significant leaf penetration of ^{14}C-glyphosate, so that the predominant effect of Silwet L77^{R} was to increase the contact area for uptake, but not necessarily the absorption per unit wetted area.

The rapid uptake of glyphosate in the presence of Silwet L77^{R} occurred only after application to the upper leaf surface. This surface is stomatous and has a dense arrangement of crystalline wax, unlike the lower surface which is astomatous and is covered with an amorphous wax sheet (1). Crystalline surfaces are difficult to wet (1); this was confirmed by the present study. Clearly the principle effect of Silwet L77^{R} was to overcome the highly hydrophobic nature of the upper leaf surface, as it reduced the contact angle from 118° to zero. Complete wetting of the lower leaf surface was not apparent, nor was there an improvement in the rate, or total mass of ^{14}C-glyphosate absorbed. The result is significant because it confirms that in the absence of complete leaf surface wetting and stomata, Silwet L77^{R} did not improve uptake by increasing the permeability of the cuticle.

The foregoing conclusion, plus the UV microscope observations following the inclusion of sodium fluorescein in the application solution, appear to confirm that a proportion of the increased uptake promoted by Silwet L77^{R} was a consequence of stomatal entry by solution rather than effects on cuticular penetration. Aqueous solutions with a surface tension approaching water do not pass through stomatal pores, although it has been demonstrated that entry may occur when the contact angle of the solution is less than the wall angle of the stomatal aperture (9). The addition of 0.1% Silwet L77^{R} reduced the solution contact angle to zero within 5 seconds and clearly meets the stated criteria. Commercial surfactants rarely reduce contact angles to values close to zero, as efficacy of the herbicide may be reduced, owing to solution run-off from the leaf, or rapid drying. However, the addition of Silwet L77^{R} to formulated glyphosate has been shown to greatly improve field performance in the full season control of perennial ryegrass and may reduce the required use rate of glyphosate (2).

The concentration of Silwet L77^{R} in spray solution is important as at 0.5% it impaired glyphosate uptake and subsequent translocation from treated leaves (Table 2). Although 0.5% Silwet L77^{R} promoted more rapid leaf wetting and initial uptake of glyphosate than 0.1%, it was phytotoxic (2), suggesting that it entered the symplast of the leaf. The mode of action of Silwet L77^{R} is confined to interactions at the leaf surface and subsequently interference with the function of leaf cells, including those involved in phloem translocation.

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