Influence of Two Fatty Amine Surfactants on Foliar Absorption, Translocation, and Efficacy of 2,4-D Triethanolamine Salt

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The influence of two fatty amine surfactants on the foliar absorption, translocation, and efficacy of 2,4-dichlorophenoxyacetic acid triethanolamine salt (2,4-D TR) was investigated. The surfactants Armoblen 557 (tallow amine block polymer containing polymerized propylene oxide and polymerized ethylene oxide) and Ethomeen T/27 [polyoxyethylene (17) tallow amine] enhanced the foliar absorption of 2,4-D TR by black nightshade (*Solanum nigrum* L.) by a factor of 4, when applied at a concentration of 0.5% (w/v). Applying the surfactants at a concentration of 0.05% (w/v) had no effect on the absorption of 2,4-D TR. The surfactants reduced the translocation efficiency of ^{14}C from 2,4-D TR in black nightshade by a factor of 1.5–2. The two surfactants (at 0.05% and 0.5%) greatly enhanced the efficacy of 2,4-D TR applied at a concentration of 2.4 mM in the spray solution. At a 2,4-D TR concentration of 11.3 mM the two surfactants enhanced efficacy at a concentration of 0.5% but not at a concentration of 0.05%. The data on the efficacy of 11.3 mM 2,4-D TR agree with the ^{14}C uptake study, which also used 2,4-D TR at 11.3 mM. It was concluded that changing the hydrophilic/lipophilic character of the fatty amine surfactant by incorporation of propylene oxide groups did not influence absorption and efficacy of 2,4-D TR.

Keywords: *2,4-D salt; surfactant; foliar uptake; efficacy*

INTRODUCTION

Adding the polyoxyethylene alkylamine type of surfactants to spray solutions enhances the efficacy of the herbicides glyphosate (Wyrill and Burnside, 1977; Turner and Loader, 1980; Sherrick et al., 1986b) and sethoxydim (Kudsk et al., 1987). This improved performance of the herbicides can partly be explained by the surfactant-induced enhancement of spray retention, which depends on the wettability of the leaf surface and the surfactant concentration (de Ruiter et al., 1992). The polyethoxylated alkyamines can also improve transcuticular uptake, as has been demonstrated for the isopropylamine salt of glyphosate (Sherrick et al., 1986a; de Ruiter et al., 1988; Gaskin and Holloway, 1992) and the triethanolamine salt of 2,4-D (de Ruiter et al., 1993).

The hydrophilic/lipophilic character of a surfactant containing ethylene oxide (EO) can be influenced by propylene oxide (PO) groups being incorporated into the hydrophilic region of the surfactant molecules via block polymerization. In a previous study (de Ruiter et al., 1993) we demonstrated that a tallow amine containing ethylene and propylene oxide groups (applied at 0.5% w/v) enhanced the foliar uptake of 2,4-dichlorophenoxyacetic acid triethanolamine salt (2,4-D TR). In this study we investigated whether modification of the hydrophilic portion of tallow amine surfactants can be exploited to optimize the foliar uptake and efficacy of the 2,4-D triethanolamine salt applied to black nightshade (*Solanum nigrum* L.). Gaskin and Holloway demonstrated that the concentration of surfactant required to achieve maximal surfactant-induced foliar uptake of the herbicide glyphosate depends on the EO content of the alkylamine type of surfactant (Gaskin and Holloway, 1992). Therefore, we used two surfactant concentrations (0.05% and 0.5% w/v) in this study.

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The predictive value of herbicide absorption data for efficacy may be obscured by surfactant-induced spray retention. In a previous study with black nightshade (de Ruiter et al., 1990) we demonstrated that surfactants used in a concentration range from 0.01% to 1% (w/v) did not have a pronounced influence on spray retention. Therefore, we considered black nightshade as an appropriate species for comparing the influence of surfactants on the absorption and efficacy of 2,4-D TR.

MATERIALS AND METHODS

Absorption and Translocation. A full description of the plant material, herbicide solutions, plant treatment, and processing of the plant fractions has been published (de Ruiter et al., 1993). Essential aspects and minor modifications of that procedure are described here.

The black nightshade plants were grown hydroponically in a growth chamber under 14 h of light, 18/12 (±0.5) °C (day/night) temperature, and 70/80 (±5)% (day/night) relative humidity. The experiments with ^{14}C-labeled herbicides were performed in an identical growth chamber under the same conditions, except that the night temperature was 18 °C.

The term 2,4-D triethanolamine salt (2,4-D TR) was used to describe the salt from 2,4-D acid and tris(2-hydroxyethyl)amine. The concentrations of 2,4-D TR (labeled plus unlabeled) were 11.3 mM (specific activity 29.47 MBq $mmol^{-1}$), which is equivalent to the molarity of 2,4-D acid when this compound is applied at a rate of 1 kg ha^{-1} at a spray volume of 400 L ha^{-1}. 2,4-Dichlorophenoxy[2-^{14}C]acetic acid triethanolamine salt (Amersham, purity >96%; specific activity 437 MBq $mmol^{-1}$) and unlabeled 2,4-D TR were dissolved in acetone plus water (1 + 3 by volume). The surfactants were added on a weight-to-volume basis at concentrations of 0.05% and 0.5% (w/v). The experiments described in this study were part of a program using several species. Acetone was added to the 2,4-D TR solutions to facilitate the application of drops to difficult to wet leaf surfaces. Acetone did not influence the foliar uptake of 2,4-D TR by black nightshade, nor did it change the leaf surface (cryo-SEM).

The solutions were applied to the leaf surface as five 1-μL drops (334 Bq μL^{-1}) to a discrete area (diameter < 1 cm) on

Table 1. Properties of Surfactants Used in This Study

trade name	chemical description[a]	structural formula[a–c]	equivalent mass[a]	cmc,[a] %, w/v	surface tension (0.01% in water) mN m^{-1}	HLB[a]	cloud point[a] (1% in water), °C	pour point[a] (pure liquid), °C
Armoblen 557	polyalkoxylated (17) tallow (C_{12}–C_{18}) amine	R–N([EO]$_x$/[PO]$_a$)([EO]$_y$/[PO]$_b$)	1150	1.5×10^{-3}	36	9	19	< −10
Ethomeen T/27	polyethoxylated (17) tallow (C_{12}–C_{18}) amine	R–N([EO]$_n$)([EO]$_m$)	1000	1.5×10^{-3}	43	12	>100	<10

[a] According to the manufacturers information (AKZO-Nobel, 1995). [b] EO, ethylene oxide; PO, propylene oxide. [c] $x + y = 5$; $a + b = 12$; $n + m = 17$; products are polydisperse preparations; see also Materials and Methods (surfactants).

the adaxial surface in the median part of the leaf, outlined with waterproof ink. It took 1 h to apply all of the herbicide solutions. After 24 h, the treated leaf was washed with acetone plus water (2 mL; 1 + 3 by volume) to remove residual chemical deposits. The efficiency of the washing procedure was extensively tested. When the leaf surface was washed immediately following application, a recovery of 100% was measured, without and with each surfactant. To determine the efficacy of washing after the drops had dried, we measured the recovery of 2,4-D TR 24 h after applying the herbicide solutions (without and with each surfactant at a concentration of 0.5%) to glass slides and Parafilm (paraffin wax film) fixed onto glass slides. On Parafilm the spreading of drops was the same as that observed on the leaf surface of black nightshade. The recovery of ^{14}C was 100%. Cryo-SEM demonstrated that the leaf surface, rinsed 24 h after treatment, was clean. The tests with inert surfaces also demonstrated that there was no loss of ^{14}C via volatilization from the leaf surface.

The absorption and translocation of ^{14}C-labeled herbicide 24 h after treatment were investigated by measuring ^{14}C in the fractions: leaf surface washing; treated area; rest of the treated leaf; shoot above the treated leaf; shoot under the treated leaf; roots; and nutrient solution. The following parameters could be defined: absorption, sum of ^{14}C in the plant tissue and ^{14}C in the nutrient solution; translocation, sum of ^{14}C in the plant tissue outside the treated area and ^{14}C in the nutrient solution; translocation efficiency, translocation expressed as percentage of amount absorbed; and recovery, sum of ^{14}C in all fractions. We did not attempt to identify possible metabolites of the 2,4-D compounds.

Efficacy of 2,4-D TR. Black nightshade was grown in 11-cm diameter plastic pots (one plant per pot) filled with a mixture of sand and humic potting compost (1:2 v/v). The pots were subirrigated with the same nutrient solution as used for the ^{14}C uptake experiments. Growing conditions and growth stage at the time of treatment were the same as for those experiments. Spray solutions were applied with a laboratory track sprayer fitted with three nozzles (Birchmeier Helico Sapphire 1.2 mm provided with a whirling pin 2F-0.6 mm perforated) to give a volume of 400 L ha^{-1}. Three weeks after treatment, the shoot fresh weight was determined. The concentrations of 2,4-D TR in the spray solution were 2.4 and 11.3 mM, and each surfactant was added at 0.05% and 0.5%. The spray solutions did not contain acetone; demineralized water was used as solvent.

Surfactants. Armoblen 557 (polyalkoxylated tallow amine) is the block polymerization reaction product (Table 1) of tallow amine (1 mol) with the alkylene oxides PO (approximately 12 mol) and EO (approximately 5 mol). Ethomeen T/27 [polyethoxylated (17) tallow amine] is the polymerization reaction product of tallow amine (1 mol) with EO (approximately 17 mol). As can be seen from the physical chemical parameters [hydrophilic/lipophilic balance (HLB), cloud point, and surface tension], this product is significantly more hydrophilic than Armoblen 557. The HLB values have been calculated for the unprotonated form of the surfactants; the effect of protonation on HLB is the same for both surfactants, and therefore the difference in HLB will not change (AKZO-Nobel, personal communication, 1995). The tallow amine surfactants are polydisperse preparations containing compounds that differ in the length of the alkyl chain (C_{12}–C_{18}) and in the number of EO and PO units.

The surface tension of surfactant-containing solutions (Table 1) was measured according to the du-Noüy ring method (Harkins and Jordan, 1930) at 20 °C.

Partition Coefficient of 2,4-D TR. The log K_{ow} of 2,4-D and 2,4-D TR was determined between mutually saturated phases of 1-octanol (Sigma-Aldrich, HPLC grade, purity >99%) and 0.1 M potassium phosphate buffer (pH 7.0) at 20 °C (±1 °C). Partition was measured with 1.5 mL Eppendorf tubes, and the volume of each phase was 0.6 mL. The 2,4-D and 2,4-D TR were dissolved in the aqueous phase at concentrations of 0.1, 1, and 10 mM. The compounds were partitioned by vortex mixing for 30 s; the mixtures were then allowed to stand for 2 h, and phase separation was completed by centrifuging at 60*g* for 120 s. Aliquots (400 μL) of both phases were carefully sampled, and the concentration of the compounds was determined by liquid scintillation spectrometry. Two experiments, each with three replicates, were carried out on different dates. The mean value of ^{14}C recovery for each log K_{ow} determination varied between 99.3% and 100.2% (n = 6; SE < 1.8%).

Experimental Design. The absorption and translocation experiment was conducted four times over a period of 2 months. The applications of one experiment were made according to a completely randomized design. The class variables within each experiment were surfactant type (no surfactant, Armoblen 557, and Ethomeen T/27) and surfactant concentration (0%, 0.05%, and 0.5%). An analysis of variance was performed on the data of the four replicates. The means of each fraction (plant parts and the parameters uptake, translocation and translocation efficiency) were compared according to Fisher's lsd ($P = 0.05$) test.

Two separate efficacy experiments were conducted, each with six completely randomized blocks. The class variables within each block were herbicide concentration (2.4 and 11.3 mM), surfactant concentration (0%, 0.05%, and 0.5%) and surfactant type (no surfactant, Armoblen 557, and Ethomeen T/27). An analysis of variance was performed on the data after logarithmic transformation (natural logarithm). The means of individual treatments were compared according to Fisher's lsd ($P = 0.05$) test. Single degree of freedom contrasts ($P = 0.05$) were investigated between groups of treatments or between a group of treatments and an individual treatment. Statistically significant differences between the individual treatments (Figure 1) were determined from the least significant ratio (lsr = e^{lsd}).

RESULTS AND DISCUSSION

Recovery of Radioactivity. The mean values of ^{14}C recovery (across replications) ranged from 85% to 100% (Table 2). Signifcant loss of ^{14}C was observed in plants treated with 2,4-D TR in the presence of Armoblen 557 at a concentration of 0.5% (recovery = 85%), and nonsignificant loss was observed with Ethomeen T/27 at 0.5% (recovery = 92%). Release of [^{14}C]carbon dioxide, caused by degradation of the active compound (Naylor, 1976), may account for this loss. Loss of ^{14}C in plants treated with 0.5% surfactant may be due to the finding that these plants absorbed much more 2,4-D TR. The same trend was also observed in our previous

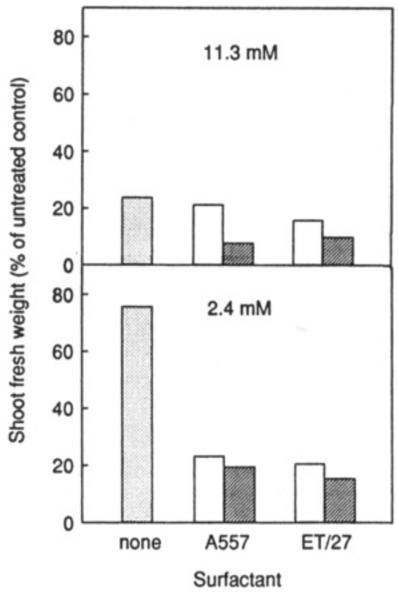

Figure 1. Influence of surfactant type (A557, Armoblen 557; ET/27, Ethomeen T/27) and concentration (open bar, 0.05%; shaded bar, 0.5%) on the efficacy of 2,4-D-triethanolamine salt against *S. nigrum*. The concentrations of 2,4-D TR in the spray solutions were 11.3 and 2.4 mM. lsr ($P = 0.05$) = 1.4.

study with black nightshade (de Ruiter et al., 1993). The ^{14}C in the nutrient solution did not exceed the amount detectable with our procedure (8% of the total ^{14}C applied). This indicates that root exudation contributed little or nothing to the loss of ^{14}C from the plants.

Octanol/Water Partition Coefficient of 2,4-D TR. At pH 7.0 and averaged over three concentrations, the log K_{ow} value was −0.72 ($n = 18$; SE = 0.02) for 2,4-D and −0.74 ($n = 18$; SE = 0.02) for 2,4-D TR. At a concentration of 0.1 mM the partition coefficients of both compounds were 0.15 log K_{ow} unit lower than the values obtained at 1 and 10 mM. The comparison of 2,4-D and 2,4-D TR showed that the triethanol ammonium ion did not influence the partition of the 2,4-D anion. Using the potentiometric titration method, a value of −0.79 was obtained for 2,4-D at pH 7.0 (Sirius Analytical Instruments, England, personal communication, 1994) which agrees with our result. The log K_{ow} we measured at pH 7.0 is much lower than the value of 2.90 reported for the undissociated 2,4-D acid (Briggs et al., 1987). This agrees with the expectation that at pH 7.0 the 2,4-D acid is almost completely dissociated, as can be calculated from its dissociation constant [pK_a = 2.87 (Kennedy and Stewart, 1980)]. The partition measurements demonstrated that the anion of 2,4-D TR is a relatively hydrophilic compound.

Influence of the Surfactants on the Absorption of 2,4-D TR. The two surfactants enhanced the foliar absorption of 2,4-D TR by black nightshade by a factor of 4 when applied at a concentration of 0.5% but had no effect when applied at 0.05% (Table 2). The modification of the hydrophilic region of the surfactant molecules did not give rise to differences between the surfactants.

The pH of the solutions containing 2,4-D TR was 6.5 (without surfactant and with surfactant at 0.05%) or 7.3 (with surfactant at 0.5%). Thus, the concentration of the more lipophilic 2,4-D acid [log K_{ow} = 2.90 (Briggs et al., 1987) and pK_a = 2.87 (Kennedy and Stewart, 1980)] in all treatment solutions was negligible. Visual assessment of drop spreading and drying time did not show differences between the surfactants and the surfactant concentrations. These observations suggest that enhanced permeability of the cuticle and/or enhanced uptake capacity of the plant tissue may be relevant in the observed influence of the surfactant concentration.

Table 2. Influence of Surfactants on Absorption and Translocation of [^{14}C]-2,4-D Triethanolamine Salt in *S. nigrum* L. after 24 h

	% of ^{14}C applied				
		A557[a]		ET/27[b]	
fraction	control	0.05%	0.5%	0.05%	0.5%
uptake	8.0 a[c]	8.0 a	32.4 b	5.4 a	30.7 b
translocation	3.2 a	2.7 a	7.9 c	2.6 a	5.9 b
trans/uptake × 100%	44.0 bc	41.5 abc	27.8 ab	54.1 c	25.2 ab
recovery	100.3 b	94.4 b	84.5 a	100.2 b	91.9 ab

[a] A557, Armoblen 557. [b] ET/27, Ethomeen T/27. [c] Horizontal rows: means followed by the same letter do not differ at the 5% probability level (Fisher's lsd test).

It was demonstrated that the EO content of C_{13}/C_{15} 1-alkanols and 4-nonylphenol surfactants had little influence on the surfactant-induced uptake of 2,4-D sodium salt (Holloway and Edgerton, 1992). In our study, partial substitution of EO by PO does not seem relevant to uptake enhancement; thus, surfactant lipophilicity does not seem to influence the extent of uptake enhancement of 2,4-D salts. This outcome and the measured log K_{ow} of −0.7 for 2,4-D TR indicate that 2,4-D salts fall into the "critical log K_{ow} range" mentioned in the description of an uptake model (Stock et al., 1993). The critical log K_{ow} range has been defined as the range in which surfactant lipophilicity (based on EO content) does not strongly influence the uptake enhancement of compounds; its upper and lower limits may vary according to plant species (Stock et al., 1993). The contribution of the nitrogen atom to the hydrophilicity of ethoxylated alkylamines need not weaken greatly the influence of EO content as indicated in a previous study (Gaskin and Holloway, 1992). These authors demonstrated that reducing of the EO content of ethoxylated alkylamines to 5 or 10 mol reduced the surfactant-induced uptake of the polar compound glyphosate by field bean and wheat. In view of this result and the proposed model (Stock et al., 1993) it is clear that uptake experiments with more polar compounds (log $K_{ow} < -3$) would elucidate whether changing the surfactant lipophilicity by partial substitution of EO by PO has the same effect as changing the EO content.

The foliar uptake of 2,4-D TR into black nightshade strongly depended on the surfactant concentration (Table 2). For a polyethoxylated (10) C_{13}/C_{15} 1-alkanol, Holloway and Edgerton (1992) found that a threshold level (>0.05% w/v) had to be exceeded to enhance the uptake of 2,4-D sodium salt by wild oat and field bean. An explanation for this dependence on concentration cannot be derived from our data, but we suspect that the surfactant concentration may influence hydration of the deposit, the mobility and partitioning of the 2,4-D anion in the cuticle-surfactant system, and the uptake capacity of the plant tissue. It was demonstrated (Schönherr, 1993) that alcohols and ethoxylated alcohols enhance the mobility of 2,4-D acid in bitter orange cuticle (*Citrus aurantium* L.), but it is doubtful whether the comparatively lipophilic 2,4-D acid behaves similarly to the 2,4-D anion. Experiments on isolated apple leaf cuticles led to the suggestion that surfactants create additional hydrophilic regions in the cuticle which enhance the passing of the 2,4-D anion (Tan and Crabtree, 1994).

Influence of Surfactants on the Translocation Efficiency of 2,4-D TR. Addition of the surfactants at a concentration of 0.5% reduced the translocation efficiency of ^{14}C from 2,4-D TR by a factor of 1.5–2 (Table 2).

The analysis of ^{14}C in the different fractions (data not shown) after black nightshade had been treated with 2,4-D TR indicates that the extent of the translocation of 2,4-D TR from the treated area to the rest of the plant is limited when the absorption is enhanced by surfactant (Table 2). This observation supports the suggestion made in a previous study (Wolf et al., 1992) on the absorption and translocation of 2,4-D dimethylamine salt in oriental mustard (*Sisymbrium orientale*). The authors suggested that localized damage to the conducting tissue of the plant caused by an increased concentration of the herbicide and/or the formulation ingredients may reduce the herbicide translocation from the treated leaf. The cationic surfactants used in this study are phytotoxic: after 48–72 h, necrotic lesions were observed on the places where 1-μL drops (without and with 2,4-D TR) were applied. The preceding penetration of surfactant into the leaf tissue may have contributed to the reduction of translocation efficiency, measured after 24 h. A surfactant similar to Armoblen 557 reduced translocation efficiency, but this was no longer observed after 48 h (de Ruiter et al., 1992). Thus, reduced translocation efficiency may be temporary and not result in a corresponding reduction in efficacy.

Influence of the Surfactants on the Efficacy of 2,4-D TR. Application of 11.3 mM 2,4-D TR without surfactants severely inhibited growth (Figure 1). The addition of either surfactant at 0.5% inhibited growth more than 2,4-D TR alone. The level of inhibition attained by adding 0.05% of either surfactant did not differ from inhibition caused by herbicide without surfactant. The individual surfactants did not differ in their ability to enhance efficacy. Despite differences in application method (individual drops vs spray) and growth medium (hydroponic vs soil), the efficacy data agree with the outcome of the ^{14}C uptake study.

Application of 2,4-D TR alone at a concentration of 2.4 mM inhibited growth less than a concentration of 11.3 mM. Both concentrations of either surfactant similarly enhanced the efficacy of 2.4 mM 2,4-D TR. Similar levels of growth inhibition were caused by 2.4 mM 2,4-D TR plus either surfactant and 11.3 mM 2,4-D TR without surfactant. This result demonstrates the capacity of the surfactants to enhance the phytotoxicity of 2,4-D TR. Application of the surfactants alone resulted in moderate (0.05%) and severe (0.5%) contact damage (necrosis) but did not inhibit growth.

The efficacy data indicate that both surfactant concentrations have the same influence on the foliar uptake when 2,4-D TR is applied at a concentration of 2,4 mM. Additional foliar uptake experiments with soil-grown plants (application of 2,4-D TR at 2.4 mM and using Ethomeen T/27) confirmed this supposition: both surfactant concentrations increased the foliar uptake by a factor of 10 (the uptake without surfactant was 2.4% of ^{14}C applied). Apparently, the influence of surfactant concentration on efficacy and foliar uptake depends on the concentration of the herbicide. On the basis of the equivalent mass of Ethomeen T/27 (Table 1), the following molar ratios of active ingredient to surfactant can be calculated (surfactant concentration in parentheses): 4.8 (0.05%) and 0.48 (0.5 %) at 2.4 mM 2,4-D TR, and 22.6 (0.05%) and 2.26 (0.5%) at 11.3 mM 2,4-D TR. We suggest that the surfactants will enhance foliar uptake when the molar ratio is below a certain threshold level. At this stage, we therefore tentatively suggest that the cationic character of the ethoxylated fatty amines enables the surfactant and the 2,4-D anion to copenetrate. This would explain why more surfactant is required to enhance uptake at a higher concentration of 2,4-D TR.

The surfactant-induced uptake and efficacy of 2,4-triethanolamine salt, demonstrated in this study, indicates that a combination of surfactant and 2,4-D triethanolamine salt could be a suitable replacement for volatile 2,4-D ester formulations. Efficacy studies in the greenhouse (unpublished data) demonstrated that Armoblen A557 and Ethomeen T/27 also enhanced the phytotoxicity of 2,4-D TR against other plant species including the weed common lambsquarters (*Chenopodium album* L.). The weed species black nightshade (*S. nigrum* L.) appeared to be a good test plant for screening combinations of adjuvant and herbicide. Its relatively low cuticle permeability and its easily wettable leaf surface make it very suitable for investigating the capacity of adjuvants to promote foliar uptake.

ABBREVIATIONS USED

EO, ethylene oxide; PO, propylene oxide; HLB, hydrophilic/lipophilic balance; 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4-D TR, 2,4-dichlorophenoxyacetic acid triethanolamine salt.

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