Bentazone – surfactant interactions in three selected weed species of differing leaf characteristics

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Summary The plant cuticle is a complex membrane, which covers the outer surface of the aerial portion of plants. It may act as a potential barrier to the penetration of foliage-applied herbicides. The beneficial effect of surfactants as adjuvants to facilitate spray droplet retention and cuticle penetration is well established, though the criteria influencing choice of surfactant is less so. This paper will report a study involving a series of fifteen non-ionic surfactants on the activity, uptake and translocation of a polar herbicide (bentazone; Log K_{ow} 0.45) when applied to three annual weed species of differing leaf surface characteristics. The surfactants belonged to the Plurafac®, Pluronic® and Lutensol® series (registered trademarks of BASF).

The influence of these surfactants on the activity of bentazone was assessed by visual symptoms, membrane leakage (conductivity) and tissue succulence (fresh-weight/dry-weight). These effects were related to the physicochemical properties of the surfactants. Effects on the uptake and translocation of ^{14}C (bentazone) were evaluated over the initial 24 h treatment period. The results showed that surface properties and uptake of ^{14}C (bentazone) were enhanced by surfactants of low to medium hydrophilic-lipophilic balance (HLB) characterised by radical groups, low cloud point, a mean ethylene oxide (EO) number of <10 and no propylene oxide (PO) groups. A series of statistical analyses including ANOVA, MANOVA, Student Newman-Keuls, Multiple Regression and Partial Least Squares were performed.

It was concluded that these surfactants may act as co-solubilisers/co-penetrators as well as influencing surface properties. Surfactant effects on systemicity of ^{14}C (bentazone) were normally indirect, resulting from enhanced absorption. When expressed as percentage of the absorbed dose of ^{14}C (bentazone) however, translocation was enhanced by selecting surfactants of higher EO number, particularly by members of the Lutensol and Pluronic groups. The study exemplifies the factors that can influence the target site delivery of such polar herbicides and underlines the importance of the appropriate choice of surfactant.

Keywords Surfactants, mode of action, selectivity, bentazone, weeds.

INTRODUCTION

The cuticle surface waxes of plants represent an initial barrier to the penetration of xenobiotics and their role has been well documented (Holloway 1993, Riederer and Schreiber 1995, Jeffree 1996, Sharma *et al*. 1996, Kirkwood 1999). The solubility and diffusivity of an active ingredient (AI) in the cuticular waxes may be enhanced and modified by non-ionic alcohol ethoxylates.

In this paper, the effect of a series of fifteen non-ionic surfactants on droplet characteristics, uptake and translocation of ^{14}C (bentazone Na^+) is reported. Three test species of differing leaf surface characteristics and susceptibility to bentazone (BASF) were used and the influence of surfactant incorporation on the activity (visual, succulence, tissue membrane permeability) of bentazone assessed. A series of statistical analyses were carried out to examine the relationships between the physicochemical characteristics of these surfactants and the measured physiological responses.

MATERIALS AND METHODS

Materials Bentazone Na^+ (3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide-[phenyl-U-^{14}C] (BASF) was used as a test herbicide. It has a molecular mass of 240.28 g mol^{-1} and a log K_{ow} of 0.45. Fifteen non-ionic surfactants, belonging to the Lutensol®, Plurafac®, and Pluronic® series (BASF) were used, varying in structure in respect to the mean number of ethylene oxide (EO) and propylene oxide (PO) units and hydrophilic:lipophilic (HLB) values. Their physicochemical properties are described in Table 1.

The test species used were *Chenopodium album* L. which has a waxy surface and many glandular hairs (trichomes), *Chrysanthemum segetum* L. which has a waxy surface and *Sinapis arvensis* L. with little surface wax and some trichomes. These species are moderately resistant (MR) moderately susceptible (MS) and susceptible (S) respectively to bentazone.

Plant growth conditions Seeds of the test species were sown in John Innes No. 2 compost and grown in glasshouse conditions during winter ('winter' plants)

Table 1. Surfactant physicochemical parameters (obtained from BASF).

Surfactant	Abbreviation	ST	CA	HLB	EO	PO	R	WeC	CP	CMC
Plurafac LF400	LF4	30.8	43	8	6.3	4	14	25	45a	0.0011
Plurafac LF600	LF6	34.4	49	10	12	6	14	65	57a	0.00074
Plurafac LF700	LF7	32.5	49	4.2	4.6	9.1	17	170	29a	0.001
Pluronic PE6200	PE62	44.4	77	4	9.7	30.1	0	300	54a	0.25
Pluronic PE6400	PE64	44.5	80	8	26	30.1	0	300	69a	0.25
Pluronic PE6800	PE68	48.5	86	16	156	30.1	0	300	95a	0.25
Pluronic PE9200	PE92	36.9	69	4	15.6	47.3	0	120	49a	0.25
Lutensol ON30	ON30	30.7	59	9	3	0	10	30	53a	0.024
Lutensol ON50	ON50	35.5	70	11.5	5	0	10	20	67a	0.073
Lutensol AO3	AO3	27.2	43	8	3	0	14	300	45a	0.0079
Lutensol AO5	AO5	28.7	40	10	5	0	14	100	62a	0.0035
Lutensol AO7	AO7	28.6	38	12	7	0	14	30	43b	0.002
Lutensol AO10	AO10	31.8	52	13.5	10	0	14	60	80b	0.0013
Lutensol AT11	AT11	37.3	68	13	11	0	17	300	87b	0.019
Lutensol AT25	AT25	45.5	79	16	25	0	17	300	100b	0.25

ST = surface tension (nN m^{-1}) with 0.25% surfactant; CA = contact angle (degrees) with 0.025% surfactant on parafilm; HLB = hydrophilic lipophilic balance value; EO= ethoxylation value; PO = propylene oxide value; R = radical value, WeC = wetting capacity; CP = cloud point in butyl diglycol (a) or in water (b); and CMC = critical micelle concentration (mM).

or outside in summer ('summer' plants). After treatment, the plants were transferred to a growth room (temperature: 25 ± 5°C day, 15 ± 0.5)°C night; relative humidity 60 ± 5%; day length 14 h in 24 h, provided by warm white fluorescent lamps (200 µE m^{-2} s^{-1})).

Droplet studies Leaves for droplet application were taken from plants at the two to three leaf stage. Freshly prepared aqueous surfactant solutions (0.5 µL) were applied to the middle of the leaf lamina of the adaxial surface and droplet diameters were measured using a light microscope with ×3 and ×20 magnification eyepieces fitted with a graticule (1 unit on scale = 34 µM). Droplet diameter was measured in mm units and the means of five replicates were taken. Surface tension and contact angle data were also assessed.

Activity studies Spraying of the test species was carried out using a laboratory track sprayer; delivery being equivalent to 400 L ha^{-1}, speed 1 m s^{-1}, nozzle pressure 2.70 bar, height 30 cm, and sward width 50 cm; between treatments the sprayer was washed with water (× 10) and with 25% acetone (× one). Visual assay of the treated plants was carried out daily until seven days after treatment (DAT). At harvest (seven DAT) fresh (FW) and dry weights (DW) were taken and succulence calculated by FW/DW. Membrane leakage of leaf tissues was assessed by measuring conductivity (Fletcher and Drexler 1980) using a PT1-18 Digital Conductivity Meter (Fison).

Radiotracer studies ^{14}C bentazone application was made using a Hamilton microsyringe (50 µL capacity). The plants were treated at the two to three leaf stage, 5 × 1 µL droplets being applied to the centre of the adaxial surface of the leaf lamina; there were five replicates per treatment. The same volume of the treatment solution was dispensed into vials containing 5 mL Ecoscint to determine the amount of ^{14}C applied to the plants.

At harvest, surface residues were removed using cellulose acetate film and placed in Ecoscint (5 mL); radioassay was carried out using a Packard Tricarb Scintillation Spectrometer (model 300C). The distribution of ^{14}C (bentazone) in the plant tissues was calculated as percentages of the applied dose per mg. Treated and untreated tissues were individually pelletised in Whatman No.1 filter paper and combusted using a Packard Tricarb Sample Oxidiser (model 306M). The ^{14}C-CO_2 formed was trapped in Luma Sorb (6 mL) to which the appropriate scintillation fluid Carbo Luma (12 mL) was added and the samples radioassayed as before. There were three fractions: A – surface residues (cellulose acetate film), B – treated tissue, and C –untreated tissue. 'Uptake' was B + C and 'translocation' was C.

Experimental design The effect of the test surfactants (*0, 0.1% and 0.5%) on the activity of bentazone was assessed using *C. album* (0.375 kg a.i. ha^{-1}), *C. segetum* (0.05 kg a.i. ha^{-1}) and *S. arvensis* (0.05 kg a.i. ha^{-1}). The 0 data equated to the bentazone control.

The effect of surfactant incorporation (0, 0.1 and 0.5%) on the uptake and translocation of ^{14}C (bentazone) (1.0 kg a.i. ha^{-1}) was assessed 24 hours after treatment (24 HAT).

The effects on succulence (FW/DW) and conductivity (seven DAT) were assessed on glasshouse grown 'winter plants' and outdoor-grown 'summer' plants.

Statistical treatment Where appropriate, statistical analyses were carried out on the data using ANOVA, MANOVA, Student Newman-Keuls (SNK) and Partial Least Squares (PLS). With the exception of the PLS, analyses were carried out using the SPSS (Windows version 7.5.1) statistical package. The PLS tests were carried out using the Simca 7.01 software package from Umetrics AB, Sweden.

RESULTS

The effect of surfactants on the physical properties of droplets The effect of surfactant treatments (0.025% and 0.1%) on the diameter of water droplets applied to the adaxial surfaces of the test species showed maximum droplet diameter tended to occur with surfactants of low mean EO number (results not presented).

The influence of surfactants on contact angle and surface tension of water droplets were also assessed. Surfactants of low mean EO particularly ON30 and ON50 were again most effective in reducing the contact angle (results not presented).

The effect of surfactants on the activity and mode of action of bentazone Generally, the greatest reductions in succulence of summer grown plants in response to the surfactants were obtained with *C. album* and *C. segetum*. The relative absence of surfactant effect on *S. arvensis* appears to reflect the greater effectiveness of bentazone *per se* on this species (S). A significant species/surfactant/concentration interaction (P = 0.05) was obtained (results not presented).

The effect of surfactant incorporation (0.5%) on uptake of ^{14}C (bentazone) by summer plants is shown in Figure 1. Uptake of bentazone was enhanced by surfactants in all species (*C. album* > *C. segetum* > *S. arvensis*), there being significant species/surfactant and species/concentration interactions (both P = 0.05). Generally the AO, AT, LF and ON series were most effective and the PE series ineffective. Uptake of ^{14}C (bentazone) was greater in summer than winter plants (*C. album* × eight and *C. segetum* × three); in *S. arvensis* there was less difference in uptake (× two). Surfactant treatments had less influence on bentazone uptake in glasshouse-grown plants compared with outdoor-grown plants.

In summer plants significant differences in ^{14}C (bentazone) translocation (% applied dose) were obtained in response to surfactants (Figure 2). Translocation response was greater in *C. segetum* > *C. album* > *S. arvensis* and a significant species/surfactant/

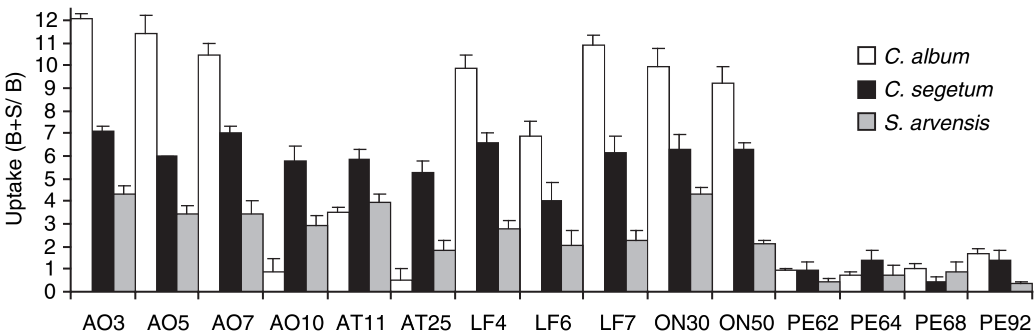

Figure 1. The effect of surfactant treatments (0.5%) on the uptake (% applied) of ^{14}C (bentazone (B+S)) by the three test species (summer plants). The results are expressed as response in terms of the bentazone controls (B).

concentration (P = 0.05) was obtained. In *S. arvensis* only the LF series and ON30 were effective.

Relative performance values The species-specific nature of bentazone/surfactant effects are evident from the above and the results for uptake, translocation and conductance showed significant season/species interactions.

Relative performance values for the three most effective surfactants for each set of results were derived using the Student-Newman Keuls (SNK) values. Overall the most successful surfactants belonged to the Plurafac (LF) series though the ON and AO series were effective in enhancing the droplet (water) surface properties; LF4 was also highly effective. There were inter-species variations in the degree of effectiveness of these surfactants.

Variable directed analyses Multivariate analysis creates new (fewer) variables containing information from the many. These new variables are then used for problem solving. Multivariate Partial Least Squares (PLS) analysis of the various surfactant groups was carried out and the results recorded as significant when the R27 (cum) value for the explained model and the Q2 (cum) for the predictive value were >44%.

Surface property responses: PLS analysis was carried out on data for surface tension, contact angle and droplet diameter of water droplets in relation to the physicochemical properties of the surfactants. The results of PLS analysis carried out on data relating to the treatments with the AO series applied to *C. album* produced a 78.3% predictive ability and 78.9% of the data was explained. The correlation structure between Y responses (c) (surface tension, contact angle, droplet diameter) and X physicochemical data (w*) (HLB, EO, Radical, PO, WETT, CMC, viscosity, CP_BDG, MMASS) showed that surface tension (s_tens) is positively correlated to EO/viscosity, with droplet diameter (dr_dia) negatively correlated to viscosity/CMC/HLB. The Variable Importance in the Projection (VIP) values derived, reflect the importance of terms in the model both with respect to Y (namely it's correlation to all the responses) and with respect to X (the projection). In *C. album* and the AO series viscosity, CMC, EO and the cloud point (in butyl diglycol) was shown to be important characteristics.

Physiological responses: PLS analysis was carried out on the data for visual assessment, succulence (reduction), conductance, uptake, and translocation (% applied, % absorbed) in relation to the physicochemical properties of the bentazone + surfactants (Y variables). As before the X variables were HLB, EO, PO, Radical, wettability, viscosity, CP-BDG, molar mass and CMC. The following observations were made.

1. Generally, uptake of ^{14}C (bentazone) was inversely related to mean EO number for surfactants of the AO, AT, LF and PE series.
2. Translocation of ^{14}C (bentazone) was enhanced in *C. album* by surfactants of the LF series; in *C. segetum* it was negatively related to HLB.

DISCUSSION

Surfactant effectiveness has been shown to depend on a complex of inter-related factors. These include: 1) the nature of the surfactant and its concentration; 2) plant factors relating to species and growth conditions; and 3) the interaction of surfactant and plant factors. Certain surfactants conveyed beneficial droplet characteristics including reduced surface tension/contact angle and enhanced diameter. These characteristics were shown by surfactants of the AO, ON and to a

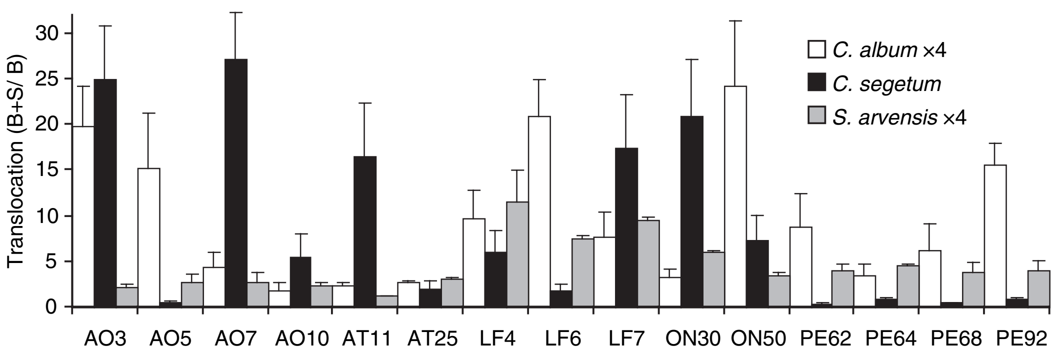

Figure 2. The effect of surfactant treatments (0.5%) on the translocation (% applied) of ^{14}C (bentazone (B+S)) by the three test species (summer plants). The results are expressed as response in terms of the bentazone controls (B); *C. album* and *S. arvensis* are ×4.

lesser degree the LF series, AO3, AO5, LF4, ON30 and ON50 being most effective. These surfactants contain a radical group and have in common low mean EO, PO and molar mass parameters.

Uptake of ^{14}C (bentazone) was particularly enhanced by low mean EO members of the AO, LF and ON series, which reduced surface tension/contact angle and increased droplet diameter. Surfactants of higher mean EO (e.g. AO7, LF7, ON50) may act as humectants, thus prolonging the period of droplet drying and absorption of polar herbicides such as bentazone (sodium salt). Such surfactants may cause swelling and hydration of the CM enhancing trans-cuticular transport of bentazone and increasing uptake and indirectly translocation of ^{14}C (bentazone). Surfactants of the Pluronic (PE) series were notable in that they enhanced translocation *per se*, PE92 being particularly effective. This surfactant has a mean EO number of 15.6, PO value of 47.3 and thus has relatively polar qualities.

The effect of surfactant treatments on the activity of bentazone was recorded by measuring effects on growth, succulence (FW/DW) and tissue conductance (membrane permeability). The LF series, especially LF4, was most effective; these surfactants have low values for all the physicochemical characteristics and contain a radical group. While uptake of ^{14}C (bentazone) increased in association with the higher surfactant concentration (AO, AT, LF and ON series), translocation (% of applied) was reduced, especially in combination with the AO and AT series. This may reflect inhibition of photosynthesis and assimilate transport at enhanced rates of bentazone uptake. At the higher surfactant concentration, enhanced effects on visual activity (e.g. AO7, AO10, ON30 and ON50) succulence (e.g. LF4 and LF6) and conductance (especially with AT11 and LF4) were noted. In the surfactants tested the higher concentration was well above the CMC.

In general the advantageous effects of the surfactants on contact angle, droplet diameter and uptake were attributed to low EO, HLB, CMC, cloud point and viscosity; these characteristics suggest that the beneficial traits relate to enhanced lipophilicity.

The effects of bentazone on succulence and conductance were greater in summer plants, particularly when formulated with certain surfactants of the AO, ON and AT series. The PE series showed most effect on visual symptoms when bentazone was applied to winter plants.

To conclude, the results of this study have demonstrated that while herbicide/surfactant relationships were species specific, some overall trends were also evident.

1. The AO and ON series (especially AO3 and ON30) were most effective in respect to effects on surface properties of droplets.
2. Surfactants of the AO, ON and LF series were effective in increasing uptake and translocation (% of applied) of ^{14}C (bentazone).
3. The effect of bentazone on growth (visual effects, succulence and conductivity) was particularly enhanced by LF surfactants.
4. Overall the most successful surfactants belonged to the Plurafac (LF) series. The Lutensol series were efficient alternatives with the ON and AO surfactants being most effective on *C. album* and *C. segetum* respectively; in *S. arvensis* the PE surfactants were generally most successful.

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