Alkoxylated fatty amines as adjuvants for herbicides

H. de Ruiter, K. R. Straatman, E. Meinen & A. J. M. Uffing

cabo-dlo

Report 175, October 1993

Report

The DLO Centre for Agrobiological Research (CABO-DLO) falls under the Agricultural Research Department (DLO) of the Dutch Ministry of Agriculture, Nature Management and Fisheries.

The aim of DLO is to generate knowledge and develop expertise for implementing the agricultural policies of the Dutch government, for strengthening the agricultural industry, for planning and management of rural areas and for the protection of the environment. At CABO-DLO experiments and computer models are used in fundamental and strategic research on plants. The results are used to:

- achieve optimal and sustainable plant production systems;
- find new agricultural products and improve product quality;
- enhance nature and environmental quality in the countryside.

Address:
CABO-DLO
P.O. Box 14
6700 AA Wageningen
The Netherlands

tel. 31.8370.75700
fax. 31.8370.23110
e-mail postkamer@cabo.agro.nl

A report for

AKZO Chemicals B.V.
Research Centre Deventer
The Netherlands

Preface

The experiments described in this report were carried out in the period from September 1989 until September 1991. I acknowledge AKZO Chemicals B.V. for their financial support and particularly Drs Robert J. Butselaar for his great interest in the matter of adjuvants added to agrochemicals. Our regular meetings in Wageningen and in Deventer provided a good base for the project. I acknowledge Kees Straatman who carried out the experiments with much enthusiasm. I want to thank Esther Meinen for her substantial contribution (statistics, additional experiments, tables and figures) to this report. I also thank André Uffing who professionally carried out the field experiments.
Finally I thank the director of CABO-DLO, dr ir J.H.J. Spiertz for his advices during the preparation of this project.

Dr ir H. de Ruiter
Wageningen, December 17, 1992

Table of Contents

Table of Contents

I The influence of a fatty amine surfactant on foliar absorption and translocation of the triethanolamine salt and iso-octyl ester of 2, 4-D; time course

Abstract. The influence of the surfactant Armoblen 600 (tallowamine blockpolymer containing a block of polymerized propylene oxide (12PO) and a block of polymerized ethylene oxide (5EO)) on the foliar absorption and translocation of 2,4-dichlorophenoxyacetic acid triethanolamine salt (2,4-D TEA) and 2,4-dichlorophenoxyacetic acid iso-octyl ester (2,4-D IOE) was investigated. Absorption and translocation were monitored for 48 h after application. During this period, without surfactant the leaves of black nightshade (*Solanum nigrum* L.) absorbed 2.4 times more 2,4-D IOE than 2,4-D TEA whereas pea leaves (*Pisum sativum* L.) absorbed 1.3 times more 2,4-D IOE than 2,4-D TEA. Pea leaves absorbed more of both compounds than black nightshade did. Addition of surfactant (0.5 % w/v) enhanced the absorption of 2,4-D TEA by black nightshade (4.8 fold after 48 h) and pea (1.7 fold after 48 h) but reduced the absorption of 2,4-D IOE. Without surfactant the absorption of 2,4-D TEA and 2,4-D IOE was not affected by the drops drying. Addition of surfactant enhanced absorption of 2,4-D TEA after the drops had dried. Translocation of 2,4-D TEA in black nightshade and in pea expressed as percentage of the amount absorbed was reduced by the presence of surfactant. At the end of the observation period this reduction was not more observed. The translocation efficiency of ^{14}C from 2,4-D IOE was not influenced by the surfactant. The effects of the surfactant are discussed in relation to its possible mode of action.

1.1 Introduction

Surfactants may enhance the efficacy of herbicides and other biocides by enhancing the retention of drops sprayed onto plants and by enhancing the foliar absorption of the active ingredient.[1]

A previous study on compounds with different water solubilities[2], and a recent study by Holloway *et al.* on two fungicides differing in lipophilicity[3] indicate that the addition of a relatively hydrophilic polyoxyethylene surfactant will probably enhance the foliar absorption of a hydrophilic compound appreciably but will enhance the absorption of a lipophilic compound much less.

We investigated the influence of the cationic fatty amine surfactant Armoblen 600 on the foliar absorption of 2,4-dichlorophenoxy acetic acid either as the water soluble triethanolamine salt (2,4-D TEA) or the lipophilic iso-octyl ester (2,4-D IOE). A cationic fatty amine surfactant with a moderate number of hydrophilic groups (average of 17 hydrophilic groups per molecule) was selected because previous studies suggested that a greater number of hydrophilic groups may reduce the foliar absorption of water soluble compounds.[2,4,5]

We measured the foliar absorption by pea and black nightshade at 12 intervals during the 48 hours of the experiment. These species were selected because of their contrasting leaf surfaces: the adaxial surface of pea leaves is covered with a layer of crystalline epicuticular waxes whereas the leaves of black nightshade have a smooth surface, as has been demonstrated by scanning electron microscopy.[6]

Surfactants may enhance the foliar absorption of 2,4-dichlorophenoxy acetic acid,[7] glyphosate,[8,9], and 2D-glucose, atrazine, and DTT,[2] but simultaneously reduce the translocation of these compounds. Therefore we also measured the translocation of ^{14}C.

1.2 Materials and methods

1.2.1 Plant material

Pea seeds (*Pisum sativum* L., cv. Finale) were germinated and after two weeks the seedlings were placed in 1 liter pots containing 1/2-strength Steiner's nutrient solution.[10] Seeds of black nightshade (*Solanum nigrum* L.) were germinated in a greenhouse in trays containing a mixture of sand and humic potting soil (1:2, v/v) and then transferred to the growth chamber. Two weeks after emergence these seedlings were also placed in 1 liter pots containing 1/2-strength Steiner's nutrient solution. To ensure good growth of black nightshade the pots were connected to an aerating system two days after the seedlings had been transferred. The pea and black nightshade plants were grown in a growth chamber under 14 h light, 18/12 °C (day/night) temperature, and 70/80 % (day/ night) relative humidity. Light was provided by high pressure sodium lamps (Phlips 400W SON/T) and fluorescent tubes (Philips TLD 58W colour 54) to give 80-120 W/m^2 (PAR) at leaf level. The experiments were done on pea plants that were 10 cm tall and had about four pairs of leaflets and two tillers after 12 days on nutrient solution, and on black nightshade plants (6 cm tall) that had six unfurled leaves and one to three tillers after 15 days on nutrient solution. In the case of pea one single unfurled leaflet of the youngest pair of leaflets was treated. In the case of black nightshade, the herbicide solutions were applied to the youngest fully expanded leaf of black nightshade.

1.2.2 Herbicides and surfactant

Unlabelled 2,4-D (Merck, purity 98 %) was mixed with water and converted to the triethanolamine salt (2,4-D TEA) by the adding triethanolamine (Merck, purity 98 %) while heating (Austria Linz, 1989, pers. comm.). Labelled 2,4-D TEA (2,4-dichlorophenoxy[2-^{14}C]acetic acid triethanolamine salt, Amersham, purity > 96 %; s.a. 437 MBq/mmol) and unlabelled 2,4-D TEA were dissolved in acetone+water (1+3 by volume). The concentration of 2,4-D TEA (labelled plus unlabelled) was 11.3 mM (s.a. 29.47 MBq/mmol), which is equivalent to the molarity of 2,4-D when this compound is applied at a rate of 1 kg/ha at a water volume of 400 L/ha. Drops of the 2,4-D TEA solution without surfactant were repelled by the waxy leaf surface of pea. To overcome this, acetone (final concentration 25 %) was added to solutions containing 2,4-D TEA with and without surfactant. To find out whether the addition of acetone affects the foliar uptake of 2,4-D TEA in pea and black nightshade we measured the uptake with and without surfactant at four acetone concentrations: 0 % (except for pea without surfactant), 25 %, 50 %, and 75 % (two separate experiments with three replicates each). Using cryo-scanning electron microscopy (cryo-SEM) we investigated whether aqueous acetone (1+3, and 3+1 by volume) modified the leaf surface.
Labelled 2,4-D IOE (2,4-dichlorophenoxy[2-^{14}C]acetic acid iso-octyl ester, Amersham, purity > 96 %,; s.a. 333 MBq/mmol) and unlabelled 2,4-D IOE (Austria Linz, technical grade, purity 97 %) were dissolved in acetone+water (3+1 by volume). The concentration of 2,4-D IOE was 11.3 mM (s.a. 29.47 MBq/mmol). The cationic fatty amine surfactant Armoblen 600

(tallowamine blockpolymer containing a block of polymerized propylene oxide (12PO) and a block of polymerized ethylene oxide (5EO)) was used in this study (supplied by AKZO Chemicals BV, The Netherlands). The product is a blend of compounds differing in the length of their alkyl chains and in content of EO and PO. The surfactant was added on a weight to volume basis; the concentration was 0.5 %. The pH of the herbicide solutions was measured using a glass electrode and was 7.0 (2,4-D TEA), 7.4 (2,4-D TEA with surfactant), 4.5 (2,4-D IOE), and 8.4 (2,4-D IOE with surfactant).

1.2.3 Absorption and translocation

The solutions were applied to the leaf surface as five 1 µl drops (1.67 kBq/5 µl) to a discrete area on the adaxial surface in the median part of the leaf, outlined with waterproof ink. To find out how much ^{14}C was applied to the leaves, five drops were dispensed directly into a scintillation vial. All applications were done with the Burkard Microapplicator PAX 100 fitted with a 50 µl syringe and needle coated with PTFE. The applications were done in the growth chamber, one (± 1/2) hour after the beginning of the photoperiod. It took less than one hour to apply the solutions of one replicate. After the treatment period the treated leaf was excised and washed with 2 ml aqueous acetone (1+3 (v/v) with 2,4-D TEA, and 3+1 (v/v) with 2,4-D IOE) to remove residual chemical deposits. We tested the efficiency of washing procedure by washing the leaf surface of both species immediately following application. Between 95 and 100 % of the 2,4-D TEA or 2,4-D IOE without surfactant was recovered.
To determine the efficiency of washing after the drops had dried we measured the recovery of 2,4-D TEA 24 hours after applying the herbicide solutions to glass slides in the climate chamber. The recovery of 2,4-D TEA varied between 98 and 100 % regardless of whether surfactant was present. Because 2,4-D IOE is volatile the glass slide test can not be used to ascertain the washing efficiency.
The epicuticular wax was removed from the treated area with a cellulose acetate strip,[11] and the treated area was excised with a cork borer (diam. 1cm). Translocation to other parts of the plant was determined by measuring radioactivity in the rest of the treated leaf and in the rest of the plant. To determine how much ^{14}C had been exuded into the nutrient solution we measured the radioactivity in 1 ml samples of that solution. The leaf surface wash and the sample from the nutrient solution were dissolved in scintillation liquid (10 ml; Packard Ultima Gold, Packard Instruments B.V., The Netherlands). The amount of ^{14}C exuded could only be measured accurately by this procedure if it was more than 8 % of the amount applied because the ^{14}C was so diluted in the nutrient solution. The cellulose acetate strips with adhering epicuticular wax were dissolved in acetone (0.5 ml) before scintillation liquid (10 ml; Packard Ultima Gold) was added. The fractions treated area and the rest of the treated leaf were oxidized using a Packard Tri-Carb Oxidizer Model 306. The fraction containing the rest of the plant including roots was dried at 70 °C and then ground. Samples of the powder (200 mg) were oxidized. The $^{14}CO_2$ was trapped in Lumasorb I (5 ml; Lumac LSC B.V., Belgium) and then scintillation liquid (10 ml; Carboluma, Lumac LSC B.V.) was added. The ^{14}C in all fractions was quantified with a scintillation counter (Packard Tri-Carb 300C). Using this procedure the following parameters could be defined:
residual deposit = ^{14}C in leaf surface washing; epicuticular wax (pea) = ^{14}C in cellulose acetate strip; absorption = sum of ^{14}C in the plant tissue plus ^{14}C in the nutrient solution (in the case of black nightshade the ^{14}C in the cellulose acetate strip was included (see Results section)); translocation = ^{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.

We did not attempt to identify possible metabolites of the 2,4-D compounds.

1.2.4 Spreading and drying of drops

The spreading of the drops and the time required for the drops to dry up was assessed visually.

1.2.5 Experimental design

Very many applications had to be done to record at 13 points of time the influence of the surfactant on the foliar absorption of 2,4-D TEA and 2,4-D IOE in pea and black nightshade. Therefore we designed the experiment so that two solutions (one 2,4-D compound with and without surfactant) were applied to one of the species on a certain day. Such a treatment was repeated at three other separate dates which means that four replicates were carried out. The applications of one replicate were made according to a completely randomized design. An analysis of variance was performed after logarithmic transformation of the data. Logarithmic transformation was necessary because the residuals were not independent from the fitted values. The LSD ($P = 0.05$) values calculated could not be used to compare the absolute values of the data. Since the difference between two logarithms is the same as the logarithm of the ratio we applied the formula $LSR = 10^{LSD}$ in which LSR means Least Significant Ratio. The LSR was used to compare the absolute values of the data.

TABLE 1.1 The absorption and translocation of [^{14}C]2,4-D triethanolamine salt in the absence and presence of surfactant (*Solanum nigrum* L.)

	Radioactivity as percentage of total ^{14}C *applied* [a]									
Time (h)	*Leaf washing*	*Cellulose acetate strip*	*Treated area*	*Rest of the treated leaf*	*Rest of the plant*	*Nutrient solution*	*Recovery*	*Absorption*	*Translocation*	*Trans/Abs * 100%*
without surfactant										
0.08	99.06 (3.26)	0.68 (0.47)	0.36 (0.17)	0.12 (0.03)	0.03 (0.01)	- [b]	100.3	1.19	0.15	12.61
0.25	99.20 (2.40)	0.11 (0.02)	0.27 (0.07)	0.11 (0.03)	0.06 (0.02)	-	99.8	0.55	0.17	30.91
0.50	101.2 (1.86)	0.24 (0.07)	0.54 (0.15)	0.09 (0.02)	0.06 (0.02)	-	102.1	0.93	0.15	16.13
0.75	101.5 (2.58)	1.14 (0.88)	0.94 (0.42)	0.11 (0.01)	0.11 (0.09)	-	103.8	2.30	0.22	9.57
1.0	99.56 (4.08)	0.32 (0.10)	1.03 (0.24)	0.13 (0.03)	0.15 (0.09)	-	101.2	1.63	0.28	17.18
2.0	99.83 (4.54)	0.26 (0.11)	1.57 (0.54)	0.16 (0.03)	0.11 (0.03)	-	101.9	2.10	0.27	12.86
2.5	97.21 (4.41)	0.92 (0.49)	2.77 (1.05)	0.59 (0.35)	0.25 (0.10)	-	101.7	4.53	0.84	18.54
3.0	90.93 (3.98)	0.63 (0.30)	1.78 (0.75)	0.18 (0.05)	0.23 (0.02)	-	93.8	2.82	0.41	14.54
5.3	95.29 (5.34)	0.68 (0.16)	1.58 (0.23)	0.22 (0.04)	0.45 (0.08)	-	98.2	2.93	0.67	22.87
8.0	88.99 (3.13)	1.39 (0.70)	2.14 (0.54)	0.25 (0.01)	0.68 (0.25)	-	93.5	4.46	0.93	20.85
12.0	91.10 (3.13)	1.31 (0.65)	1.30 (0.42)	0.97 (0.28)	2.40 (1.40)	-	97.1	5.98	3.37	56.35
24.0	91.35 (3.05)	0.87 (0.20)	1.87 (0.38)	0.64 (0.14)	1.40 (0.58)	-	96.1	4.78	2.04	42.68
48.0	78.87 (2.69)	4.65 (1.79)	2.27 (0.32)	1.03 (0.12)	1.69 (0.92)	-	88.5	9.64	2.72	28.22

Continue Table 1.1

	Radioactivity as percentage of total ^{14}C applied [a]																					
Time (h)	*Leaf washing*		*Cellulose acetate strip*		*Treated area*		*Rest of the treated leaf*		*Rest of the plant*		*Nutrient solution*	*Recovery*	*Absorption*	*Translocation*	*Trans/Abs * 100%*
with surfactant															
0.08	99.99	(1.82)	0.04	(0.02)	0.18	(0.03)	0.12	(0.06)	0.07	(0.03)	-	100.4	0.41	0.19	46.34*
0.25	101.5	(2.86)	0.03	(0.01)	0.23	(0.08)	0.08	(0.02)	0.09	(0.05)	-	102.0	0.43	0.17	39.53
0.50	99.13	(6.23)	0.57	(0.37)	0.38	(0.07)	0.21	(0.12)	0.04	(0.01)	-	100.3	1.20	0.25	20.83
0.75	88.69	(7.74)	8.96	(2.53)*	1.02	(0.19)	1.24	(0.53)*	0.05	(0.01)	-	100.0	11.27*	1.29*	11.45
1.0	81.79	(10.5)	14.52	(2.75)*	1.61	(0.31)	0.83	(0.36)*	0.05	(0.00)	-	98.8	17.01*	0.88 *	5.17*
2.0	85.22	(9.90)	9.89	(1.83)*	2.42	(0.53)	0.74	(0.09)*	0.11	(0.04)	-	98.4	13.16*	0.85*	6.46
2.5	66.91	(7.77)	11.05	(4.55)*	1.35	(0.22)	0.87	(0.15)	0.18	(0.10)	-	80.4	13.45*	1.05	7.81*
3.0	58.98	(6.42)	10.64	(2.68)*	2.84	(0.30)	1.63	(0.70)*	0.23	(0.05)	-	74.3	15.34*	1.86*	12.13
5.3	64.50	(6.33)	7.02	(1.56)*	3.38	(0.77)*	0.94	(0.29)*	0.26	(0.03)	-	76.1	11.60*	1.21	10.43*
8.0	46.08	(7.45)	18.02	(4.69)*	6.25	(1.37)*	1.45	(0.45)*	0.79	(0.13)	-	72.6	26.51*	2.24*	8.45*
12.0	23.81	(0.76)	21.46	(2.20)*	14.19	(2.46)*	4.57	(0.81)*	2.42	(0.30)	-	66.4	42.64*	6.99	16.39*
24.0	37.47	(10.7)	14.89	(1.26)*	15.36	(1.26)*	2.52	(0.45)*	4.41	(0.54)*	-	74.7	37.18*	6.93*	18.64*
48.0	23.22	(4.76)	20.48	(4.12)*	15.78	(2.99)*	3.99	(1.77)*	11.21	(1.35)*	-	74.7	51.46*	15.20*	29.54
LSR[c] (P=0.05)			3.49		1.98		2.67		2.92				1.93	2.10	2.09

a) Mean values (n=4), standard errors in parentheses

b) < 8%, see Materials and Methods

c) Least Significant Ratio; see Materials and Methods

*) Differs (P=0.05) from the value without surfactant measured at the same point of time

TABLE 1.2 The absorption and translocation of [^{14}C]2,4-D iso-octyl ester in the absence and presence of surfactant (*Solanum nigrum* L.)

	Radioactivity as percentage of total ^{14}C applied [a]									
Time (h)	*Leaf washing*	*Cellulose acetate strip*	*Treated area*	*Rest of the treated leaf*	*Rest of the plant*	*Nutrient solution*	*Recovery*	*Absorption*	*Translocation*	*Transl/Abs * 100%*
without surfactant										
0.08	91.47 (5.22)	0.31 (0.10)	6.58 (5.13)	0.15 (0.03)	0.05 (0.02)	- [b]	98.6	7.09	0.20	2.82
0.25	93.51 (0.38)	0.35 (0.15)	3.50 (1.56)	0.27 (0.11)	0.04 (0.02)	-	97.7	4.16	0.31	7.45
0.50	92.52 (1.57)	0.59 (0.06)	4.84 (1.65)	0.24 (0.12)	0.02 (0.01)	-	98.2	5.69	0.26	4.57
0.75	88.95 (4.72)	0.58 (0.20)	8.20 (5.21)	0.29 (0.17)	0.08 (0.03)	-	98.1	9.15	0.37	4.04
1.0	86.80 (5.93)	3.14 (3.35)	5.88 (1.90)	1.42 (1.33)	0.15 (0.07)	-	97.4	10.59	1.57	14.83
2.0	88.23 (3.00)	0.58 (0.32)	7.48 (3.97)	0.25 (0.02)	0.15 (0.03)	-	96.7	8.46	0.40	4.73
2.5	88.68 (4.68)	0.96 (0.36)	8.55 (4.28)	0.43 (0.10)	0.18 (0.06)	-	98.8	10.12	0.61	6.03
3.0	90.74 (2.10)	0.46 (0.10)	7.65 (1.87)	0.16 (0.03)	0.24 (0.06)	-	99.3	8.51	0.40	4.70
5.3	87.52 (3.31)	1.14 (0.40)	8.97 (2.28)	0.65 (0.39)	0.24 (0.01)	-	98.5	11.00	0.89	8.09
8.0	80.75 (5.05)	2.12 (1.64)	9.55 (5.11)	0.93 (0.22)	0.69 (0.28)	-	94.0	13.29	1.62	12.19
12.0	76.73 (2.57)	2.89 (1.53)	9.73 (1.94)	1.95 (0.57)	2.10 (0.49)	-	93.4	16.67	4.05	24.30
24.0	80.64 (2.42)	0.54 (0.40)	9.91 (2.18)	0.94 (0.20)	4.47 (0.56)	-	96.5	15.86	5.41	34.11
48.0	72.69 (4.34)	0.78 (0.25)	9.71 (3.68)	1.91 (0.53)	9.77 (1.23)	-	94.9	22.17	11.68	52.68

Continue Table 1.2

	Radioactivity as percentage of total ^{14}C applied [a]									
Time (h)	*Leaf washing*	*Cellulose acetate strip*	*Treated area*	*Rest of the treated leaf*	*Rest of the plant*	*Nutrient solution*	*Recovery*	*Absorption*	*Translocation*	*Transl/Abs * 100%*
with surfactant										
0.08	98.75 (0.55)	0.13 (0.06)	0.69 (0.13)*	0.08 (0.04)	0.03 (0.01)	-	99.7	0.93*	0.11	11.83*
0.25	97.86 (0.34)	0.36 (0.15)	0.79 (0.17)*	0.08 (0.02)	0.03 (0.01)	-	99.1	1.26*	0.11*	8.73
0.50	92.00 (5.50)	0.22 (0.05)	1.26 (0.10)*	0.08 (0.02)*	0.04 (0.01)	-	93.6	1.60*	0.12	7.50
0.75	92.48 (4.23)	0.49 (0.19)	1.79 (0.62)*	0.14 (0.04)	0.04 (0.01)	-	94.9	2.46*	0.18	7.32
1.0	89.52 (4.08)	1.45 (1.19)	2.24 (0.35)*	0.43 (0.25)*	0.05 (0.02)*	-	93.7	4.17*	0.48*	11.51
2.0	93.76 (2.50)	0.31 (0.06)	3.23 (0.27)*	0.21 (0.05)	0.11 (0.02)	-	97.6	3.86*	0.32	8.29
2.5	96.06 (0.52)	0.46 (0.15)	2.83 (0.45)*	0.20 (0.05)	0.15 (0.03)	-	99.7	3.64*	0.35	9.62
3.0	95.08 (0.33)	0.35 (0.08)	3.50 (0.50)	0.23 (0.09)	0.17 (0.06)	-	99.3	4.25*	0.40	9.41
5.3	93.04 (1.65)	0.41 (0.21)	4.61 (1.19)	0.62 (0.44)	0.39 (0.05)	-	99.1	6.03	1.01	16.75
8.0	88.64 (1.22)	0.85 (0.22)	5.43 (1.02)	0.56 (0.31)	1.02 (0.32)	-	96.5	7.86	1.58	20.10
12.0	75.09 (4.86)	1.14 (0.76)	12.09 (4.23)	1.01 (0.17)	2.82 (0.81)	-	92.2	17.06	3.83	22.45
24.0	80.11 (5.11)	0.46 (1.25)	10.90 (3.08)	0.51 (0.16)	3.12 (0.21)	-	95.1	14.99	3.63	24.22
48.0	74.16 (3.82)	0.93 (0.25)	12.12 (3.39)	0.85 (0.11)	4.11 (1.31)*	-	92.2	18.01	4.96	27.54
LSR[c] (P=0.05)		3.01	2.30	3.18	2.33			2.02	2.51	2.57

a) Mean values (n=4), standard errors in parentheses

b) < 8%, see Materials and Methods

c) Least Significant Ratio; see Materials and Methods

*) Differs (P=0.05) from the value without surfactant measured at the same point of time

TABLE 1.3 The absorption and translocation of [^{14}C]2,4-D triethanolamine salt in the absence and presence of surfactant (*Pisum sativum* L.)

	Radioactivity as percentage of total ^{14}C applied [a]																					
Time (h)	*Leaf washing*		*Cellulose acetate strip*		*Treated area*		*Rest of the treated leaf*		*Rest of the plant*		*Nutrient solution*		*Recovery*	*Absorption*	*Translocation*	*Transl/Abs * 100%*						
without surfactant																						
0.08	99.96	(0.71)	0.06	(0.01)	0.30	(0.15)	0.13	(0.10)	0.11	(0.04)	-[b]		100.6	0.54	0.24	44.40						
0.25	75.89	(14.3)	0.06	(0.02)	0.37	(0.19)	0.28	(0.17)	0.49	(0.41)	-		77.1	1.14	0.77	67.50						
0.50	100.8	(0.78)	0.13	(0.04)	0.54	(0.28)	0.29	(0.26)	0.74	(0.67)	-		102.5	1.57	1.03	65.60						
0.75	99.61	(0.42)	0.09	(0.02)	0.78	(0.32)	0.18	(0.14)	0.27	(0.20)	-		100.9	1.23	0.45	36.60						
1.0	98.83	(0.72)	0.08	(0.04)	1.21	(0.46)	0.36	(0.26)	0.25	(0.16)	-		100.7	1.82	0.61	33.50						
2.0	97.45	(1.26)	0.12	(0.05)	1.87	(0.70)	0.21	(0.13)	0.58	(0.23)	-		100.2	2.66	0.79	29.70						
2.5	94.80	(1.84)	0.15	(0.06)	4.73	(1.85)	0.24	(0.13)	0.37	(0.09)	-		100.3	5.34	0.61	11.40						
3.0	91.87	(2.43)	0.25	(0.13)	5.07	(2.07)	0.38	(0.26)	0.35	(0.06)	-		97.9	5.80	0.73	12.60						
5.3	79.04	(9.30)	3.06	(2.71)	7.59	(2.30)	0.61	(0.29)	0.62	(0.15)	-		90.9	8.82	1.23	13.90						
8.0	80.77	(6.47)	1.72	(1.16)	11.75	(4.07)	1.37	(0.54)	1.28	(0.32)	-		96.9	14.40	2.65	18.40						
12.0	68.56	(6.20)	3.74	(1.97)	18.31	(4.22)	1.38	(0.34)	2.72	(0.94)	-		94.7	22.41	4.10	18.30						
24.0	61.34	(9.24)	5.35	(1.86)	17.55	(5.48)	2.19	(0.67)	7.84	(2.82)	-		94.3	27.58	10.03	36.40						
48.0	37.07	(3.13)	10.12	(1.79)	13.49	(2.34)	3.24	(0.44)	17.91	(1.75)	8.0	(5.3)	89.8	42.64	29.15	68.36						

Continue Table 1.3

	Radioactivity as percentage of total ^{14}C applied [a]																					
Time (h)	*Leaf washing*		*Cellulose acetate strip*		*Treated area*		*Rest of the treated leaf*		*Rest of the plant*		*Nutrient solution*	*Recovery*	*Absorption*	*Translocation*	*TranslAbs * 100%*
with surfactant															
0.08	95.83	(1.93)	0.98	(0.97)	0.37	(0.19)	0.16	(0.12)	0.34	(0.22)	-	97.7	0.86	0.50	57.33
0.25	97.62	(2.49)	0.85	(0.82)	0.55	(0.24)	0.21	(0.12)	0.13	(0.04)	-	99.4	0.89	0.34	37.92
0.50	99.45	(0.72)	0.04	(0.01)	0.72	(0.13)	0.14	(0.09)	0.09	(0.02)	-	100.4	0.95	0.23	24.39*
0.75	98.03	(1.17)	0.15	(0.04)	1.87	(0.35)	0.19	(0.10)	0.18	(0.06)	-	100.4	2.24	0.37	16.58*
1.0	94.95	(0.87)	0.53	(0.42)	4.23	(0.89)*	0.21	(0.09)	0.11	(0.02)	-	100.0	4.56*	0.33	7.15*
2.0	83.83	(1.88)	0.31	(0.06)	11.68	(1.01)*	0.31	(0.10)	0.38	(0.09)	-	96.5	12.37*	0.69	5.56*
2.5	76.73	(2.68)	0.45	(0.11)	17.24	(1.00)*	0.50	(0.14)	0.51	(0.04)	-	95.4	18.25*	1.01	5.54*
3.0	72.44	(2.92)	0.41	(0.11)	21.81	(1.46)*	0.77	(0.33)	0.85	(0.05)	-	96.3	23.42*	1.61	6.89
5.3	72.60	(1.32)	0.42	(0.13)*	21.16	(3.90)*	0.45	(0.08)	1.14	(0.11)	-	95.8	22.74*	1.59	6.98*
8.0	63.11	(1.17)	0.55	(0.13)	29.03	(0.43)*	1.14	(0.18)	3.23	(0.61)	-	97.1	33.40*	4.37	13.08
12.0	45.73	(2.60)	0.90	(0.21)	33.04	(1.88)	3.19	(0.35)	8.35	(2.24)	-	91.2	44.58	11.54*	25.88
24.0	47.80	(4.25)	1.18	(0.35)	37.12	(3.13)	2.39	(0.46)	11.37	(2.20)	-	99.9	50.88	13.76	27.05
48.0	29.44	(3.01)	1.16	(0.23)*	24.88	(1.64)	4.65	(2.89)	27.94	(3.76)	13.3 (4.5)	101.4	70.77	45.88	64.80
LSR[c] (P=0.05)			4.76		2.44		2.73		3.44				2.30	2.61	1.90

[a] Mean values (n=4), standard errors in parentheses
[b] < 8%, see Materials and Methods
[c] Least Signifant Ratio; see Materials and Methods
*) Differs (P=0.05) from the value without surfactant measured at the same point of time

TABLE 1.4 The absorption and translocation of [^{14}C]2,4-D iso-octyl ester in the absence and presence of surfactant (*Pisum sativum* L.)

	Radioactivity as percentage of total ^{14}C applied [a]									
Time (h)	*Leaf washing*	*Cellulose acetate strip*	*Treated area*	*Rest of the treated leaf*	*Rest of the plant*	*Nutrient solution*	*Recovery*	*Absorption*	*Translocation*	*TranslAbs * 100%*
without surfactant										
0.08	94.86 (0.73)	0.85 (0.06)	3.68 (0.99)	0.12 (0.02)	0.14 (0.05)	- [b]	99.7	3.94	0.26	6.60
0.25	93.99 (0.48)	1.09 (0.22)	3.32 (0.67)	0.17 (0.08)	0.09 (0.05)	-	98.7	3.58	0.26	7.26
0.50	91.03 (1.05)	1.62 (0.11)	5.66 (1.21)	0.22 (0.12)	0.16 (0.08)	-	98.7	6.04	0.38	6.29
0.75	90.10 (0.96)	1.33 (0.44)	6.57 (1.19)	0.23 (0.07)	0.25 (0.13)	-	98.5	7.05	0.48	6.81
1.0	89.22 (1.79)	1.15 (0.15)	4.18 (0.60)	0.19 (0.07)	0.16 (0.01)	-	94.9	4.53	0.35	7.73
2.0	84.15 (3.99)	1.28 (0.35)	8.95 (2.37)	1.22 (0.82)	0.45 (0.11)	-	96.1	10.62	1.67	15.73
2.5	82.12 (3.19)	1.46 (0.10)	12.97 (2.71)	0.43 (0.08)	0.51 (0.04)	-	97.5	13.91	0.94	6.76
3.0	83.41 (2.93)	1.52 (0.16)	13.67 (2.72)	0.66 (0.12)	0.81 (0.12)	-	100.1	15.14	1.47	9.71
5.3	75.88 (5.83)	1.56 (0.47)	17.02 (4.47)	1.35 (0.59)	1.50 (0.16)	-	97.3	19.87	2.85	14.34
8.0	70.05 (5.84)	1.34 (0.19)	19.65 (3.66)	1.35 (0.34)	2.42 (0.33)	-	94.8	23.42	3.77	16.10
12.0	45.62 (10.1)	2.29 (0.84)	29.40 (3.56)	3.27 (0.90)	7.58 (1.79)	-	88.2	40.25	10.85	26.96
24.0	53.66 (6.80)	1.77 (1.00)	24.73 (4.62)	2.67 (0.92)	11.30 (1.23)	-	94.1	38.70	13.97	36.10
48.0	39.34 (11.6)	0.80 (0.20)	22.21 (8.07)	3.45 (2.14)	20.68 (2.74)	8.4 (4.3)	94.9	54.77	32.56	59.50

Continue Table 1.4

	Radioactivity as percentage of total ^{14}C applied [a]									
Time (h)	*Leaf washing*	*Cellulose acetate strip*	*Treated area*	*Rest of the treated leaf*	*Rest of the plant*	*Nutrient solution*	*Recovery*	*Absorption*	*Translocation*	*Transl/Abs * 100%*
with surfactant										
0.08	99.56 (2.12)	0.58 (0.14)	1.11 (0.20)*	0.11 (0.06)	0.05 (0.02)*	-	101.4	1.27*	0.16	12.60
0.25	97.01 (1.08)	0.65 (0.11)	2.26 (0.53)	0.06 (0.01)	0.20 (0.12)	-	100.2	2.52	0.26	10.32
0.50	96.74 (1.43)	0.98 (0.35)	4.66 (1.82)	0.47 (0.37)	0.25 (0.19)	-	103.1	5.38	0.72	13.38*
0.75	92.18 (1.41)	0.99 (0.11)	4.35 (1.02)	0.27 (0.11)	0.14 (0.03)	-	97.9	4.76	0.41	8.61
1.0	93.17 (1.85)	0.73 (0.25)	4.14 (1.25)	0.16 (0.08)	0.12 (0.03)	-	98.3	4.42	0.28	6.33
2.0	86.86 (2.21)	1.22 (0.39)	7.42 (1.06)	1.13 (0.33)	0.29 (0.06)	-	96.9	8.84	1.42	16.06
2.5	89.21 (2.30)	0.92 (0.16)	7.82 (1.51)	0.57 (0.24)	0.35 (0.10)	-	98.9	8.74	0.92	10.53
3.0	81.22 (3.45)	1.96 (0.61)	10.80 (2.90)	0.81 (0.31)	0.69 (0.19)	-	95.5	12.30	1.50	12.20
5.3	83.58 (2.88)	1.31 (0.27)	10.23 (0.93)	0.56 (0.14)	0.80 (0.07)	-	96.5	11.59	1.36	11.73
8.0	84.27 (5.76)	1.34 (0.60)	9.93 (2.56)*	1.21 (0.93)	1.29 (0.29)	-	98.0	12.43	2.50	20.11
12.0	71.15 (6.67)	2.28 (1.25)	13.66 (1.78)*	2.31 (1.02)	4.31 (0.59)	-	93.7	20.28*	6.62	32.64
24.0	51.79 (4.60)	3.26 (0.84)	22.63 (2.11)	4.44 (1.73)	14.74 (1.39)	-	96.9	41.81	19.18	45.87
48.0	55.75 (4.22)	3.43 (1.74)*	15.10 (2.74)	1.19 (0.51)	16.23 (3.00)	-	91.7	32.52	17.42	53.57
LSR[c] (P=0.05)		2.23	1.77	3.79	2.29			1.75	2.32	1.95

[a] Mean values (n=4), standard errors in parentheses

[b] < 8%, see Materials and Methods

[c] Least Significant Ratio; see Materials and Methods

*) Differs (P=0.05) from the value without surfactant measured at the same point of time

Table 1.5 The spreading of drops of the herbicide solutions [a]

Solution	*Solanum nigrum* L.	*Pisum sativum* L.
aqueous acetone (1+3, v/v)	±	-
2,4-D TEA [b]	±	-
2,4-D TEA + surfactant	+	+
aqueous acetone (3+1, v/v)	+	+
2,4-D IOE [c]	+	++
2,4-D IOE + surfactant	++	++

a - = no spreading; ± = little spreading (contact angle $\theta = \pm 90°$); + = spreading ($\theta < 90°$); ++ = drop flattens ($\theta << 90°$)

b TEA = triethanolamine salt

c IOE = iso-octyl ester

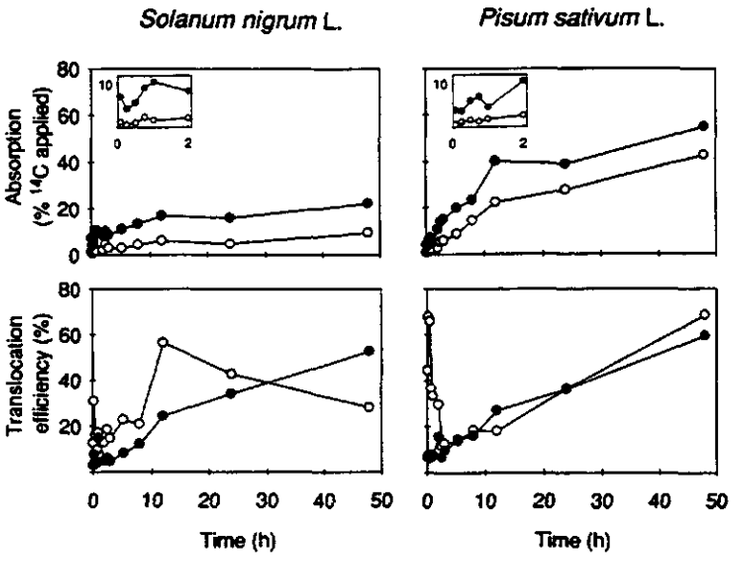


Figure 1.1 Foliar absorption and translocation efficiency of 2,4-D triethanolamine salt (o) and 2,4-D iso-octyl ester (•) in black nightshade and pea. Inserts, absorption during the first two hours after application.

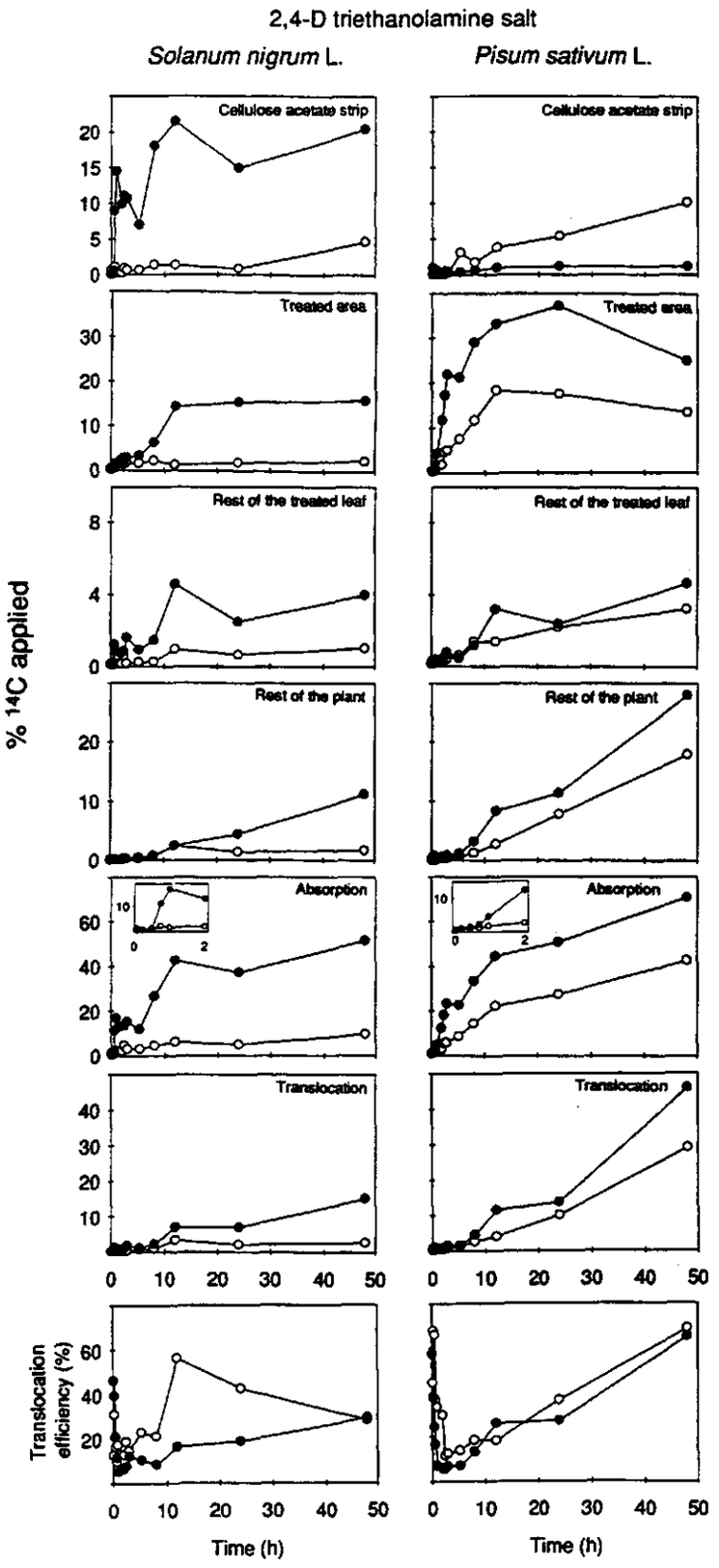


Figure 1.2 Influence of surfactant on the absorption, the distribution, the translocation, and translocation efficiency of ^{14}C from 2,4-D triethanolamine salt in black nightshade and in pea. Without surfactant (o); with surfactant (●). Inserts, influence of surfactant on foliar absorption during the first two hours after application.

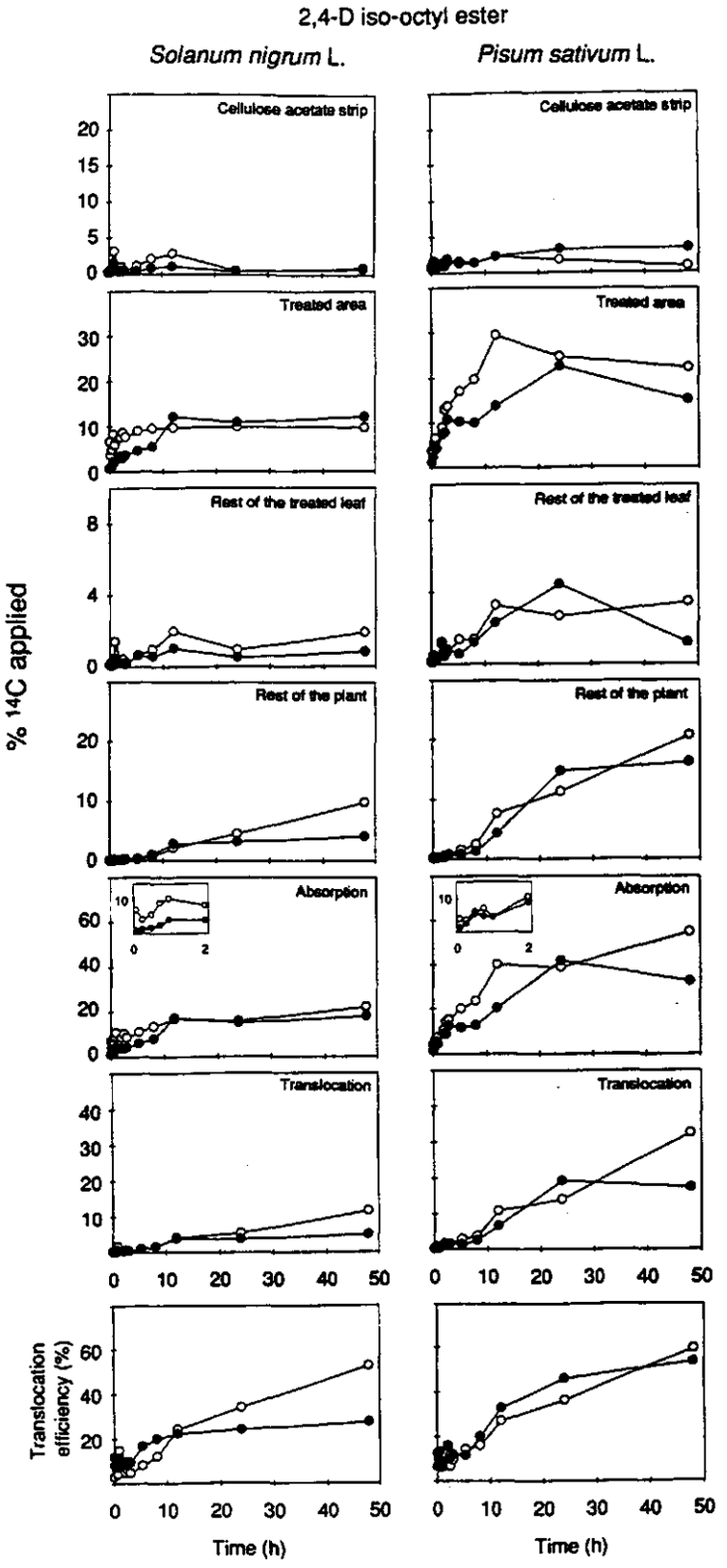


Figure 1.3 Influence of surfactant on the absorption, the distribution, the translocation, and translocation efficiency of ^{14}C from 2,4-D iso-octyl ester in black nightshade and in pea. Without surfactant (o); with surfactant (•).
Inserts, influence of surfactant on foliar absorption during the first two hours after application.

1.3 Results

1.3.1 Influence of acetone on foliar absorption of 2,4-D TEA

Acetone did not change the leaf surface of black nightshade, nor did it influence the foliar absorption of 2,4-D TEA by black nightshade in the absence and presence of surfactant. Acetone slightly damaged the leaf surface of pea; locally some "melting" of the epicuticular waxes could be observed. With and without surfactant there was no influence of acetone on the absorption.

1.3.2 Cellulose acetate film stripping

Cryo-SEM showed that the cellulose acetate stripping technique removed the crystalline epicuticular wax of pea efficiently. Removing the cellulose acetate film from the leaf surface of black nightshade (no epicuticular crystalline waxes present) did not affect the appearance of the surface, but, surprisingly these films were radioactive (Figs 1.2 and 1.3, Tables 1.1 and 1.2). Given the recovery of the washing procedure, we suggest that this radioactivity was either caused by the active ingredients diffusing from the cuticle into the solution of cellulose acetate in acetone while the acetone was evaporating, or by the film removing some waxes from the cuticular surface in a way that cannot be revealed by cryo-SEM. In the case of black nightshade we concluded that the ^{14}C activity found in the cellulose acetate film must have been part of the foliar uptake.

1.3.3 Recovery of radioactivity

The mean values of ^{14}C recovery calculated from all the recovery data from the treatments per series (= time course) varied between 95 and 100 % (SE < 2 %). The only loss of ^{14}C was when 2,4-D TEA plus surfactant was applied to black nightshade, and this loss began 2.5 hours after the application. In black nightshade much more 2,4-D TEA was taken up when surfactant was present. Release of $^{14}CO_2$ may account for this loss of ^{14}C, because it is well known that degradation of the side chain of ^{14}C labelled 2,4-D by the plant may occur.[12] This implies that in this case the uptake of 2,4-D TEA was greater (up to 25 %) than indicated. The good recovery of ^{14}C from 2,4-D IOE means that there is no great loss as a result of volatilization from the leaf surface or evaporation of the ester or its metabolites during the further processing of the different fractions after harvest. The glass slide test done to measure the washing efficiency showed that 15 % of 2.4-D IOE applied had been lost after 24 hours. If is assumed that there is no adsorption of 2,4-D IOE to the glass then the good recovery in the experiments with the plants indicate that adsorption of 2,4-D IOE to the lipophilic leaf surface prevents volatilization.

1.3.4 Absorption and translocation of 2,4-D TEA and 2,4-D IOE (without surfactant)

When evaluating the influence of the type of 2,4-D compound alone on absorption and translocation in both species, we excluded the data obtained after addition of surfactant. Without surfactant the leaves of black nightshade absorbed 2.4 times more 2,4-D IOE than 2,4-D TEA and pea leaves absorbed 1.3 times more 2,4-D IOE than 2,4-D TEA in the 48 hours after application (Fig. 1.1). The absorption of 2,4-D IOE was significantly greater (LSR = 2.0, P = 0.05) in black nightshade at all intervals and significantly greater (LSR = 2.0, P = 0.05) in pea until 8 hours after application. Pea leaves absorbed much more of both compounds than did the leaves of black nightshade. The translocation efficiency of 2,4-D IOE and that of 2,4-D TEA did not differ significantly in pea (LSR = 1.9, P = 0.05) after the first hour and the curve recorded for 2,4-D IOE in black nightshade (Fig. 1.1) was similar to that in pea. The irregular course of the curve for 2,4-D TEA without surfactant in black nightshade made a comparison with the other curves difficult. Analysis of variance showed that until 8 hours after application there is a significant (LSR = 2.3, P = 0.05) greater translocation efficiency for 2,4-D TEA. An autoradiographic study (autoradiographs not shown) demonstrated that in both species 24 hours after application there was no difference between the two 2,4-D compounds in the distribution of ^{14}C over the plant. The autoradiographs of both species clearly indicated basipetal translocation of ^{14}C via the phloem.

1.3.5 Influence of surfactant on absorption and translocation of 2,4-D TEA

Addition of surfactant (0.5 % w/v) enhanced the absorption of 2,4-D TEA in black nightshade (4.8 fold after 48 hours) and in pea (1.7 fold after 48 hours). In both species the surfactant effect could be observed from about 30 min. after treatment; the effect was sustained during the period of observation (Fig. 1.2) and was most significant in black nightshade (Table 1.1) and less significant in pea (Table 1.3) The absorption rate (absorption per unit of time) in pea is maximal until about 700 min. after treatment (with and without surfactant). Thereafter the absorption continues much more slowly. The absorption of 2,4-D TEA in black nightshade in the presence of surfactant shows the same pattern as in pea. Without surfactant the absorption was very poor even after two days of observation. The addition of Armoblen 600 reduced the translocation efficiency of ^{14}C from 2,4-D TEA in black nightshade and in pea (Fig. 1.2). This reduction was significant during several intervals (Tables 1.1 and 1.3). The surfactant reduced the efficiency of translocation in black nightshade but after 48 hours there was no difference in translocation efficiency. Both species accumulated more ^{14}C in the rest of the plant as a result of the adddition of surfactant.

1.3.6 Influence of surfactant on the absorption and translocation of 2,4-D IOE

The surfactant reduced the absorption of 2,4-D IOE in black nightshade and pea (Fig. 1.3). This effect was most significant in black nightshade (Table 1.2). The absorption rate in black nightshade decreased 10 to 11 hours after treatment in the presence and absence of surfactant (Fig. 1.3). The absorption rate in pea in the absence of surfactant shows a similar pattern (Fig. 1.3) to that in black nightshade. The surfactant did not influence the translocation efficiency of ^{14}C from 2,4-D IOE in pea and in black nightshade (Fig. 1.3). Using autoradiography (data not shown) we demonstrated that the addition of surfactant did not influence the distribution of ^{14}C from either 2,4-D compound over the different parts of the plant.

1.3.7 Spreading and drying times of drops

The visual assessment of drop spreading showed (Table 1.5) a small difference between the two species per herbicide solution. Generally the ester-containing solutions spread more than the salt-containing solutions.
In both species the drying time of drops containing 2,4-D TEA (with or without surfactant) was about one hour following application. The drying time for the 2,4-D IOE (with or without surfactant) was about 20 minutes.

1.4 Discussion

1.4.1 Absorption and translocation of 2,4-D TEA and 2,4-D IOE (without surfactant)

The herbicide solutions contained the same solvent per type of 2,4-D compound. This and the similarity of drop spreading (Table 1.5) indicate that the permeability of the cuticle predominantly determines the difference in foliar absorption between the two species.
The data on foliar absorption without surfactant indicate that the crystalline waxes of pea are not a serious barrier to foliar absorption of the two 2,4-D compounds and secondly that the cuticle of pea is more permeable than the cuticle of black nightshade (Fig. 1.1). A great permeability of the pea cuticle was also observed by Silcox and Holloway when recording the penetration of a surfactant into the leaves.[13] The amount of herbicide translocated in the species we examined seems to be largely dependent on the observed difference in uptake, as there were no pronounced differences in translocation efficiency between the two species (Fig. 1.1). The greater absorption of 2,4-D IOE in both species agrees with other observations: the iso-octyl ester of 2,4-D was more readily absorbed by the leaves of bigleaf maple than the 2,4-D acid and the 2,4-D triethanolamine salt.[14] When Price and Anderson measured the foliar uptake of ten compounds by ten species,[15] they found that in five of these species the absorption of the iso-octyl ester of 2,4-D was greater than the absorption of the sodium salt, in one species it was less, and in the other four there was no difference. The presence of surfactants in the herbicide solutions of the studies cited, possible differences in pH between the

herbicide solutions, and differences between the drop deposits may have affected the findings. Nevertheless we contend that the results of these other studies indicate an inverse relationship between the polarity of the 2,4-D compound and its absorption. Assuming that in our study the difference in pH between the 2,4-D IOE (pH 4.5) and the 2,4-D TEA (pH 7.0) solution does not contribute to the results, then it can be concluded that our results fit in the described relationship. Pea absorbed both 2,4-D formulations rapidly during the first 10-11 hours after application (Fig. 1.1). Black nightshade absorbed the 2,4-D IOE rapidly during the first 10-11 hours (Fig. 1.1). A reduction of the absorption rate was observed after this first phase. A similar pattern has been demonstrated for the absorption of the sodium salt of MCPA by the leaves of sunflower.[16] It has also been shown that 2,4-D acid penetrated rapidly into *Silene vulgaris* (Moench) Garcke during a period of 24 hours following application and that thereafter absorption ceased.[17] We suggest that the concentration gradient in the cuticle decreased 10-11 hours after application. This concentration gradient is thought to be the force driving the movement of compounds through the cuticle.[18] The reduction of concentration gradient may be achieved by active ingredient accumulating in the apoplast compart-ment between the cell membrane of the epidermal cells and the inner side of the cuticle or by immobilization of the active ingredient in the drop deposit residue on the leaf surface. Knoche gave a similar explanation for the uptake of gibberelin A_3 into the leaves of sour cherry.[19]

Neither 2,4-D compound accumulated further in the treated area of pea and black nightshade after the first 10-11 hours. We also observed that the translocation of ^{14}C to the rest of the plant began at about 10-11 hours after application. A similar time course for uptake and translocation was found for 2,4-D acid applied to *Asclepias syriaca* L.: only during the first six hours did the treated leaves of the common milkweed seedlings absorb a large amount of 2,4-D (45 %), and thereafter the translocation of ^{14}C to the rest of the plant accelerated.[20] This pattern may result from limitations in the uptake of the compounds into the symplast and/or further translocation, either or both of which result in a higher concentration of the compound in the apoplast nearby the inner side of the cuticle. As a consequence the absorption will decrease.

Autoradiography (photographs not shown) indicated that the ^{14}C of the two 2,4-D compounds followed a symplastic route in accordance with the phloem-mobile character of 2,4-D.[21] In both species the ^{14}C from 2,4-D TEA and 2,4-D IOE showed the same translocation pattern. In a quantitative study Norris and Freed found a similar result when they compared the translocation of 2,4-D TEA and 2,4-D IOE in bigleaf maple;[14] there was a much greater accumulation of 2,4-D IOE in the treated area but the distribution of ^{14}C over the other plant fractions was similar. Crafts demonstrated the hydrolysis of 2,4-D iso-propyl ester in the treated leaf of barley.[22] His results indicate that after hydrolysis, 2,4-D translocates through the symplast. Considering the intermediate permeability hypothesis it is very unlikely that a lipophilic compound is retained well enough in the symplast to permit phloem transport.[23] Our results and those in the reports cited above suggest that the ^{14}C of the 2,4-D IOE was translocated after hydrolysis in the treated area, but are not conclusive proof of this. This implies that for 2,4-D esters the hydrolysing capacity in the treated area may seriously limit further translocation of the 2,4-D.

1.4.2 Influence of surfactant on absorption and translocation of 2,4-D TEA

The greater foliar absorption of 2,4-D TEA in the presence of surfactant supports the suggestion derived from previous studies[2,3] that a relatively hydrophilic surfactant enhances the foliar uptake of water soluble compounds. The surfactant increased the spreading of drops containing 2,4-D TEA. This effect was most pronounced in pea. The influence of surfactant on foliar absorption was much greater in black nightshade than in pea, which indicates that influence on drop spreading is not the most relevant factor in our experiment. In both the species we studied drop drying had no effect on the absorption of 2,4-D TEA without surfactant (Fig. 1.2, inserts). Therefore it is unlikely, that the surfactant effect is primarily attributable to a better wetting of the residual deposit as result of an enhanced hygroscopicity. Therefore we suggest that the surfactant influenced the permeability of the cuticle. The influence of the surfactant starts abruptly when the drop deposit is dry (visual assessment). As the drop dries the concentrations of active ingredient and surfactant increase rapidly and this may enhance the partitioning of the surfactant into the cuticle. A similar product (Ethomeen T/25: polyoxyethylene (15) tallowamine) increased the water permeability of isolated cuticles[4], which indicates that the surfactant must have penetrated the cuticle. This supports the view that partition of the surfactant into the cuticle may have caused the greater uptake of 2,4-D TEA. Previous studies on the foliar penetration of NAA in cowpea discs and the foliar penetration of diflufenican in *Galium aparine* L. showed that the surfactants induced a relatively great increase of penetration after the drops had dried up[24,25] The authors mention hydroscopicity as a possible explanation for their results. However the continuous penetration of 2,4-D TEA alone in pea and black nightshade after drop drying as observed in our study indicates, certainly in the case of pea, that drop drying did not limit penetration much. In both species addition of surfactant reduced the translocation of ^{14}C from 2,4-D TEA, because the absorption increased more than the translocation did. Our experiments did not reveal the causes of this observation. In the results section we pointed out that in black nightshade the ^{14}C in the cellulose acetate strip can be considered to have come from the cuticle and not from the residual deposit. In black nightshade the amount of ^{14}C in the cellulose acetate strip, in the treated area, and in the rest of the treated leaf attained a constant level after 10 to 11 hours. However the amount of ^{14}C in the rest of the plant increased thereafter. After 48 hours this resulted in a translocation efficiency similar to the translocation efficiency without surfactant.

1.4.3 Influence of surfactant on the absorption and translocation of 2,4-D IOE

In pea and black nightshade the addition of surfactant resulted in a reduction of the foliar absorption of 2,4-D IOE. During the first two hours after application this effect was most pronounced in black nightshade. Numerous factors may cause the surfactant effect. Addition of surfactant increased the pH of the 2,4-D IOE solution (from pH 4.5 to pH 8.4) and this may have contributed to the effect. A previous study indicated the presence of dissociable carboxyl groups in the cuticle.[26] A higher pH of the drop may lead to a more negatively charged and thus a more polar cuticle which may reduce the partition of the lipophilic 2,4-D IOE into

the cuticle. Reduced partition and thus a reduced concentration of 2,4-D IOE in the cuticle reduces the force driving the flow rate through the cuticle.[18] Reduced partition might also be caused by: accumulation of the active ingredient in surfactant micelles (thereby decreasing the concentration of biocide available for penetration),[4,27] and the sorption sites in the cuticle being blocked by surfactant partition into the cuticle.[27] Further, the partition of a relatively hydrophilic surfactant into the cuticle may result in a more hydrophilic cuticle-surfactant system and this may also reduce the partition of a lipophilic compound.[28]
In black nightshade and in pea the surfactant had no significant influence on the translocation efficiency of ^{14}C from 2,4-D IOE (Fig. 1.3, Tables 1.2 and 1.4).
It is remarkable that the surfactant reduces the translocation efficiency of 2,4-D TEA in both species whereas the translocation efficiency of ^{14}C from 2,4-D IOE was not influenced. This indicates that there was no direct effect of the surfactant on the translocation. In that case it could be expected that the translocation efficiency of ^{14}C from 2,4-D IOE was reduced too. It seems more likely that there is a limitation in black nightshade to translocate 2,4-D TEA from the treated leaf to the rest of the plant (Fig. 1.2 and Table 1.1) and a limitation in pea to translocate 2,4-D TEA from the treated area to the rest of the treated leaf and to the rest of the plant (Fig. 1.2 and Table 1.3) when the absorption is enhanced by the surfactant.
However after 24 hours (black nightshade) and after 5.3 hours (pea) there was no more significant reduction of translocation efficiency which may mean that the observed reduction of translocation efficiency does not necessarily lead to a reduced efficacy of the herbicide. This also indicates the importance of measuring absorption and translocation of herbicides at more than one interval.

Acknowledgements

The authors thank AKZO Chemicals BV (Deventer, the Netherlands) for providing the surfactant and financial support to this study.

References

1. Hull, H.M., D.G. Davis & G.E. Stolzenberg, 1982.
   In: Adjuvants for herbicides, R.H. Hodgson ed. Weed Science Society of America, Champaign, 26-67
2. Stevens, P.J.G., & M.J. Bukovac, 1987.
   Pesticide Science 20, 37-52
3. Holloway, P.J., W.C. Wong & H.J. Partridge, 1992.
   Pesticide Science 34, 109-118.
4. Schönherr, J. & H. Bauer, 1989.
   In: Proc. 2nd International Symposium on Adjuvants for Agrichemicals, C.L. Foy ed., CRC Press, Boca Raton, 17-35
5. De Ruiter, H., E. Meinen & M.A.M. Verbeek, 1992.
   In Proc. 2nd International Symposium on Adjuvants for Agrichemicals, C.L. Foy ed., CRC Press, Boca Raton, 109-116.
6. De Ruiter, H., A.J.M. Uffing, E. Meinen & A. Prins, 1990.
   Weed Science 38, 567-572
7. Coble, H.D., F.W. Slife & H.S. Butler, 1970.
   Weed Science 18, 653-656
8. Sherrick, S.L., H.A. Holt & F.D. Hess, 1986.
   Weed Science 34, 811-816
9. McWorther, C.G., T.N. Jordan & G.D. Wills, 1980.
   Weed Science 28, 113-118
10. Steiner, A.A., 1984.
    ISOSC Proc. 6th Int. Congress on Soilless Culture, 633-650
11. Silcox, D. & P.J. Holloway, 1986.
    Aspects Applied Biology 11, 13-17
12. Naylor, A.W., 1976.
    In: Herbicides, Physiology, Biochemistry, Ecology, L.J. Audus ed., Academic Press, London, 2nd edn. 397-426
13. Silcox, D., & P.J. Holloway, 1986.
    Aspects of Applied Biology 11, 19-28
14. Norris, L.A., & V.H. Freed, 1966.
    Weed Research 6, 203-211
15. Price, C.E. & N.H. Anderson, 1985.
    Pesticide Science 16, 369-377
16. Holly, K., 1956.
    Ann. Applied Biology 44, 195-199
17. Wall, D.A., J.C. Hall, & I.N. Morrison, 1991.
    Weed Research 31, 81-88
18. Schönherr, J., & M. Riederer, 1989.
    Rev. Environment Cont. and Toxicol.108, 1-70.
19. Knoche, M., N.K. Lownds, & M. Bukovac, 1992.
    Crop Protection 11, 57-63
20. Bhowmik, P.C., 1985.
    Proceedings Northeastern Weed Science Society 39, 92-97

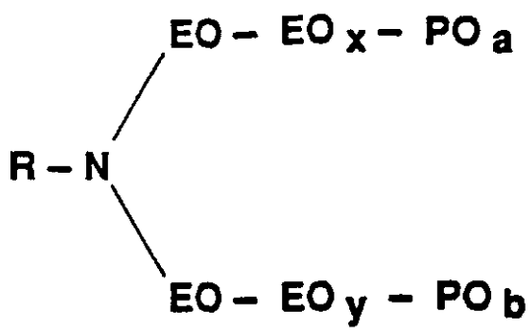
21. Ashton, F.M., & A.S. Crafts, 1981.
In: Mode of Action of herbicides, John Wiley and Sons, New York, 1981, 2nd edn., 20-39
22. Crafts, A.S., 1960.
Weeds 8, 19-25.
23. Tyree, M.T., C. Peterson, & L.V. Edgington, 1979.
Plant Physiology 63, 367-374
24. Lownds, N.K., `J.M. Leon, & M.J. Bukovac, 1987
Journal of American Horticultural Science 112, 554-560
25. Knight, H., & R.C. Kirkwood, 1991.
Pesticide Science 33, 305-317
26. Schönherr, J., M.J. Bukovac, 1973.
Planta 109, 73-93
27. Shafer, W.E. & M.J. Bukovac, 1989.
Journal of Agriculture and Food Science 37, 486-492
28. Valkenburg, J.W. van, 1982.
In: Adjuvants for Herbicides, R.H. Hodgson ed. Weed Science Society of America, Champaign, 1-9

Table of Contents

II The influence of the type and concentration of surfactant on the absorption and translocation of 2,4-D compounds

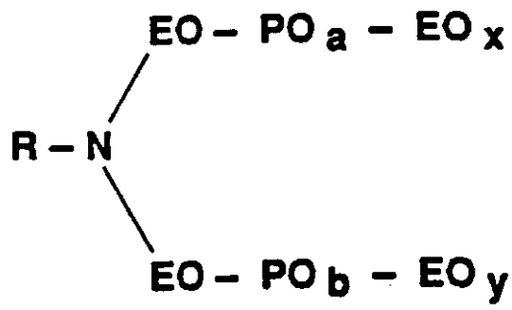
2.1 Introduction

Surfactants may enhance the foliar uptake of herbicides and other biocides. In how far this surfactant effect is influenced by the chemical and physical properties of surfactant and active ingredient is not well known for the fatty amine surfactants. Therefore in this study we compared the influence of three cationic tallowamine surfactants on the foliar uptake of 2,4-D triethanolamine salt (2,4-D TEA) and 2,4-D iso-octy ester (2,4-D IOE). The surfactants Armoblen 557 and Armoblen 600 (both tallowamine blockpolymers containing a block of polymerized propylene oxide (12PO) and a block of polymerized ethylene oxide (5EO); see Fig. 2.1) and Ethomeen T/27 (polyoxyethylene (17) tallowamine) were selected. The variation in chemical structure of the hydrophilic regions of the surfactants (Fig. 2.1) causes differences between the hydrophilicity of the surfactants. The two 2,4-D compounds were selected to have active ingedients with a water soluble (2,4-D TEA) and a lipophilic character (2,4-D IOE). In relation to the influence on foliar absorption the performance of a surfactant is generally better at higher concentrations of surfactant (0.5 %). To find out whether one ore more of the selected surfactants are very good penetration enhancers we maesured the foliar uptake at surfactant concentrations of 0.05 % and 0.5 %.



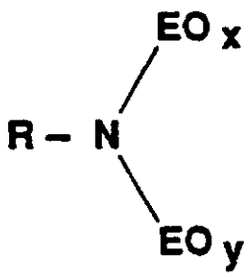
a+b=12 (a)

x+y=4



a+b=12 (b)

x+y=5



x+y=17 (c)

Figure 2.1 Structure of the tallowamine surfactants; a = Armoblen 557, b = Armoblen 600 and c = Ethomeen T/27. Abbreviations: R= tallow, EO = ethlene oxide and PO = propylene oxide.

2.2 Materials and methods

2.2.1 Plant material

Pea seeds (*Pisum sativum* L., cv. Finale) were germinated and after two weeks the seedlings were placed in 1 liter pots containing 1/2-strength Steiner's nutrient solution.[10] Seeds of black nightshade (*Solanum nigrum* L.) were germinated in a greenhouse in trays containing a mixture of sand and humic potting soil (1:2, v/v) and then transferred to the growth chamber. Two weeks after emergence these seedlings were also placed in 1 liter pots containing 1/2-strength Steiner's nutrient solution. To ensure good growth of black nightshade the pots were connected to an aerating system two days after the seedlings had been transferred. The pea and black nightshade plants were grown in a growth chamber under 14 h light, 18/12 °C (day/night) temperature, and 70/80 % (day/ night) relative humidity. Light was provided by high pressure sodium lamps (Phlips 400W SON/T) and fluorescent tubes (Philips TLD 58W colour 54) to give 80-120 W/m^2 (PAR) at leaf level. The experiments were done on pea plants that were 10 cm tall and had about four pairs of leaflets and two tillers after 12 days on nutrient solution, and on black nightshade plants (6 cm tall) that had six unfurled leaves and one to three tillers after 15 days on nutrient solution. In the case of pea one single unfurled leaflet of the youngest pair of leaflets was treated. In the case of black nightshade, the herbicide solutions were applied to the youngest fully expanded leaf of black nightshade.

2.2.2 Herbicides and surfactant

Unlabelled 2,4-D (Merck, purity 98 %) was mixed with water and converted to the triethanolamine salt (2,4-D TEA) by the adding triethanolamine (Merck, purity 98 %) while heating (Austria Linz, 1989, pers. comm.). Labelled 2,4-D TEA (2,4-dichlorophenoxy[2-^{14}C]acetic acid triethanolamine salt, Amersham, purity > 96 %; s.a. 437 MBq/mmol) and unlabelled 2,4-D TEA were dissolved in acetone+water (1+3 by volume). The concentration of 2,4-D TEA (labelled plus unlabelled) was 11.3 mM (s.a. 29.47 MBq/mmol), which is equivalent to the molarity of 2,4-D when this compound is applied at a rate of 1 kg/ha at a water volume of 400 L/ha. Drops of the 2,4-D TEA solution without surfactant were repelled by the waxy leaf surface of pea. To overcome this, acetone (final concentration 25 %) was added to solutions containing 2,4-D TEA with and without surfactant. Labelled 2,4-D IOE (2,4-dichlorophenoxy[2-^{14}C]acetic acid iso-octyl ester, Amersham, purity > 96 %,; s.a. 333 MBq/mmol) and unlabelled 2,4-D IOE (Austria Linz, technical grade, purity 97 %) were dissolved in acetone+water (3+1 by volume). The concentration of 2,4-D IOE was 11.3 mM (s.a. 29.47 MBq/mmol). The cationic fatty amine surfactants Armoblen 557 and Armoblen 600 (both tallowamine blockpolymers containing a block of polymerized propylene oxide (12PO) and a block of polymerized ethylene oxide (5EO); see Fig. 2.1)) and Ethomeen T/27 (polyoxyethylene (17) tallowamine) were used in this study. The product are blends of compounds differing in the length of their alkyl chains and in content of EO and PO. The surfactant was added on a weight to volume basis; the concentrations were 0.05 % and 0.5 %.

2.2.3 Absorption and translocation

The solutions were applied to the leaf surface as five 1 µl drops (1.67 kBq/5 µl) to a discrete area on the adaxial surface in the median part of the leaf, outlined with waterproof ink. To find out how much ^{14}C was applied to the leaves, five drops were dispensed directly into a scintillation vial. All applications were done with the Burkard Microapplicator PAX 100 fitted with a 50 µl syringe and needle coated with PTFE. The applications were done in the growth chamber. It took one and half hour to apply the herbicide solutions to one of the two species. One of the species was treated two hours after the beginning of the photoperiod and the other one was treated 6 hours after the beginning of the photoperiod. After the treatment period the treated leaf was excised and washed with 2 ml aqueous acetone (1+3 (v/v) with 2,4-D TEA, and 3+1 (v/v) with 2,4-D IOE) to remove residual chemical deposits. The epicuticular wax was removed from the treated area with a cellulose acetate strip,[11] and the treated area was excised with a cork borer (diam. 1cm). Translocation to other parts of the plant was determined by measuring radioactivity in the rest of the treated leaf and in the fractions: shoot above the treated leaf, shoot under the treated leaf and the roots. To determine how much ^{14}C had been exuded into the nutrient solution we measured the radioactivity in 1 ml samples of that solution. The leaf surface wash and the sample from the nutrient solution were dissolved in scintillation liquid (10 ml; Packard Ultima Gold, Packard Instruments B.V., The Netherlands). The amount of ^{14}C exuded could only be measured accurately by this procedure if it was more than 8 % of the amount applied because the ^{14}C was so diluted in the nutrient solution. The cellulose acetate strips with adhering epicuticular wax were dissolved in acetone (0.5 ml) before scintillation liquid (10 ml; Packard Ultima Gold) was added. The fractions treated area and the rest of the treated leaf were oxidized using a Packard Tri-Carb Oxidizer Model 306. The fractions shoot above the treated leaf, shoot under the treated leaf and the roots were dried at 70 °C and then ground. Samples of the powder (150 mg) were oxidized. The $^{14}CO_2$ was trapped in Lumasorb I (5 ml; Lumac LSC B.V., Belgium) and then scintillation liquid (10 ml; Carboluma, Lumac LSC B.V.) was added. The ^{14}C in all fractions was quantified with a scintillation counter (Packard Tri-Carb 300C). Using this procedure the following parameters could be defined:
residual deposit = ^{14}C in leaf surface washing; epicuticular wax (pea) = ^{14}C in cellulose acetate strip; absorption = sum of ^{14}C in the plant tissue plus ^{14}C in the nutrient solution (in the case of black nightshade the ^{14}C in the cellulose acetate strip was included (see Results section of Chapter I)); translocation = ^{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.
We did not attempt to identify possible metabolites of the 2,4-D compounds.

2.2.4 Experimental design

Four replicates were carried out. The applications of one replicate were made according a completely ranomized design (arrangements of plants per species, the sequence of application of the herbicide solutions per species and the sequence of species). An analysis of variance was performed on the data of the four replicates. The LSD (P = 0.05) was used to compare the results of the different treatments.

Table 2.1 "The absorption and translocation of [^{14}C]2,4-D triethanolamine salt in the absence and presence of surfactants"
(black nightshade)

	Radioactivity as percentage of total ^{14}C applied [1]						
	Surfactant (%w/v) [2]						
	none	0.05% A600	0.05% A557	0.05% ET/27	0.5% A600	0.5% A557	0.5% ET/27
Leaf washing	92.3 a [3]	73.8 bc	86.3 ab	94.7 a	40.4 e	52.1 de	61.2 cd
Cellulose acetate strip	3.2 a	8.8 ab	3.8 a	1.8 a	19.9 b	13.5 ab	18.5 b
Treated area	1.61 ab	1.55 ab	1.51 ab	0.97 a	14.03 d	11.06 cd	6.38 bc
Rest of the treated leaf	0.97 a	1.72 ab	0.79 a	0.72 a	3.33 c	2.15 bc	2.50 bc
Shoot above treated leaf	0.225 a	0.150 a	0.175 a	0.380 a	0.332 a	0.237 a	0.248 a
Shoot below treated leaf	1.00 a	1.05 a	0.98 a	0.94 a	2.91 c	2.89 c	1.96 b
Roots	0.97 a	0.62 a	0.75 a	0.58 a	2.23 b	2.57 b	1.15 a
Recovery	100.25 b	87.66 ab	94.37 b	100.15 b	83.21 a	84.45 a	91.88 ab
Uptake	7.95 a	13.89 a	8.03 a	5.42 a	42.78 b	32.40 b	30.70 b
Translocation	3.16 a	3.54 a	2.69 a	2.62 a	8.80 c	7.87 c	5.86 b
Trans/Uptake x 100%	43.99 bc	35.78 abc	41.50 abc	54.12 c	20.41 a	27.82 ab	25.17 ab

1) Mean values (n=4)
2) A600=Armoblen 600; A557=Armoblen 557; ET/27=Ethomeen T/27
3) Means followed by the same letter are not different at the 5% level

Table 2.2 "The absorption and translocation of [^{14}C]2,4-D triethanolamine salt in the absence and presence of surfactants"
(pea)

	Radioactivity as percentage of total ^{14}C applied [1]						
	Surfactant (%w/v) [2]						
	none	0.05% A600	0.05% A557	0.05% ET/27	0.5% A600	0.5% A557	0.5% ET/27
Leaf washing	49.3 c [3]	35.2 b	37.1 b	41.1 bc	33.6 ab	24.5 a	42.5 bc
Cellulose acetate strip	3.33 ab	4.50 b	2.51 ab	3.32 ab	0.84 a	0.97 a	1.04 a
Treated area	24.62 a	24.35 a	30.45 a	27.41 a	38.47 b	39.05 b	27.22 a
Rest of the treated leaf	3.19 a	8.91 c	8.56 bc	5.40 abc	6.15 abc	7.65 abc	3.49 ab
Shoot above treated leaf	0.558 a	0.670 ab	0.910 b	0.663 ab	0.642 ab	0.768 ab	0.763 ab
Shoot below treated leaf	8.02 a	9.61 a	10.51 a	8.7 a	11.14 a	11.67 a	10.13 a
Roots	6.55 a	5.59 a	10.12 a	5.25 a	6.82 a	6.95 a	9.04 a
Recovery	95.53 ab	88.85 a	100.14 b	91.87 ab	97.70 ab	91.52 ab	94.21 ab
Uptake	42.94 a	49.12 a	60.55 b	47.43 a	63.22 b	66.09 b	50.64 a
Translocation	18.32 a	24.77 abc	30.10 c	20.02 ab	24.75 abc	27.04 bc	23.43 abc
Trans/Uptake x 100%	42.97 a	50.53 a	49.57 a	42.36 a	39.16 a	40.84 a	44.73 a

1) Mean value (n=4)
2) A600=Armoblen 600; A557=Armoblen 557; ET/27=Ethomeen T/27
3) Means followed by the same letter are not different at the 5% level

Table 2.3 "The absorption and translocation of [^{14}C]2,4-D iso-octyl ester in the absence and presence of surfactants"
(black nightshade)

	Radioactivity as percentage of total ^{14}C applied [1]						
	Surfactant (%/v) [2]						
	none	0.05% A600	0.05% A557	0.05% ET/27	0.5% A600	0.5% A557	0.5% ET/27
Leaf washing	82.3 ab [3]	81.4 ab	81.0 ab	75.9 a	81.2 ab	84.8 b	85.5 b
Cellulose acetate strip	0.81 a	3.24 b	1.71 ab	2.39 ab	1.66 ab	2.58 ab	1.44 ab
Treated area	10.28 a	7.79 a	9.16 a	8.58 a	8.03 a	6.37 a	11.95 a
Rest of the treated leaf	1.45 a	1.33 a	1.93 a	1.93 a	2.88 a	1.3 a	1.45 a
Shoot above treated leaf	0.358 a	0.375 a	0.377 a	0.252 a	0.300 a	0.300 a	0.455 a
Shoot below treated leaf	2.60 a	2.44 a	2.44 a	1.91 a	1.71 a	1.34 a	2.14 a
Roots	1.87 a	1.78 a	1.97 a	1.93 a	1.36 a	1.11 a	2.06 a
Recovery	99.63 bc	98.32 ab	98.56 abc	92.86 a	97.14 ab	97.75 ab	105.02 c
Uptake	17.36 a	16.96 a	17.59 a	16.98 a	15.93 a	12.98 a	19.49 a
Translocation	6.27 a	5.93 a	6.71 a	6.02 a	6.25 a	4.03 a	6.11 a
Trans/Uptake x 100%	42.35 a	38.61 a	39.35 a	36.02 a	38.48 a	32.22 a	32.28 a

1) Mean values (n=4)
2) A600=Armoblen 600; A557=Armoblen 557; ET/27=Ethomeen T/27
3) Means followed by the same letter are not different at the 5% level

Table 2.4 "The absorption and translocation of [^{14}C]2,4-D iso-octyl ester in the absence and presence of surfactants"
(pea)

	Radioactivity as percentage of total ^{14}C applied [1]						
	Surfactant (%w/v) [2]						
	none	0.05% A600	0.05% A557	0.05% ET/27	0.5% A600	0.5% A557	0.5% ET/27
Leaf washing	29.5 a [3]	41.2 ab	51.8 b	41.4 ab	46.6 b	43.2 b	43.6 b
Cellulose acetate strip	1.12 a	1.51a	0.66 a	1.28 a	1.46 a	1.28 a	1.10 a
Treated area	35.4 c	24.7 abc	19.7 a	23.4 ab	23.9 ab	24.0 ab	32.9 bc
Rest of the treated leaf	9.03 c	7.87 bc	5.36 abc	4.77 abc	5.64 abc	3.73 ab	3.35 a
Shoot above treated leaf	0.795 ab	0.910 b	0.845 b	0.897 b	0.555 a	0.670 ab	0.580 a
Shoot below treated leaf	13.56 ab	13.74 ab	9.90 ab	15.06 b	11.93 ab	13.22 ab	8.85 a
Roots	5.39 abc	7.10 abc	9.75 c	7.94 bc	2.74 a	4.04 ab	4.03 ab
Recovery	94.76 ab	96.98 b	98.00 b	94.36 ab	92.77 ab	90.17 a	94.39 ab
Uptake	64.19 b	54.32 ab	45.58 a	51.72 a	44.76 a	45.70 a	49.72 a
Translocation	28.78 bc	29.62 bc	25.85 bc	28.66 bc	20.87 ab	21.66 ab	16.80 a
Trans/Uptake x 100%	46.67 ab	55.29 b	58.04 b	55.73 b	46.35 ab	46.32 ab	35.14 a

1) Mean values (n=4)
2) A600=Armoblen 600; A557=Armoblen 557; ET/27=Ethomeen T/27
3) Means followed by the same letter are not different at the 5% level

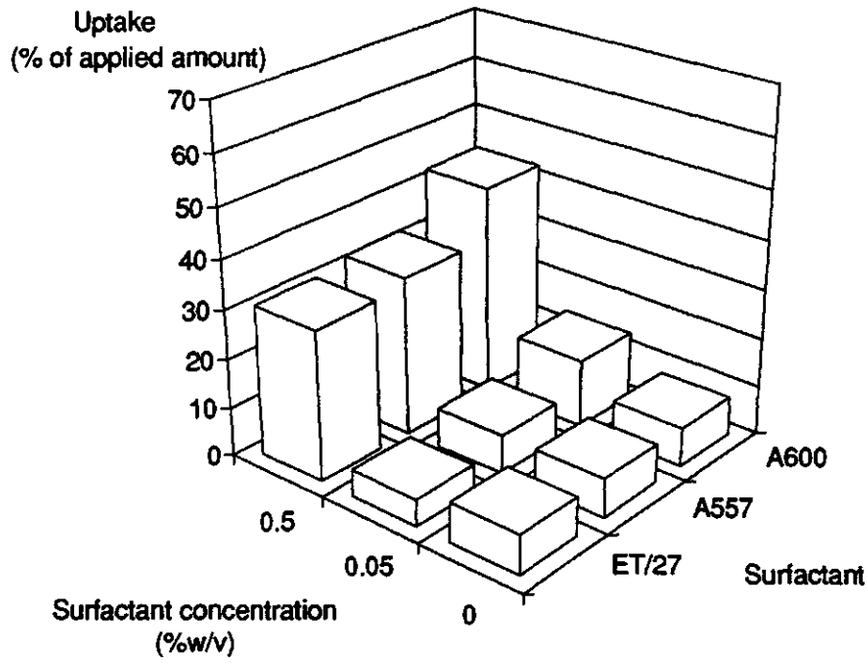


Figure 2.2 The influence of surfactant type and concentration on the foliar absorption of 2,4-D TEA (black nightshade)

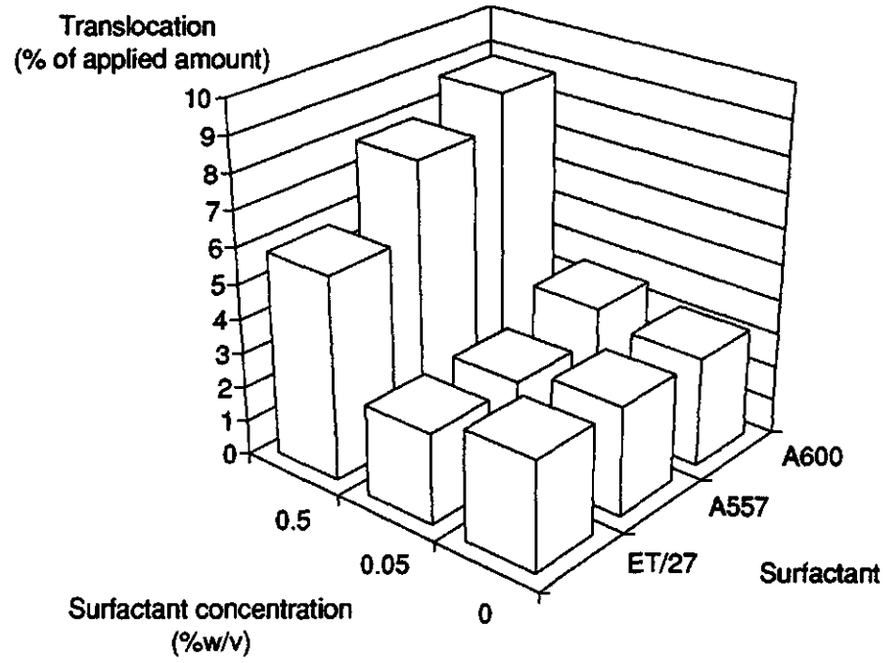


Figure 2.3 The influence of surfactant type and concentration on the translocation of 2,4-D TEA (black nightshade)

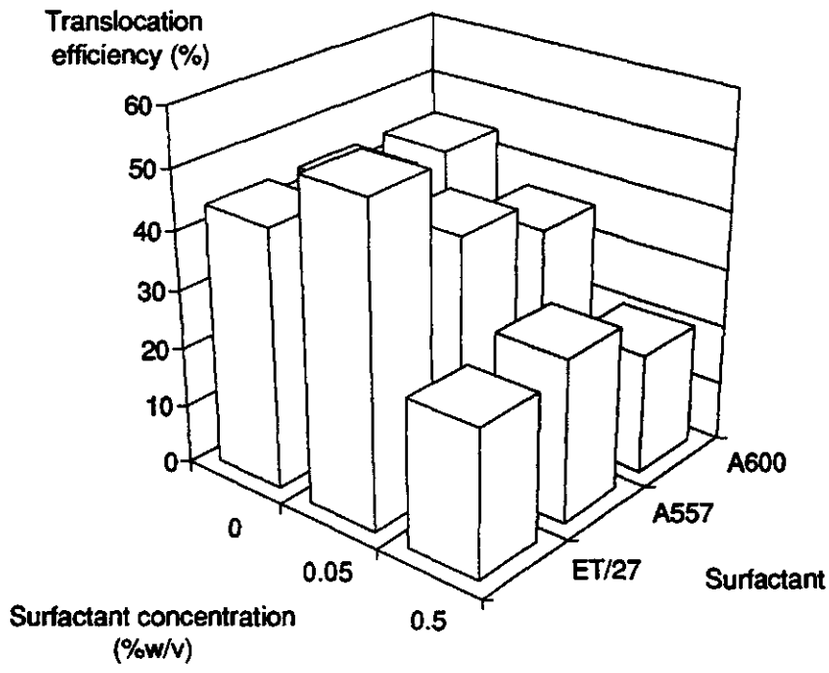


Figure 2.4 The influence of surfactant type and concentration on the translocation efficiency of 2,4-D TEA (black nightshade)

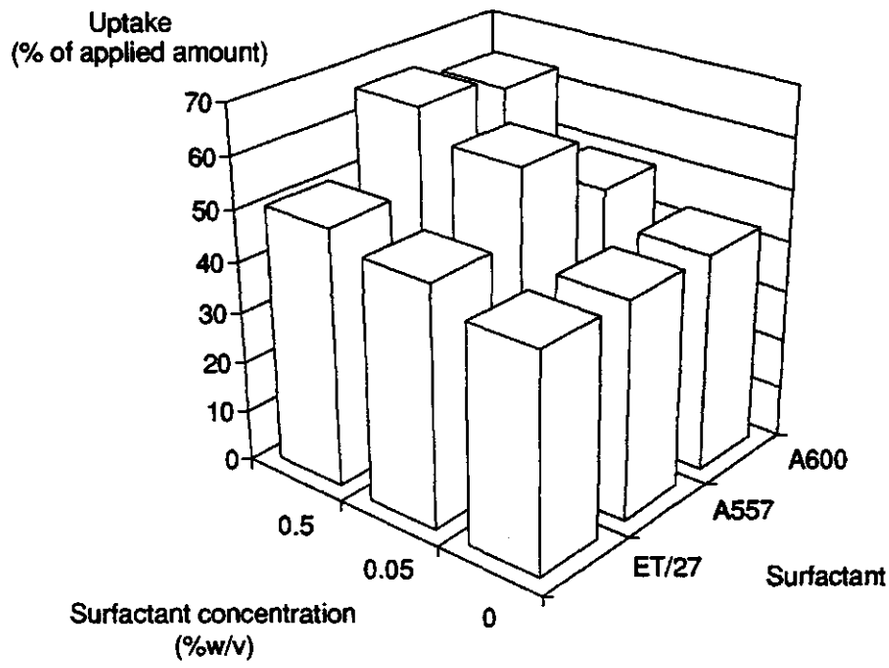


Figure 2.5 The influence of surfactant type and concentration on the foliar absorption of 2,4-D TEA (pea)

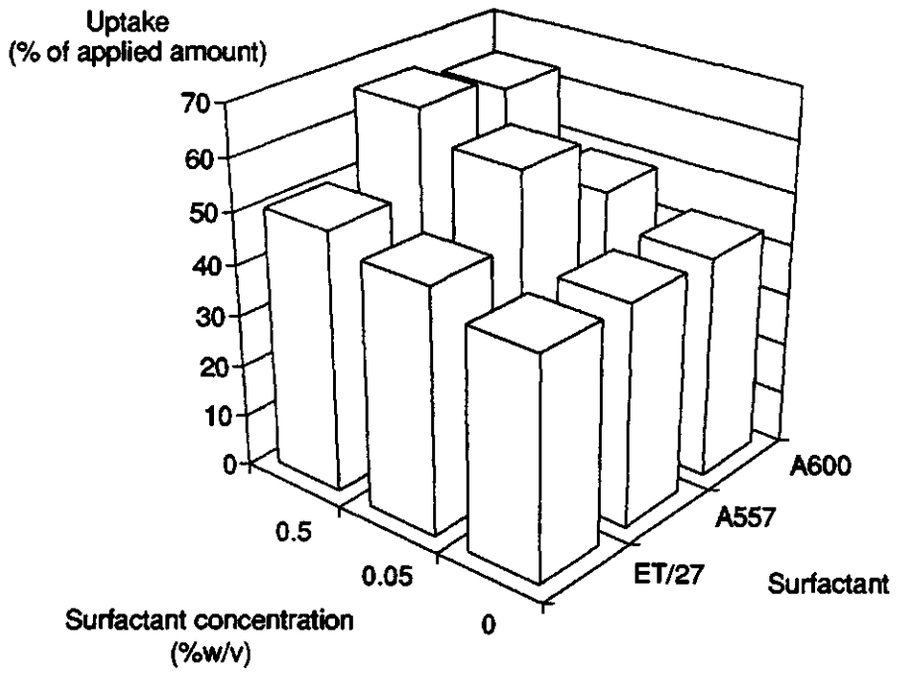


Figure 2.6 The influence of surfactant type and concentration on the translocation of 2,4-D TEA (pea)

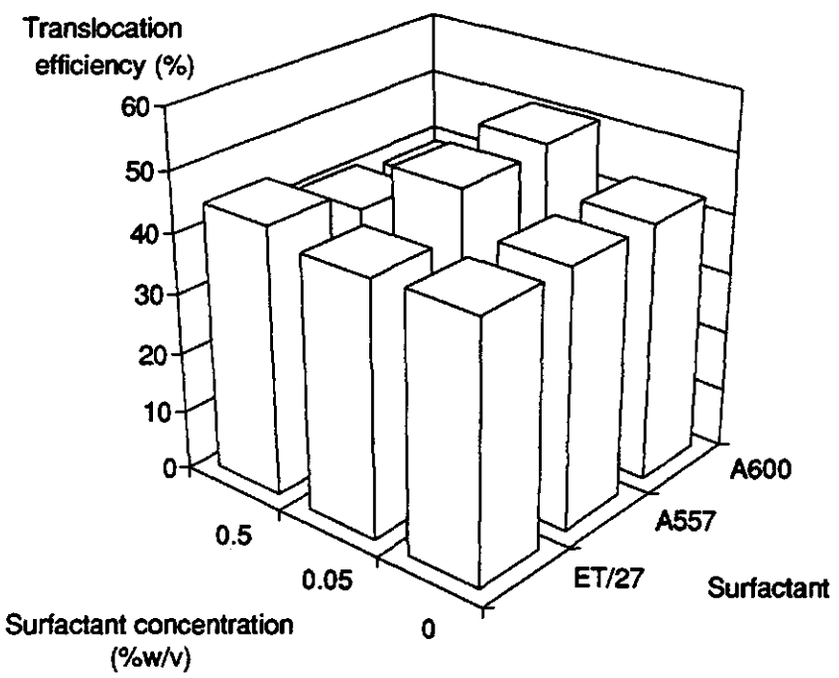


Figure 2.7 The influence of surfactant type and concentration on the translocation efficiency of 2,4-D TEA (pea)

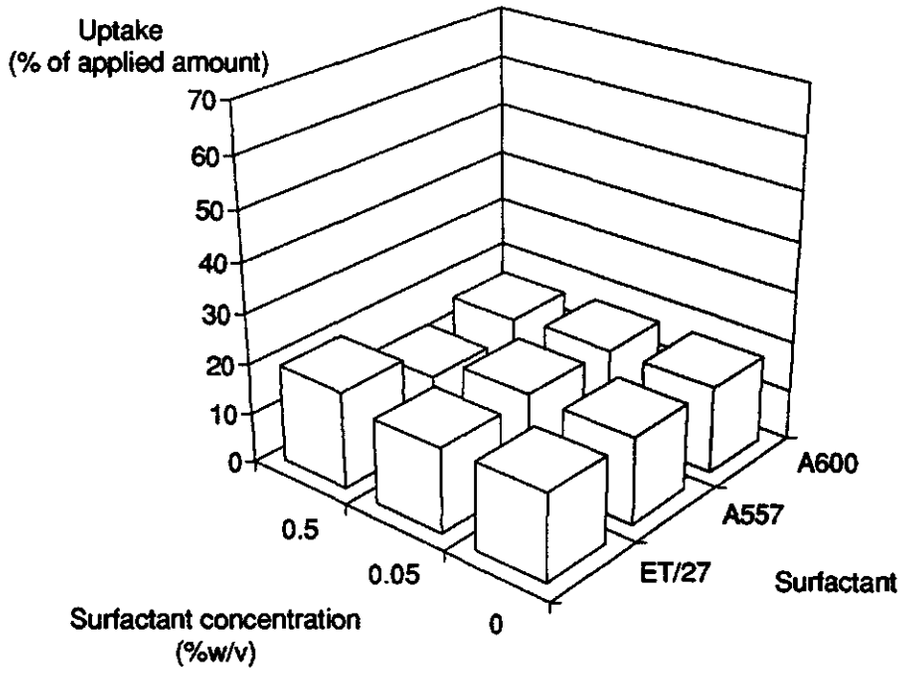


Figure 2.8 The influence of surfactant type and concentration on the foliar absorption of 2,4-D IOE (black nightshade)

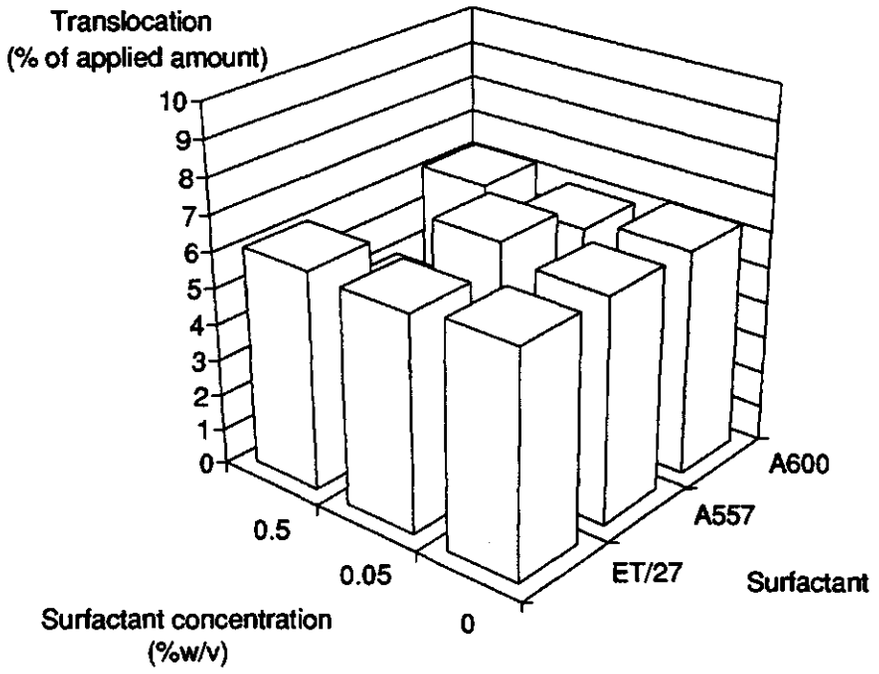


Figure 2.9 The influence of surfactant type and concentration on the translocation of 2,4-D IOE (black nightshade)

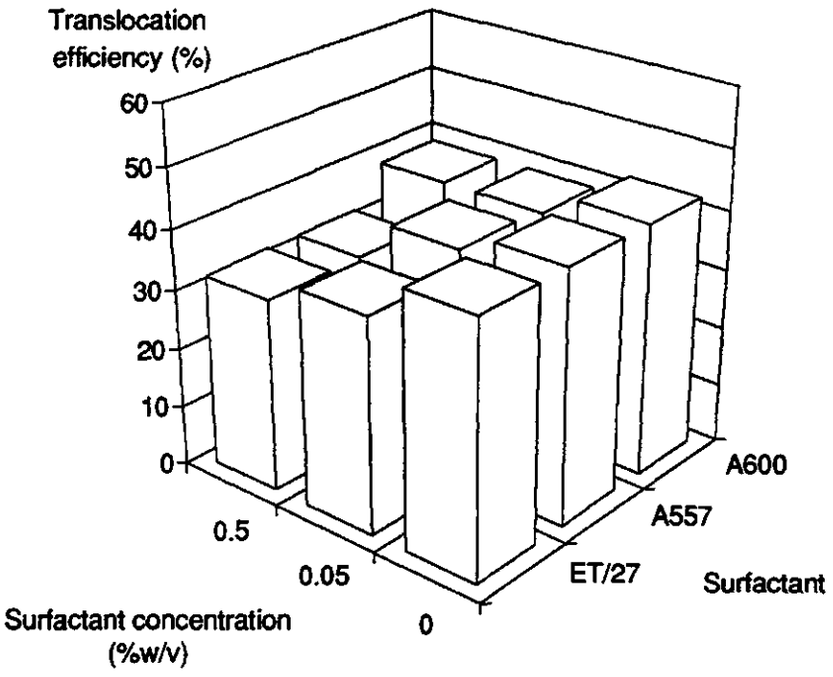


Figure 2.10 The influence of surfactant type and concentration on the translocation efficiency of 2,4-D IOE (black nightshade)

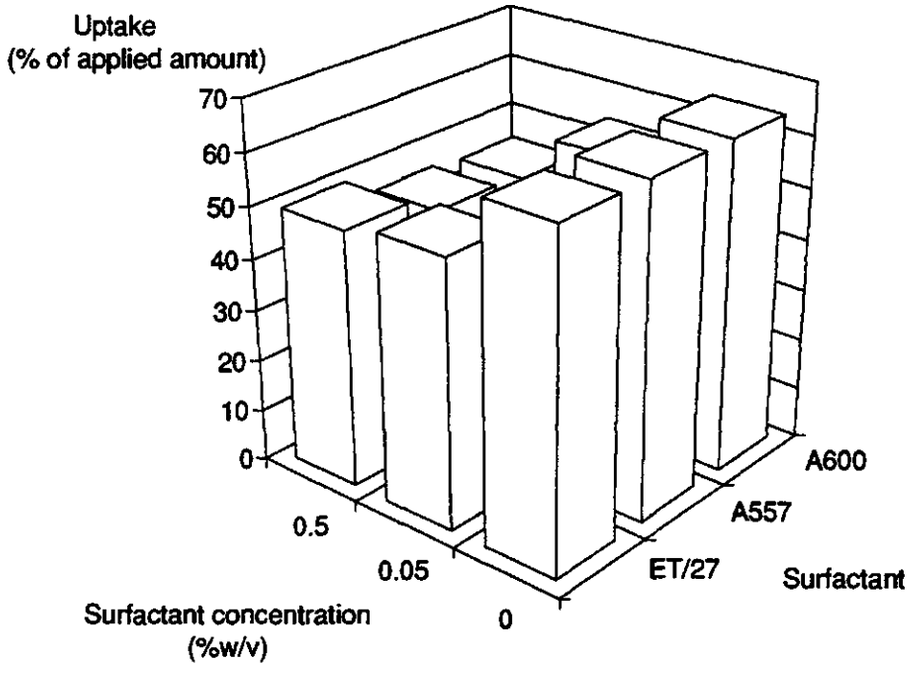


Figure 2.11 The influence of surfactant type and concentration on the foliar absorption of 2,4-D IOE (pea)

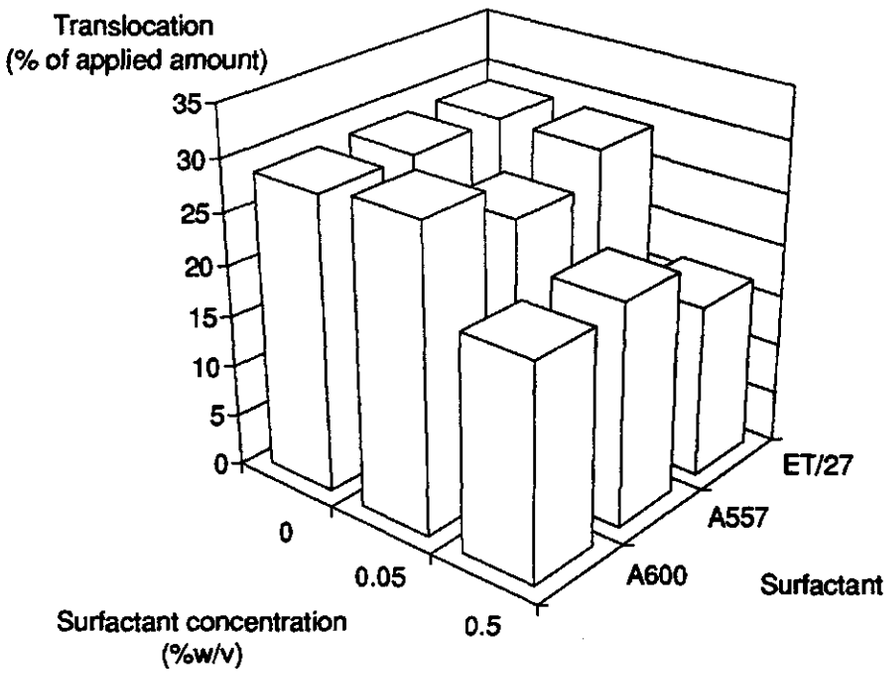


Figure 2.12 The influence of surfactant type and concentration on the translocation of 2,4-D IOE (pea)

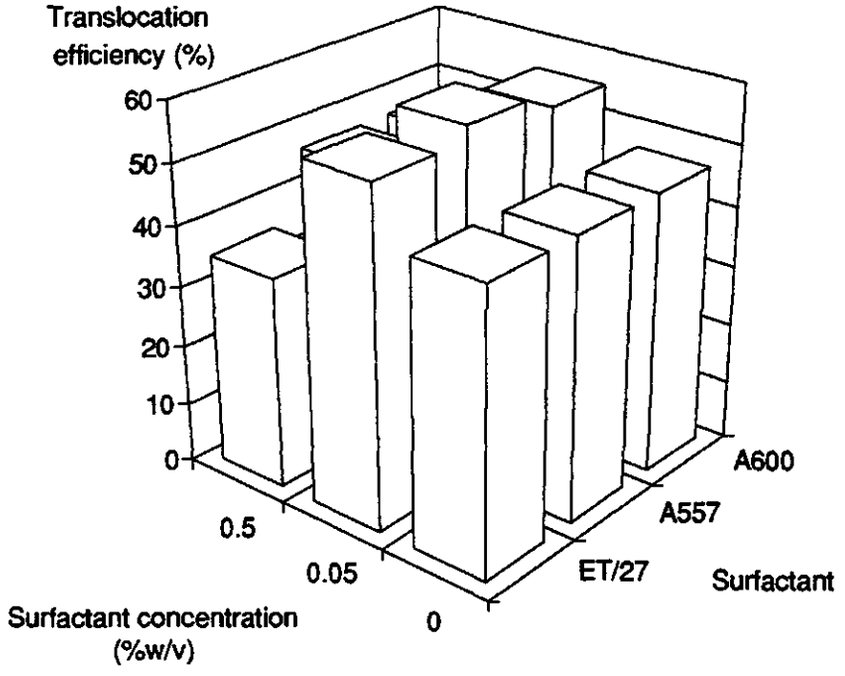


Figure 2.13 The influence of surfactant type and concentration on the translocation efficiency of 2,4-D IOE (pea)

2.3 Results and discussion

2.3.1 Absorption and translocation of 2,4-D TEA and 2,4-D IOE (without surfactant)

Pea leaves absorbed more of both compounds than black nightshade did. The translocation efficiency was similar (range: 42-44 %) in the four plant-herbicide combinations tested.

2.3.2 Influence of surfactants on absorption of 2,4-D TEA (Tables 2.1 and 2.2)

In both species addition of the surfactants Armoblen 557 and Armoblen 600 enhanced the absorption of 2,4-D TEA at the surfactant concentration of 0.5 % but generally not at the surfactant concentration of 0.05 %. Alone in pea addition of 0.05 % Armoblen 557 enhanced the absorption of 2,4-D TEA. At the surfactant concentration of 0.5 % there was no difference between the penetration enhancing effects of Armoblen 557 and Armoblen 600. Addition of Ethomeen T/27 at 0.05 % did not enhance or reduce the foliar absorption of 2,4-D TEA in both species. Alone in black nighsthade the addition of Ethomeen T/27 at 0.5 % enhanced the foliar absorption. The results indicate that that the propylene oxide containing, thus more lipophilic surfactants are effective penetration enhancers whereas the only ethylene oxide containing surfactant was much less effective in the tested combinations. If is assumed that passage through the cuticle is the greatest barrier for foliar penetration then it can be suggested that the more lipohilic surfactants partition more easily into the lipophilic cuticle. This partitioning may lead to a more hydrophilic cuticle which facilitates the passage of 2,4-D TEA.

2.3.3 Influence of surfactants on absorption of 2,4-D IOE (Table 2.3 and 2.4)

In black nightshade the surfactants had no effect on the foliar absorption of 2,4-D IOE at both surfactant concentrations. In pea addition of the three surfactants at 0.5 % and addition of Armoblen 557 and Ethomeen T/27 at 0.05 % reduced the foliar absorption of 2,4-D IOE. An efect of armoblen557 at the concentration of 0.05 % was also observed with the foliar penetration of 2,4-D TEA in pea. This supports the suggestion that Armoblen 557 partitions into the cuticle giving the cuticle a more hydrophilic character which may explain the reduction of the absorption of the lipophilic 2,4-D IOE.

2.3.4 The influence of surfactants on translocation efficiency

The translocation efficiency of 2,4-D TEA in pea was not influenced by the addition of surfactant. In black nightshade alone addition of Armoblen 600 at 0.5 % reduced the

translocation efficiency of 2,4-D TEA. This apparent inhibition of translocation may result from a limited ability of black nightshade to translocate the greater amount of 2,4-D TEA in the cuticular waxes (cellulose acetate strip), in the treated area and in the rest of the treated leaf in the case that Armoblen 600 was added.
The translocation efficiency of 2,4-D IOE in pea and black nightshade was not influenced by addition of the surfactants.

2.3.5 Recovery of ^{14}C

The recovery of ^{14}C was generally between 90 and 100 % of the applied amount. Alone in black nightshade addition of the surfactants Armoblen 600 and Armoblen 557 to 2,4-D TEA at 0.5 % gave a lower recovery. We suggest that metabolism of 2,4-D TEA with release of $^{14}CO_2$ caused this loss (see also chapter I).

Table of Contents

III The influence of three adjuvants on the phytotoxicity of phenmedipham, difenoxuron and sethoxydim

Abstract. In 1990 field trials were performed to measure the influence of three adjuvants on the phytotoxicity of phenmedipham (Betanal), difenoxuron (Lironion), and sethoxydim (Fervinal). The adjuvants Schering 11E oil (mineral oil with emulsifier), Armoblen 600 (fatty amine type of surfactant) and Atplus 258 (nonionic surfactant) were selected for this study. The herbicide-adjuvant combinations were applied in sugarbeets (phenmedipham and sethoxydim) and in onions (difenoxuron and sethoxydim). Barley was sown as a model 'weed' between the rows, so that the phytotoxicity of the sethoxydim treatments could be estimated. The efficacy of phenmedipham was enhanced by addition of mineral oil, whereas the two surfactants had a minor influence. The three adjuvants all enhanced the efficacy of sethoxydim and difenoxuron at the recommended rate and at one-quarter of that rate.

3.1 Introduction

Adding of adjuvants to agrochemicals may be a way of reducing the dose required to control pests and weeds adequately. However, there are few general rules on how best to do this. Field trials need to be performed under conditions similar to the standard application of the biocide, before recommendations on the use of adjuvants can be made.

In 1990 we performed field experiments to measure the influence of three adjuvants on the phytotoxicity of the herbicides phenmedipham, sethoxydim and difenoxuron. A mineral oil (Schering 11E) and two surfactants (Armoblen 600 and Atplus 258) were selected as adjuvants for this study. In field and greenhouse experiments, Miller and Nalewaja (1973), found that the efficacy of phenmedipham could be enhanced by adding oils (linseed oil, sunflower oil and petroleum oil) but not by adding a surfactant (ethoxylated alcohol with isopropanol). Their study also demonstrated that weed species differ in their response to the addition of oils to phenmedipham.

The efficacy of sethoxydim had found to be enhanced by the following adjuvants: ammonium sulphate (Chow, and MacGregor 1983, York, Jordan, and Wilcut 1990); nonionic surfactants (Chow, and MacGregor 1983); a cationic surfactant (Kudsk, Thonke, and Streibig 1987); an oil emulsifiable adjuvant (Buhler, and Burnside 1984, Chernicky, Gossett, and Murphy 1984, Chow, and MacGregor 1983, Harzler, and Foy 1983); vegetable oil (Chow, and MacGregor 1983, Hatchard, Ashford, and Reed 1989, Manthey Nalewaja, and Szelezniak 1989, Nalewaja, Skrzypczak, and Gillespie 1986) and petroleum oil (Manthey, Nalewaja, and Szelezniak 1989, Nalewaja, Skrzypcszak, and Gillespie 1986). Kudsk and colleagues (1987) measured the dose-response curve of sethoxydim on winter barley, applied the parallel-line assay and found that the cationic surfactant Atplus 221 (ethoxylated alkylamine) was a more effective adjuvant than the mineral oil Sun Spray Plus. In turn, that oil was more effective than the nonionic surfactant Sandovit (alkyl-aryl-polyglycolether).

Substituted urea herbicides like difenoxuron predominantly act on the soil. There is little published information on how adjuvants affect the foliar action of these herbicides. Hill and colleagues (1985) found that the foliar action of diuron and linuron was enhanced by adding a nonionic surfactant (trade name Surfactant WK; dodecylether of polyethylene glycol). West and Clay (1988) found that the foliar action of metoxuron and isoproturon was enhanced by

adding the nonionic surfactant Agral (ethoxylated nonylphenol), Codacide oil (rape seed oil) and Actipron (paraffinic oil).
In our study we applied the formulated herbicides phenmedipham, sethoxydim and difenoxuron, alone and with adjuvants, at the recommended application rate and at a quarter of this rate.
Our aim was to answer three questions: is there an adjuvant effect at both application rates? Does the result after treatment at the reduced rate combined with an adjuvant differ from the result after treatment at the recommended rate alone? Are there differences in the efficacy of the different adjuvants we used?

3.2 Materials and methods

Herbicides and adjuvants
Commercially formulated phenmedipham (Betanal), sethoxydim (Fervinal) and difenoxuron (Lironion) were used in this study. The adjuvants Schering 11E oil (mineral oil), Armoblen 600 (cationic fatty amine type of surfactant) and Atplus 258 (nonionic surfactant) were combined with the three herbicides.

Field experiments
The field experiments were performed at two experimental farms: Droevendaal (Wageningen) and De Bouwing (Randwijk). Phenmedipham and sethoxydim were applied in sugarbeets (cv. Univers) grown at Droevendaal (on sandy soil). Difenoxuron and sethoxydim were applied in onions (cvs Robusta and Augusta respectively) grown at De Bouwing (on alluvial clay).
Phenmedipham was applied at rates of 0.24 and 0.94 kg a.i./ha alone and in combination with Schering 11E oil (2 % v/v), Armoblen 600 (0.25 and 0.05 % w/v) and Atplus 258 (0.25 % w/v). Sethoxydim was applied at rates of 0.095 and 0.38 kg a.i./ha alone and in combination with Schering 11E oil (1.25 % v/v), Armoblen 600 (0.05 and 0.25 % w/v) and Atplus 258 (0.25 % w/v). Difenoxuron was applied at rates of 0.63 and 2.5 kg a.i./ha alone and in combination with Schering 11E oil (1.25 % v/v), Armoblen 600 (0.05 and 0.25 % w/v) and Atplus 258 (0.25 % w/v).
The herbicides were applied with an air-pressured sprayer (Birchmeier Helico Sapphire 1.2-mm nozzles fitted with a perforated (0.6-mm) whirling pin 2F) delivering 250 L/ha at 182 kPa (phenmedipham) and 400 L/ha at 182 kPa (sethoxydim and difenoxuron). The experiments consisted of a randomized block design with four replications. The experimental plots were 4 x 2 m (sugarbeets) and 6 x 2 m (onions). To assess the toxicity of sethoxydim, barley (cv. Apex) was seeded in rows between the rows of sugarbeets and onions, to represent a weed susceptible to sethoxydim.
The treatments were applied when the sugarbeets were in the two-leaf stage, the onions were 9 to 12 cm tall (sethoxydim) and 7 to 10 cm tall (difenoxuron). The barley was at the 2 to 4-leaf stage at the time of treatment.
The control of weeds and barley was assessed both qualitatively and quantitatively. The control was estimated visually, using a scale of 1= no control to 7= complete kill, either at three to four weeks (phenmedipham and sethoxydim) or at five weeks (difenoxuron) after treatment. Six weeks after treatment the weeds or barley (sethoxydim experiments) were harvested from a 1 m^2-sample (phenmedipham and sethoxydim) or from the complete plot (difenoxuron).

An analysis of variance was done on the data. To find the significance of the differences between means, a pairwise comparison of the means was done using the LSD value at the 5 % level.

3.3 Results and discussion

Phenmedipham in sugarbeets

The following weeds were found in the plots with sugarbeets (ranked in order of decreasing frequency): fat hen (*Chenopodium album* L.), common chick-weed (*Stellaria media* (L.) Vill), couch grass (*Agropy- ron repens* (L.)P. Beauv.), annual meadowgrass (*Poa annua* L.), and cockspur (*Panicum Crus-galli* L.). Comparisons were made between phenmedipham alone and phenmedipham with all adjuvants as measured at both application rates (Tables 3.1 and 3.2) of phenmedipham.

Table 3.1 Adjuvant effect at both application rates of phenmedipham (visual estimation; 1-7)

Phenmedipham rate (kg/ha)	No adjuvant (weed control)	All adjuvants (weed control)
0.24	2.5 a[1)]	3.3 b
0.94	5.8 c	6.0 c

Table 3.2 Adjuvant effect at both application rates of phenmedipham (fresh weight measurement)

Phenmedipham rate kg/ha)	No adjuvant (g/1 m^2)	All adjuvants (g/1 m^2)
0.24	1611 b [1)]	1159 b
0.94	251 a	273 a

Untreated: 2440 g/1 m^2

Table 3.3. Influence of the different types of adjuvant on the efficacy of phenmedipham (visual estimation; 1-7)

Phenmedipham rate (kg/ha)	Adjuvant type				
	None	Schering 11E (2 %)	A600 (0.25 %)	A600 (0.05 %)	Atplus 258 (0.25 %)
0.24	2.5 a [1)]	3.8 b	3.8 b	3.0 a	2.5 a
0.94	5.8 cd	6.5 e	5.5 c	5.8 cd	6.3 de

1) Means followed by the same letter are not different at the 5 % level.

Table 3.4. Influence of the different types of adjuvant on the efficacy of phenmedipham (fresh weight measurement; g/1 m^2).

Phenmedipham rate (kg/ha)	Adjuvant type None	Schering 11E (2 %)	A600 (0.25 %)	A600 (0.05 %)	Atplus 258 (0.25 %)
0.24	1611 c[1)]	734 ab	1401 c	1031 bc	1470 c
0.94	251 a	147 a	368 a	373 a	209 a

Untreated 2440 g/1 m^2

Table 3.5. Adjuvant effect at both application rates of sethoxydim sugarbeets in (visual estimation)

Sethoxydim rate (kg/ha)	No adjuvant (barley control)	All adjuvants (barley control)
0.095	1.0 d [1)]	4.2 b
0.38	4.5 b	5.9 c

Table 3.6 Adjuvant effect at both application rates of sethoxydim in sugarbeets (fresh weight measurement)

Sethoxydim rate (kg/ha)	No adjuvant (g/1 m^2)	All adjuvants (g/1 m^2)
0.095	3463 c [1)]	1248 b
0.38	105 a	18 a

Untreated: 3743 g/1 m^2

Table 3.7. Influence of the different types of adjuvant on the efficacy of sethoxydim in sugarbeets (visual estimation; 1-7)

Sethoxydim rate (kg/ha)	Adjuvant type None	Schering 11E (1.25 %)	A600 (0.25 %)	A600 (0.05 %)	Atplus 258 (0.25 %)
0.095	1.0 a [1)]	4.3 c	4.8 c	4.3 c	3.5 b
0.38	4.5 c	6.3 e	6.3 e	5.5 d	5.8 de

Table 3.8. Influence of the different types of adjuvant on the efficacy of sethoxydim in sugarbeets (fresh weight measurement; g/1 m^2)

Sethoxydim rate (kg/ha)	Adjuvant type None	Schering 11E (1.25 %)	A600 (0.25 %)	A600 (0.05 %)	Atplus 258 (0.25 %)
0.095	3463 c[1)]	1398 b	952 b	1366 b	1275 b
0.38	105 a	19 a	10 a	32 a	11 a

Untreated 3743 g/1 m^2

1) Means by followed by the same letter are not different at the 5 % level

The visual assessment indicated that the adjuvants improved the weed control at the phenmedipham rate of 0.24 kg/ha but not at the recommended phenmedipham rate. The harvest of all weeds gave a less clear result (Table 3.2); there seems to be an adjuvant effect but it is not significant at the 5 % level.
Generally, the harvest of weeds or barley gave a less clear result than the visual assessment. This was also found with sethoxidim and difenoxuron. There are two possible explanations for this: Firstly, the visual assessments were done about two weeks before the harvest. At the time of harvest there was more recovery of weed growth. Secondly, the visual assessment, being a qualitative method, is not hindered by differences in weed density per plot.
The results also indicated that application of phenmedipham alone at the recommended rate gave a better weed control than application at a quarter of the recommended rate with and without adjuvants.
When the types of adjuvant are compared (Tables 3.3 and 3.4) then the Schering 11E oil appeared to be the most effective. This mineral oil improved the weed control at both application rates according to the visual estimations (Table 3.3) and improved the control at the reduced application rate according to the harvest data (Table 3.4). The harvest data also indicated a reduction of weed growth as a result of addition of adjuvants to the recommended rate of phenmedipham, but this was not significant.
The addition of the two surfactants did not greatly improve the efficacy of phenmedipham. A similar result was reported by Miller and Nalewaja (1973); oils enhanced the efficacy of phenmedipham but the surfactant they used had no influence.
Addition of the adjuvants to phenmedipham applied in sugarbeets did not result in more visible injury to the crop.

Sethoxydim in sugarbeets
A great adjuvant effect was observed both visually and in the harvest of barley (Tables 3.5 and 3.6). The visual assessment showed that the adjuvant had a significant effect at the recommended dose of herbicide, but this effect was not picked up in the harvest of barley. The data on fresh weight measurements of the barley harvested (Table 3.6) indicated that the reduced application rate plus adjuvants is much less effective than the recommended rate alone; whereas these treatments did not differ in the visual estimation (Table 3.5). This is because the barley regrew after the visual estimation. The differences in the efficacy of the adjuvant types are not great (Tables 3.7 and 3.8). Only Armoblen 600 was also applied at a reduced rate (0.05 % w/v), and it is remarkable that this small amount of surfactant is very effective in enhancing the toxicity of sethoxydim when applied at the reduced rate (0.095 kg/ha). This indicates that this type of surfactant (cationic fatty amine) is rather effective in combination with sethoxydim, as was also demonstrated by Kudsk, Thonke, and Streibig (1987).
Addition of the adjuvants to sethoxidim applied in sugarbeets did not result in visible injury to the crop.

Sethoxydim in onions
In onions the distribution of barley seeds was irregular because of faulty seeding machinery. Therefore the barley control was assessed by the visual estimations alone. An adjuvant effect was apparent at both application rates (Table 3.9). The reduced application rate with adjuvants was less effective than the recommended rate alone. Comparison of the different types of adjuvant (Table 3.10) indicated that Armoblen 600 is the most effective.
Addition of the adjuvants to sethoxydim applied in onions did not result in visible injury to the onions.

Table 3.9 Adjuvant effect at both application rates of sethoxydim in onions (visual estimation; 1-7)

Sethoxydim rate (kg/ha)	No adjuvant (barley control)	All adjuvants (barley control)
0.095	1.3 a [1)]	2.9 b
0.38	3.8 c	5.6 d

Table 3.10. Influence of the different types of adjuvant on the efficacy of sethoxydim in onions (visual estimation; 1-7).

Sethoxydim rate (kg/ha)	Adjuvant type				
	None	Schering 11E (1.25 %)	A600 (0.25 %)	A600 (0.05 %)	Atplus 258 (0.25 %)
0.095	1.3 a [1)]	2.5 b	3.8 c	2.8 b	2.5 b
0.38	3.8 c	5.3 c	6.0 d	5.5 cd	5.8 cd

The field experiments with sethoxydim in sugarbeets and onions indicate that the efficacy of sethoxydim can be greatly enhanced by the addition of adjuvants and that the recommended rate can be reduced.

Difenoxuron in onions

The following weeds were found in the plots of the experiment with application of difenoxuron (ranked in order of decreasing frequency): wild chamomile (*Matricaria Chamomilla* L.), redshank (*Polygonum persicaria* L.), red chickweed (*Anagallis arvensis* L.), field sowthistle (*Sonchus arvensis* L.), knotgrass (*Polygonum aviculare* L.), common groundsel (*Senecio vulgaris* L.) and cleavers (*Gallium aparine* L.).

Results from the visual estimation (Table 3.11) and the fresh weight measurements (Table 3.12) indicate an adjuvant effect at both application rates. With both methods of estimation of weed control there was no difference between the control after application of the reduced rate with adjuvants and the control after the application of the recommended rate of difenoxuron alone. Comparison of the different types of adjuvant did not reveal pronounced differences (Tables 3.13 and 3.14). Armoblen 600 was also effective at a reduced application rate (0.05 % w/v).

The same experiment had also been performed in 1989 (application of surfactants at 0.5 % w/v) and a similar result had been obtained: an adjuvant effect at both application rates of difenoxuron and no pronounced differences between the different types of adjuvant.

It can be concluded that the efficacy of difenoxuron can be greatly improved by adding an adjuvant. This result agrees with the results of studies on other urea substituted herbicides (Hill,Belasco, and Ploeg 1965, West, and Clay 1988).

In the 1990 experiment the addition of Schering 11E oil and Armoblen 600 to difenoxuron inhibited the growth of the onions and caused necrosis at the leaf tips. However, later in the season, the crop growth did not lag behind that in the untreated plots.

1) Means followed by the same letter are not different at the 5 % level.

Table 3.11 Adjuvant effect at both application rates of difenoxuron (visual estimation; 1-7)

Difenoxuron rate (kg/ha)	No adjuvant (weed control)	All adjuvants (weed control)
0.63	1.5 a [1)]	2.9 b
2.5	3.0 b	4.8 c

Untreated: 5450 g/12 m^2

Table 3.12 Adjuvant effect at both application rates of difenoxuron (fresh weight measurement)

Difenoxuron rate (kg/ha)	No adjuvant (g/12 m^2)	All adjuvants (g/12 m^2)
0.63	4990 c	3229 b
2.5	3770 bc	1414 a

Table 3.13 Influence of the different types of adjuvant on the efficacy of difenoxuron (visual estimation; 1-7)

Difenoxuron rate (kg/ha)	Adjuvant type				
	None	Schering 11E (1.25 %)	A600 (0.25 %)	A600 (0.05 %)	Atplus 258 (0.25 %)
0.63	1.5 a [1)]	3.0 b	2.8 b	2.8 b	3.3 b
2.5	3.0 b	4.8 c	5.3 c	4.5 c	4.5 c

Table 3.14 Influence of the different types of adjuvant on the efficacy of difenoxuron (fresh weight measurement; g/12 m^2)

Difenoxuron rate (kg/ha)	Adjuvant type				
	None	Schering 11E (1.25 %)	A600 (0.25 %)	A600 (0.05 %)	Atplus 258 (0.25 %)
0.63	4990 e [1)]	3435 cde	3500 cde	3445 cde	2535 bcd
2.5	3770 de	415 a	1073 ab	1668 abc	2500 bcd

Untreated: 5450 g/12 m^2

3.4 Concluding remarks

The results of this study confirm that the use of adjuvants is a promising technique for improving the efficacy of herbicides. The results presented here can be used as a rough indication to of how much the recommended rates of the selected (commercially formulated) herbicides can be reduced.
Under the experimental conditions described in this study the adjuvants did not differ very much in their performance with sethoxydim and difenoxuron. Experiments with the addition

1) Means followed by the same letter are not different at the 5 % level.

of different amounts of an adjuvant may provide more information about the potency of an adjuvant to improve the efficacy of a herbicide.

Acknowledgements

The authors acknowledge AKZO Chemicals (The Netherlands) for providing Armoblen 600 and ICI Specialty Chemicals (Belgium) for providing Atplus 258.

Literature cited

Buhler, D. D., & O.C. Burnside, 1984.
Effect of application factors on post-emergence phytotoxicity of fluazifop-butyl, haloxyfop-methyl, and sethoxydim. Weed Science 32: 574-583

Chernicky, J.P., B.J. Gossett, & T.R. Murphy, 1984.
Factors influencing control of annual grasses with sethoxydim or RO-13-8895. Weed Science 32: 174-177

Chow, P.N.P., & A.W. MacGregor, 1983.
Effect of ammonium sulfate and surfactants on activity of the herbicide sethoxydim. Journal of Pesticide Science 8: 519-527.

Hartzler, R.G., & C.L. Foy, 1983.
Efficacy of three postemergence grass herbicides for soybeans. Weed Science 31: 557-561

Hatchard, K.G., R. Ashford, & W.B. Reed, 1989.
The effect of vegetable oil carriers and adjuvants on herbicide efficacy. In: P.N.P.Chow, C.A. Grant, A.M. Hinshalwood, & E. Simundsson, eds. Adjuvants and Agrochemicals, volume II. CRC Press, Inc., Boca Raton, Florida, 149-155

Hill, Jr., G.D., I.J. Belasco, & H.L. Ploeg, 1965.
Influence of surfactants on the activity of diuron, linuron, and bromacil as foliar sprays on weeds. Weeds 13: 103-106

Kudsk, P., K.E. Thonke, & J.C. Streibig, 1987.
Method for assessing the influence of additives on the effect of foliar-applied herbicides. Weed Research 27: 425-429

Manthey, F.A., J.D. Nalewaja,& E.F. Szelezniak, 1989.
Esterified seed oils with herbicides. In: P.N.P. Chow, C.A. Grant, A.M. Hinshalwood & E. Simundsson, eds. Adjuvants and Agrochemicals, volume II. CRCPress, Inc., Boca Raton, Florida, 139-148

Miller, S.D., & J.D. Nalewaja, 1973.
Effect of additives upon phenmediphamfor weed control in sugarbeets. Weed Science 21: 67-70

Nalewaja, J.D., G.A. Skrzypczak, & G.G. Gillespie, 1986.
Absorption and translocation of herbicides with lipid compounds. Weed Science 34: 564-568.

West, T.M., & D.V. Clay, 1988.
Effect of additives on the toxicity of three herbicides to Bromus sterilis. Proc. EWRS Symp. Factors affecting herbicidal activity and selectivity, 151-156.

York, A.C., D.L. Jordan, & J.W. Wilcut, 1990.
Effects of (NH4)2SO4 and BCH 81508 S on efficacy of sethoxydim. Weed Technology 4: 76-80

Table of Contents

IV The influence of surfactants on the efficacy of 2,4-D triethanolamine salt

4.1 Introduction

To find out in how far the results of the uptake experiments using ^{14}C labelled 2,4-D compounds can be used to predict the influence of the surfactants on the phytotoxicity of the herbicides we carried out efficacy studies. Alone 2,4-D TEA was used in these studies because the 2,4-D IOE cannnot be used in water without addition of an emulsifier which makes it difficult to estimate the effect of the fatty amine surfactants.

4.2 Materials and methods

Seeds of different species: lettuce (cv. Mirena), pea (cv. Finale), savoy cabbage (cv. Wirosa F1), garden cress (cv. Cressida), winter wheat (cv. Arminda), red fescue, black nightshade and fat hen were sown in trays (50 cm x 30 cm) filled with sandy soil ("Born Zuid" soil). The plants were grown in the green house under additional light (12h) provided by high pressure mercury lamps and with 18/12 °C (light on/light off). The plants were treated about two weeks after emergence. The concentrations of 2,4-D TEA in the herbicide solutions were 11.3 mM and 1.4 mM which were equivalent to the molarity of 2,4-D when this compound is applied at rates of 1 kg/ha and 0.125 kg/ha at a water volume of 400 L/ha. The surfactants Armoblen 557, Armoblen 600 and Ethomeen T/27 were added at concentrations of 0.05 % (w/v) and 0.5 %(w/v). Per experiment the treatments are indicated in the tables related to the separate experiments. The herbicide solutions were applied with an air-pressured sprayer fitted with three nozzles (Birchmeier Helico Sapphire 1.2 mm provided with a whirling pin 2F-0.6 mm perforated) delivering 400 L/ha at 235 kPa. The phytotoxicity of the treatments was recorded at several days after the date of spraying.

4.3 Results and discussion

Experiment nr. 1: Application of 2,4-D TEA at 11.3 mM affected the growth of all species (Table 4.1). Addition of Armoblen 600 (0.5 %) to 2,4-D TEA (11.3 mM) enhanced the phytotoxicity of 2,4-D TEA to pea, savoy cabbage, fat hen and garden cress. A much greater surfactant effect was observed when 2,4-D TEA was applied at 1.4 mM. The effect was again most pronounced with pea, savoy cabbage, fat hen and garden cress. It is remarkable that Armoblen 600 reduced the toxicity of the herbicide to lettuce when applied at the concentration of 1.4 mM.
Visual estimation of the coverage of the leaves with spray solution (Table 4.2) shows that the species most susceptible to surfactant induced enhancement of 2,4-D TEA toxicity generally (except fat hen) showed a much better coverage of the leaves. This indicated that enhanced retention of spray solution as result of the addition of surfactant is a very relevant factor to explain enhanced phytotoxicity of 2,4-D TEA. In the case of fat hen increased foliar absorption as result of surfactant addition may be more relevant than enhanced retention. The

reduction of herbicide effect in lettuce induced by the surfactant may result from run-off of the spray solution.

Experiment 2: This experiment shows that addition of Armoblen at 0.05 % and 0.5 % to 2,4-D TEA at 11.3 mM gives a similar but relatively minor enhancement of herbicide toxicity (Table 4.3). Two monocotyledons (winter wheat and red fescue) were included to find out whether addition of surfactant (resulting in enhanced spray retention by the monocotyledons) gives damage to these species. Monocotyledons were selected because 2,4-D is used for weed control in the growth of monocotyledons. The results show that no herbicide toxicity symptoms were observed with the monocotyledons (Table 4.3).

Experiment 3: Addition of the surfactants Armoblen 557, Armoblen 600 and Ethomeen T/27 at a concentration of 0.05 % improved the efficacy of 2,4-D TEA when applied at concentrations of 11.3 mM (black nightshade, pea, garden cress and fat hen) and 1.4 mM (black nightshade, pea, fat hen and savoy cabbage) (Table 4.4). This effect was most pronounced at the herbicide concentration of 1.4 mM. Alone at this concentration Armoblen 600 seemed to be a more effective surfactant than Armoblen 557 and Ethomeen T/27 as was observed with black nightshade, savoy cabbage and pea. At the herbicide concentration of 11.3 mM no differences between the surfactants were observed.

General remarks: The experiments showed that the surfactants Armoblen 557, Armoblen 600 and Ethomeen T/27 enhanced the phytotoxicity of 2,4-D TEA. This was most pronounced when the herbicide was applied at a reduced rate (1/8 of the recommended rate). The influence of the surfactants on spray retention seemed to be a relevant factor to explain the influence of the surfactants. This implies that it is difficult to use results from uptake studies for prediction of the influence of surfactants on herbicide efficacy. Quantification of the spray retention will help to
make a better estimation of the relevance of surfactant induced foliar penetration to increased herbicide efficacy.

To make an accurate comparison between uptake studies and efficacy studies it is also necessary to use plants plants grown under the same conditions. Further it is necessary to know in how far uptake depends on the leaf selected and on the selected area of a leaf.

Table 4.1 "Influence of Armoblen 600 on the phytotoxicity of 2,4-D triethanolamine salt (TEA)" (Experiment 1)

Treatment	Species	Phytotoxicity [1]			
		2.5 h	24 h	7 days	15 days
"2,4-D TEA (11.3 mM)"	black nightshade	x	x	x	xxx
	lettuce	xxx	xxx	xxx	xxx
	pea	xx	xx	xx	xx
	savoy cabbage	x	x	xx	xx
	fat hen	xx	x	x	xx
	garden cress	x	xx	x	xx
"2,4-D TEA (11.3 mM) +" A600 (0.5%)	black nightshade	xxx	xx	xxx	xxx
	lettuce	xxx	xxx	xxx	xxx
	pea	xxx	xxx	xxx	xxx
	savoy cabbage	xxx	xx	xxx	xxx
	fat hen	xxx	xx	xxx	xxx
	garden cress	xx	xx	xxx	xxx
"2,4-D TEA (1.4 mM)"	black nightshade	x	x	x	x
	lettuce	x	x	xxx	xxx
	pea	x	-	xx	xx
	savoy cabbage	-	-	n.d. [2]	xx
	fat hen	xx	x	x	x
	garden cress	x	x	-	x
"2,4-D TEA (1.4 mM) +" A600 (0.5%)	black nightshade	xx	x	-	x
	lettuce	-	-	-	-
	pea	x	xx	xxx	xxx
	savoy cabbage	x	xx	xxx	xx
	fat hen	xx	xx	xx	xxx
	garden cress	x	xx	x	xx

1) - = no effect; x = little effect; xx = medium effect; xxx = strong effect

2) n.d. = not determined

Table 4.2 The influence of surfactant on the coverage of leaves by spray solution (Experiment 1)

Treatment	Species	Coverage [1]
"2,4-D TEA " (11.3 mM and 1.4 mM)	black nightshade	xx
	lettuce	xxx
	pea	-
	savoy cabbage	-
	fat hen	xx
	garden cress	x
"2,4-D TEA (11.3 mM and" 1.4 mM) + A600 (0.5%)	black nightshade	xxx
	lettuce	xxx
	pea	xx
	savoy cabbage	xx
	fat hen	xx
	garden cress	xx

1) - = no coverage; x = little coverage; xx = medium coverage; xxx = large coverage

Table 4.3 "The influence of Armoblen 600 on the phytotoxicity of 2,4-D triethanolamine salt (TEA)" (Experiment 2)

Treatment	Species	Phytotoxicity 1) 1 day	5 days	12 days
"2,4-D TEA (11.3 mM)"	black nightshade	xx	xx	xx
	pea	xx	xx	xxx
	lettuce	xx	xxx	xxx
	wheat	-	-	-
	red fescue	-	-	-
"2,4-D TEA (11.3 mM) +" A600 (0.05%)	black nightshade	xxx	xxx	xxx
	pea	xxx	xxx	xxx
	lettuce	xxx	xxx	xxx
	wheat	-	-	-
	red fescue	-	-	-
"2,4-D TEA (11.3 mM) +" A600 (0.5%)	black nightshade	xxx	xxx	xxx
	pea	xxx	xxx	xxx
	lettuce	xxx	xxx	xxx
	wheat	-	-	-
	red fescue	-	-	-

1) - = no effect; x = little effect; xx = medium effect; xxx = strong effect

Table 4.4 "Influence of the surfactants on the phytotoxicity of 2,4-D triethanolamine salt (TEA)" (Experiment 3)

Treatment	Species	Phytotoxicity 1)			
		1 day	7 days	14 days	21 days
"2,4-D TEA (11.3 mM)"	black nightshade	xx	xxx	xx	x
	savoy cabbage	xx	xx	xxx	xxx
	pea	xxx	x	x	x
	lettuce	xxx	xxx	xxx	xxx
	garden cress	x	x	x	x
	fat hen	x	xx	xx	xx
"2,4-D TEA (11.3 mM) +" A600 (0.05%)	black nightshade	xx	xxx	xxx	xxx
	savoy cabbage	xx	xxx	xxx	xxx
	pea	xxx	xxx	xxx	xxx
	lettuce	xxx	xxx	xxx	xxx
	garden cress	x	xx	xx	xxx
	fat hen	x	xxx	xxx	xxx
"2,4-D TEA (11.3 mM) +" A557 (0.05%)	black nightshade	xx	xxx	xxx	xxx
	savoy cabbage	xx	xxx	xxx	xxx
	pea	xxx	xxx	xxx	xxx
	lettuce	xxx	xxx	xxx	xxx
	garden cress	x	xx	xx	xxx
	fat hen	x	xxx	xxx	xxx
"2,4-D TEA (11.3 mM) +" ET/27 (0.05%)	black nightshade	xx	xxx	xxx	xxx
	savoy cabbage	xx	xxx	xxx	xxx
	pea	xxx	xxx	xxx	xxx
	lettuce	xxx	xxx	xxx	xxx
	garden cress	xx	x	xxx	xxx
	fat hen	x	xxx	xx	xxx
"2,4-D TEA (1.4 mM)"	black nightshade	xx	-	x	x
	savoy cabbage	x	x	x	x
	pea	xx	-	-	-
	lettuce	xx	x	xxx	xxx
	garden cress	x	x	x	xxx
	fat hen	xx	x	x	x
"2,4-D TEA (1.4 mM) +" A600 (0.05%)	black nightshade	xx	xxx	xxx	xxx
	savoy cabbage	x	xx	xxx	xxx
	pea	xxx	xxx	xxx	xxx
	lettuce	xxx	xx	xx	xx
	garden cress	x	xx	x	x
	fat hen	xx	xxx	xxx	xxx
"2,4-D TEA (1.4 mM) +" A557 (0.05%)	black nightshade	xx	xx	xx	x
	savoy cabbage	xx	xx	xx	xx
	pea	xxx	xx	xxx	xx
	lettuce	xxx	xx	xx	xx
	garden cress	x	x	xx	xx
	fat hen	xx	xx	xx	xx
"2,4-D TEA (1.4 mM) +" ET/27 (0.05%)	black nightshade	xx	xx	xx	xx
	savoy cabbage	xx	xx	xx	xx
	pea	xxx	xx	xx	xx
	lettuce	xxx	xx	xx	xx
	garden cress	xx	x	x	x
	fat hen	x	xxx	xxx	xxx

1) - = no effect; x = little effect; xx = medium effect; xxx = strong effect

Table of Contents

V The influence of surfactants and a mineral oil on the retention of spray solution by pea and black nightshade

5.1 Introduction

In South-Africa the mineral oil Actipron (BP) is used as an adjuvant in the crop protection of the citrus growth. In 1989 AKZO introduced the surfactant Armoblen 600 followed by Armoblen 650 in 1990. Both surfactants were effective and addition of Armoblen 650 gave better results than addition of armoblen 600. So far there is no explanation for this difference. In this study we measured the influence of the mineral oil and the surfactants on the retention of spray solution. because it was impossible to obtain appropriate citrus shoots as test plants we selected pea (rough cuticular surface) and black nightshade (smoots cuticular surfaca) as test plants.

5.2 Materials and methods

Black nightshade was sown in the greenhouse and transferred to the growth chamber after emergence. Pea was sown and grown in the growth chamber under the following conditions: 14 h light (80-120 W/m^2 at leaf level), 18/12 °C (day/night) temperature, and 70/80 % (day/-night) relative humidity. Pea and black nightshade plants were used for the retention measurements when they were respectively 24 and 27 days old.
Two experiments with each four replicates were carried out. The mineral oil Actipron was added to demineralized water at the concentrations 0.05, 0.5 and 5 %(w/v) and the surfactants Armotan PML-20, Armoblen 600L80 and Armoblen 650 were added at concentrations of 0.01, 0.1 and 1 %(w/v). The solutions were applied with an air-pressured sprayer fitted with three nozzles (Birchmeier Helico Sapphire 1.2 mm provided with a whirling pin 2F-0.6 mm perforated) delivering 400 L/ha at 235 kPa.
The retention of the spray solution was quantified by spectrofluorometry. The spray solutions contained Na-fluorescein (0.002 %(w/v)) as a fluorescent dye.
Fifteen minutes after the spray application the fluorescent dye was washed off the plants with 0.005 M NaOH. The concentration of the dye in the washing solution was determined by using a spectrofluorometer.

5.3 Results and discussion

Two experiments were carried out with each species. The data of each experiment are given (Figs 5.1, 5.2, 5.3 and 5.4) because the absolute values were different per experiment.
The surfactants had a great influence on the spray solution by pea. Retention increased when the surfactant concentration was enhanced. There were no differences between the surfactants. The mineral oil was much less effective. Even at a concen tration of 5 % the retention was much less than was measured at a surfactant concentration of 0.1 %. The results with pea

support the suggestions coming from South-Africa that the surfactants had a much better influence on wetting of citrus leaves than the oil did. However it is important to realize that results with pea can not be representive for citrus leaves.
The retention of spray solution by black nightshade was not influenced by addition of the adjuvants. This results agrees with previous publications: adjuvants do not have great influence on spray retention by leaves with a smooth cuticular surface.

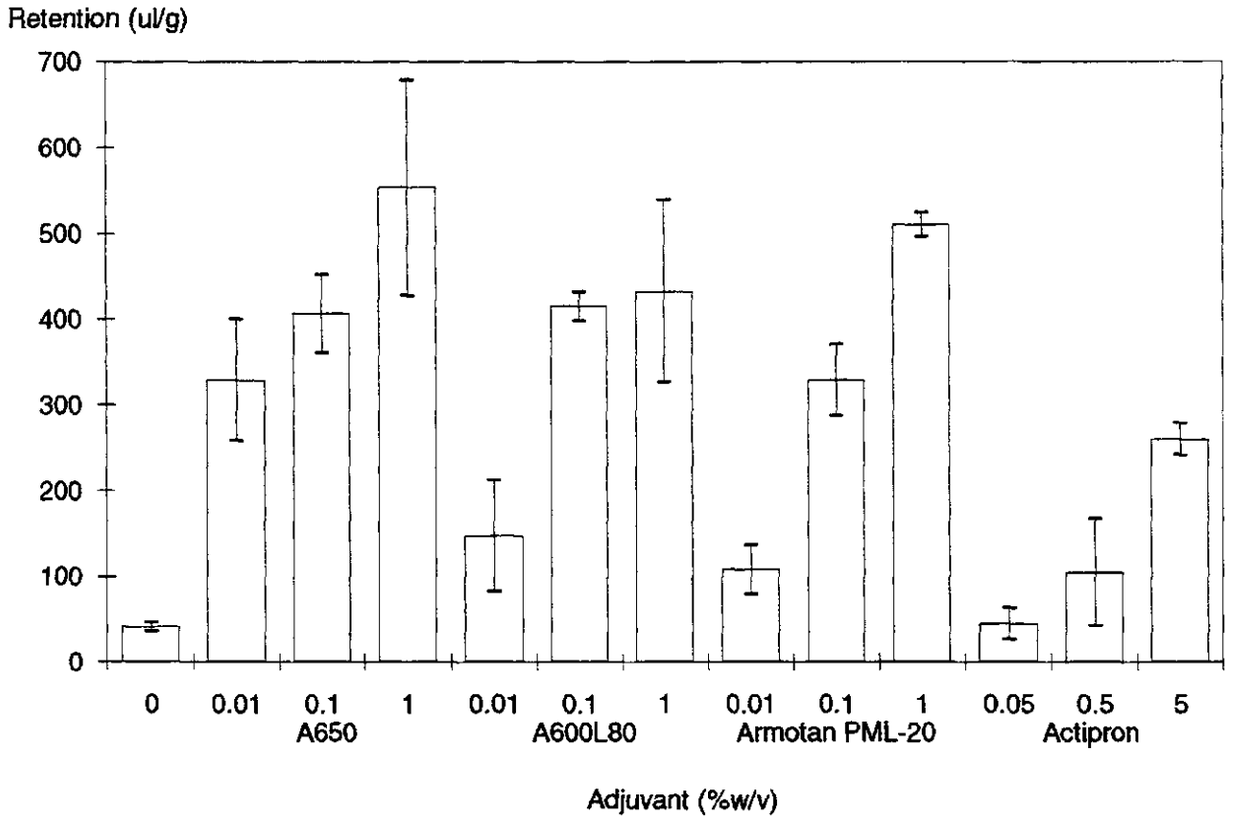


Figure 5.1 The influence of adjuvants on the retention of spray solution by pea (Experiment 1); bars represent S.D.

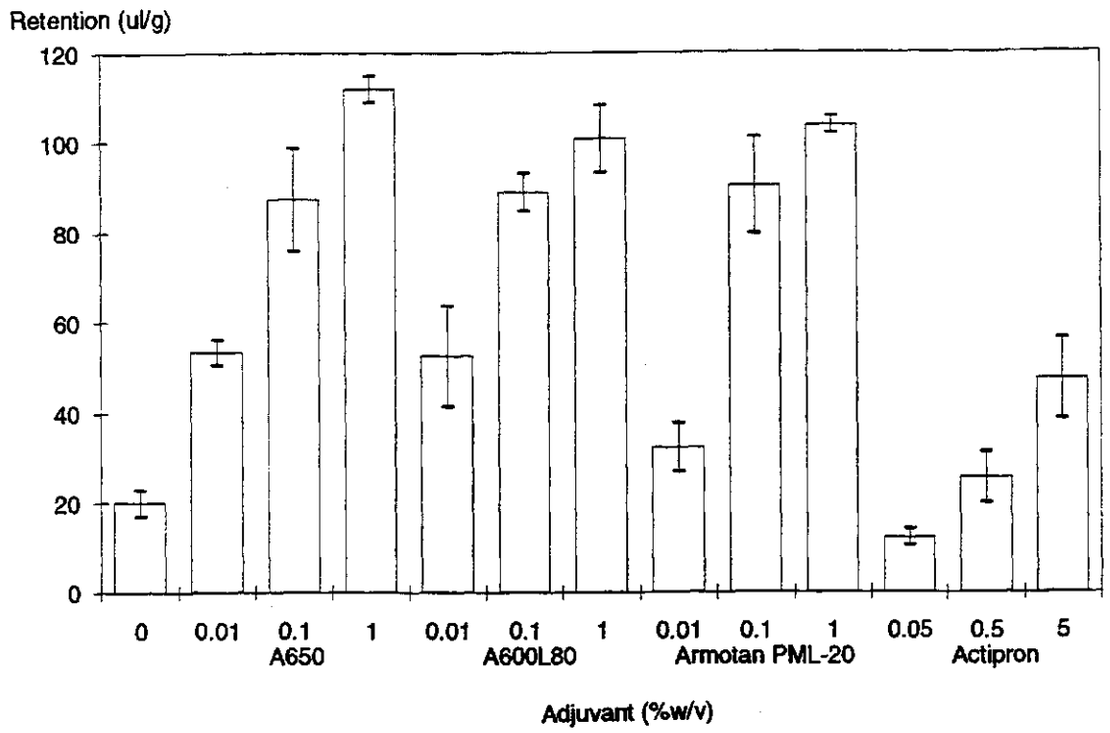


Figure 5.2 The influence of adjuvants on the retention of spray solution by pea (Experiment 2); bars represent S.D.

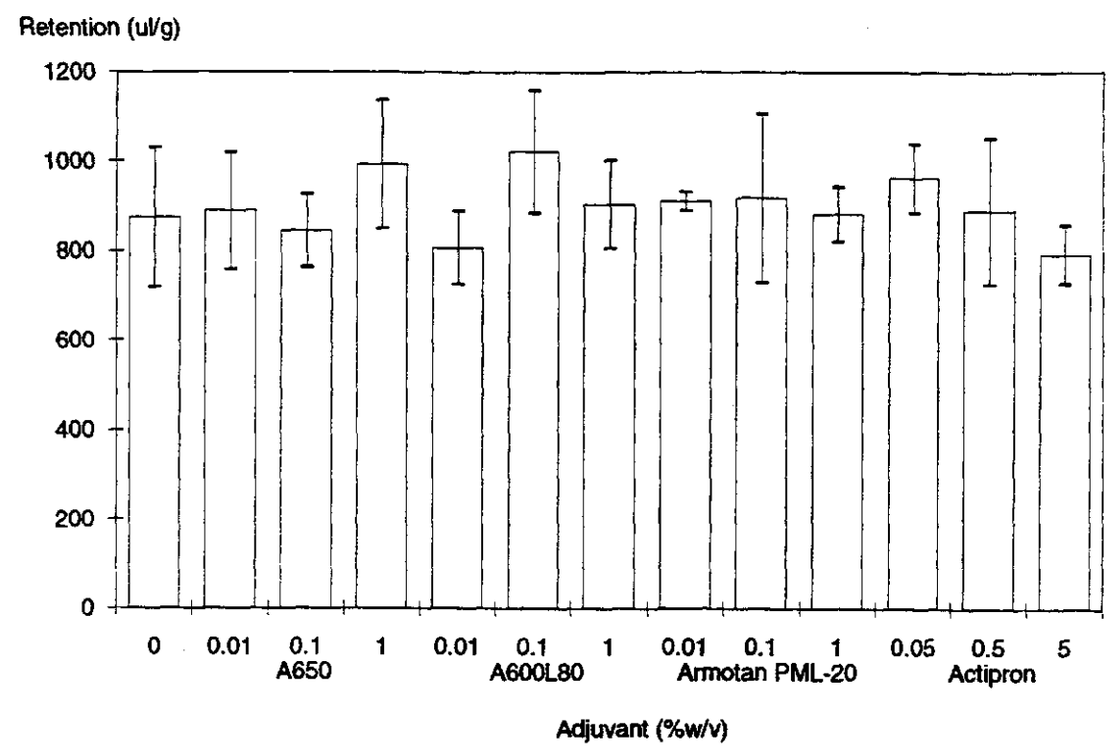


Figure 5.3 The influence of adjuvants on the retention of spray solution by black nightshade (Experiment 1); bars represent S.D.

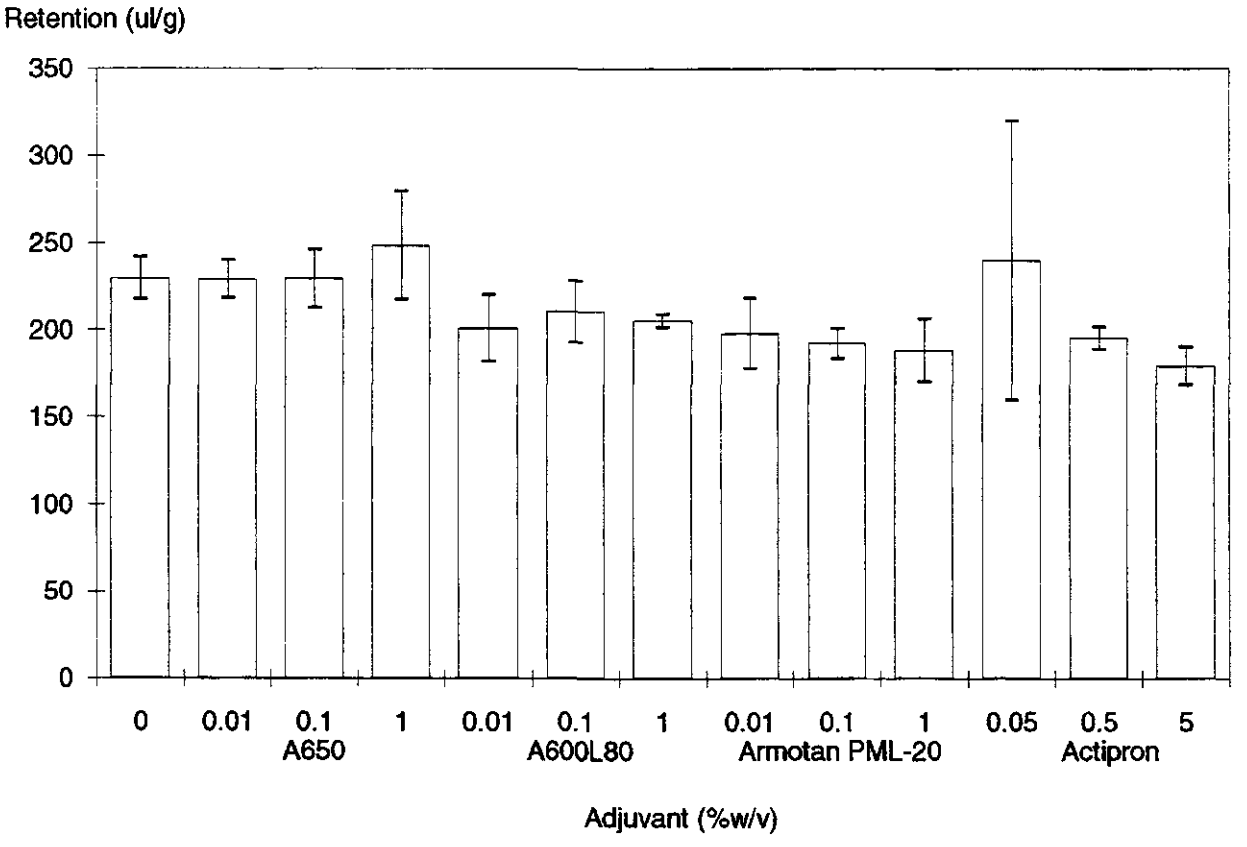


Figure 5.4 **The influence of adjuvants on the retention of spray solution by black nightshade (Experiment 2); bars represent S.D.**

VI Recommendations

The results in this report indicate that fatty amine surfactants are good penetration enhancers for water soluble compounds. The differences between the chemical structures of the selected fatty amine surfactants appeared to be of minor importance to the foliar absorption.
In the literature there is some evidence that more lipophilic surfactants are required to enhance the foliar penetration of lipophilic compounds. It should be interesting to see in how far very lipohilic fatty amine surfactants can enhance the penetration of lipophilic active ingredients.
If one want to explain the penetration enhancing properties of the fatty amines then more basic research with isolated cuticles is necessary.
Worlwide more people start to realize that the fatty amines have rather unique properties in the sense that they seem to be very effective enhancers of the permeability of the leaf cuticle. To take more advantages of this type of surfactants more research with different compounds and at different levels (basic work and efficacy tests) is required.