SESSION 3C

NEW CONCEPTS AND METHODS

SESSION ORGANISER DR G. G. BRIGGS

RESEARCH REPORTS (Poster Papers) 3C-1 to 3C-13

SD 95481 A VERSATILE NEW HERBICIDE WITH WIDE SPECTRUM CROP USE

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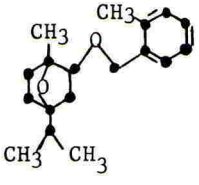
ABSTRACT

SD 95481 (Weed Science Society of America [WSSA] common name, cinmethylin) 7-oxabicyclo[2.2.1]heptane, 1 methyl-4-(1-methylethyl)-2-[(2-methylphenyl)-methoxy]-,exo-, is a novel preemergence herbicide. It offers selective control of many economically important annual grass weeds in a wide range of temperate and tropical crops, including soya bean, cotton, groundnuts, dry beans (and other legumes), sunflower, sugarcane, tobacco, and vegetables. It inhibits meristematic growth in both roots and shoots of plants and is readily metabolized. The compound has a low order of mammalian toxicity and shows no tendency to accumulate in the environment. SD 95481 has moderate soil persistence and data indicate a minimal risk of carryover to subsequent, susceptible rotation crops.

INTRODUCTION

SD 95481 (trademark, CINCH$^{(R)}$) is a novel herbicide developed by Shell Chemical Company, U.S.A., and Shell International Chemical Company, England, for the selective preemergence control of many annual grass weeds in a wide range of temperate and tropical crops. Representing new herbicide chemistry, SD 95481 is in the cineole family.

Chemical and Physical Properties

Chemical Name	7-oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-2-[(2-methylphenyl)-methoxy]-,exo-
Common Name	cinmethylin (WSSA)
Code Number	SD 95481
Molecular Formula	$C_{18}H_{26}O_2$
Structural Formula	

Molecular Weight	274.16
Colour and Physical State	amber liquid
Boiling Point	110-119°C at 13.33 Pa 313°C ± 2° at 1.013 x 10^5 Pa
Solubility	miscible in all proportions with a large range of organic solvents. Water solubility at 20°C is 63 mg/l.
Vapour Pressure at 20°C	1.013 x 10^{-2} Pa
n-Octanol/Water Partition Coefficient	k_{ow} is 6,850.
Formulation	839 g/l (7.0 lb/US gal) emulsifiable concentrate
Hydrolytic and Photostability	SD95481 is hydrolytically stable from pH 3 to pH 11 at 25°C. Light-catalyzed decomposition can occur in the presence of air; therefore, protect technical ingredient from sunlight and air.

Toxicology

SD 95481 has a low order of mammalian toxicity:

	Technical Ingredient	SD 95481, 839 g/l EC
Acute Oral LD_{50} (Rat)	4500 mg/kg	1600 mg/kg
Acute Dermal LD_{50} (Rabbit)	>2000 mg/kg	>2000 mg/kg
Skin Irritation (Rabbit)	Moderate	Moderate
Eye Irritation (Rabbit)	Mild	Moderate

Mode of Action

Herbicidal Effect

SD 95481 has herbicidal effects which result primarily from disruption of meristematic development in shoots and roots of susceptible species. Physiological effects on older, more mature plant parts or tissues have not been observed.

Uptake of SD 95481 from the soil solution or in the vapour phase occurs through the shoots and roots of germinating or emerging weeds. The parent material has limited apoplastic mobility in both broadleaved and grass species. Polar metabolites of SD 95481 have extensive apoplastic mobility.

Edaphic Characteristics

In the soil SD 95481 is readily metabolized to weakly herbicidal polar compounds that further degrade to CO_2 and other inactive metabolites. Carryover of herbicidal activity from one cropping season to the next has not been observed.

Leaching experiments have indicated that SD 95481 has limited downward mobility in soil. SD 95481 sorption properties cause the herbicide to sorb tightly to soil colloids and, as a result, remain in the top 2.5 to 5 cm of most soil profiles.

The potential for SD 95481 or its metabolites to migrate into groundwater is minimal because of the compound's sorption properties and ready metabolism when incorporated into soil.

MATERIALS AND METHODS

SD 95481 has been tested extensively in a total of more than 40 countries worldwide in the years 1981 to 1985. This large-scale testing programme has involved in excess of 1000 field trials and has included evaluation of SD 95481 in a range of broadleaved and monocotyledonous crops.

Results presented in this paper were obtained from replicated field trials. Herbicide applications generally were made using gas-pressurized small-plot sprayers or small tractor equipment using volumes around 500 l/ha.

RESULTS

Results are summarized by crop tolerance and weed control of annual grasses and broadleaved weeds.

Crop Tolerance

SD 95481 has shown good selectivity in a range of crops, principally broadleaved, at doses up to two times that required for grass weed control. Crops exhibiting excellent tolerance to preemergence applications of SD 95481 are:

Cotton	Soya bean
Dry bean	Sunflower
Groundnut	Sweet potato
Potato	Tobacco
Sesame	Sugar cane (plant)
Snap bean	Turf

Numerous horticultural crops, including transplanted species (e.g., brassicae, aubergine, pepper, tomato) and many established woody ornamentals, vines and trees are tolerant.

Preliminary evidence indicates that SD 95481 is selective for use in lupins and established lucerne and as directed-postemergence sprays in many crops to control late-emerging annual grasses.

Tolerance that depends upon seed depth and time of emergence is exhibited by the following seeded crops:

Aubergine
Brassicae (seeded - broccoli, brussels sprout, cabbage)
Cucumber
Lentil
Lucerne
Maize
Melon (cantaloupe, water)
Oil seed rape
Pea
Squash
Tomato
Wheat

Certain small seeded crops like carrots, onions, sugar beet, and turnip are not tolerant to SD 95481.

Weed Control

Annual Grasses

Most annual grasses are well controlled by SD 95481 applied pre-emergence at doses of 0.75 to 1.25 kg/ha.

Genera of Annual Grass Weeds Susceptible to SD 95481

Alopecurus	Echinochloa	Lolium
Avena*	Eleusine	Panicum
Brachiaria	Eragrostis	Poa
Bromus	Eriochloa	Rottboellia
Cenchrus	Festuca	Setaria
Dactyloctenium	Leptochloa	Sorghum**
Digitaria	Leptocoriphium	Urochloa

* Control is dependent upon weed seed depth and time of emergence.
** Sorghum halepense (seedling).
SD 95481 gives good control of annual sedges.
(eg. Cyperus difformis, C. diffusus)

Annual Broadleaved Weeds

At doses used for grass weed control, SD 95481 exhibits good activity on some broadleaved weeds. In addition, suppression of some other species is achieved, although efficacy on these species tends to be inconsistent.

Annual Broadleaved Weeds Susceptible to SD 95481

Amsinckia spp.	Mollugo verticillata
Calandrinia caulescens	Polygonum aviculare
Capsella bursa-pastoris	Portulaca oleracea
Chamomilla suaveolens	Richardia scabra
Galinsoga spp	Stellaria spp.

Annual Broadleaved Weeds Suppressed by SD 95481

Amaranthus spp.	Chenopodium spp.
Abutilon theophrasti	Hibiscus trionum
Acanthospermum hispidum	Lamium amplexicaule
Ambrosia artemisiifolia	Polygonum pensylvanicum
Bidens spp.	Sida spinosa
Bilderdykia convolvulus	Veronica spp.

Perennial Weeds. No control of perennial weeds - broadleaved, grass or sedge - is given by SD 95481. However, some suppression of infestations of Cyperus esculentus, C. rotundus, and Sorghum halepense (rhizome) has been observed.

Examples of the performance of SD 95481 in different crop outlets are illustrated in Tables 1-4 from research conducted in the United States, Brazil and France.

TABLE 1.
Summary of SD 95481 performance in U.S.A. soya bean, cotton and groundnut field trials - 1981-1984

Treatment	Rate kg/ha	Percentage Weed Control: Soya bean (336) Grass	Soya bean (336) Broadleaf	Cotton (78) Grass	Cotton (78) Broadleaf	Groundnut (52) Grass	Groundnut (52) Broadleaf
SD 95481	0.50	78	42	85	47	81	46
	0.75	81	44	84	41	83	47
	1.0	84	47	88	46	85	50
	1.5	92	60	97	54	93	60
SD 95481 + metribuzin		87	82	-	-	-	-
SD 95481 + fluometuron		-	-	88	77	-	-
SD 95481 + vernolate		-	-	-	-	86	79

( ) number of trials summarised

TABLE 2.
Summary of SD 95481 performance in preemergence soya bean trials conducted in Brazil during the period 1982-1984 (51 trials)

Treatment	Rate, kg/ha	Percentage Weed Control: Annual Grass Weeds	Broadleaved Weeds
SD 95481	0.75	90	53
	1.0	94	56
	1.25	96	69
SD 95481 + metribuzin *		96	88

* Rate adjusted to soil type (0.2 to 0.4 kg/ha).

TABLE 3.
Summary of the performance of SD 95481 in sunflowers in France in 1984 (9 trials)

(a) Percentage control of weed species

Species	SD 95481 1.0 kg/ha	SD 95481 2.0 kg/ha	Terbutryne 2.0 kg/ha
Echinochloa crus-galli	98	99	55
Lolium multiflorum	85	91	61
Setaria viridis	100	100	67
Amaranthus retroflexus	60	75	100
Chenopodium album	71	91	76
Mercurialis annua	52	81	77
Veronica hederifolia	85	90	90

(b) Crop Effects 2-14 Weeks after SD 95481 Application (CEB 0-10 Scale)*

Weeks after Applic.	SD 95481 1.0 kg/ha	SD 95481 2.0 kg/ha	Terbutryne 2.0 kg/ha
2-4	1.2	1.5	0.3
10-14	0.0	0.3	0.2

* The scale accepted for use in French trials

DISCUSSION

SD 95481 is a highly effective herbicide for the control of many economically important annual grasses. There is evidence of a high level of activity of SD 95481 on Rottboellia exaltata, a weed of increasing importance and this may be of major significance.

The compatibility of SD 95481 with broadleaved weed herbicides affords the opportunity to extend the activity of the compound to provide broad spectrum weed control in many crops.

The range of temperate and tropical crops, which are amenable to the selective usage of SD 95481, provides many outlets for commercial development of the compound worldwide.

The environmental safety aspects of SD 95481 are of importance. The compound has a low order of mammalian toxicity. It has moderate persistence and shows no tendency to accumulate in the environment. The potential for SD 95481 or its metabolites to migrate into groundwater is minimal.

ACKNOWLEDGEMENTS

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PP 005 - THE R-ENANTIOMER OF FLUAZIFOP-BUTYL

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ABSTRACT

Fluazifop-butyl (PP 009), a post-emergence graminicide selective to broad-leaf crops, is a racemate comprising R and S enantiomers. In a glasshouse study, post-emergence herbicidal activity of the racemate was attributed almost entirely to the R-enantiomer (PP 005, fluazifop-P-butyl); the S-enantiomer was practically inactive. However, the, albeit weaker, pre-emergence activity of PP 009 was matched by both enantiomers. Transformation studies in soil showed that, following rapid hydrolysis, the S-enantiomer inverted to the R-acid (fluazifop-P) which, once formed, retained high optical purity. Rapid hydrolysis, but not substantial inversion, also occurs in plants; the acid is translocated and is the toxophore. Wide-scale field testing has shown that the post-emergence performance of PP 005 generally permits use at half the rate recommended for PP 009. PP 005 is already registered as a post-emergence graminicide in several countries, for use over the range of 105-420 g ha^{-1}. It is also an effective sugar cane ripener, and is registered in Southern Africa for this purpose at a use rate of 40 g ha^{-1}.

INTRODUCTION

In herbicide development, there appears to be only one documented precedent for the exploitation of an optical isomer possessing superior activity to that of its corresponding racemate. For flamprop-isopropyl, Scott et al. (1976) showed that WL 43425, the laevorotatory enantiomer, possessed twice or more the activity of the racemate against wild oats, coupled with an enhanced selectivity margin for barley; subsequently the racemate of this ester was superseded by its laevorotatory (R) enantiomer.

In 1980, Plowman et al. reported the development by ICI and Ishihara SK of fluazifop-butyl (coded PP 009). This post-emergence graminicide, selective to broad-leaf crops, is a pyridinyloxyphenoxypropionate, possessing an asymmetric carbon atom in the propionate moiety (see below); thus properties previously described are those of the racemate. Subsequent research by ICI Plant Protection Division has led to the development of the R-enantiomer (coded PP 005) of fluazifop-butyl. PP 005 is already registered in several

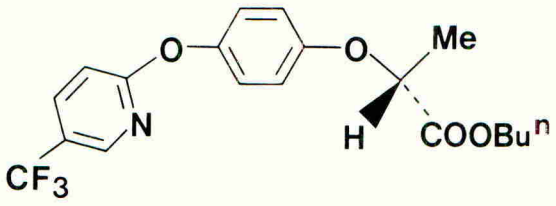
countries with retention of the trademark 'Fusilade', appropriately suffixed. This paper describes some properties of PP 005; where appropriate, relationships between the R- and S- enantiomers and the racemate (RS) are displayed.

CHEMICAL AND PHYSICAL PROPERTIES OF PP 005

Chemical name : butyl (R)-2-[4-(5-trifluoromethyl-2-pyridyloxy) phenoxy] propionate [IUPAC]

Common name : fluazifop-P-butyl (BSI approved; ISO provisional)

Structural formula :



Physical appearance : colourless, odourless liquid; technical grade is of light straw colour

Freezing point : becomes glass-like at - 20°C

Boiling point : no boiling point at atmospheric pressure (decomposing at ~100°C)

Vapour pressure : 1×10^{-7} kPa at 30°C

Density : 1.22 g cm^{-3} at 20°C

Rotation : dextrorotatory

Solubility : solubility in water at 20°C is 1 mg l^{-1}. The compound is completely miscible with methanol, acetone, hexane, dichloromethane, xylene, toluene and ethyl acetate

Stability : Stable for at least 12 months at ambient temperature (15-25°C)

Formulations : 12.5% e.c. (major); 25% e.c. (minor)

TOXICOLOGY OF PP 005

Results from acute toxicological studies indicate that PP 005 is of a low order of toxicity. The acute oral LD_{50} value to the rat is between 2,712 and 4,096 mg kg^{-1} and the 24 hour dermal LD_{50} to the rat and rabbit is in excess of 2,400 mg kg^{-1}. PP 005 is slightly irritant to rat skin and rabbit eyes and is not a sensitiser of guinea pig skin. PP 005 is also of low toxicity to birds and invertebrates. The acute oral LD_{50} for mallard ducks is 3,528 mg kg^{-1}. No effect was seen on bees when administered both

orally and by contact at 200 mg a.i. per bee. The EC_{50} to Daphnia magna was >1 mg a.i. l^{-1} after 48 hours. PP 005 is moderately toxic to fish; the LC_{50} of a 12.5% e.c. formulation at 96 hours for rainbow trout (Salmo gairdneri) was 5.4 mg l^{-1}.

MODE OF ACTION

No differences in symptoms of phytotoxicity from PP 009 and PP 005 application have been observed. Although normally associated with chlorosis, and often reddening, of foliage, the first observable and critical symptom is a cessation of shoot (aerial shoots, rhizomes, stolons) growth, leading to necrosis and death of meristematic regions of the shoot system.

PP 009 and, by inference, PP 005 are both xylem- and phloem-mobile. A detailed study of the behaviour of esters of radiolabelled pyridinyloxy-phenoxypropionates, including PP 009, in Elymus repens has established that after foliar absorption, rapid de-esterification releases the free acid (Hendley et al., 1985). Parent ester was not found in organs (rhizomes, untreated tillers) distant from the site of application; free acid (along with its polar conjugates) was the major metabolite. It was concluded that the acid is the translocated form of these herbicides, and is the toxophore. The acids both of PP 009 (fluazifop) and PP 005 (fluazifop-P) are effective in control of annual and perennial grass weeds (authors' unpublished observations). The primary site of action of PP 005 is not yet firmly established. However, a recent study by Carr et al. (1985) with the acid of PP 009 (fluazifop) led the authors to conclude that it inhibits lipid biosynthesis, through interference with synthesis of fatty acids and/or phospholipids.

MATERIALS AND METHODS

Glasshouse experiments

Enantiomer ratios of preparations employed were as follows: PP 009 (R:S, 50:50), R-enantiomer (PP 005) (R:S, 94:6), S-enantiomer (R:S, 10:90). They were all applied as 12.5% w/v e.c. formulations. Application was by track sprayer at rates from 5 to 320 g ha^{-1} in 200 l ha^{-1} of water containing 0.1% Agral 90. Two test species, wild oats (Avena fatua) and maize (cv Pioneer) were grown in 10 cm diam. pots for the post-emergence experiment. Both were treated at the 3.5-leaf growth stage. Post-emergence treatments included 'foliage and soil', 'foliage only' (soil surface covered with alumino-silicate (Perlite) during spraying and removed once the spray had dried), and 'soil only' (plants covered during spraying). The pre-emergence experiment was conducted in half-size seed trays. Eight maize seeds were sown at a depth of 1 cm and the soil surface sprayed. All experiments were carried out in a standard compost (45% loam, 55% 'Cracker' grit) with added fertiliser. Plants were top-watered as required with tap water. Two assessment methods were used as appropriate; visual assessment on a percentage scale (where 0 = no effect and 100 represents complete kill) and fresh weights. Both assessments were made 21 days after treatment.

Statistical analysis of pre-emergence data comprised the fitting of three parallel dose-response curves to the visual assessments, which were used to calculate relative activities, with confidence limits, of the three herbicide treatments. For post-emergence data, two factorial analyses were carried out on results for PP 005 and PP 009. The first compared effects of soil cover, herbicide and rate; the second was performed assuming PP 005 to be twice as active as PP 009 (cf Table 1). Dose-response curves were fitted to the maize data in order to calculate relative activities. Such curves could not be fitted to data for A. fatua as there were insufficient data points for mid-response. For maize, the true relative activity of R- and S-enantiomers was calculated from the equation:

$$x = \frac{q_1 - rhq_2}{rhp_2 - p_1}$$, where x = relative activity of pure R- and S-enantiomers
rh = relative activity of impure enantiomers
p_1 = % rate of R-enantiomer
q_1 = % rate of S-enantiomer
Experimental R is in ratio R:S of $p_1:q_1$
Experimental S is in ratio R:S of $p_2:q_2$

The rate for 50% growth reduction achieved by the pure S-enantiomer is simply 'x' multiplied by the rate required for the R-enantiomer.

Field trials

Replicated field trials in USA in 1983 compared a 48% (4E) formulation of PP 009 with a 24% (2E) e.c. formulation of PP 005, in a volume range of 135-290 l ha^{-1} at a pressure of 200-400 kPa. All treatments included oil concentrate at 1% of the final spray volume and were timed in accordance with PP 009 recommendations against various grass weed species. Visual assessments of percentage control were made 14-35 DAT. Sugar cane ripening trials were conducted with a 12.5% e.c. formulation of PP 005 incorporating 25% non-ionic surfactant.

HERBICIDAL ACTIVITY OF THE ENANTIOMERS (GLASSHOUSE)

Post-emergence treatments

No herbicidal activity was detected when the compounds were applied only to soil. In accord with this, 'foliar and soil' treatments were not different from 'foliar only' treatments; they were combined to obtain a more accurate estimate of phytotoxicity of the enantiomers. Table 1 shows the activity of the enantiomers compared with that of the racemate (PP 009). Insofar as there was no significant difference between a given rate of PP 005 and twice that rate of PP 009, these data are consistent with the hypothesis that the activity of PP 009 resides in the R-enantiomer. Further supporting evidence was provided by the lack of activity of the S-enantiomer on A. fatua.

It was possible to calculate the doses required to produce 50% and 90% growth reductions in maize (Table 2). These also indicated that the activity of the racemate was very largely due to its R-component. However, there was some activity from the S-enantiomer treatments on this species which could not be attributed entirely to R-enantiomer contamination of the preparation. The S-enantiomer itself appeared to be active, albeit almost x 16 less active than PP 005. It is unknown whether the S-enantiomer is

active per se; conceivably, it partially inverts to the R-form in maize. If this were so, then it only occurred to a limited extent, calculated to be 6.4% from figures in Table 2. Certainly, there is no evidence for either rapid or substantial enantiomer inversion in broad-leaf crops which have been examined (B.D. Cavell, pers. comm.). In any event, the S-enantiomer had at most only a small effect on the relative activity of PP 009 to PP 005.

TABLE 1

Effect of the S- and R (PP 005) -enantiomers and racemate (PP 009) on fresh weights (g) of Avena fatua and maize, post-emergence

Avena fatua					Maize			
Rate ($g\ ha^{-1}$)	S*	R	RS	Rate ($g\ ha^{-1}$)	RS	R	S*	Rate ($g\ ha^{-1}$)
5	14.4	14.5	13.5	10	43.8	39.2	43.0	5
10	14.5	13.5	12.6	20	43.6	40.0	42.8	10
20	12.8	13.7	13.0	40	29.8	27.2	43.5	20
40	12.2	11.9	13.9	80	9.8	15.0	41.7	40
80	12.8	3.2	4.4	160	1.8	1.7	35.4	80
160	13.3	1.6	0.9	320	1.4	0.9	19.6	160
Untreated (13.0 g)					Untreated (39.9 g)			
LSD (P = 0.05) 2.1					LSD (P = 0.05) 6.1			

Each value is a mean of 8 replicates
* Not included in the statistical analysis

TABLE 2

Rates ($g\ ha^{-1}$) for 50% and 90% reductions in fresh weight in maize, post-emergence

	50% (± S.E.)	90% (± S.E.)
RS (PP 009)	56 (3)	141 (5)
R (PP 005)	30 (2)	76 (3.5)
S-enantiomer	182 (6.5)	455 (10.5)
	(470)*	(1200)*
PP 005:PP 009 activity ratio	1.85	
95% Confidence limits	1.53, 2.17	

* Estimates for activity of S-enantiomer alone (i.e. contribution from R-enantiomer contamination discounted)

Pre-emergence treatments

Erratic germination made fresh weight determinations of doubtful validity and this experiment was assessed visually. There was no significant difference between the activities of the enantiomers when applied to maize (Table 3). This contrasts very markedly with their relative performance post-emergence. Originally, it was thought that both enantiomers were racemised in soil by microbial activity.

TABLE 3

Pre-emergence activity of enantiomers and racemate (maize)

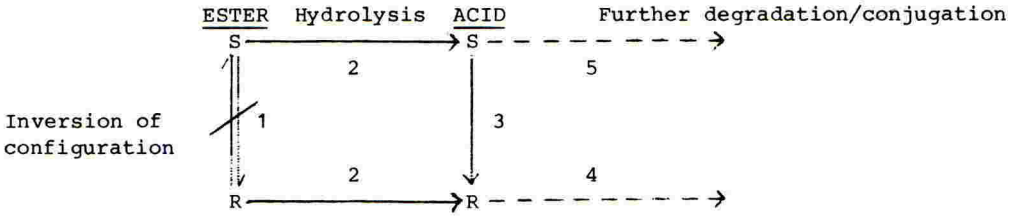
	Visual assessments of % age damage*		
Rate (g ha^{-1})	RS	R	S
40	12	0	6
80	38	36	48
160	70	86	82
320	80	97	78

Relative activities		95% Confidence limits
RS:R	0.99	0.51, 1.47
RS:S	1.02	0.58, 1.46
S:R	0.97	0.45, 1.49

* Each value is the mean of 5 replicates

STEREOCHEMISTRY OF TRANSFORMATIONS IN SOIL

The data above imply some 'activation' of the S-enantiomer in the soil and the virtual absence of such a mechanism in the grass weed. Consequently, the stereochemistry of transformations of the two enantiomers in soil was examined (Bewick, 1985). A freshly sampled sandy loam soil was treated separately with ^{14}C-R- and ^{14}C-S-enantiomers of fluazifop-butyl at 1 kg ha^{-1}. Under aerobic conditions at 20°C, both enantiomers were rapidly hydrolysed (half life, <2 hours) to their corresponding acids, with complete retention of their optical configurations. After hydrolysis, however the S-enantiomer was found to undergo inversion of configuration (half-life, 1-2 days under these conditions) to yield, ultimately, a residue of the corresponding R-enantiomer. Whether formed by inversion from the S-acid or by hydrolysis of the R-ester, the R-acid retained a high optical purity, although the residue declined with time. The half life of the acid in this study was ~7 days. An extension to this work, conducted with the ^{14}C-racemic ester confirmed that rapid hydrolysis produced an acid residue in which the R-enantiomer greatly predominated (R:S ratio ~3:1 after 2 days). In sterilised soil (γ-irradiation) the enantiomer ratio remained essentially unchanged, suggesting that the inversion in non-sterile soil was microbially-mediated. Transformations of PP 009 and its enantiomers, described in this and preceding sections of the paper, are summarised in Figure 1.



1. Absent in soil and plant.
2. Rapid in soil and plant.
3. Fairly rapid in soil; absent or very slow in plant.
4. Predominant in soil; significant in plant.
5. Very limited in soil; significant in plant.

Fig. 1. Transformations of PP 009 and its enantiomers in soil and plant.

FIELD PERFORMANCE OF PP 005

Crop tolerance

The high degree of selectivity of PP 009 towards a wide range of broad-leaf crops (Plowman et al., 1980) is also possessed by PP 005. No differences in crop tolerance towards the racemate and R-enantiomer have been observed.

Herbicidal efficacy

Many field trials have been conducted in which post-emergence efficacy of PP 005 and PP 009 have been compared. The main objective was to determine whether PP 005 could be introduced as a formulation change, in which the S-enantiomer, viewed essentially as an impurity in PP 009, has been largely removed. Hence, for most markets, 25% e.c. formulations of PP 009 have been compared with 12.5% formulations of PP 005 at equivalent applications in terms of 1 ha^{-1} of product. Typically, these trials employed a limited rate range based on the recommended rates of PP 009. This approach has generally substantiated equivalence of PP 009 and PP 005 formulations, and hence a factor of 2 between the R-enantiomer and the racemate in terms of kg ha^{-1} 'a.i.'. For example, recently published results from UK trials undertaken in 1982/1983 (Anon, 1985; Barrett and Sutton, 1985), cover comparisons against Avena fatua, Bromus sterilis, Alopecurus myosuroides, Lolium perenne, Elymus repens, volunteer wheat and barley out-of-crop, in sugar beet and oilseed rape.

A detailed examination was undertaken in USA in 1983. Data from thirty-four development trials in which PP 009 and PP 005 were compared at 3-5 rates were subjected to linear regression analysis. Some grouping of species was done to strengthen the data bases for development of models. Regression lines representing the log dose-response relationships for PP 009 and PP 005 within any group were not significantly non-parallel. Those for perennial species (eight trials with Sorghum halepense, three with Elymus repens and one with Cynodon dactylon) and for Digitaria (D. sanguinalis and D. ischaemum, 3 trials) are illustrated in Fig. 2.

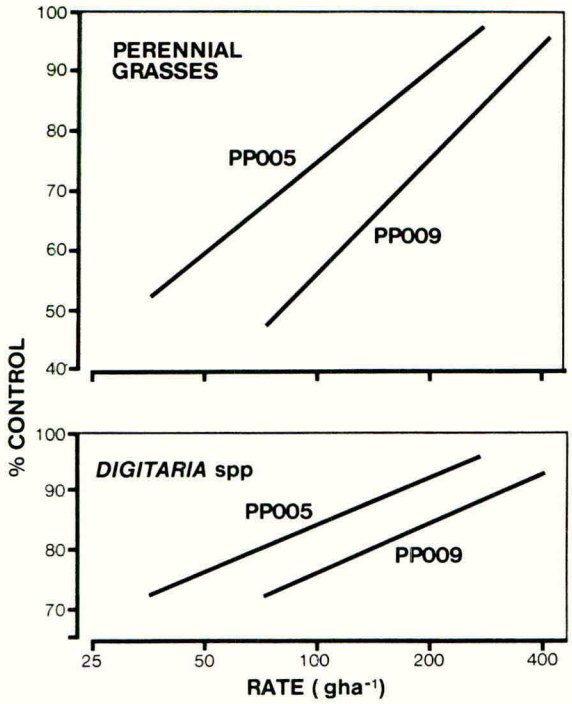


Fig. 2. Rate-response regression lines for PP 005 and PP 009 on (a) perennial grasses and (b) Digitaria spp.

TABLE 4

Mean level of grass control achieved by PP 009, and by PP 005 at half the rate (USA, 1983)

Grouping*	% Control			
	PP 009	(0.28 kg ha^{-1})	PP 005	(0.14 kg ha^{-1})
Perennials	85	(81, 90)$^{+}$	83	(78, 87)
Setaria spp	85	(80, 91)	82	(78, 86)
Digitaria spp	ID		ID	
Other annuals	93	(88, 98)	91	(87, 95)

$^{+}$ 95% confidence limits for the means in parenthesis

* Setaria spp (10 trials; S. faberii, S. viridis, S. lutescens); other annuals (10 trials; species included Sorghum bicolor, Brachiaria platyphylla, Echinochloa crus-galli, Panicum texanum, Eragrotis spp, wheat, barley). Details for perennials and Digitaria are given in text.

ID Insufficient data for a reliable estimate

From this analysis, it is clear that the efficacy of PP 009 at 0.28 kg/ha differs little from PP 005 at half that rate (Table 4). However, this difference is large enough in some of these warm-climate species, especially growing under hot, dry conditions, to necessitate PP 005 rates higher than half of the PP 009 recommendation.

SUGAR CANE RIPENING WITH PP 005

Field experiments in Southern Africa with PP 005 have demonstrated it to be similar to, or better than, standard ripeners at much lower rates of application. It was effective at both the beginning and the end of the milling season; best results were obtained in young, well-grown crops not suffering from moisture stress. Smaller, but economic, yield increases also occurred under less favourable conditions. Cane sucrose percentage and juice purity improved between four and six weeks after treatment, resulting in large yield increases. The average yield response to PP 005 in ten experiments over two years was 1.1 t ha^{-1} sucrose or 1.5 t ha^{-1} estimated recoverable sugar. Most varieties responded in a similar manner and the maximum increase in sugar yield in two varieties was obtained at application rates of 39-53 g ha^{-1} a.i. (Table 5).

TABLE 5

The ripening response of two sugar cane varieties to PP 005

		Yield		
Ripener	Rate (g ha^{-1})	Ers (% Cane)	Cane (t ha^{-1})	Ers (t ha^{-1})
Variety NCo 376 sprayed early season				
Control	0	9.8	133	13.0
PP 005	39	11.0**	129	14.2
PP 005	44	10.7**	128	13.7
PP 005	53	11.6**	129	15.0
PP 005	97	11.7**	117	13.7
glyphosate^{+}	406	10.8**	135	14.5
Variety N14 sprayed late milling season				
Control	0	6.4	116	7.3
PP 005	17	7.4	112	8.2
PP 005	35	8.6*	113	9.7*
PP 005	51	10.0*	102	10.3**
glyphosate^{+}	380	7.1	119	8.5

Ers : estimated recoverable sugar. $^{+}$, as Polado
Statistically significant * P = 0.05; ** P = 0.01

At optimum rates of application, PP 005 often killed stalk tissue just behind the growing point, resulting in blackening and ring barking of the stalk. However, there was no rotting of stalk tissue, even fourteen weeks after treatment, because damaged cells appeared to be cauterised. Death of young leaves and damage to the growing point often reduced cane fresh weight, particularly at high rates of application. This improved both harvest and transport efficiency because of better crop burns and higher cane sucrose percentages. The reduction in cane yield was a disadvangage only if harvest was delayed so long after treatment that it negated the beneficial ripening effect. PP 005 was recently registered as a sugar cane ripener in South Africa and Swaziland.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge many colleagues for contributions, particularly Mr. S.J. Salter (glasshouse tests), Dr. C. Doty and Mr. K. White (USA field results), Dr. D. Cartwright (synthesis of S-enantiomer), Dr. B.D. Cavell (crop metabolism), Dr. P. Chapman and Mr. M. Greenwood (statistical analysis of glasshouse results); also Dr. H. Rostron of Farmers Organisation (Natal) (Pty) Ltd (sugar cane ripening).

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DPD-20027, A NEW RESIDUAL HERBICIDE FOR ANNUAL GRASS AND BROAD-LEAVED WEED CONTROL IN CEREALS

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ABSTRACT

DPD-20027 is a residual herbicide for all winter and spring cereals composed of Orbencarb and Linuron. In 5-years' field trials in W.-Germany DPD-20027 proved effective against annual grasses and weeds. On an average of all trials we reached an efficacy of 92 % against *Alopecurus myosuroides* and 98,5 % against *Apera spica-venti*. Beyond that it shows a good efficacy against special weeds like *Galium aparine, Viola ssp.* and *Veronica spp.* which can hardly be controlled by residual herbicides. DPD-20027 can be applied in all soils. On extremely heavy soils as well as on soils with a high humus content efficacy specially against *A. myosuroides* is decreased. The compatability is good in all cereals proved by the yield.

INTRODUCTION

The spread of *A. myosuroides, Apera spica-venti* and *Poa ssp.* was favoured by crop rotations with high cereal frequency. The increase of winter cereals, particularly winter barley has made pre-emergence treatment necessary in several parts of W.-Germany. As some pre-emergence herbicides have no effect against *Galium aparine, Viola ssp.* and *Veronica spp.*, early sowing leads to a greater competition of these species with the crop. The development of a new pre-emergence herbicide which is effective against grasses and dicotyledonous weeds including *Galium aparine, Viola ssp.* and *Veronica spp.* to be applied in all cereals seems to be of a great promise. For that reason Orbencarb, an active ingredient of Kumiai Chemical Industries, Tokyo, has been developed. Under Central European conditions Orbencarb shows some gaps against dicotyledonous weeds. Linuron proved to be the most effective partner.
Extended field trials have been carried out in cereals with the combination under the code DPD-20027 in the years 1980/81 to 1984/85.

MATERIALS AND METHODS

DPD-20027 (trademark LANRAY[R] L) is an EC with 540 g Orbencarb and 107 g Linuron /l. The standard application rate is 7 l/ha. DPD-20026 (trademark LANRAY[R]) is an EC with 500 g Orbencarb /l. Application rate is 8 l/ha. Both products were applied during the pre-emergence stage of the crop.

[R] = reg. trademark of Kumiai Chemical Industry Co., Ltd., Tokyo, Japan

3C—3

Orbencarb is the common name of S-(2-chlorphenyl)methyl) diethylcarbamothioate and was synthesized in the laboratories of Kumiai Chem. Ind., Tokyo. The biological features have been reported (Iori et al. 1975, Takeucki 1983, Takahashi & Iskizuka 1985).

Orbencarb shows selectivity in all cereals, maize, sugarcane, potatoes, soybeans, kidney bean, cotton, sunflower, carrots and sugarbeet. Application is made during the pre-emergence stage of the crop. Trials were carried out in accordance with BBA-guidelines. Plot-size 20-25 m^2 in randomized blocks with four replicates. Application was done with mobile plot sprayers at a volume rate of 400 l/water ha, pressure 2,5 - 3 bar.

Herbicidal effect and crop safety (damage / thinning out), were assessed in % and No. of spikelets / m^2 at the following times.

1. EC13-21 stage of the crop in late autumn.
2. EC25-32 stage of the crop in spring.
3. Emergence of spikelets of grasses.
4. Lodging before harvest.

The plots were harvested by a plot combine harvester. Reported yields are based on 86 % of dry matter.

RESULTS AND CONCLUSIONS

Efficacy spectrum

Fig. 1 shows a comparison of the average grass and weed control of the combination (DPD-20027) with the straight Orbencarb formulation (DPD-20026).
Orbencarb alone performs well to adequate against *A. myosuroides, A. spica-venti* as well as the dicots *Anthemis arvensis, G. aparine, Lamium spp., Myosotis arvensis, Papaver rhoeas, Stellaria media, Thlaspi arvense* and *V. arvensis* but it shows gaps in efficacy against *Matricaria spp., Polygonum spp., Veronica spp.* and most of the cruciferae. Therefore, as indicated in the graph, the trial work with Orbencarb alone was not so extensive as with the combination. The addition of Linuron to Orbencarb increases the effect and enlarges the weed spectrum considerably. With DPD-20027 most of the weeds appearing in the trials could be controlled up to 95 - 100 %, the herbicidal efficacy being largely independent of soil and weather conditions. But *Centaurea cyanus* remains a gap and the effect against *P. aviculare* and *B. convolvulus* is inadequate.

The 7 l rate per ha of DPD-20027 performs optimal control. Table 1 shows a comparison of the herbicidal effect of the rates 5 l, 6 l and 7 l DPD-20027 per ha. At the lower dosages the control of most weed species remains good. The effect against *A. myosuroides* and *G. aparine* is obviously decreased but remains adequate. Against *G. tetrahit* the effect at the 5 l and 6 l rate is unacceptable.

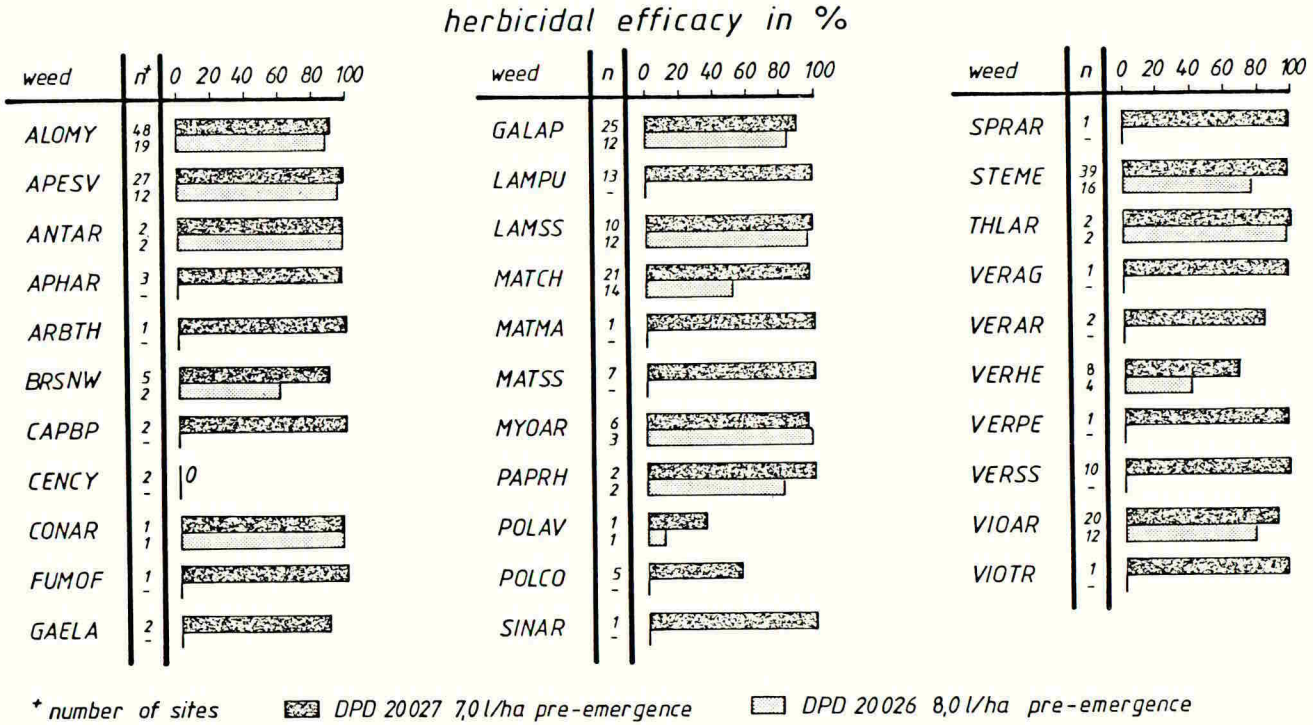

Fig. 1: Herbicidal effect (% control) of DPD-20027 (Orbencarb + Linuron) against grasses and broad-leaved weeds compared with DPD-20026 (Orbencarb straight) (1981 - 1985)

TABLE 1

Herbicidal effect (% control) of DPD-20027 against main grass and weed species at different application rates (1981 - 1985)

	n	DPD-20027 5 l/ha	DPD-20027 6 l/ha	DPD-20027 7 l/ha
Alopecurus myosuroides	14	83	87	93
Apera spica-venti	6	100	100	100
Capsella bursa-pastoris	2	100	100	100
Galeopsis tetrahit	3	57	67	88
Galium aparine	6	78	84	95
Lamium spp.	5	100	100	100
Matricaria spp.	5	98	98	100
Myosotis arvensis	3	99	100	100
Bilderdykia convolvulus	2	92	88	90
Stellaria media	12	100	100	100
Veronica spp.	8	92	98	100
Viola arvensis	10	85	94	99

n = No. of sites

Grass control

On an average of all trials a control rate of 92 % against *A. myosuroides* and 98,5 % against *A. spica-venti* was achieved. Fig. 2 and 3 show the efficacy of DPD-20027 against *A. myosuroides* and *A. spica-venti* in comparison to reference products graded into groups of efficacy. The columns indicate the cumulated frequency of the efficacy groups. Against *A. myosuroides* a very good to complete control was achieved in 50 %, a good control in 60 %, a sufficient control in more than 70 %, and an at least adequate control (> 85 % effect) in more than 80 % of all trials.
As against *A. spica-venti* a very good to complete control was achieved in 90 % of all trials. In no trial was an effect of less than 90 % found.

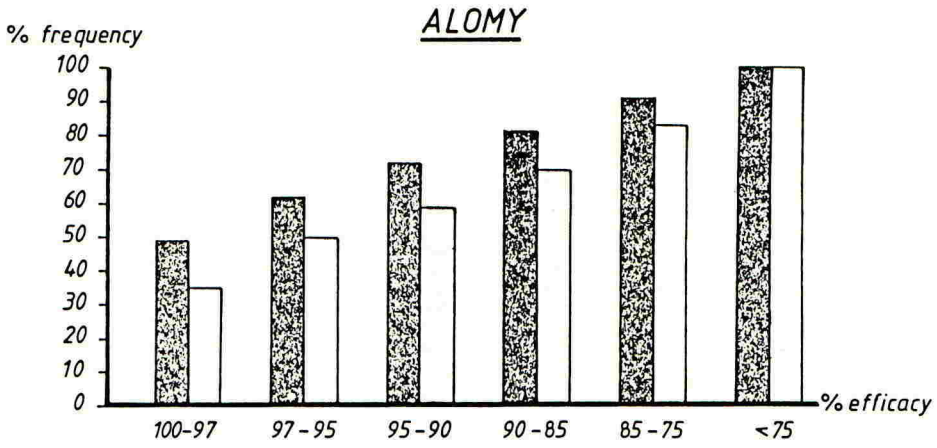

Fig. 2: Control of *Alopecurus myosuroides* after application of DPD-20027 compared with reference products - cumulated frequency of control graded into groups of efficacy.

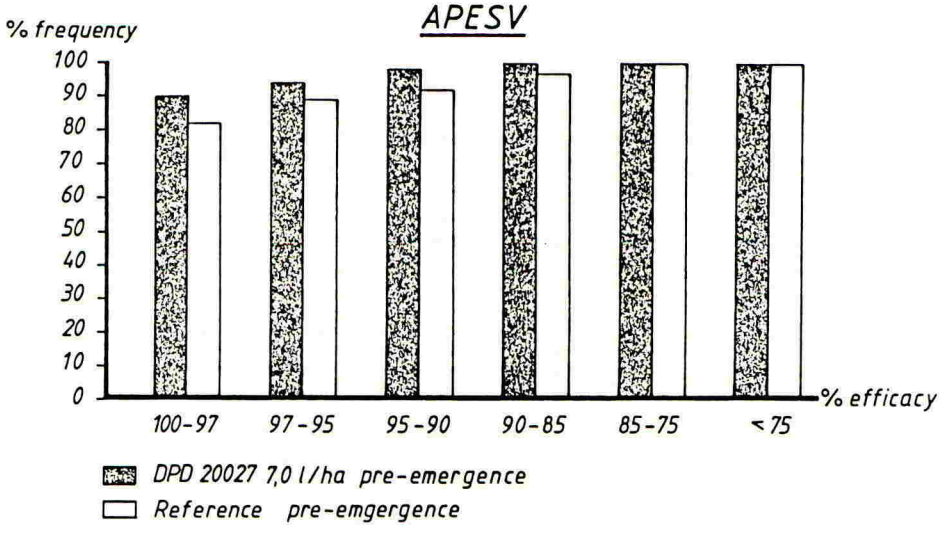

Fig. 3: Control of *Apera spica-venti* after application of DPD-20027 compared with reference products - cumulated frequency of control graded into groups of efficacy.

Influence of clay and organic matter content of soil

In many cases the effect of residual herbicides is affected by various soil conditions. Figs. 4 and 5 show the interdependency of *A. myosuroides* control by DPD-20027 on the organic matter and clay content of soil.

Humus content of > 3 % reduces the efficacy partially. In spite of that soil condition an effect of > 80 % control was achieved. The low effect at 1 trial site with < 1 % of organic matter can hardly be correlated to the humus content. The influence of the clay content of soil is more efficient. Up to a content of 35 - 45 % of clay, as in loam soils, the efficacy is retained. At higher clay contents of > 45 % a satisfactory control cannot be guaranteed.

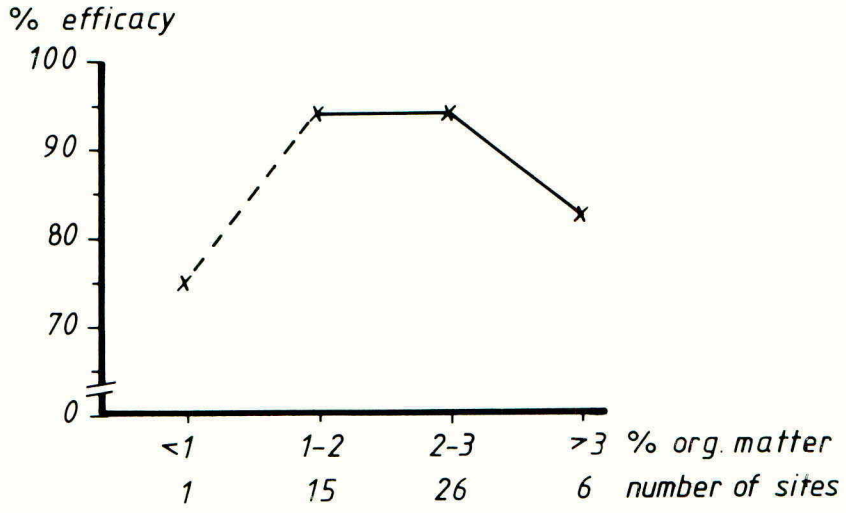

Fig. 4: Efficacy of DPD-20027 against *A. myosuroides* in relation to organic matter content of soil

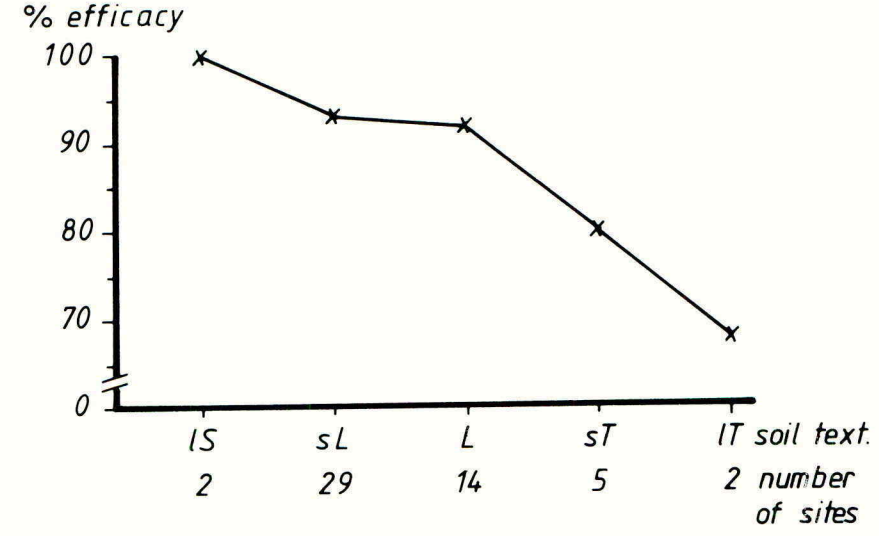

Fig. 5: Efficacy of DPD-20027 against *A. myosuroides* in relation to clay content of soil

Crop safety and yield

DPD-20027 showed generally a good compatability with all winter cereals under various weather conditions. Crop damage occurred only in 10 % of all trials.
Fig. 6 shows the crop damage to winter wheat, winter barley and winter rye scored at 34, 42 and 22 sites respectively.

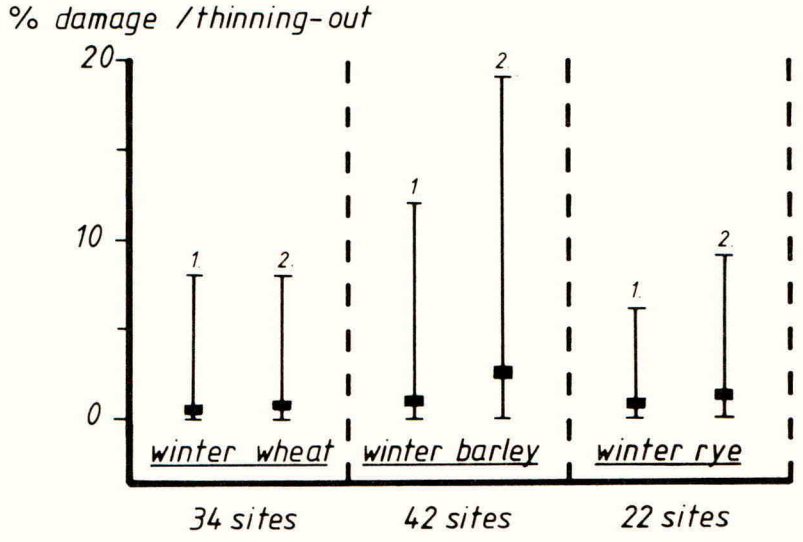

Fig. 6: Crop damage of winter cereals following DPD-20027 application with 7 l/ha.

= average damage

= variation of damage

1. = 1st assessment (autumn)

2. = 2nd assessment (may, june)

Winter wheat and winter rye are highly compatible with DPD-20027 as indicated by the average of about 1 % damage. In winter barley initial yellowing at some places disappeared fairly soon in autumn and had no influence on yield. Exceptionally in one trial considerable crop damage and thinning out occurred in autumn and grew in spring to an extent of 19 % total damage. However, the average of 1 % damage in autumn and 2.5 % in spring indicate a good compatability to winter barley, too.

Yields

Fig. 7 shows the effect of the application of DPD-20027 on yield of winter cereals in comparison to 3 reference products mainly used with respect to typical regional conditions.

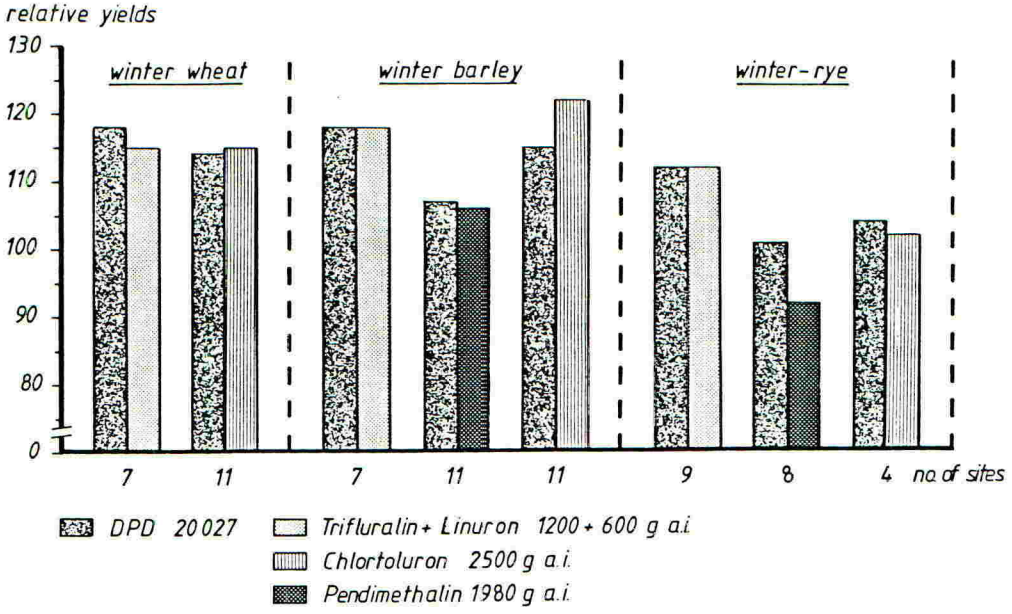

Fig. 7: Yields of winter cereals following application of DPD-20027 with 7 l/ha in comparison to reference products (field trials 1981 - 1985)

In none of the trials could a significant reduction of yield be observed with DPD-20027 application. As Fig. 7 indicates the application of DPD-20027 was followed by considerable yield increases in winter wheat and winter barley In winter rye the beneficial effect of the treatment was observed in about 50 % of the trials. The increase in yield following treatment with DPD-20027 was due to an increase in crop density following the elimination of weed and grass competition.

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INVESTIGATIONS OF A GLYCYL FORMULATION OF MECOPROP WITH VERY LOW VAPOUR ACTION

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ABSTRACT

Three trials were conducted over two years to investigate the vapour movement of a new ester formulation of mecoprop. Mecoprop ethylene glycyl diester was compared with the potassium salt and the iso-octyl ester, applied at normal herbicidal effective rates to test crops under sealed polythene tunnels. Sensitive species of crop plants were placed in the tunnels after application, under conditions capable of inducing high vapour release. The test plants were exposed to the atmosphere in the tunnels for 0-24, 0-48 and 24-48 hours after application. Severe effects were noted from the vapour released in the iso-octyl treatment; some effects were noted at very high temperature conditions from the potassium salt, but no serious effects were noted in the glycyl diester treatment. Whilst displaying this resistance to vapour movement in extreme circumstances and exhibiting very good crop safety, the glycyl diester has in field trials been proven to be highly comparable to the iso-octyl ester in herbicidal activity.

INTRODUCTION

As has been reported by Eagle (1982), there is the possibility of some damage from vapour drift to adjacent susceptible crops after spraying cereals with the iso-octyl ester of mecoprop.

J. D. Campbell & Sons Ltd. have developed a mecoprop ethylene glycyl diester formulation that initial trials show as having the herbicidal properties of the potassium salt and the iso-octyl ester and is apparently of low volatility.

To investigate this three trials were laid down to examine the relative level of vapour release, post spraying, from a glycyl diester formulation of mecoprop, compared with a formulation of known high potential vapour release and a formulation of known low potential vapour release.

MATERIALS & METHODS

Three separate unreplicated tests were carried out over two years.

TABLE 1

Products and treatments used in the three tests.

Product	% a.i.	Rate (l/ha) Test 1 1983	Test 2 1983	Test 3 1984
Potassium Salt	57%	4.2	4.2	4.2
Iso-octyl Ester	60%	2.8	2.3	2.8
Glycyl diester	73%	2.0	2.0	3.0

Individual plots measured 1m x 5m and were bordered by areas of freshly mown crop. The formulations under test were applied using one pass of a propane pressurised AZO sprayer fitted with a rear mounted two metre boom. Spraying was carried out at a pressure of 2.5 kg/sq.cm and at a volume of 200 l/ha. The equipment was washed thoroughly with clean water between treatments. Weather conditions were recorded before, during and after application.

When the treated crop had completely dried after application (approximately $1\frac{1}{2}$ hours) the plots were covered with enclosures in order to contain any vapour released. The enclosures measured 3.8m x 6.5m at the base and were curved in shape with a maximum height at the centre of 0.7m, giving an approximate volume of $8.65m^3$. They were constructed of tubular steel frames covered with polythene sheeting, the edges being sealed with straw bales. One enclosure was used for each of the test products.

Test plants were introduced into the enclosures and remained exposed to the contained air and any released vapour for pre-determined periods of time. These were 0-24 hours, 24-48 hours and 0-48 hours.

The enclosures were ventilated completely by the removal of the polythene during the change of test plants twenty four hours after initiation. All plants were watered at this time. In each test on the afternoon following ventilation and the change of test plants, hot weather caused the temperature under the polythene to rise considerably. In test 3 by 1500 hours the temperature had reached 38°C and it was decided to roll back the polythene for one hour to prevent severe heat damage to the plants which might prevent them growing on sufficiently to show symptoms of vapour damage.

TABLE 2

Application & Crop Details

Details	Test 1	Test 2	Test 3
Date	13.4.83	27.4.83	20.7.84
Time	11.30 am	10.00 am	9.00 am
Volume	200 l/ha	200 l/ha	200 l/ha
Soil temp.	7°C	9°C	-
Air temp.	14°C	11°C	20°C
Max temp.			38°C
Min temp.			10°C
Crop	W.Barley	Rye grass mix	Spring barley
Variety	Igri	-	Triumph
Growth stage	30-31	10-20cm height	45

TABLE 3

Test Plants

Plant	Variety	No pots per tunnel/plants per pot	Growth Stage
Test 1			
Cauliflower	All year round.	8/1	Cotyledons plus 2 small true leaves
Cabbage	Hispi	8/2	Cotyledons plus 2 full true leaves
Oilseed Rape	Lingot	26/1	10cm high plus flowerbuds
Oilseed Rape	Lingot	8/3	20cm high plus flowerbuds*
Test 2			
Cauliflower	Nevada	12/1	Cotyledons plus 2 true leaves
Cabbage	Greyhound	12/1	Cotyledons plus 2 true leaves
Oilseed Rape	Lingot	24/1	10-15cm high plus flowerbuds
Lettuce	Favourite	2/1 tray	4-6 leaves
Test 3.			
Tomato	Sonatina	2/1	6" height,6 leaves not yet flowering
Field bean	Maris Bead	4/1	10-16" height, first flower buds appearing
Brussel sprout	Bedford type	4/1	8" height, 6-8 leaves

*The triple rape plants were etiolated due to having been kept a few days in poor light conditions.

Following exposure the test plants were removed to grow on. The day after removal, the test plants were assessed individually on a 1-10 scale of damage.

TABLE 4.

Scale of damage scores used in the three tests.

Score	Symptoms
1	Plant unaffected
2	Twisting and/or leaf scorch very slight
3	Twisting and/or leaf scorch slight
4	Twisting and/or leaf scorch light-moderate
5	Twisting and/or leaf scorch moderate
6	Twisting and/or leaf scorch heavy
7	Less than 60% green tissue remaining
8	Less than 30% green tissue remaining
9	Small amount of green tissue remaining
10	Plant dead

Further assessments were made at intervals. Visual observations were also made of crop damage and herbicidal efficacy.

RESULTS

The first visible vapour drift symptoms were seen at the time of removal of the plants.

Test 1

Potassium salt - no effect noticed at any stage.

Glycyl diester - no effect noticed at any stage.

TABLE 5

Results obtained from plants exposed to vapour from the iso-octyl ester (Scale 1 - 10, 10 = dead).

Exposure Time:	0 - 24 hrs				0 - 48 hrs				24 - 48 hrs			
Days after Removal.	TR	SR	C	Cb	TR	SR	C	Cb	TR	SR	C	Cb
2	4.2	3.5	2.0	2.3	5.0	4.2	3.3	3.5	3.9	3.4	2.5	2.3
5	4.5	4.5	2.5	3.0	5.3	4.4	4.3	4.3	4.5	3.7	2.8	3.0
9	7.0	3.6	5.0	3.8	7.0	4.5	5.0	4.3	7.0	3.4	5.0	3.3
12	7.0	5.5	4.5	5.1	7.0	7.0	4.8	4.9	7.0	3.8	4.0	4.1
16	8.0	9.0	6.0	6.1	8.0	9.0	6.0	6.1	8.0	9.0	6.0	6.0
19	8.0	9.0	6.0	6.1	8.0	9.0	6.0	6.1	8.0	9.0	6.0	6.0
30	9.3	9.1	7.8	7.4	10.0	9.0	7.0	7.4	8.7	8.2	7.8	6.0

TR = Rape 3/pot. SR = Rape 1/pot. C = Cauliflower. Cb = Cabbage

Test 2

Potassium salt - no effect noticed at any stage.

Glycyl diester - no effect noticed at any stage.

TABLE 6

Results obtained from plants exposed to vapour from the iso-octyl ester (Scale 1 - 10, 10 = dead).

Exposure Time:	0 - 24 hrs				0 - 48 hrs				24 - 48 hrs			
Days after Removal.	L	R	C	Cb	L	R	C	Cb	L	R	C	Cb
2	2.0	3.8	2.8	2.6	3.0	4.9	3.0	3.3	2.0	3.8	2.0	2.3
5	4.0	4.2	3.4	3.3	4.0	4.9	3.5	3.5	2.0	4.3	2.6	2.6
10	4.0	4.3	4.0	4.0	4.0	4.9	4.3	3.8	2.0	4.3	3.2	2.8
16	4.0	7.8	6.0	6.5	4.0	7.6	6.0	5.8	2.0	7.9	6.0	5.6
23	4.0	8.4	6.7	8.7	5.0	8.6	8.5	7.5	2.0	8.3	6.7	6.5
26	4.0	8.4	8.0	8.6	5.0	8.6	8.5	7.5	2.0	8.3	6.7	6.5
33	4.0	8.5	9.5	9.0	5.0	8.9	9.3	9.0	3.0	8.4	7.3	6.5
39	5.0	9.2	9.5	9.5	5.0	9.3	10.0	9.8	4.0	8.4	7.3	6.8

L = Lettuce. R = Rape. C = Cauliflower. Cb = Cabbage

Test 3

TABLE 7

Results obtained from plants exposed to vapour from the glycyl diester, the potassium salt and the iso-octyl ester (Scale 1-10, 10 = dead).

Exposure Time:	0 - 24 hrs			0 - 48 hrs			24-48 hrs		
Days After Removal	T	S	B	T	S	B	T	S	B*
Glycyl diester									
1	1.5	1.3	9.3	3.5	5.0	10.0	2.0	2.0	5.5
3	2.0	1.5	9.5	5.0	5.8	10.0	2.0	2.3	5.5
6	3.0	1.5	9.5	6.0	7.5	10.0	2.0	2.8	4.5
14	3.0	1.8	10.0	6.0	7.8	10.0	2.0	2.3	4.5
21	2.5	2.0	10.0	7.5	8.0	10.0	2.0	2.0	4.3
Mean	2.4	1.6	9.7	5.6	6.9	10.0	2.0	2.3	4.9
Potassium salt									
1	4.0	6.3	9.0	7.0	7.5	10.0	4.0	3.5	5.3
3	6.0	6.8	9.8	8.0	7.8	10.0	5.0	2.8	5.3
6	8.5	7.5	10.0	9.0	8.5	10.0	4.5	2.8	5.0
14	9.0	8.8	10.0	10.0	9.0	10.0	4.0	2.5	5.0
21	9.0	9.0	10.0	10.0	9.5	10.0	3.5	2.5	5.0
Mean	7.3	7.7	9.8	8.8	8.5	10.0	4.2	2.8	5.1
Iso-octyl ester									**
1	9.0	9.3	10.0	10.0	10.0	10.0	7.0	5.0	5.5
3	9.0	9.8	10.0	10.0	10.0	10.0	7.5	5.5	5.8
6	10.0	10.0	10.0	10.0	10.0	10.0	6.5	5.8	5.5
14	10.0	10.0	10.0	10.0	10.0	10.0	6.5	5.8	5.5
21	10.0	10.0	10.0	10.0	10.0	10.0	6.0	5.8	5.2
Mean	9.6	9.8	10.0	10.0	10.0	10.0	6.7	5.6	5.5

T = Tomato. S = Brussel sprout. B = Field bean.

* Bean plants having some curling at top, probably due to tall fast growing plants coming into contact with the polythene whilst in the tunnel.

** Bean plants shortened and epinastic.

At the change of test plants 24 hours after application, visual observations were made of the weed control and crop safety. The crop treated with the iso-octyl ester showed very severe scorch on 90% of the leaves, and the crop treated with the potassium salt showed severe scorch on 50% of the leaves. The crop treated with the glycyl diester, however, showed no scorch at all. The reaction of the crop to the various products was accentuated by the extreme heat, and it would therefore appear that the glycyl diester was extremely safe in use. Weeds in all three enclosures were severely affected, and there was no apparent difference in herbicidal efficacy between the three products.

Five replicated field trials were conducted in 1984 to investigate herbicidal activity. The glycyl diester at 3.0 l/ha product gave high levels of weed control equivalent to that afforded by the potassium salt and the iso-octyl ester at double normal use rates. Results were only marginally improved by applying 6.0 l/ha of the glycyl diester product. Visual assessments of ear/grain formation showed no adverse effect in any trial.

TABLE 8

Weed control from five replicated field trials conducted in 1984.

Weed	Mecoprop Product					
	K-salt		Iso-octyl ester		Glycyl diester	
	l/ha		l/ha		l/ha	
	4.2	8.4	2.8	5.6	3.0	6.0
Urtica urens	89.5	96.0	89.0	93.8	95.0	95.3
Bilderdykia convolvulus	94.3	98.0	91.0	95.8	98.0	98.0
Stellaria media	92.8	97.2	89.6	96.2	95.0	97.0
Galium aparine	90.7	93.6	81.3	90.5	94.3	94.8
Chenopodium album	96.5	98.0	95.0	97.3	98.0	98.0
Myosotis arvensis	80.0	90.0	77.8	90.3	92.0	95.0
Fumaria officinalis	92.3	98.5	93.3	98.5	99.0	99.5
Senecio vulgaris	86.8	93.5	90.0	93.8	92.5	95.5
Chamomilla recutita	81.3	89.3	80.7	88.2	88.0	92.6
Viola arvensis	84.2	88.5	81.7	88.4	89.3	91.2
Papaver rhoeas	84.5	92.5	65.0	88.8	90.0	94.0
Lamium purpureum	81.7	90.1	79.2	87.9	88.2	92.0
Veronica spp.	82.0	88.8	79.2	86.9	82.5	90.5
Mean overall % weed control	87.4	93.4	84.1	92.0	92.4	94.9

DISCUSSION

The principle objective of the test was to create conditions which would facilitate vapour production from treated plots and thereby give assessable levels of damage to potted test plants.

Whilst covering the treated plots with polythene enclosures was necessary to contain the vapour evolved, it also raised both temperature and humidity above the levels normally encountered under field conditions. These factors would be likely to render the test plants more susceptible to the effects of damaging vapour since the tests had to be run in high summer. The temperature rose to high levels inside the enclosures and this caused sun scorch and heat prostration on the test plants, the field beans being the most severely affected. For this reason the field beans showed apparently high damage levels even with the glycyl diester and the potassium salt, but the damage could not be directly attributable to vapour drift.

Assessments of the test plants were made by comparison with untreated controls placed outside the tunnels. The results showed that the glycyl diester was considerably less damaging to the test plants than the iso-octyl ester, and slightly less damaging than the potassium salt.

For each test product, the most severely damaged plants were those which had remained under the enclosure for the full 48 hours. Plants which had been in the enclosures for the 0-24 hour period were slightly less affected, whilst those which had been introduced at 24 hours showed considerably less damage. This suggests that most of the vapour given off was produced during the first 24 hours, and that during the second 24 hour period the vapour given off was very much less, but still at potentially damaging levels.

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BEHAVIOUR OF THREE NONIONIC SURFACTANTS FOLLOWING FOLIAR APPLICATION

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ABSTRACT

Foliar uptake from droplets of dilute aqueous solutions of three ^{14}C-labelled nonionic surfactants, C12E8 (1-dodecanol octaethoxylate), C18E8.5 (1-octadecanol polyethoxylate) and NPE5.5 (nonylphenol polyethoxylate), varied considerably between ten plant species. The most rapid rates of uptake were observed for C12E8 and the slowest for C18E8.5. Penetration into waxy leaves was generally faster than that into less waxy ones. Following uptake there was little movement of radiolabel away from the treated area; only in cereal leaves could significant translocation be detected and this was predominantly acropetal. Metabolism of the surfactants occurred within the treated area of leaves with both the rates and products of metabolism differing amongst the plants examined. There was evidence, mainly from C12E8, for the existence of two main metabolic routes (i) de-ethoxylation yielding products less polar than the parent compound and (ii) conjugation, probably with sugars, to produce a number of polar metabolites.

INTRODUCTION

Most pesticide formulations for use in water-based foliar sprays contain surfactants which serve two main functions, (i) as formulants for the dispersion of water insoluble ingredients in emulsion, wettable powder, suspension and granule preparations and (ii) as adjuvants to increase target coverage (spray-modifiers), uptake and ultimate biological efficacy of active ingredients (activators). Several different types of surfactant may be included for these purposes in the same formulation. However, despite their widespread use and proven effectiveness in commercial practice very little is known about the role of surfactants in the second category apart from their obvious effects on the physical properties of the spray solution (reviews Norris 1982, McWhorter 1985). In addition the fate of surfactants following foliar application has largely been ignored.

As the first stage in a study of the mode of action of adjuvants in increasing uptake of pesticidal active ingredients we have examined the uptake, translocation and metabolism of three radiolabelled surfactants following droplet application of dilute aqueous solutions to leaves of a range of economically important plant species. The linear alcohol and alkylphenol polyethoxylates used in the work are representative of the classes of nonionic surfactants commonly used as herbicide activators having an HLB value (Griffin 1954) between 10 and 13 (McWhorter 1985).

MATERIALS AND METHODS

Plants

Barley (*Hordeum vulgare* cv. Golden Promise), field bean (*Vicia faba* cv. Maris Bead), chickweed (*Stellaria media*), corn marigold (*Chrysanthemum segetum*), maize (*Zea mays* cv. LG11), pea (*Pisum sativum* cv. Meteor), rape

(Brassica napus cv. Rafal), sugar beet (Beta vulgaris cv. Nomo), wheat (Triticum aestivum cv. Avalon) and wild oat (Avena fatua) plants were raised from seed in pots of Levington peat-based compost in a controlled environment (CE) of 20/15°C with a 16 h photoperiod. Lighting was provided by fluorescent tubes supplemented with tungsten bulbs to give 425 $\mu E/m^2/s$. There was no control of humidity which ranged from 74-81% r.h. (day) to 88-93% r.h. (night). Plants were treated with surfactants 3-4 weeks from sowing.

Surfactants

C12E8 (1), a homogeneous [1-^{14}C] labelled 1-dodecanol octaethoxylate, purchased from CEA, France (sp. act. 46 mCi/mmol) was diluted with unlabelled C12E8 (Nikko Chemicals Co., Japan) to give a treatment solution containing c. 10,000 dpm and 1 µg per µl of water.

1 $CH_3(CH_2)_{10}$-$\overset{*}{C}H_2$-$(OCH_2CH_2)_8OH$ MW 538, HLB value 13.1

C18E8.5 (2), an oligomer mixture of [U-^{14}C] ethoxylate chain labelled 1-octadecanol ethoxylates average ethylene oxide (EO) content 8.5, purchased from Hoechst AG, West Germany (sp. act. 4.71 mCi/g) was used as supplied in a solution containing c. 20,000 dpm and 1 µg per µl of water.

2 $CH_3(CH_2)_{17}(O\overset{*}{C}H_2\overset{*}{C}H_2)_{\overline{8.5}}OH$ MW mean 644, HLB value 11.6

NPE5.5 (3), an oligomeric mixture of [U-^{14}C] phenyl ring labelled nonyl phenol ethoxylates average EO content 5.5, kindly supplied by ICI PLC, England (sp. act. 7 mCi/mmol) was diluted with unlabelled PP222 (a nonyl phenol ethoxylate mixture, average EO content 5.5, ICI PLC) to give a treatment solution containing c. 10,000 dpm and 1 µg of surfactant per µl of water.

3 brC_9H_{19}-$\overset{*}{C}_6H_4$-$(OCH_2CH_2)_{\overline{5.5}}OH$ MW mean 462, HLB value 10.5

Foliar application

Dilute aqueous solutions (0.1% w/v) were applied as ten 0.2 µl (c. 700 µm diameter) droplets to the central region of the adaxial surface of fully expanded leaves avoiding the major veins. All applications were made inside the CE room at the commencement of the light period using a Burkard PAX 100 Programmable Microapplicator fitted with a 50 µl syringe and PTFE coated needle. Treated areas were marked immediately with drops of black waterproof drawing ink.

Uptake and translocation

At intervals up to 144 h (6 days) any surfactant remaining on the leaf surface was recovered from the marked treated area either by an acetone wash (5 ml) or by using the cellulose acetate film stripping technique; radioactivity was determined by scintillation counting using a dioxan based cocktail (10 ml). Uptake was subsequently assessed after excision of the solvent washed or stripped areas of treated leaves followed by combustion analysis (Harvey Biological Oxidiser) or by scintillation counting of the extract obtained by exhaustive extraction with boiling methanol. The extent of translocation of surfactant was determined when necessary by combustion analysis of the remaining parts of the treated leaves and other regions of the plant. Five replicates were employed at all stages of the procedure.

Metabolism

Radio-tlc analysis (Isomess IM-3000 Linear Analyzer) of methanol extracts of treated leaf areas was used to monitor any metabolic changes in surfactant composition at intervals following foliar application. For

C12E8 both adsorption and reverse-phase methods were carried out; because they were complex mixtures of oligomers it was only possible to utilise the latter technique for C18E8.5 and NPE5.5.

Adsorption tlc: silica gel 60 precoated (Merck), water saturated 2-butanone (Bürger 1963), two developments, Rf C12E8 = 0.45

Reverse-phase tlc: Whatman KC-18 precoated, methanol-water (9 : 1) (Stolzenberg et al. 1984, Rf C12E8 = 0.1, NPE 5.5 = 0.3; ethanol-water (19 : 1), Rf C18E8.5 = 0.5

RESULTS

Uptake

The uptake of C12E8, C18E8.5 and NPE5.5 into ten plant species was examined over a time course of 0-144 h. Values recorded after 24 h are summarized in Table 1. In each case there was an initial period of rapid uptake followed by a longer period of slower uptake, best described by an exponential curve. The rates and asymptote values varied according to plant species. Penetration into waxy leaved plants, e.g. rape, pea, C. segetum, was generally much faster than that into the less waxy ones, e.g. bean, beet, S. media. However, such differences were less marked for C18E8.5 where uptake was slow for all plants, a maximum value of c. 65% being recorded for maize at 144 h. Uptake rates were greatest for C12E8; values >90% were observed after 2 h on pea and C. segetum leaves. The corresponding rates for NPE5.5 were intermediate between those of C12E8 and C18E8.5.

TABLE 1

Comparison of uptake of 0.1% w/v aqueous solutions of C12E8, C18E8.5 and NPE5.5, 24 h after foliar application

Plant	% Applied radioactivity		
	C12E8	C18E8.5	NPE5.5
A. fatua	91.0 (5.0)	*	30.3 (5.2)
Barley	80.3 (1.5)	20.4 (2.5)	55.7 (3.4)
Bean	75.1 (7.6)	14.1 (3.1)	42.6 (6.5)
Beet	92.3 (4.2)	17.8 (3.8)	26.9 (11.2)
C. segetum	92.0 ≠	23.1 (2.7)	80.1 (2.2)
Maize	93.0 ≠	32.4 (3.3)	47.9 (10.4)
Pea	89.4 (1.3)	22.7 (5.9)	93.9 (1.1)
Rape	99.4 (3.1)	24.3 (7.7)	56.2 (7.0)
S. media	53.6 (18.4)	27.3 (5.9)	16.7 (12.0)
Wheat	86.6 (3.7)	19.6 (3.3)	15.0 (3.7)

Results are mean values for five replicates, standard deviations are given in parentheses.

* Data not yet available. ≠ 24 h result calculated from uptake curve.

Considerable differences were also noted between plants in the spreading behaviour and dry-down times of droplets of the three surfactants. However, these did not appear to be correlated with either the rates or amounts of uptake observed. For all three surfactants phytotoxicity was generally low at the 0.1% concentration used except in plants where there were extremely rapid or high uptake values (>80%). On waxy species there

was little evidence from scanning electron microscopy of any disruption or dissolution of the microcrystalline deposits of wax during or after surfactant uptake.

Translocation

For all the plants and surfactants examined the bulk of the applied radioactivity that penetrated into the leaf remained in the treated area, even after rapid or considerable uptake of a particular surfactant. However, detectable amounts of translocation were recorded in cereal leaves, where it was mainly acropetal. For example, in barley c. 7% of the applied radioactivity was recovered in the distal region of the leaf 5 d after treatment with C12E8. It was not possible to determine whether this radioactivity was attributable to parent surfactant and/or metabolites.

Metabolism

Following foliar uptake, the three labelled surfactants were metabolised within the treated area of leaf tissue. Usually the radioactive products of metabolism could be completely recovered from the tissue by subsequent extraction with boiling methanol although in cereal leaves a small proportion (c. 10%) of the radioactivity remained bound. Both the rates and products of metabolism varied according to the plant species (Table 2); there was no obvious relationship between the rates of uptake of the surfactant and metabolism. Biodegradation was slow in most of the species studied with substantial amounts of unchanged surfactant being detectable several weeks after foliar application, e.g. in bean. However, C12E8 was completely metabolised in barley leaves within 48 h and in chickweed 6 d after foliar application.

TABLE 2

Metabolism of C12E8 in treated areas of eight plant species 24 h after foliar application

Plant	Non-polar*	Parent*	Polar*
A. fatua	6.6	56.7	36.7
Barley	19.6	15.1	65.3
Bean	16.2	62.0	21.8
Beet	29.7	63.1	7.2
Pea	21.9	53.5	24.7
Rape	17.5	68.8	13.7
S. media	33.9	40.0	26.1
Wheat	0.0	67.3	32.7

* Relative amounts of radioactivity (as%) determined by electronic integration of radio-tlc scans of corresponding methanol extracts.

Most of the metabolic studies were carried out with C12E8 because the surfactant is homogeneous and therefore it was comparatively easy to detect any degradation by simple tlc analysis. Results obtained from different plants indicated that there were two main routes for metabolism of C12E8, one yielding products less polar, the other producing metabolites more polar than the parent compound. The main non-polar metabolite in all species was tentatively identified as a de-ethoxylation product corresponding to C12E4. At least four different polar metabolites, most probably conjugates with sugars, could be detected by reverse-phase tlc. Further work is necessary in order to fully characterise all these metabolic products.

In sugar beet leaves metabolism of C12E8 proceeded mainly by the non-polar route, the major metabolite reaching a maximum level after 8 h. The polar route predominated in bean, pea, rape and cereal leaves whereas in those of C. segetum and S. media degradation products from both pathways were formed. For S. media, the relative proportion of the non-polar metabolite reached a maximum at 8 h and then declined with a concommitant increase in the proportion of polar metabolites; there was a regular decrease in the amount of parent compound.

The formation of polar metbolites from C18E$\overline{8.5}$ and NPE$\overline{5.5}$ was confirmed in barley, maize, S. media and wheat using reverse-phase tlc. However, it was not possible to detect de-ethoxylation metabolites of these surfactants because they are not resolved from parent product on reverse-phase tlc or from the oligomeric constituents of the mixture by adsorption tlc.

DISCUSSION

So far there have been few systematic investigations of the foliar uptake of nonionic surfactants mainly due to the scarcity of chemically defined radioactive materials and, until recently, to the lack of sufficiently sensitive microanalytical methods for residue analysis. Only a small number of compounds and plant species have been examined. Nevertheless, previous work using HPLC analysis (McCann 1982) has shown that the penetration of commercial octylphenol (OP) polyethoxylates up to c. E9 into wheat leaves is essentially complete after 24h and that further increases in EO content above this value progressively reduces the rate of uptake. The ability of this class of sufactant to penetrate foliage was simultaneously confirmed by Stolzenberg et al. (1982) for barley leaves using homogeneous ^{14}C-labelled OPE6 and OPE9. Likewise Anderson & Girling (1983) were able to demonstrate >80% uptake within 48 h of four linear alcohol (C13-C18) polyethoxylates (E12-E17) into wheat leaves using a colorimetric method of analysis. However, not all classes of nonionic surfactant appear to be taken up by leaves. Very low levels of entry of ^{14}C-sorbitan based surfactants are reported for the leaves of barley, cotton, bean (Smith & Foy 1966), pear cuticles (Norris 1971), Sorghum halepense and soybean (McWhorter 1985) and of ^{14}C-ethylene oxide-propylene oxide copolymer surfactants in barley and cotton (Smith & Foy 1966) and bean (Smith & Foy 1966, Norris & Freed 1962) leaves.

Our quantitative work on the uptake of three radiolabelled surfactants has shown that considerable quantities of linear alcohol and alkylphenol polyethoxylates can enter plants by foliar absorption and that there is only limited translocation after foliar uptake of these surfactants. However, the rate of uptake is strongly influenced by the particular plant species. Penetration may be extremely rapid in some plants with as much as 90% uptake within 2 h of foliar application, e.g. C12E8 on rape and pea leaves. Rather surprisingly the presence of microcrystalline deposits of epicuticular wax on some of the plants studied did not present a significant barrier to the penetration of the surfactants, in fact the converse was true. It is likely that the surfactants are penetrating into leaves by passive diffusion; monomers rather than micelles are probably the form of transport solely from a consideration of molecular size. In addition the rates of surfactant uptake will probably be modified in the presence of pesticidal active ingredients but to what extent remains to be determined. However, the fact that many surfactants are capable of substantial foliar penetration on their own does provide some insight into their role as activator adjuvants.

The metabolism of surfactants following foliar absorption is poorly documented. The present work, however, has demonstrated that linear alcohol and nonylphenol ethoxylates are degraded at different rates and to various extents within the treated areas of different leaves. Most of the metabolites formed are soluble in methanol and the results from radio-tlc analyses of such extracts lend support for the two main metabolic routes of de-ethoxylation and conjugation (with sugars) proposed by Stolzenberg and co-workers from a study of the breakdown of OP surfactants mainly in excised leaves and tissue cultures of various plants (Stolzenberg et al. 1982, 1984; Stolzenberg & Garg 1984). Soluble metabolites within the treated leaf tissue may have biological effects different from those of the parent surfactant and they could have important implications for phytotoxicity and transport of pesticidal active ingredients.

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CONTROLLED FOLIAR PENETRATION WITH SELECTED SOLVENTS OR SURFACTANTS

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ABSTRACT

The potential of a rapid screening technique to allow the selection of solvents or surfactants with certain plant wax solubilization capabilities is reported.

Leaves of Convolvulus arvensis, Ambrosia artemisiifolia, and Asclepias syriaca were stripped of epicuticular wax. Solvents were then evaluated in contact with each wax, and rated with the ratings of solubilizing capabilities to be used to predictably increase or decrease foliar penetration. Similarly, several surfactants were rated on the waxes of Cyperus rotundus and Abutilon theophrasti.

The solvent ratings were used to choose five solvents to study the relative penetration into the abovementioned weeds. Solvents selected were: Aromatic 150 and amyl acetate ("good"), DMSO ("intermediate"), and methanol and water ("poor"). The penetration of imazapyr (carbon-14 radiolabeled herbicide) was used as the indicator on plants mentioned earlier.

Results indicate that the solvents performed relatively as predicted although amyl acetate sometimes performed worse, and DMSO sometimes better than expected.

In the surfactant portion of the study, the proposed rating system was used to choose ten surfactants to test against two weeds. Five surfactants predicted to give more penetration and five predicted to give less penetration on those weeds were selected. Then, each of the ten surfactants was sprayed with imazapyr onto C. rotundus, and with imazaquin onto A. theophrasti. The reduction in shoot fresh weights was measured and ranked. The correlation between wax rating numbers and relative herbicidal activity enhancement is reasonable.

These results support the use of the proposed technique as a rapid preliminary screen for selecting solvents or surfactants to obtain desired penetrating properties.

3C—6

INTRODUCTION

Plant cuticle waxes are a penetration barrier to foliar applied active ingredients (a.i.). The ability to briefly solubilize these foliar waxes may have an effect on the ability of an a.i. to penetrate that initial barrier. Thus, the potential of a rapid, preliminary screening technique for the selection of solvents or surfactants in relation to foliar plant wax solubilization is being examined. The technique involves the visual examination and rating of the solubilizing capabilities of solvents or surfactants in contact with waxes taken from various plant leaves. It is proposed that these ratings be used to select solvents or surfactants to predictably influence foliar penetration of an a.i.. If found useful, the technique has potential for lowering the amount of a.i. necessary to achieve a desired result, for achieving more consistent product results, or perhaps even for increasing selectivity of an a.i..

MATERIALS AND METHODS

Solvents

Mature plant leaves were stripped of epicuticular waxes by a ten second chloroform dip (Martin and Juniper, 1970). Each plant wax was spread on a glass plate as a stripe and after the chloroform was evaporated, a drop of neat solvent was pipetted onto the wax, and a visual observation made. Surfactants were tested similarly, but as 0.25% aqueous solutions. Based on the visual observation, a rating number was assigned according to the solvent's or surfactant's ability to solubilize the particular wax. The ratings assigned are as follows: 1 - does not appear to wet wax, but beads and rolls off; 2 - appears to wet wax, but then rolls off easily; 3 - appears to wet wax and partially solubilize wax; and 4 - appears to wet wax and solubilize wax. Wax rating results of solvents on C. arvensis, A. artemisiifolia, and A. syriaca were used to choose five solvents to attempt to influence the penetration of imazaquin (Orwick et al., 1983) into these weeds. Aromatic 150 (Exxon) and amyl acetate were selected as "good", while dimethylsulfoxide (DMSO) was selected as "intermediate", and methanol and water as "poor" for increasing penetration of the a.i. into these weeds. The solvents were tested at a final dilution in water of 1:30, with carbon-14 radiolabeled imazapyr, using 0.25% Igepal DM-710 (GAF Corp.) in the final dilution. The amyl acetate and Aromatic 150 solutions also contained N-methyl-2-pyrrolidone (m-pyrol), 2:1; solvent:m-pyrol, in order to get the a.i. into solution. The m-pyrol is poor at solubilizing two of the three waxes in the earlier ratings, but this approach was considered to be better than the potentially confounding use of different emulsifiers.

Each test solution was applied foliarly with a cotton-tipped applicator to a leaf. At sampling, duplicate treated leaves were taken, rinsed with methanol, and combusted in a TRI-CARB Model 306 oxidizer (Packard Instrument Co., Downers Grove, IL). The methanol rinses were sampled and counted directly in AQUASOL-2 liquid scintillation fluid (New England Nuclear Co., Boston, MA).

Surfactants

Ten surfactants were chosen, five with wax ratings of 3 or 4 on C. rotundus and A. theophrasti, and five with wax ratings of 1 or 2 on those same weeds. The surfactants with a 3 or 4 rating are: Tergitol 15-S-9 and Tergitol TMN-6 (Union Carbide), Makon 8 (Stepan), Sterox AJ (Monsanto), and Triton N-101 (Rohm and Haas). The surfactants with a 1 or 2 rating are: Aerosol A-103 (American Cyanamid), Ethomeen S/25 (Armak), Igepal DM-710 and Igepal DM-730 (GAF), and Sterox DF (Monsanto). Each surfactant (at 0.25%) was then sprayed with imazapyr onto C. rotundus or with imazaquin (Lignowski, 1985; O'Neil et al., 1985) onto A. theophrasti. Shoot fresh weights were taken 22 days after treatment (in triplicate) and the data ranked based on the percent reduction in fresh weight for each surfactant. These rankings were then assigned to one of these groups: poor, fair, good or excellent, with excellent having the highest percent reduction.

RESULTS

Table 1 shows the results of the solvent experiment.

TABLE 1

Percent penetration of different solvent-containing treatment solutions measured by carbon-14 labeled imazapyr

Solvent	Percent Penetration*		
	C. arvensis	A. artemisiifolia	A. syriaca
		2 Hours	
Water	9	36	3
Methanol	21	24	2
DMSO	55	51	13
Amyl acetate	80	41	7
Aromatic 150	65	52	8
		4 Hours	
Water	35	34	22
Methanol	52	33	6
DMSO	80	56	20
Amyl acetate	83	50	19
Aromatic 150	76	58	27

*Data are the average of two replicates.

At two hours, for C. arvensis, the trend is as predicted. For A. artemisiifolia, there appears to be an inversion of predicted results for DMSO and amyl acetate, although the "intermediate" and "good" solvents clearly outperform the "poor" ones. For A. syriaca, the same is true, except that DMSO outperforms both amyl acetate and Aromatic 150 (DMSO has a wax rating of 3 on this weed). At four hours, C. arvensis data are still in good order, with DMSO performing better than expected. For A. artemisiifolia, the same is true, while for A. syriaca, water seems to perform better and amyl acetate a bit worse than expected. Thus, the trend is generally as predicted.

Even more penetration may have occurred with Aromatic 150 and amyl acetate if the a.i. had happened to have better inherent solubility in these solvents. The addition of m-pyrol, which is categorized as "poor" at penetrating two of the three waxes originally looked at, to these two treatment solutions may have lessened the penetrating capabilities of the two solutions. In addition, the comparatively rapid volatilization of amyl acetate may have hindered a better performance for this solvent. Overall, these results would encourage use of the proposed technique.

The results for surfactants, to date, may be seen in Table 2.

TABLE 2

Performance rankings of surfactants selected by wax ratings

Surfactant	Performance Rankings*	
	C. rotundus with imazapyr	A. theophrasti with imazaquin
Tergitol 15-S-9**	E	G
Tergitol TMN-6**	E	E
Makon 8**	G	F
Sterox AJ**	G	E
Triton N-101**	E	P
Aerosol A-103***	P	P
Ethomeen S/25***	P	G
Igepal DM-710***	E	P
Igepal DM-730***	G	F
Sterox DF***	P	G

*E = excellent, G = good, F = fair, P = poor; from rankings of shoot weights (see Materials and Methods section).
**These surfactants had a wax rating of 3 or 4, expect E or G here.
***These surfactants had a wax rating of 1 or 2, expect F or P here.

From Table 2, the five surfactants predicted to provide more penetration (those having wax ratings of 3 or 4 on these weeds), show good agreement, except for Makon 8 and Triton N-101 on A. theophrasti. For the five surfactants expected to provide less penetration (those having wax ratings of 1 or 2 on these weeds), there is somewhat less agreement, with Igepal DM-710 and Igepal DM-730 on C. rotundus, and Ethomeen S/25 and Sterox DF on A. theophrasti giving better than expected results. However, in this experiment, foliar penetration is not what is being directly measured and certainly other factors, such as translocation, could be influential. Nonetheless, those predicted to give better penetration did, in general, provide better activity in this experiment.

CONCLUSIONS

Overall, these data support the use of the proposed technique as a rapid, preliminary screen for selecting solvents or surfactants to obtain desired foliar penetration properties.

Many factors are considered when selecting a solvent or surfactant for use with active ingredient. We hope that this proposed technique will be used as one guideline by scientists who want to achieve more controlled penetration into plants.

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PROPERTIES OF OCTYLPHENOXY SURFACTANTS AND THEIR EFFECTS ON FOLIAR UPTAKE

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ABSTRACT

Octylphenol and its poly(oxyethylene) derivatives (2 g l^{-1}) increased the uptake of three chemicals (1 g l^{-1} in aqueous acetone) into maize leaves. Uptake of 2-deoxy-D-glucose (water solubility c. 50 g l^{-1}) was directly related to the water content of the surfactants at 80% r.h. Water content increased both with r.h. and with the log oxyethylene (EO) content of the surfactants. Uptake of atrazine (water solubility 40 mg l^{-1}) and of DDT (water solubility 17 ug l^{-1}) increased with the uptake of the surfactants, being inversely related to their hydrophile: lipophile balance (HLB). Phytotoxicity, as measured by ethylene production, was inversely related to surfactant EO content. Aqueous solubilities of atrazine, DDT and pentacosane were increased up to eight-fold by surfactants (20 g l^{-1}) and were maximal at intermediate HLB's. The solubility of pentacosane (max. 220 ug l^{-1}) suggested that the surfactants would not solubilise >0.1% of the epicuticular wax on maize leaf. No visible modifications to epicuticular wax structure were evident after 24 h exposure to any of the surfactants. The appearance of chemical deposits on the leaf surface varied markedly among the surfactants, with similar trends for all three chemicals and without visible evidence for infiltration of the stomatal pores.

INTRODUCTION

Surfactants are almost ubiquitous in formulations for foliar applications of pesticides, being incorporated for a wide range of purposes (Seaman 1982). The effects of surfactants are commonly the result of specific interactions with both the a.i. and the leaf surface (Stevens 1984). The increases in foliar absorption generally observed on addition of surfactant have been variously attributed to enhanced wetting of the leaf surface (Sands & Bachelard 1973), penetration of the epicuticular wax (Cantliffe & Wilcox 1972) and its disruption/solubilisation (Takeno & Foy 1974, Bukovac *et al* 1983). Enhanced uptake may also be related to the hygroscopic nature of surfactants (McCann & Whitehouse 1983) and their ability to solubilise the a.i. (Temple & Hilton 1963).

Empirical data on the effects of surfactants on foliar uptake are numerous but correlations with the chemistry and properties of surfactants are limited (Jansen 1964, Smith *et al* 1966, Bland & Brian 1975). Those studies have been restricted to water soluble a.i.'s with few exceptions (McCann 1982).

This paper reports the relationships between the chemistry of an homologous series of nonionic surfactants and their properties and effects on foliar uptake. Three chemicals with water solubilities in the ug, mg and g l^{-1} ranges were employed as model a.i.'s, with the economically important species maize as a test plant.

MATERIALS AND METHODS

Plants

Maize (Zea mays, B73 x M017 hybrid) was grown in a 1:1 mixture of sterilized loam soil and Baccto planting mixture (Michigan Peat Co.). Growth conditions were 25°/20°C (day/night) with r.h. averaging 65%/80% and with a photosynthetic photon flux (400-700 nm) of 350-400 μmoles s^{-1} m^{-2} supplied for 16 h daily. Uptake studies were conducted with the third leaf at full expansion, 15 d after planting (10-11 d post-em). This coincided with emergence of the fifth leaf.

Chemicals and formulation

The chemicals employed were 2-deoxy-D-glucose (2D-glucose), atrazine and o,p'-DDT. The surfactants were 4-(tert)-octylphenol (OP) and its poly(oxyethylene) derivatives OP+5EO, +9.5EO, +12.5EO, +16EO, +30EO and +40EO (Fig. 1) of the Triton X series (Rohm & Haas). Chemicals (1 g l^{-1}; equivalent to c. 250 g ha^{-1} at medium volume) and surfactants (2 g l^{-1}; above aqueous critical micelle concentration (CMC) in all cases) were dissolved in acetone+water (3+2 by vol).

OP+nEO

Fig. 1. Generalized structure of Triton X series of surfactants

Foliar uptake determinations

^{14}C-chemicals were applied (10 x 0.24 μl drops) alone and with "cold" surfactants to the adaxial surface in the median portion of the leaf to either side of the midrib. ^{14}C-surfactants were similarly applied (20 x 1 μl drops) with "cold" atrazine or DDT. Applications were made at the midpoint (± 30 min.) of the photoperiod and the plants returned to the growth cabinet for the treatment period (24 h). The treated leaf was then excised and the surface of the treated portion was washed with aqueous acetone (1 ml) to remove residual chemical deposits (>98% recovery of ^{14}C immediately after drop drying). The leaf tissue was oxidized and the ^{14}C in the leaf and in the wash were quantified by liquid scintillation counting. Uptake was calculated as ^{14}C(leaf/leaf+wash) x 100.

Hygroscopicity of surfactants

Approximately 100 mg of each surfactant was weighed (± 0.1 mg) into 30 preweighed plastic petri dishes. Ten replicate samples were main-

tained at each of three r.h.'s by stacking them in sealed containers at 20°C over distilled water (100% r.h.), saturated aqueous $ZnSO_4.7H_2O$ (90% r.h.) and saturated aqueous NH_4Cl (80% r.h.); (Weast 1970). Samples were removed and reweighed at intervals until equilibrium was achieved and water contents were calculated on a % wt basis.

Solubility in aqueous surfactant

^{14}C-DDT and ^{14}C-atrazine in acetone solution and ^{14}C-pentacosane in chloroform were evaporated to dryness in polypropylene centrifuge tubes (15 ml). Aqueous surfactant solutions (20 g l^{-1}) (OP+5EO = emulsion) were added (10 ml) to 10 replicate tubes for each chemical. Water (10 ml) was added to additional tubes in which 0.2 g OP had been evaporated to dryness with the ^{14}C-chemicals. The tubes were shaken at 25°C and samples were removed periodically for radioassay. At equilibrium, the tubes were centrifuged at 1000g for 30 minutes, to sediment suspended particulate, and aliquots (1 ml) were sampled for ^{14}C quantitation.

Promotion of ethylene biosynthesis

Surfactants (2 g l^{-1}) in aqueous acetone were applied (12 x 5 µl drops) to 20 maize leaves (1st leaf, adaxial surface, seven days post-planting). Three h after application the leaves were excised at the base and two leaves were transferred to each of 10 replicate glass tubes (10 ml), with their cut ends immersed in water (0.5 ml). The tubes were capped and illuminated for 24 h at 25°C before removal of headspace samples (1 ml) and determination of ethylene content by gc on an alumina column.

RESULTS

See Table 1.

TABLE 2

Correlations between properties and effects of octylphenoxy surfactants

Least squares regression		R^2	p =
1) Ethylene (nl)	= 10.32 - 0.23(EO)	0.947	0.01
Solubility[a] (l^{-1})			
2) atrazine (mg)	= 36(HLB) - 1.5(HLB)^2 - 99	0.999	0.001
3) DDT (µg)	= 123(HLB) - 4.2(HLB)^2 - 751	0.964	0.01
4) pentacosane (µg)	= 36(HLB) - 1.7(HLB)^2 + 40	0.989	0.001
5) Water content[b] (% wt)	= LogEO[(3.86 x %r.h.) - 265] -[(0.9 x %r.h.) -41.7]	0.994	0.001
Uptake (%)			
6) 2D-glucose	= 2.77(% wt H_2O) - 0.83	0.972	0.01
7) atrazine	= -1.19(HLB) + 24.8	0.989	0.001
8) DDT	= -3.08(HLB) + 64.3	0.921	0.01

[a] excluding OP
[b] excluding OP+40EO at 80% r.h.

TABLE 1

Properties and effects of octylphenoxy surfactants

Parameter	Units	Surfactant							Control	LSD (p=0.05)
		OP	OP+5EO	OP+9.5EO	OP+12.5EO	OP+16EO	OP+30EO	OP+40EO		
HLB[a]		1.8	10.4	13.5	14.6	15.8	17.3	17.9	20	(NA)
Ethylene	nl	11.5	8.1	7.7		6.9		1.4	0.9	0.9
Solubility of:										
atrazine	mg l^{-1}	57	113	118		106		77	40	4.9
DDT	µg l^{-1}	17	72	136		131		115	17	4.2
pentacosane	µg l^{-1}	27	221	190		173		123	55	19
Water content at:										
100%		0	37	69		96	130	138	0	7.2
90% r.h.	% weight	0	16	37		55	85	99	0	8.4
80%		0	4.7	13		21	35	12	0	5.9
Uptake of:	%									
2D-glucose		1.0	4.6	37		57		34	1.9	15
atrazine		25	13	7.1		6.6		4.1	0.7	3.8
surfactant (+atrazine)				18	14	6.8		6.4		3.9
DDT		61	36	12		12		11	8.2	7.6
surfactant (+DDT)				21	19	14		10		6.3

[a] calculated as % wt $EO/_5$ (Griffin 1954) except OP: calculated as % wt $hydroxyl/_5$ and control: assigned value

(NA) not applicable

DISCUSSION

Phytotoxicity of surfactants

All the surfactants caused stress to treated leaf tissue, as evidenced by ethylene production (Table 1), although the effect of OP+40EO was not significantly different from the untreated control. Ethylene is apparently produced at a membraneous site (Yang & Hoffman 1984), and octylphenoxy surfactants of intermediate EO content caused maximum disruption of mitochondrial membranes (Egan et al 1976). However, ethylene production by treated maize leaves was significantly and inversely related to the EO content of the surfactants (Table 2, Equation 1). This relationship may indicate the innate toxicities of this series of surfactants but it might also result from differential cuticular penetration, since the foliar uptake of these surfactants is also inversely related to their EO content (Table 1, McCann 1982).

Solubility in aqueous surfactants

Aqueous solubilities of atrazine, pentacosane and DDT were increased in the presence of surfactant (20 g l^{-1}; simulating the final stages of drop drying) by, respectively, up to 3-, 4- and 8-fold. Maximum solubilities were observed at intermediate HLB values; pentacosane: c. 10, atrazine: 13, DDT: 14.5. Data for OP were excluded when fitting the quadratic equations (Table 2; Equations 2,3 & 4), since partitioning between the solution and the excess solid OP prevented direct comparisons with data for the other surfactants. Solubilities of atrazine, DDT and pentacosane in saturated aqueous OP were, relative to water, increased, unchanged and decreased, respectively.

The epicuticular wax of maize is predominantly composed of long chain (C_{27}-C_{33}) alkanes (10%) and primary alcohols (70%) (Stevens 1984) and is thus likely to have a similar solubility to that of pentacosane. Using the data for this compound, the area of the maize leaf surface wetted (not reported) and assuming a very low wax deposit of one $\mu g\ cm^{-2}$ on the leaf surface, it is calculated that none of the surfactants would solubilise >0.1% of the epicuticular wax underlying a drop deposit. This conclusion was supported by scanning electron microscopic observations: leaf surfaces were exposed to surfactants for 24 h, residual surfactant removed with an aqueous acetone wash and the area originally beneath the deposit examined. No modifications to the fine-structure of the wax were evident in comparison to untreated leaf surfaces.

The water solubility determined for atrazine (40 mg l^{-1}) compared favorably with a literature value of 30 mg l^{-1} (Worthing 1979) but the solubility of DDT (17 ug l^{-1}) was 14-fold higher than a previous report of 1.2 ug l^{-1} (Bowman et al 1960). These variations are of limited importance in this context, since similar trends in solubility existed between the surfactants for these three diverse compounds ranging in solubility over three orders of magnitude.

Hygroscopicity of surfactants and uptake of 2D-glucose

The equilibrium water content of the surfactants increased both with the log of their EO content and with increasing humidity (Table 1; Table 2, Equation 5), with a max. of 1.4 times its own wt of water for OP+40EO at 100% r.h. The only exception to this trend was observed for OP+40EO at 80% r.h.; its water content (12%) was lower than that of both OP+16EO and OP+30EO (21% & 35% respectively) and not significantly different

from that of OP+9.5EO (13%). At equilibrium the surfactants were viscous liquids in all cases, with the exception of OP+40EO at 80% r.h. which was a firm gel. The anomalous behavior of this surfactant was thus presumed to result from the formation of a stable liquid crystalline state under these conditions.

The foliar uptake of 2D-glucose (Table 1) was highly correlated with the water content of the surfactants at 80% r.h. (Table 2, Equation 6). This implied that the surfactants enhanced the uptake of 2D-glucose by maintaining the chemical in solution on the leaf surface. Although 2D-glucose is itself hygroscopic (equilibrating to be completely dissolved in >4 times its own wt of water at 100% r.h.), it retained <1% of its own wt of water at 80% r.h. This was in keeping with the low uptake (1.9%) observed for the control treatment. The low uptake of this chemical in the presence of OP (1.0%) indicates that phytotoxic adjuvants do not necessarily enhance uptake, as was the case for both atrazine and DDT with OP (Table 1). Note that the uptake of the highly water soluble (740 g l^{-1}) growth regulator chlormequat chloride was enhanced when its foliar deposits were rewetted (Hunt & Baker 1983).

Uptake of atrazine, DDT and surfactants

The uptake of both atrazine and DDT was inversely related to the EO content of the surfactants (Table 1), although the levels of uptake observed in the presence of OP+9.5EO, OP+16EO and OP+40EO were not significantly different for either chemical. Nonetheless, highly significant negative correlations with surfactant HLB existed for the uptake of both compounds (Table 2, Equations 7 & 8). The uptake of both atrazine and DDT was approximately paralleled by the uptake of the surfactants in the range OP+9.5 to +40EO, when the surfactants were applied with these chemicals (Table 1). The increase in the uptake of the surfactants as their EO content decreased is in accord with the findings of McCann (1982) and suggests that the enhanced uptake of atrazine and DDT may have been associated with co-penetration of the surfactants. How this effect was mediated remains to be established but it is notable that the same general responses were observed for these two differing chemicals with water solubilities ranging from mg to $\mu g\ l^{-1}$.

CONCLUSIONS

The octylphenoxy series of surfactants increased the uptake into maize leaf of chemicals with water solubilities ranging over six orders of magnitude. This was the result of two apparently distinct modes of action. The uptake of 2D-glucose was enhanced by the surfactants maintaining the chemical in solution on the leaf surface. To maximize this effect, surfactants with long EO chains would probably be the adjuvants of choice for similarly water soluble a.i.'s. There was evidence that the uptake of atrazine and DDT was related to the penetration of the surfactants. The mode of action involved remains unknown but the solubility of pentacosane suggests that it was not attributable to solubilisation of the leaf surface waxes by the surfactants. Short EO chain surfactants would thus be the adjuvants of choice to maximize uptake of these non-polar a.i.'s. The phytotoxicity of the short EO chain surfactants might be advantageous for herbicidal a.i.'s but surfactants with intermediate EO chain lengths may provide a preferable compromise, between minimal adjuvant phytotoxicity and maximal uptake, for non- or selectively herbicidal a.i.'s

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THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE METABOLIC FATE OF ^{14}C-FLUAZIFOP-BUTYL IN ELYMUS REPENS

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ABSTRACT

The metabolic fate of ^{14}C-fluazifop-butyl in rhizomatous E. repens (L.) Gould was studied in controlled environment rooms. ^{14}C-herbicide was applied to the foliage of plants for 48 h under contrasting conditions of temperature, soil moisture and light intensity. Components in the plant extracts were separated using single-dimension thin-layer chromatography. In all experiments metabolism was characterised by : 1) relatively high levels of radio-activity remaining in the tissue residues after extraction; 2) small amounts of metabolites with low Rf values (assumed to be polar conjugates); 3) variable amounts of radioactivity associated with compounds with higher Rf values than fluazifop acid (presumed to be lipoidal conjugates in tissues other than shoots); and 4) relatively high levels of ^{14}C-fluazifop acid (e.g. in the shoots, approximately twice as much of this acid than all of the other extractable metabolites, and in the rhizomes, four times more). The influence of environmental factors on herbicide metabolism was varied and depended upon the factor and the type of tissue. However, more of the phytotoxic fluazifop acid was found in the target rhizome tissues under warmer, brighter conditions and for plants not subjected to a soil moisture deficit.

INTRODUCTION

Environmental factors influence the susceptibility of plants to herbicides (Muzik, 1976). In many cases, these effects are due to changes in the amounts of herbicide retained, absorbed, or translocated to the sites of action. However, this altered susceptibility can be due, in part, to changes in the way the plant metabolises the herbicide. These latter effects may be qualitative and/or quantitative so that different metabolites and/or different amounts of metabolites are formed. Although the term 'herbicide metabolism' frequently is used to describe a type of detoxification or degradation process, changes in the chemical structure of the applied herbicide forming metabolites with increased phytotoxicity are known to occur (Hatzios & Penner 1982). Furthermore, in specific cases, this type of 'bioactivation' is necessary in order to produce the biologically active ingredient. Such 'bioactivation' is required for fluazifop-butyl since the des-butyl acid, formed through carboxylesterase activity, is thought to be the principal phytotoxic compound (Hendley et al. 1985).

This paper describes the metabolic fate of ^{14}C-fluazifop-butyl applied to rhizomatous E. repens under contrasting temperatures, soil moistures and light intensities; conditions that are known from field, glasshouse and controlled environment studies, to have significant effects on herbicide performance (Plowman et al. 1980; Kells et al. 1984; Coupland (in press)).

MATERIALS AND METHODS

Plant Material

Plants were grown from single-node rhizome fragments (Headington clone 31) in a sandy loam soil in 9 cm pots under glasshouse conditions (April and May 1984). Plants for the soil moisture experiment were watered differently to produce two groups of plants with 'low' or 'medium' soil moistures (approximately 50% and 100% field capacity respectively) as described by Coupland (in press). This took approximately 7 days. Plants for the temperature and light experiments were watered normally. Plants were chosen for uniformity of growth having 4 tillers and 1 or 2 rhizomes, and the main stems were jointing (growth stage 32, 24 (Zadoks 1974)).

TABLE 1

Environmental conditions

Variable		Light[a] intensity (Wm^{-2})	Temperature[b] (°C, day/night)	Humidity[c] (%rh, day/night)	Soil[d] moisture
Temperature	(High)	90	26/16	85/93	e
	(Medium)/	90	16/10	75/90	e
	(Low)	90	10/6	64/86	e
Soil moisture	(Moist)	90	16/10	80/94	100(22)
	(Dry)	90	16/10	80/94	50(11)
Light	(High)	128	16/10	80/94	e
	(Low)	13	16/10	80/94	e

/ 'Intermediate' conditions under which plants were kept or grown prior to and during treatment with ^{14}C-herbicide.

a Light intensities were constant throughout the experiments, and photoperiod was 14 h maintained by fluorescent lights supplemented with tungsten lamps (approximately 4% of total light output).

b Temperatures did not vary by more than 2°C. Relative humidities were adjusted in the temperature experiments so that approximately the same vapour-pressure deficit was obtained for each temperature regime.

c Relative humidities did not vary by more than 5% from their set points.

d Values are % of field capacity (g water/100 g dry soil in parenthesis).

e Soil moistures were not measured. Plants were watered daily.

Treatments and Conditions

Plants were conditioned in controlled environment rooms set at 16/10°C, 75/90% rh (day (90 Wm^{-2})/night) for 4 days prior to treatment. Plants were first sprayed with 0.4 kg ai/ha fluazifop-butyl in 0.1% w/v Agral solution using a laboratory pot sprayer fitted with a single Spraying Systems 8001 'T-jet' nozzle delivering 200 l/ha. They were then returned to the growth room (described above) and when the spray deposits had dried (within 10 mins) the plants were treated with the ^{14}C-herbicide. Fluazifop-butyl labelled in the carboxyl position (original specific activity 0.795 GBq/mmol, 50 µCi) was dissolved in methanol (1 ml) and a small quantity of this was mixed with a volume of unlabelled herbicide solution (equivalent in concentration and composition to the spray solution) to make a 'stock' solution having 200 Bq/µl (12,000 dpm/µl). This solution was used to treat all shoots, each one receiving 10 µl dispensed in between the leaf sheath

and the rest of the 'stem' of the youngest, fully-expanded leaf (50 µl/plant, approx. 10^4 Bq). This method of application is known to effect rapid and efficient absorption of other herbicides (Coupland et al. 1978; Coupland 1983).

After application of ^{14}C-herbicide, the plants were placed into their respective environments (Table 1).

Sampling Procedure

After two days, the shoots were cut off at soil level, put into plastic bags, sealed and stored at -18°C. The 'crowns' (a conglomeration of shoot and rhizome bases, often with rhizome initials), roots and rhizomes were removed from the soil, washed carefully and blotted dry, then separated and stored at -18°C until further analysis.

Samples were dried under vacuum in desiccators containing anhydrous silica gel. The gases from each desiccator passed through a series of traps designed to absorb any volatile herbicide and losses of ^{14}C as $^{14}CO_2$. The first two traps contained toluene (10 ml each) and a final trap contained a proprietary CO_2-absorbing scintillation liquid (15 ml). Vacuum was maintained for 4 days and the desiccators were kept at ambient temperatures. Aliquots from the toluene traps, and the contents of the final traps were taken for radioassay. After drying, the samples were weighed, then homogenised using a micro-hammer mill. Depending on sample size, either all or 200 mg aliquots of the powder were taken for extraction, all operations being carried out in a cold room at 2 to 4°C. For each sample, the material was suspended in 4 ml of solvent consisting of acetone containing 15% v/v water and 25 µg/ml fluazifop-butyl (technical material 95% pure, to aid desorption) centrifuged (10 min, 3000 x g), and the supernatant decanted off. This procedure was repeated a further 3 times. Separate experiments (data not presented) confirmed that no further radioactivity could be extracted from the tissue residues using aqueous acetone, pure acetone or water. For each sample the supernatants were combined and, apart from the shoot samples, were taken to dryness under a stream of compressed air at ambient temperatures. Shoot samples were reduced in volume to 5 ml. Sample residues were washed in pure acetone, filtered through cellulose papers, and after drying, the whole filter paper plus residue was burnt in a biological oxidiser and the $^{14}CO_2$ absorbed in a suitable scintillant for radioassay by liquid scintillation spectrometry.

Thin-layer Chromatography (TLC) Analysis

Dried extracts were re-dissolved in 150 µl of the extraction solvent, using ultrasonic agitation, and then applied as 20 x 5 mm bands to the pre-absorbent zones on silica gel TLC plates (Merck, number 11798). The same volume of the shoot extracts was used. The TLC plates were developed in a diethyl ether:hexane:glacial acetic acid solvent (12 : 8 : 1), then viewed under UV light to locate any UV - quenching bands. Radioactive areas were located by scanning (Berthold TLC Scanner), and these, together with the remainder of the silica gel constituted the following samples: the area of pre-absorbent to which the extracts were applied (Origin 1, no Rf); a 5 mm band of absorbent extending from the junction of the pre-absorbent material with the silica gel (Origin 2, Rf = 0) the area corresponding the fluazifop acid (indicated using a co-chromatographed standard (Acid, Rf = 0.35)); and finally the remainder of the silica gel; (Remainder, no Rf). These samples were scraped from the plates, added to 5 ml Picofluor scintillant (Packard Inst. Co.), disintegrated using ultrasound, and allowed to elute overnight before radioassay.

Statistical Design and Analysis

Plants were arranged as complete randomised blocks within the growth rooms. Where possible, two aliquots from each powdered sample were analysed separately. No significant differences were found between these aliquots, accordingly one set of data is presented. Amounts of each component of the TLC analyses were expressed as percentages of the total radioactivity recovered.

RESULTS

Vapour trap counts: No radioactivity was found in the CO_2 - traps for any of the samples, and none was found in the toluene traps for the crown, rhizome or root samples. However, a small amount, no more than 100 dpm (i.e. approximately 0.02% of the applied radioactivity), was found in the first toluene trap when the shoot samples were dried. As these were very small amounts, no further data are presented.

Metabolism Studies (Table 2)

General: There were relatively large amounts of ^{14}C retained in the tissue residues, the shoot samples containing the most (53%, averaged over the three experiments) with the other tissues containing an average of 19%.

Apart from the shoot residues, the majority of ^{14}C was recovered as fluazifop acid, identified by thin-layer co-chromatography. However, in some experiments, appreciable quantities of radioactivity, which chromatographed in the same region as the ester, were found in tissues distant from the application sites.

Only a few per cent of the extracted radioactivity was associated with the origin zones (assumed to be polar metabolites such as conjugates).

Effect of Temperature: There was a significant effect of temperature on the metabolism of ^{14}C-herbicide in the shoots and rhizomes, but not for crown or root tissues. In the shoots, an increase in temperature (above $16^{o}C$) caused an increase in the amount of ^{14}C recovered from the residue samples with a concomitant decrease in the amount of ^{14}C-acid. There was also a small increase in the amount of polar materials recovered from the origin zones. Almost the opposite effect was found in the rhizomes, with a decrease in the amount of polar metabolites and an increase in the acid fraction, with an increase in temperature. There was no effect of temperature on the proportion of radioactivity recovered in the rhizome residue samples.

Effect of Soil Moisture: The main effect was a decrease in the amount of ^{14}C-acid recovered from the rhizomes of plants growing under a soil moisture deficit. Associated with this was a rise of similar magnitude in the amount of ^{14}C in the residue samples.

Effect of Light Intensity: There was little influence of contrasting light intensities on herbicide metabolism in the shoot and crown tissues. With both roots and rhizomes however, significantly more ^{14}C-acid was present in the extracts of plant growing under the 'high light' conditions. For both of these tissues, these increases were parallelled by decreases of similar magnitude in the amounts of ^{14}C associated with the tissue residue samples.

DISCUSSION

Fluazifop-butyl belongs to a relatively new class of herbicides - the pyridinyloxyphenoxypropionates. In several respects, this group is similar

TABLE 2

Effects of environmental factors on the metabolic fate of ^{14}C-fluazifop-butyl in E. repens after 48 h.

Tissue	Environmental factor[a]	DPM (as % of recovered ^{14}C) Extractable ^{14}C Origin[b]	Acid	Remainder[c]	^{14}C in residue[d]
	Temperature				
SHOOTS	26/16	14	24	11	51
	16/10	7	46	13	34
	10/6	4	46	15	35
	LSD[e]	2.1	5.4	3.1	5.5
CROWNS	26/16	6	71	5	18
	16/10	5	78	4	13
	10/6	4	78	8	10
	LSD	1.0	4.9	1.8	4.4
ROOTS	26/16	6	67	10	17
	16/10	6	76	7	11
	10/6	6	67	14	13
	LSD	1.2	6.4	7.4	5.5
RHIZOMES	26/16	5	79	6	10
	16/10	7	73	8	12
	10/6	16	57	16	11
	LSD	5.5	10.6	4.4	3.2
	Soil Moisture				
SHOOTS	100	3	26	8	63
	50	3	22	9	66
	LSD	1.6	7.6	3.6	8.9
CROWNS	100	3	51	28	18
	50	3	54	20	23
	LSD	1.5	4.6	3.3	6.6
ROOTS	100	1	70	24	5
	50	1	78	15	6
	LSD	0.6	8.2	11.5	2.0
RHIZOMES	100	5	65	11	19
	50	4	44	14	38
	LSD	1.9	3.2	3.6	5.2
	Light				
SHOOTS	128	3	25	9	63
	13	3	26	10	61
	LSD	1.4	7.3	4.9	11.4

Cont'd ...

TABLE 2 Cont'd.

Tissue	Environmental factor[a]		DPM (as % of recovered ^{14}C) Extractable ^{14}C Origin[b]	Acid	Remainder[c]	^{14}C in residue[d]
CROWNS	128		6	56	11	27
	13		2	64	7	27
		LSD	0.4	7.7	3.8	6.7
ROOTS	128		2	57	23	18
	13		3	36	30	31
		LSD	1.1	11.1	12.2	7.2
RHIZOMES	128		3	61	6	30
	13		6	46	8	40
		LSD	2.3	5.0	2.6	5.7

a = Units temperature (^{o}C, day/night); soil moisture (approximate % of field capacity of the soil used); light intensity (Wm^{-2} during the photoperiod).
b = Non-migratory material on pre-absorbent band plus that at origin of silica gel.
c = Radioactivity in parts of the silica gel after removal of 'Origin', and 'Acid' regions.
d = Tissue residue after extraction.
e = LSD at P = 0.05.

to the previously-discovered phenoxyphenoxypropionates (e.g. diclofop-methyl). Both are esters, effective against grass weeds, and are predominantly foliage-acting, although both have some soil activity (Roberts 1982). In turn, these two groups have characteristics in common with the more simple esters of the halogenated alkanoic acids (e.g. chlorfenprop-methyl) and the esters of the N-arylalanines (e.g. benzoylprop-ethyl). All of the above herbicides rely to a large extent (entirely with some) on their de-esterification to the parent organic acid to form the biologically-active compound. Furthermore, with some (e.g. benzoylprop-ethyl) this structural change is necessary before the herbicide is effectively translocated to the meristematic sites where these compounds are thought to exert their main effect (Jeffcoat & Harries 1973). Thus de-esterification is a key process in the mode of action of fluazifop-butyl (Hendley et al. 1985) and many other important herbicides.

In these studies there was considerable de-esterification to fluazifop acid. The majority of extractable ^{14}C was identified as this acid by thin-layer co-chromatography. Averaging the temperature, light, and soil moisture experiments, the following acid to 'other metabolites' ratios were obtained; shoots (1.9), crowns (4.0), roots (2.9), rhizomes (3.7). Another characteristic of these studies was the appreciable quantities of ^{14}C retained in the tissue residue samples. Shoot samples contained the most, 53% of the recovered radioactivity (averaged over the three experiments)

with the other tissues containing an average of 19%. There could be several reasons for this variability: a) the various tissues may have metabolised the herbicide differently and certain metabolites may not have been extracted; b) the drying procedure under vacuum at ambient (hence variable) temperatures could have produced different levels of binding; and c) there could have been differential binding of the herbicide (or metabolites) associated with the four types of tissue. The techniques used in these studies were unable to distinguish between these possibilities and further work is required to identify the nature of this bound radiochemical. However, Hendley et al. (1985) found similar binding of pyridinyloxyphenoxypropionate herbicides also in E. repens. Unfortunately, data for fluazifop-butyl was not presented but a similar compound (an ethoxyethyl ester with a chlorine substitution on the pyridine ring) and its corresponding acid were bound to tissue residues to 15% and 30% respectively. Treatment with HCl released the parent acid. Whitehouse (1981) had similar results using diclofop-methyl (a phenoxyphenoxy propionate herbicide) in wild oats. With this compound, up to 19% (of the applied ^{14}C) was bound to cell debris, but could be released with alkali treatment. It was concluded that this provided evidence for binding through esterification to structural plant components.

The radioactivity chromatographing in the same area of the TLC plates as the butyl ester standard was thought to be due to metabolites rather than the parent herbicide (Hendley et al, 1985). There is strong evidence to show that these metabolites are lipoidal conjugates, perhaps fatty acid triglycerides, having similar Rf values to the butyl ester in this type of TLC system (B. Cavell, pers. comm.). The single dimension TLC procedure using only one type of solvent system was unable to resolve the parent ester from these conjugates.

Individual effects of the three environmental factors were varied and depended upon the type of tissue. The contrasting effect of temperature on the levels of fluazifop acid in shoots and rhizomes may well reflect different types of metabolism associated with these two tissues. Unfortunately, this effect cannot be analysed further because the type of experimentation used in these studies does not enable the distinction to be made between different amounts of fluazifop acid in the rhizomes resulting from the rate of its metabolism (i.e. conjugation), and different amounts of acid being translocated to the rhizomes. Nevertheless, the reduction in the amount of fluazifop acid in this tissue with higher temperatures may help to explain the poorer performance of the herbicide when sprayed plants are kept under warm conditions throughout the post-spraying period (Coupland, in press).

Imposing a soil moisture deficit on treated plants had little effect on the overall pattern of metabolism. The most pronounced effect was a reduction in the amount of fluazifop acid recovered from the rhizomes. Again, this agrees with previous reports that a soil moisture deficit reduces herbicide efficacy (Ready & Wilkerson 1982; Coupland, in press).

Light intensity had slightly more of an effect on herbicide metabolism than did soil moisture, these effects being evident in the roots and rhizomes. Translocation of ^{14}C-metabolites again may be an important factor, since Kells et al. (1984) found more extensive transport of radioactivity out of treated leaves growing under bright compared to shady conditions.

In conclusion, the effects of environmental factors on the metabolic fate of fluazifop-butyl in E. repens were varied and depended upon the factor and the type of tissue. However, more of the phytotoxic fluazifop acid was found in the rhizomes, the main target tissue for this herbicide, under warmer, brighter conditions and for plants not subjected to a soil moisture deficit.

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EFFECT OF SIMULATED RAIN ON THE ACTIVITY, RETENTION, DISTRIBUTION, UPTAKE AND MOVEMENT OF PHENMEDIPHAM APPLIED TO VERONICA PERSICA

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ABSTRACT

In studies conducted in controlled environment rooms, control of Veronica persica increased with all post-spray rain treatments of between 0.25 and 6.0 mm, even when rainfall commenced only 5 minutes after spraying, and despite substantial quantities of the herbicide being removed from the plant. Rainfall redistributed some of the spray deposit from the leaves and cotyledons to the petioles and stem. Application of phenmedipham to these areas resulted in increased herbicide performance. Less ^{14}C-phenmedipham was absorbed by the stem than by the leaves, although translocation from the stem to other parts of the plant was far greater than from the leaves. It is suggested that the increased phytotoxicity of phenmedipham after rain is partly due to its redistribution, principally from the leaves to the petioles and stem where entry of herbicide leads to greater translocation throughout the plant.

INTRODUCTION

Rainfall soon after the application of many foliage-applied herbicides, especially those with little or no soil activity, is known to reduce their performance. This is due to removal of active ingredient from the foliage before a lethal dose can be absorbed by the plant (Bovey and Diaz-Colon 1969, Doran and Andersen 1975, Skuterud and Caseley 1980).

Phenmedipham is a post-emergence herbicide used to control many broad-leaved weed species in sugarbeet (Arndt & Kötter 1968, Detroux et al. 1967). It has no soil activity acting solely via the foliage and thus might be expected to be susceptible to removal by rainfall occurring soon after spraying.

In this study the formulation of phenmedipham used is claimed to be 'rainfast' one hour after application (FBC technical leaflet FEB/3/8335). The objectives of this investigation were to determine the effect of (a) amount of rain and its intensity and (b) the rainfree period required for effective control of Veronica persica after application of phenmedipham.

MATERIALS AND METHODS

Plant material and growing conditions

Veronica persica seeds were sown into trays containing sterilized Begbroke sandy loam and germinated in a controlled environment (C.E.) room where the day/night temperatures and humidity regimes were 16/10°C and 75/86% r.h. respectively. Maximum light intensity was 100 Wm^{-2}, using cool white fluorescent lamps supplemented with tungsten lamps, and the daylength was 14 h. Once the plants had reached the 'first true-leaves just emerging'

stage of growth they were transplanted, 4 per pot, into non-sterilized Yarnton sandy loam. Approximately one week later they were thinned to 3 plants per pot.

The plants were treated when the first true leaves were almost fully expanded and the second set of true leaves had emerged, but were only just expanding. Plants were assessed 21 days after treatment and their fresh weights recorded.

Statistical design

The number of replicates ranged from four to ten, as shown under individual tables. The experiments were arranged in a randomized block design and where appropriate, data were subjected to a standard analysis of variance. The plants remained in the C.E. room throughout the experiment.

Herbicide treatment

Phenmedipham formulated as an emulsifiable concentrate 'Betanal E' (114 g ai/l) was used throughout these experiments. Herbicide solutions of 1.14 kg ai/ha in 200 l were applied with a laboratory pot sprayer fitted with a single Spraying Systems 8001 'Tee-Jet' nozzle operating at 210 kPa pressure.

Application of phenmedipham solution to specific sites of Veronica persica were made using a Burkard automatic syringe. A concentration of 37.6 µg a.i. in 2 µl of solution, based on spray retention data, was applied as ten 0.2 µl drops to the following locations:

a) 'Leaves' - 5 drops spaced evenly on the adaxial surface of lamina of each of the first pair of true leaves and avoiding any major veins.
b) 'Cotyledons' - 5 drops spaced evenly over the adaxial surface of each of the cotyledons.
c) 'Petioles plus stem' - A 0.25 µl drop placed on the petioles of the cotyledons and the first true leaves, plus five 0.2 µl drops placed on that part of the stem between the petioles of the cotyledons and first true leaves.

For the 'soil only' treatments a concentration of 1.2 mg in 2 ml was applied as ten 200 µl drops evenly distributed over the soil surface avoiding contact with the plant shoots. This amount approximated to twice the total amount of herbicide retained by the foliage and soil.

Rain treatments

Rainfall of 0.25, 1.0, 2.5 and 6.0 mm were each applied with the rain simulator (Simmons 1984) in a period of 0.5 h, all at intervals of 5, 30, 60 and 120 minutes after spraying.

Spray retention and redistribution

After treatment, plants were returned to the C.E. room for 15 minutes before being placed under the rain simulator and subjected to 0.5 mm h^{-1} rainfall for either 3, 5, 15, 20, 30 or 40 minutes. The foliage was then divided into: 1) true leaves; 2) cotyledons; 3) petioles plus the stem. Herbicide was washed from these using either 2 ml (for petioles plus stem) or 5 ml (for leaves and the cotyledons) of methanol (50% v/v aqueous) and shaking for 15-20 seconds in plastic vials. The concentration of phenmedipham in the resultant solutions was determined by hplc (Byast et al. 1977).

Herbicide uptake

A 2.0 μCi sample of ^{14}C-labelled phenmedipham (specific activity 59.0 μCi mg^{-1}) was dissolved in a small volume (100 μl) of herbicide solution to produce an emulsion containing 0.02 μCi μl^{-1} and 30 μg a.i. μl^{-1}. Plants were treated with 1 μl of this emulsion using a hand-held 5 μl Hamilton syringe. A 1 μl drop was placed on either the adaxial surface of the lamina of one of the first pair of true leaves, or onto the stem just below the first pair of true leaves. After 6, 24, and 96 h the treated parts were carefully removed and the surface deposit removed by a sequence of 2 washes; first 5 ml of an emulsion of phenmedipham (2.5 g ai/l of distilled water), and second, 0.5 ml chloroform. Previous tests had shown that this sequence of washes would remove the maximum amount of label from the surface of the plants. The herbicide wash was considered to remove the maximum quantity of herbicide displaceable by the action of water, and the chloroform wash would remove that material remaining on the surface but not removable by water.

The chloroform washes were air dried and 4 ml of Picofluor scintillation cocktail added to each for radioassay. The herbicide washes were each made up to 10 ml with distilled water and 1 ml aliquots were added to 4 ml of Picofluor scintillation cocktail for liquid scintillation counting.

After washing, the treated parts of the plant were solubilized in 0.75 ml 'Soluene' (Packard Instrument Co.) decolourized with 0.2 ml of a saturated solution of benzoyl peroxide in toluene, then 5 ml Triton X-100 scintillation cocktail (acidified with HCl) was added for liquid scintillation counting.

The remaining plant material was divided into untreated leaf(ves) the new pair of leaves, cotyledons, side shoots, stem and the roots. These were solubilized, decolourized and assayed as described above.

RESULTS

Effect of time interval between spraying phenmedipham and the incidence of 'rain' (Table 1)

The phytotoxicity of phenmedipham against Veronica persica was significantly increased with all 'rain' treatments, compared to the 'no rain' treatment. The lower rainfalls of 0.25 and 1.0 mm were not as effective in reducing plant weights.

Effect of simulated rain on retention of phenmedipham (Table 2)

Rainfall of 0.25, 1.0, 2.5 and 6.0 mm was applied after the spray deposit had dried, approximately 15 minutes after spraying. The lowest amount of rain (0.25 mm) removed approximately 49% of the herbicide whereas the highest rainfall (6.0 mm) removed 96% of the herbicide (Table 2).

Redistribution of phenmedipham on the foliage of Veronica persica following 0.5 mmh^{-1} rainfall (Table 3)

Phenmedipham was readily washed from the leaves by the action of the 'rain' with approximately 27% of the original amount of herbicide being removed after only 5 minutes. The herbicide was also washed from the cotyledons, but at a much slower rate. Increased amounts of herbicide were recovered from the petioles and stems with increased duration of the rainfall up to 30 min, after which a slight decrease was observed.

TABLE 1

Effect of four amounts of rain applied at four intervals after spraying on the activity of 1.14 kg ai/ha phenmedipham against Veronica persica

Rain (mm)	Interval from spraying until the onset of rain (min) 5	30	60	120	
		(% of control fresh wt.)			
0	25.2	S.E. ± 5.39			
0.25	6.7	3.0	4.7	8.5	
1.0	3.3	2.4	3.0	1.4	
2.5	1.2	1.4	1.5	1.7	
6.0	1.4	1.9	3.7	1.4	S.E. ± 0.08
Control (g)	7.79				

Values are means of 5 replicates

TABLE 2.

Retention of phenmedipham (1.14 kg/200 l/ha) on foliage after 0, 0.25, 2.0 and 6.0 mm simulated rain

Rain (mm)	retention (µg/g dry wt.)	± S.E.	% removed
0	1137.1	193.2	0
0.25	581.7	32.4	48.8
1.0	553.9	138.3	51.3
2.5	99.3	18.1	91.3
6.0	45.4	14.1	96.0

Values are means of 5 replicates

TABLE 3

Redistribution of phenmedipham (1.14 kg/200 l/ha) on Veronica persica following 0.5 mm h^{-1} rainfall

Position	Duration (min) of 0.5 mm h^{-1} rainfall Total rain (mm) in parenthesis						
	0(0)	3(0.02)	5(0.04)	15(0.12)	20(0.17)	30(0.25)	40(0.33)
	µg of phenmedipham						
Laminae	72.3	61.0	52.4	56.5	42.3	43.7	32.8
Cotyledons	18.3	18.8	18.5	18.2	14.4	13.7	11.5
Petioles + stem	2.5	2.8	3.9	6.1	6.5	9.8	8.9
Total	93.1	82.6	74.8	80.8	63.2	67.2	53.2

Values are means of 5 replicates.

Effect of applying phenmedipham to specific areas of Veronica persica

Application of 37.6 µg of phenmedipham to the adaxial surface of the cotyledons had no effect on the plant fresh weight, whereas application to either the first true leaves or the petioles plus stem reduced plant fresh weight by approximately 38 and 91% respectively.

Effect of applying phenmedipham to the soil of pots containing Veronica persica

A slight, though insignificant (F. ratio, $P > 6\%$) reduction in plant fresh weight was observed after 14 days when 1.0 mm of rain was applied to pots containing soil treated with 1.2 mg a.i. phenmedipham. All other rain treatments had no effect on plant weight.

Uptake and movement of ^{14}C-phenmedipham (Table 4)

Uptake of ^{14}C-phenmedipham by the leaves of Veronica persica over the first six hours was greater than by the stems (23.5% of the total activity recovered compared to 5.9%). However, the subsequent rate of penetration into the leaves was far less than penetration into the stems, although the total uptake by the leaves was still greater than that by the stems after 96 hours.

Translocation of the herbicide out of treated leaves was almost negligible, although some radiolabel was detected in the opposite leaf and the roots. However, movement of ^{14}C from the stem was more extensive. The percentage of radiolabel taken up by the stem that was translocated was 18.6, 42.9 and 32.9 after 6, 24 and 96 h respectively. Leaf tissues contributed the strongest 'sinks' for the radiolabel with smaller amounts of ^{14}C being recovered from the cotyledons and roots.

TABLE 4
Effect of site of application on uptake and translocation of ^{14}C-phenmedipham

Sample	Site of application	Time (h) 0	6	24	96	S.E. ±
		Radioactivity in samples (% of total recovered)				
Leaf[a]	Leaf	99.9	76.4	79.2	72.2	4.03
stem[a]	stem	99.9	94.1	92.2	79.0	6.80
Leaf[b]	Leaf	0.1	23.4	19.8	27.4	3.12
stem[b]	stem	0.1	4.8	4.4	14.1	1.83
Untreated[b]	Leaf	-	0.0	0.7	0.1	0.25
mature leaves	stem	-	0.8	2.0	4.1	0.45
New pair[b]	Leaf	-	0.0	0.0	0.0	-
of leaves	stem	-	0.1	0.8	1.8	-
Cotyledons[b]	Leaf	-	0.0	0.0	0.0	-
	stem	-	0.1	0.2	0.7	-
Side[b]	Leaf	-	0.0	0.0	0.0	-
shoots	stem	-	-	-	-	-
stem[b]	Leaf	-	0.0	0.0	0.0	-
	stem	-	-	-	-	-
roots[b]	Leaf	-	0.1	0.2	0.2	-
	stem	-	0.1	0.3	0.3	-
% recovery	Leaf	-	81.4	96.6	100.7	-
	stem	-	107.5	103.7	83.5	-
Total absorbed	Leaf	0.1	23.5	20.7	27.7	3.1
	stem	0.1	5.9	7.7	21.0	1.6
Total trans-located out of treated area	Leaf	-	0.1	0.1	0.3	0.2
	stem	-	1.1	3.3	6.9	0.5

[a] Radioactivity on surface removed with dilute herbicide and chloroform.
[b] Total radioactivity within sample determined by digestion.

DISCUSSION

That all rain treatments caused a significant increase in the performance of phenmedipham against Veronica persica was unexpected, especially in view of the large amounts of herbicide washed from the plants and considering the lack of soil activity of this herbicide.

Rainfalls of 0.25 and 1.0 mm were generally not as effective in reducing plant fresh weights as rainfalls of 2.5 and 6.0 mm. This is contrary to previous reports for other herbicides (Behrens and Elakkad 1981, Nalewaja et al. 1975, Skuterud & Caseley 1980).

More phenmedipham entered the leaves within the first 6 h than from the stems. However, by 96 h there was little difference in total radiolabel absorbed between the two sites of application. Furthermore, accumulation of herbicide in plant parts remote from the treated area was much higher following application to the stems than the leaves at all times after treatment. This may account both for the greater rate of uptake by the stem after the first six hours, because the concentration gradient across the cuticle would be maintained due to the translocation of the herbicide away from the point of entry and for the increased activity after rainfall. When the leaves were treated, only that part of the leaf distal to the treated area became chlorotic suggesting that movement of the herbicide is primarily in the transpiration stream, an observation also made by Kassebeer (1969) for Sinapis alba. If this is so for Veronica persica it would explain why little radiolabel was detected outside of the treated leaf. Rain mediated redistribution of herbicide from the lamina to the ligule and inner leaf sheath, to regions from which greater amounts of active ingredients are taken up and translocated, resulted in increased phytotoxicity both for bentazone against Festuca pratensis (Skuterud & Caseley 1980) and for difenzoquat against Avena fatua (Caseley & Coupland 1980). In this study a rain intensity of 0.5 mm h^{-1}, redistributed some of the spray deposit from the leaves and cotyledons to the petioles and stem regions (Table 3) which were found to enhance the performance of phenmedipham.

Only light (0.5 mm) rainfall caused the increased phytotoxicity observed by Skuterud and Caseley (1980) and Caseley and Coupland (1980), whereas in this study the heavier rainfalls of 2.5 and 6.0 mm resulted in the best control. Wash-off from the leaf lamina of Veronica persica first gathers at the petiole/stem junction until sufficient volume accumulates to cause the droplets to run down the stem and eventually onto the soil. Greater amounts of rainfall may therefore be required to redistribute herbicide from the leaves to the stem of Veronica persica than to move herbicide to the ligule and inner sheaf regions of grasses, also, both bentazone and difenzoquat are far more water soluble than phenmedipham (500 mg/l and 765 g/l as opposed to 4.7 mg/l respectively). Both the petioles and stems of Veronica persica are particularly hairy which causes the water droplets to be trapped and prevents them being easily dislodged, making them difficult to remove by further washing. The initial wash-off solution from the leaves would probably contain the greatest amount of both active ingredient and surfactant from the spray deposit. The presence of surfactant may be sufficient to allow this dilute herbide solution to permeate between the hair cells and thus penetrate into the plant via the stem. Subsequent wash-off may contain too little surfactant to reduce the surface tension sufficiently and this solution may not be as effectively absorbed. Enhanced absorption of herbicide due to rewetting of the spray deposit has been shown due to this effect (Prasad et al. 1967, Coupland et al. 1978).

It is concluded that relatively high levels of rainfall enhance the performance of phenmedipham against Veronica persica due to more herbicide being redistributed to the stems and petioles than with lower amounts of rainfall. The presence of large numbers of trichomes may well contribute to greater retention of the ingredient facilitating enhanced absorption and translocation.

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ADSORPTION AND MOVEMENT IN SOILS OF CHLORSULFURON AND OTHER WEAK ACIDS

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ABSTRACT

The adsorption and movement on soil tlc plates of chlorsulfuron and five phenoxyacetic acids was measured using soils with pH in the range 4.0-7.5 taken from long term liming field experiments. Chlorsulfuron (pK_a~3.8) was moderately adsorbed at pH~4.0 but was weakly adsorbed and very mobile at higher pH. This indicated that it was largely dissociated at the higher pH's, the anion being only very weakly adsorbed. Adsorption of phenoxyacetic acids (pK_a~3.0) similarly decreased with increasing soil pH, and increased with their lipophilicity. The degree of adsorption of chlorsulfuron and 4-fluorophenoxy acetic acid was greater than that predicted from calculations using the pK_a value and equilibrium adsorption coefficients for the unionised molecule and the anion, the calculated values being displaced to pH values 1-2 units more acidic. Hence for weak acids, the degree of adsorption is greater and so the mobility and hence availability to organisms was less than was predicted from pK_a, adsorption constants and measured soil pH. It is concluded that there is a need to replace pH with a more satisfactory function to predict the effect of acidity on adsorption.

INTRODUCTION

The commercial introduction of sulphonylurea herbicides was a dramatic stride forward in the development of chemicals which are applied deliberately to the soil. Their very low application rates makes quantitative analysis of residues, even with modern physical methods, extremely difficult. Although very high biological activity makes quantitative analysis by bioassay possible, this approach is unsatisfactory because the biological response is only linear over a small range of concentrations and there is uncertainty about which chemical species is causing the response. The exceptional biological activity of sulphonylureas together with their moderately long persistence in soil (Thirunarayanan et al. 1985) and the paucity of accurate residue measurements makes it crucial that we have a thorough understanding of their behaviour in the environment. The acquisition of this knowledge is particularly urgent because increasing numbers of related chemicals with similarly high levels of activity are currently being developed.

Most studies on the influence of soil pH on adsorption have been made either by artificially adjusting the pH with strong acid or base or by comparing soils from different sites (Harvey et al. 1985). Both approaches are open to criticism. The present studies used field soil from adjacent plots which had over twenty years to equilibrate following application of different levels of lime. (Table 1).

MATERIALS AND METHODS

Soils

Top soil was taken from plots on long-term liming experiments started at Rothamsted and Woburn in 1961 and which had received similar fertiliser treatments. After air-drying the samples were sieved (2 mm). Table 1 shows the properties of these soils measured by the following methods. pH was determined by a glass electrode using a 1:2.5 suspension of soil in water or 0.01M $CaCl_2$ solution. Organic carbon was determined by dichromate oxidation (Kalembasa & Jenkinson 1973).

TABLE 1

Properties of soils

	Rothamsted (silty clay loam)				Woburn (sandy loam)			
	A	B	C	D	A	B	C	D
Organic carbon (%)	1.3	1.4	1.3	1.3	0.8	0.8	0.7	0.8
pH (0.01M $CaCl_2$)	4.0	4.5	6.1	7.1	4.2	4.9	6.2	6.9
pH (distilled water)	4.3	4.8	6.0	7.5	4.8	5.5	6.8	7.4

Chemicals

Chlorsulfuron [1-(2-chlorophenylsulphonyl)-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea] was supplied by E.I Du Pont de Nemours & Co. [^{14}C] Chlorosulfuron, labelled in the phenyl ring, was purified by tlc to a radiochemical purity of greater than 97% (specific activity 3.4 uCi $umol^{-1}$); unlabelled material was 95% pure. [^{14}C] Phenoxyacetic acids (Table 2), labelled in the carboxyl group, and [^{14}C] 4-chlorophenylurea, labelled in the carbonyl group, were synthesised by Dr. G.G. Briggs with radio chemical purities of at least 96% as assessed by tlc and specific activities in the order of 1 μCi $umol^{-1}$.

Chlorsulfuron adsorption isotherms

Soil (5.0 g) was weighed into a 30 ml Pyrex centrifuge tube, and a solution of chlorsulfuron in 0.01M $CaCl_2$ (5.0 ml containing 0.01 μCi chlorsulfuron in the range 0-80 ug ml^{-1}) was added. The tubes were gently shaken overnight and then centrifuged (1700 rev/min for 20 min). An aliquot of supernatant liquid (0.5 ml) was removed and added to scintillation fluid (2 ml, L.K.B. Rialuma), and the radioactivity measured by liquid scintillation counting. All adsorption measurements are the mean of three replicate experiments.

Adsorption of phenoxyacetic acids

Measurements were made as above except that no unlabelled chemical was added to the 0.01M $CaCl_2$ solution and equilibration was reduced to 1h to minimise the influence of degradation. The linear adsorption coeffient (K) was calculated from the concentration of chemical in soil divided by the solution concentration at equilibrium.

TABLE 2

Physical properties of chemicals

	$\log_{10} K_{ow}$ undissociated form [a]	pKa [b]	Mobility on Rothamsted soil tlc plates (Rf). A	B	C	D
Chlorsulfuron	1.54	3.8 [c]	0.70	0.89	0.97	0.97
4-Chlorophenylurea	1.80	-	0.47	0.53	0.49	0.51
Phenoxyacetic acids						
3-methyl-4 methylsulphonyl	0.54	2.97	0.94	0.97	0.95	0.98
4-fluoro	1.64	3.15	0.87	0.94	0.96	0.99
4-chloro	2.25	3.10	0.74	0.88	0.93	0.93
3,5-dichloro	2.90	2.95	0.64	0.82	0.88	0.84
4-(2,4-dichlorophenoxy)	4.51	3.14	0.09	0.22	0.37	0.38

a Calculated using data of Hansch & Leo (1979).
b Calculated using methods of Perrin et al. (1981).
c Zahnow (1982).

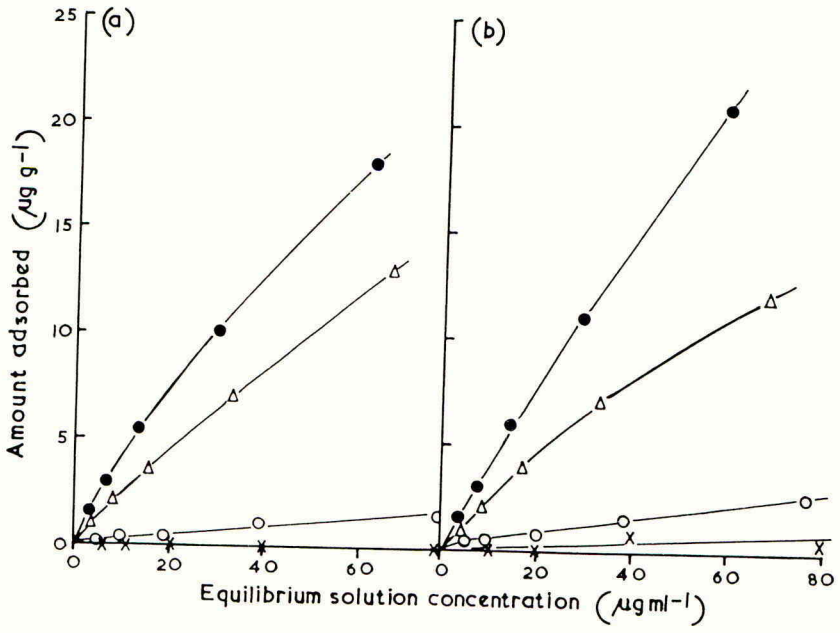

Fig. 1. Chlorsulfuron adsorption isotherms on two series of soils of varying pH: (a) Rothamsted silty clay, (b) Woburn sandy loam.
●—●, A; △—△, B; ○—○, C; ×—×, D.

Soil thin-layer chromatography

Rothamsted soils were ground in a mortar and pestle and passed through a sieve (0.5 mm). The soils were applied as a water slurry to glass plates (20 x 20 cm) to give a thickness of 1 mm. After drying, solutions of ^{14}C-labelled chemicals (c. 0.05 µCi) in acetone or chloroform were spotted and the plates developed for a distance of 12 cm in water. The plates were then dried, covered in Melinex film and the chemicals located by autoradiography with Kodak Omat S, X-ray film (Helling 1971).

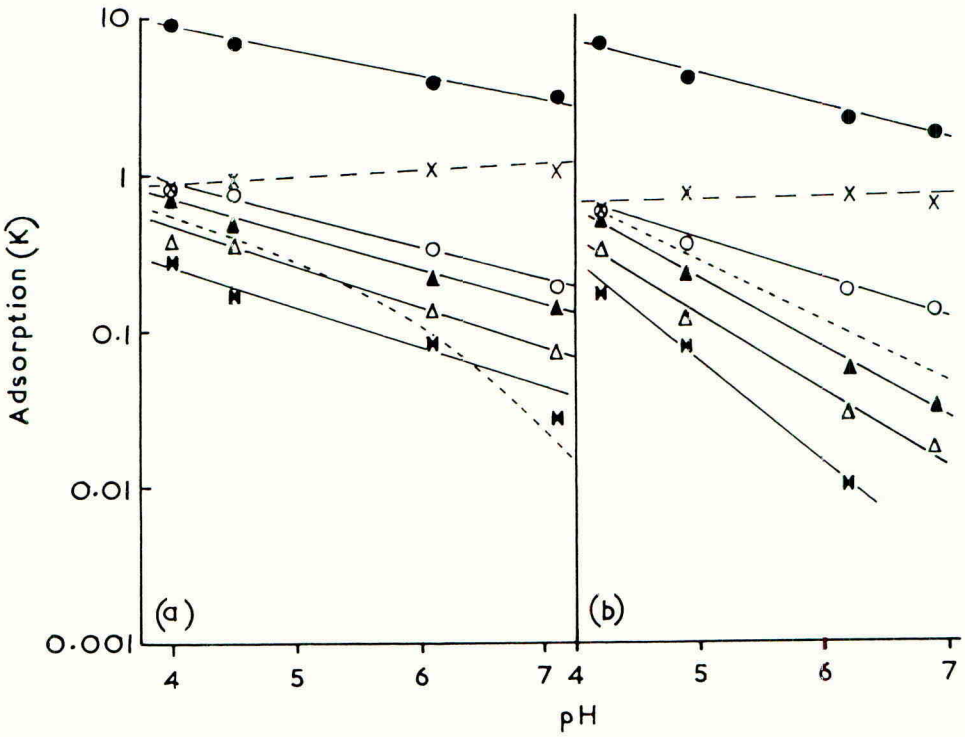

Fig. 2. Adsorption of phenoxyacetic acids and 4-chlorophenyl urea on soils of different pH: (a) Rothamsted silty clay, (b) Woburn sandy loam: Phenoxyacetic acids. —⋈— , 3-methyl-4-methyl sulphonyl; —△— , 4 fluoro; —▲— , 4-chloro; —○— 3, 5-dichloro; —●— 4-(2,4-dichloro phenoxy); – – X – – , 4 chlorophenyl urea; – – – – , chlorsulfuron.

RESULTS

Adsorption of chemicals by soil

There was little difference in chlorsulfuron adsorption between the two soil types despite the Rothamsted soil having more organic matter and generally a slightly lower pH than the Woburn soil (Fig. 1). However, in soils from both sites, adsorption decreased markedly as pH increased until there was almost no adsorption at c. 7.0. This is attributed to dissociation of the imino group adjacent to the sulphonyl group (pKa c.3.8) at higher pH. The balance of electric charge near soil surfaces is negative so anions are much more weakly absorbed than the unionised parent molecule. Also anions are more polar than the unionised molecules by 3-4 $\log_{10}K_{ow}$ units. The consequences of such weak adsorption are, a great potential for movement through soil (possibly with rapid movement below the root zone in the wet conditions of a UK winter), and hence, a high availability for uptake by plants (perhaps giving improved weed control under drier conditions which might occur in early autumn in the U.K.).

The pK_a's of the phenoxyacetic acids are similar (Table 2) and so the influence of increasing pH on adsorption is very similar, as shown by the slopes of the plots of K versus pH (Fig. 2). Adsorption decreased strongly with increasing soil pH, although once the pH was greater than 5.0 less than 2% of the acid in solution would be expected to be ionised. Adsorption increased with increasing lipophilicity ($\log_{10}K_{ow}$) of the unionised molecule. The most lipophilic compound, 4-(2,4 dichlorophenoxy) phenoxy acetic acid, was moderately strongly adsorbed even at pH 7.0, because it has a relatively lipophilic anion ($\log_{10}K_{ow}$ = 1.1).

Adsorption of the compound 4-chlorophenyl urea, which is unionised at all the soil pH's, was almost constant with pH which indicates that the adsorbing capacity of the soil organic matter was not changing with pH.

Soil thin-layer chromatographs

Mobility (Table 2) on the layers of soil followed a similar pattern to that of soil adsorption. Mobility decreased as the chemicals became more lipophilic and increased as soil pH rose, this latter effect being most marked for the most lipophilic chemical. Acids with $\log_{10}K_{ow}<$, 2, including chlorsulfuron, were very mobile and moved almost with the water front at the higher pH's. Chlorsulfuron was slightly less mobile than the comparably lipophilic 4-fluorophenoxyacetic acid at pH-4 because it is a weaker acid and would be less ionised at lower pH. The mobility of 4-chlorophenyl urea was almost constant irrespective of the soil pH, as would be expected from its adsorption being independent of soil pH.

DISCUSSION

In a two phase water-organic system containing a dissociated weak acid, the amount of acid associated with the organic phase is greater than predicted from pK_a alone because initially unionised acid in the aqueous phase preferentially partitions into the organic phase which causes formation of more unionised acid in the aqueous phase until both partition and dissociation equilibria are reached. For soil, this equilibrium can be calculated from the pK_a and the adsorption coefficients for the unionised acid and anion. Fig. 3 shows adsorption as a function of pH for chlorsulfuron and 4-fluorophenoxyacetic acid, which have similar lipophilicities. Calculated values are at 1-2 pH units more acidic than those measured. It has been suggested that pH close to the

soil surface is lower than in bulk solution (Goring & Hamaker 1972). Subsequent adsorption measurements made at high ionic strength (0.2M $CaCl_2$) were not greatly different from those measured in 0.01M $CaCl_2$. At high ionic strength the differences in pH at the surface and in bulk solution are diminished as the result of the suppression of the electrical double layer; so the differences between calculated and measured values cannot be attributed to the pH at the soil surface being greater than that in bulk solution.

The discrepancy might also be partly accounted for if the adsorption of the unionised acids was underestimated. Measurement of chlorsulfuron adsorption at pH <2.0 is not helpful because of hydrolysis and the formation of strongly adsorbed cations by protonation of the triazine ring. The adsorption coefficient of 4-fluorophenoxyacetic acid on Woburn A soil at pH 1.35, was 0.29, which is slightly less than the calculated value of 0.44 (Briggs 1981). Therefore the adsorption coefficient of the unionised acid was probably not grossly underestimated.

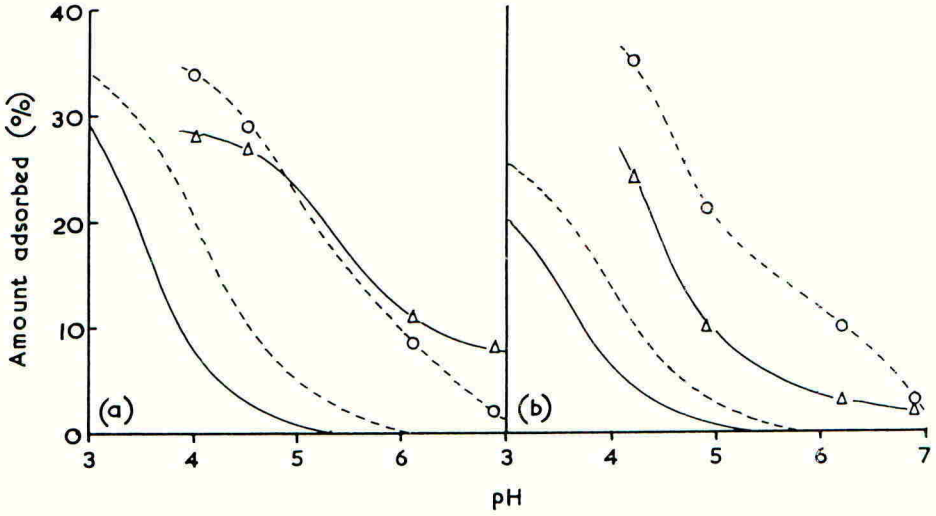

Fig. 3. Measured and calculated soil adsorption. (a) Rothamsted silty clay, (b) Woburn sandy loam; —○— chlorsulfuron measured, —△— 4-fluorophenoxyacetic acid measured, - - - - chlorsulfuron calculated, —— 4-f P.O.A.A. calculated.

The difficulty in accounting for this pH displacement may be because pH is a measure of proton concentration in solution in contact with the soil surface and not a direct measure of the number of protons on the surface. Further, as pH decreases protons displace organic and inorganic cations so changing the ionic environment at the surface. Hence there is a great necessity to replace pH with a more satisfactory function to predict the effect of acidity on adsorption (Hamaker & Goring 1972).

The important point is that adsorption of moderately lipophilic acids, whether nitrogen or carboxylic, is weak and their potential mobility very high when net movement of water is downward: e.g., for the U.K. in autumn and winter or during a wet spring.

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THE POTENTIAL OF ISOLATED CELLS FOR THE METABOLIC SCREENING OF HERBICIDES

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ABSTRACT

The metabolic activity of atrazine and ioxynil Na salt is investigated in metabolically competent mesophyll cells, isolated from Glycine max (L) Merr. cv Fiskeby V by a rapid mechanical procedure (Rees et al. 1985). Atrazine is shown to be a potent and specific inhibitor of photosynthetic electron flow with little, or no effect on other metabolic processes. Ioxynil Na inhibited electron flow, but was less potent than atrazine. Inhibition of electron flow was confirmed by a stimulation of relative fluorescence intensity, which occurred more rapidly with atrazine and revealed a rapid binding of this herbicide to the thylakoid membrane. Additionally, with ioxynil Na photophosphorylation and oxidative phosphorylation were uncoupled, ^{14}C-thymidine incorporation was stimulated and ^{14}C-leucine and uridine uptake were stimulated, revealing a possible membrane permeability action of ioxynil Na. A time-course study revealed that the stimulation of uridine uptake occurred during the first hour of treatment and that uridine incorporation progressively declined after 40 min, presumably due to reduced energy supply. These data suggest that the isolated cell system is a useful and valid system to screen the overall metabolic action of herbicides.

INTRODUCTION

All foliar applied herbicides come into contact with leaf mesophyll cells and will therefore exert some influence on their metabolism. Indeed, the primary site of action of most, if not all, contact herbicides is located in leaf mesophyll cells. Furthermore, many translocated, foliar applied herbicides have been shown to accumulate in treated leaves, thus exerting secondary metabolic effects (fluazifop-butyl, Carr et al. 1986, difenzoquat, Pallett 1982), whereas others rely on the metabolic activity of these cells for the long distance translocation of an active derivative such as ester to acid transformations (chlorfenprop-methyl, Fedtke & Schmidt 1977, benzoylprop-ethyl, Hill et al. 1978, diclofop-methyl, Shimabukuro et al. 1979). Consequently, the metabolism of leaf mesophyll cells is invariably a crucial aspect of herbicide efficacy. Metabolically competent isolated mesophyll cells would therefore be a useful and highly relevant system in which to investigate the metabolic activity of many herbicides.

It is essential for these types of investigations to use cells of maximum metabolic integrity and to ensure metabolism is relevant to a functional leaf. A system has been developed in this laboratory which allows the rapid isolation of metabolically competent mesophyll cells of Glycine max (Rees et al. 1985). This paper uses this isolated cell system to present data on two established herbicides, atrazine and ioxynil Na salt. Atrazine inhibits PSII electron transport in the chloroplast, whilst ioxynil, in addition to an inhibition of PSII electron transport, also uncouples oxidative and photo-phosphorylation (Moreland 1980, Fedtke 1982). This information has been obtained largely from isolated chloro-

plasts or mitochondria (Kerr & Wain 1964 a, b, Sanders & Pallett 1982 & 1985, Thompson et al. 1974) and no information is available from intact cell systems which could reveal other relevant metabolic effects that may contribute to a complete herbicidal action. Hence, the aim of this paper is to test an isolated cell system and so establish an overall action of two major herbicides on various metabolic processes, with a view of using this system for the metabolic screening of new compounds as potential herbicides.

MATERIALS AND METHODS

Mesophyll cells were isolated from the first trifoliate leaves of 27 day old G.max (L) Merr. cv Fiskeby V, grown under the conditions described by Rees et al. (1985). A rapid, mechanical isolation procedure was employed which produced high yields of intact and metabolically competent cells (Rees et al. 1985). Prior to all metabolic studies cells were maintained for 10 min in a photosynthetic assay medium containing 0.3 M sorbitol, 10 mM $MgCl_2$, 10 mM $MnCl_2$ and 10 mM MES-KOH buffer pH 7.6, at a photon flux density (PFD) of 100 µmol/m^2/s, photosynthetically active radiation (PAR) at 25°C.

Photosynthesis in isolated mesophyll cells was determined by measuring bicarbonate-dependent O_2 evolution in a Hansatech DW electrode. 3 x 10^6 cells/ml in the photosynthetic assay medium were incubated in a stirred reaction vessel and after the addition of 100 µl 100 mM $NaHCO_3$ were subjected to 500 µmol/m^2/s (PAR) at 25°C (Rees et al. 1985). A photosynthetic rate was obtained for 5 min prior to herbicide addition. Percentage inhibition due to herbicide was calculated when a linear rate was attained. Atrazine was diluted from a 1 mM stock solution made up in ethanol (final concentration in the reaction vessel 0.2% vol/vol) which was also included in the control. Ioxynil Na was made up in distilled water. The chlorophyll content of cells was determined by the method of Arnon (1949).

The effect of atrazine and ioxynil Na on relative fluorescence intensity (R.f.i.) was determined in a Turner model 112 filter fluorometer as described by Rees et al. (1985). 3 x 10^6 cells/ml were isolated and maintained as above and given a dark pre-incubation period for 3 min prior to addition of various herbicide concentrations. Respiration of cells was determined by monitoring O_2 uptake in a Hansatech DW electrode in darkness during the photosynthetic O_2 evolution assay.

Macromolecular synthesis was monitored using a modified method of Porter and Bartels (1977). 3 x 10^6 cells/ml were incubated with 0.5 µCi of the following radiolabelled precursors: ^{14}C-leucine (sp. act. 348 m Ci/mmol, 12.9 G.Bq/mmol); ^{14}C-uridine (sp. act. 450 mCi/mmol, 19.6 G.Bq/mmol); ^{14}C-thymidine (sp. act. 497 mCi/mmol, 18.4 G.Bq/mmol); ^{14}C-acetate (sp. act. 58 mCi/mmol, 2.14 G.Bq/mmol) or mevalonic acid (MVA) lactone (51.4 mCi/mmol, 1.9 G.Bq/mmol) and various concentrations of herbicides under the photosynthesis assay conditions.

After incubation times ranging from 10-120 min, 0.5 ml aliquots of cell suspension were taken and filtered under gentle vacuum onto 2.5 cm GF/C (Whatman) discs until just dry. Cells incubated with nucleic acid or protein precursors were washed three times with 2 ml 0.35 M sorbitol containing 1 mM unlabelled precursor. The discs were placed in a glass scintillation vials, treated with 200 µl 30% (vol/vol) hydrogen peroxide and incubated for 12 h at 30-40°C to bleach the samples prior to scintillation counting. A further 0.5 ml aliquot was filtered onto a 0.1 µm cellulose nitrate (Whatman) filter washed as previously described

but also washed three times with 1 ml of ice cold 10% (wt/vol) TCA followed by three washes with 1 ml 50% vol/vol ethanol. The filter discs were placed in glass scintillation vials prior to counting. Scintillation counting was carried out by adding 5 ml Fisofluor 2 (Fisons) scintillant and dpm in unwashed filters corresponded to precursor uptake and dpm remaining in the discs after washes with TCA corresponded to precursor incorporation into nucleic acids or proteins.

Aliquots (0.5 ml) of cells incubated with lipid precursors, after initial filtration onto GF/C discs, were washed twice with 2 ml of 0.35 ml sorbitol containing 1 mM unlabelled precursor. After 3 washes with 1 ml ice cold 10% (wt/vol) TCA, lipid was extracted by 3 washes with 1 ml ice cold chloroform:methanol, 1:1. The GF/C filters and chloroform/methanol washes were bleached with 200 µl 30% (vol/vol) hydrogen peroxide. The filters, chloroform/methanol washes and TCA washes were dried overnight prior to the addition of 5 ml Fisofluor scintillant for counting. Precursor uptake corresponded to dpm in filters plus chloroform/methanol and TCA washes, whereas precursor incorporation corresponded to dpm in chloroform/methanol washes only.

RESULTS

Fig. 1 illustrates the inhibition of bicarbonate-dependent O_2 evolution by intact G.max cells in the presence of atrazine or ioxynil Na. Atrazine is clearly a potent inhibitor of cell photosynthesis with an I_{50} value of 0.47 µM, which is similar to values obtained with isolated thylakoid studies (Thompson et al. 1974) on the other hand, ioxynil Na gave an I_{50} value of 1.9 µM with a shallower dose-response curve. Furthermore, this I_{50} value is > than that obtained with thylakoids (I_{50} ≈ 1 µM, Fedtke 1982, Sanders & Pallett 1985) which reflects other possible

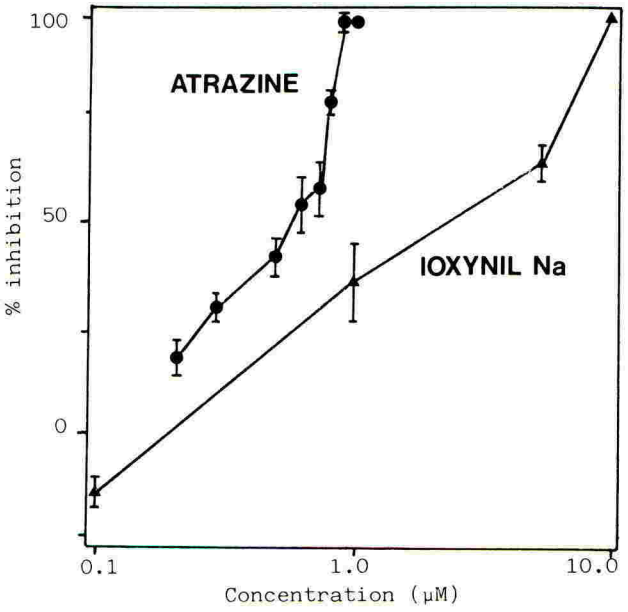

Fig. 1. The effect of atrazine and ioxynil Na on bicarbonate-dependent O_2 evolution in G.max cells. Bars represent S.E.'s from 8 replicates.

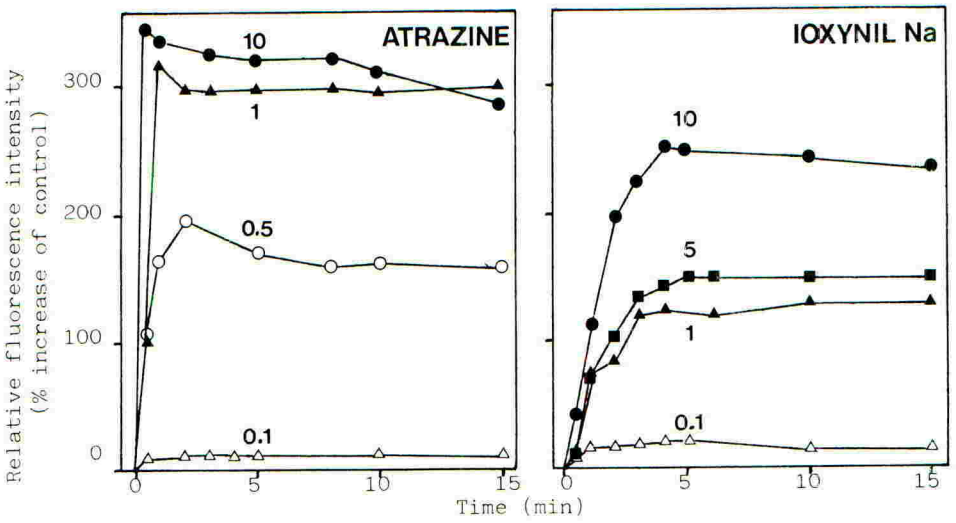

Fig. 2. The effect of atrazine and ioxynil Na on Relative fluroescence intensity (R.f.i.) in G.max cells. Data is expressed as % increase of control and was obtained over a 15 min incubation period. Numbers represent µm concentrations. Data was obtained from 2 experiments each containing 4 replicates. S.E. bars did not exceed symbol size.

sites of action within isolated cells. This observation is supported by a significant stimulation of bicarbonate-dependent O_2 evolution in the presence of 0.1 µM ioxynil Na, which probably resulted from an uncoupling of photosynthetic electron flow, and confirms the observations of others in the literature (Moreland 1980, Fedtke 1982, Sanders & Pallett 1985).

These findings are further supported by Fig. 2 which shows increases in cell R.f.i. values induced by herbicide treatment. In this experimental system the blockage of photosynthetic electron flow results in an increased fluorescence yield proportional to the degree of inhibition. Thus, in the presence of atrazine maximum R.f.i. values are rapidly observed (345% control in 0.5 min by 10 µM), whereas the response to ioxynil Na is slower and less marked (250% control in 4 min by 10 µM). These differences in response may indicate several factors of herbicide action within the cell, principally (a) speed of entry, (b) speed of distribution in the cell, (c) penetration of chloroplast envelope, (d) binding affinity to the thylakoid membranes (e) number of relevant binding sites and (f) the degree of coupling of electron flow to phosphorylation in situ. This data therefore indicates a specific action of atrazine at the thylakoid membrane, whereas ioxynil Na may interact with other metabolic processes, such as an implied uncoupling of mitochondrial electron flow, as evidenced by increased rates of O_2 uptake in darkness by 0.1 µM ioxynil Na (Table 1).

In Fig. 3 macromolecular synthesis is examined in the presence of atrazine or ioxynil Na, as determined by the uptake and incorporation of specific radiolabelled precursors into isolated cells over a 40 min period of study. Herbicide concentrations employed in this study were chosen to be within the range inactivating photosynthetic electron flow. Apart from a stimulation of uridine uptake at 0.1 µM concentration, and inhibition at higher concentrations, atrazine had only a marginal effect on precursor uptake and incorporation. On the other hand, ioxynil Na had a profound

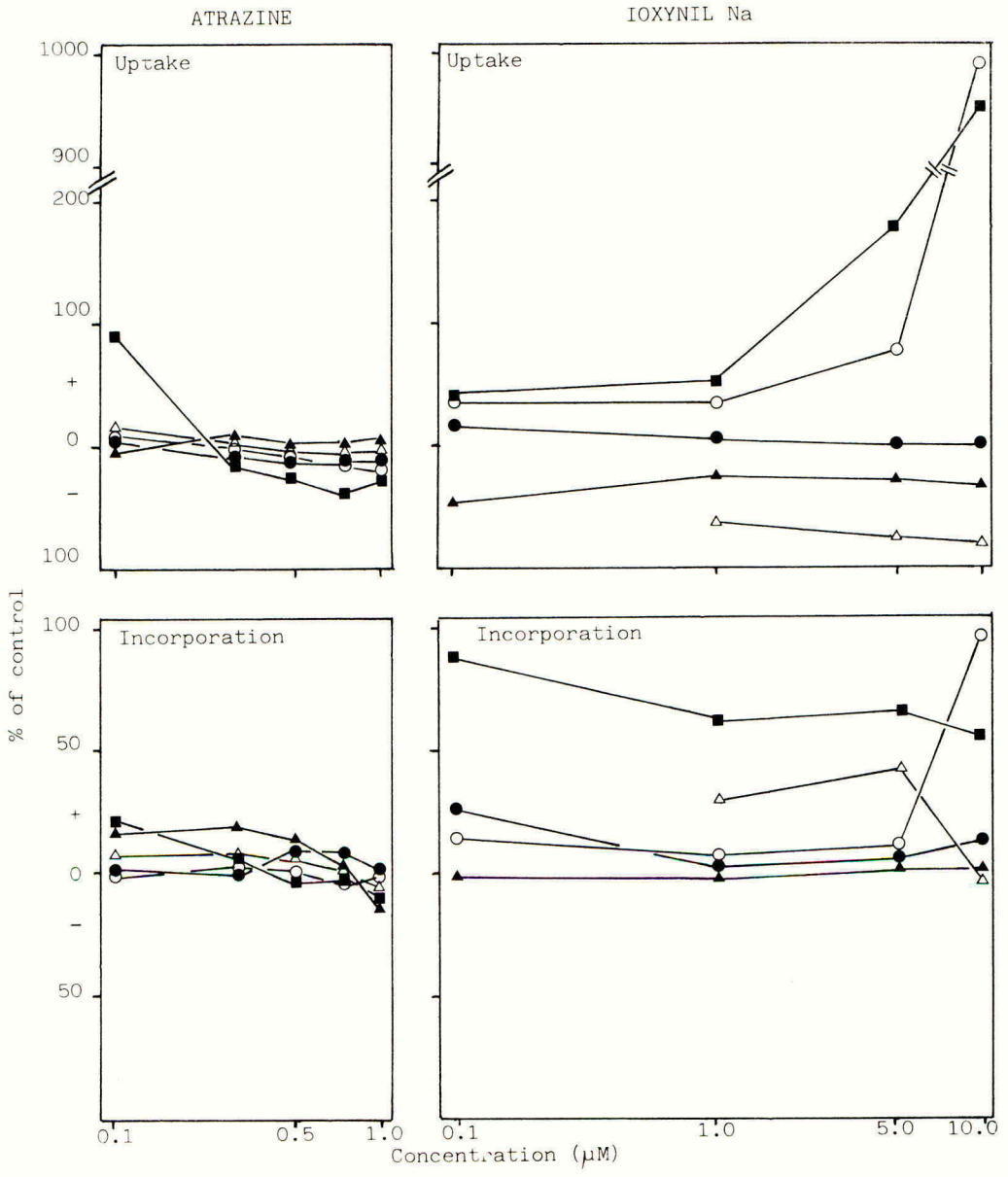

Fig. 3. The effect of atrazine and ioxynil Na on the uptake and incorporation of ^{14}C-acetate (●), leucine (O), MVA lactone (▲), thymidine (△) and uridine (■) in G.max cells expressed as % of control. Data was obtained from 2-4 experiments each containing 4 replicates and was subjected to multiple comparison t-test. In general, a difference of > 25% of control values for precursor suptake was significant at the 1% level, as was a difference of > 15% of control values for precursor incorporation.

TABLE 1

Effect of atrazine and ioxynil Na on O_2 uptake in darkness in mesophyll cells of G.max

Concentration	μmoles O_2 uptake/mg chl/h				
	0	0.1	1.0	5.0	10.0
Atrazine	19.0 ± 2.3	19.3 ± 0.1	19.2 ± 0.1	-	19.2 ± 0.1
Ioxynil Na	19.0 ± 2.3	21.6 ± 0.3	19.9 ± 0.1	18.5 ± 0.1	18.6 ± 0.1

effect on macromolecular synthesis in this system. ^{14}C-uridine and leucine uptake and incorporation by G.max cells were significantly enhanced at each concentration studied, acetate and MVA lactone metabolism were only marginally affected, whereas although thymidine uptake was drastically reduced, its incorporation was stimulated by 1 and 5 μM ioxynil Na. These observations clearly indicate a marked interaction of ioxynil Na with cell metabolism, whereas atrazine is far more specific in its action as a photosynthetic inhibitor.

Fig. 4 shows a time-course on ^{3}H-uridine uptake and incorporation in the presence and absence of 5 μM ioxynil Na. These data confirm the 40 min uptake and incorporation values presented in Fig. 3 but give a greater insight into the time-dependancy of this response. Thus, ioxynil Na initially stimulates uridine uptake during the first hour of cell incubation but no significant difference is evident between control and ioxynil Na

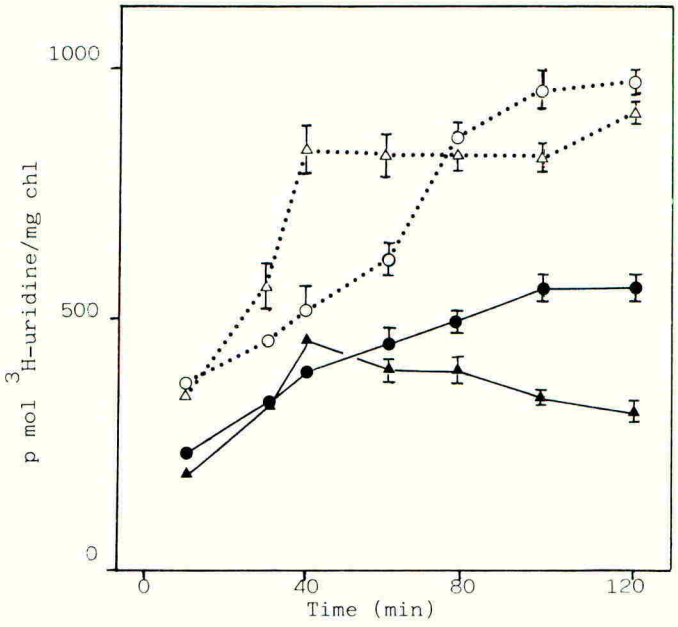

Fig. 4. A time-course of ^{3}H-uridine uptake (open symbols) and incorporation (closed symbols) in the presence (triangles) and absence (circles) of 5 μm ioxynil Na. Data was obtained from 2 experiments each containing 4 replicates. Bars representing S.E.'s are presented when larger than symbol size.

values after 2 h. Conversely, uridine incorporation into TCA-insoluble cell precipitates (presumably polyribonucleotides) is progressively inhibited by the presence of the herbicide.

DISCUSSION

This study has clearly demonstrated the value of isolated metabolically competent G.max cells for studies of herbicide action. These cells are relatively simple to prepare, highly intact and of high and reproducible metabolic integrity, since they can support linear bicarbonate dependent O_2 evolution rates of up to 140 µmol/mg chl/h for at least 2 h in vitro (Rees 1985).

The photosynthetic studies presented in Figs. 1 and 2 reveal that atrazine is a potent and specific inhibitor of electron flow, whereas although ioxynil Na similarly inhibits this process it takes longer and also uncouples electron flow. These responses were obtained with the novel use of a fluorometer which, in the opinion of the authors, is a technique of considerable promise. This is because R.f.i. values are both proportional to herbicide concentration and the efficiency of herbicide binding to thylakoids, which in itself reflects speed of movement to the thylakoid and also binding affinity. Consequently, the response obtained with atrazine suggests that this herbicide rapidly reaches its binding site to inhibit electron flow, whereas the relative delay in ioxynil Na response may enable this compound to interfere with other cellular processes.

Figs. 3 and 4 illustrate the effects of atrazine and ioxynil Na on precursor uptake and macromolecular syntheses within metabolically active G.max cells, and several important observations can be made from this data. Differences in precursor uptake relative to control values may indicate several possible actions of the herbicide at the cell membrane causing permeability changes, eg ^{14}C-leucine and uridine uptake in the presence of ioxynil Na (Fig. 3). Caution must be used in interpreting this data since uptake effects may be due to (a) pH dependency, molecular charge and lipophilicity (Rees 1985), (b) may reflect an interference with either active or passive membrane transport sites (Cobb et al. 1985) or (c) membrane damage.

Similarly, differences in precursor incorporation relative to control values require close scrutiny. Reduced uptake can lead to reduced incorporation (Rees 1985). However, Fig. 3 indicates that ^{14}C-thymidine incorporation was stimulated by 1 and 5 µM ioxynil Na even though its uptake was substantially reduced. This apparently conflicting observation may indicate a specific stimulation of incorporation by ioxynil Na and clearly suggests the need for further study. On the other hand, Fig. 4 demonstrates that although ^{3}H-uridine uptake is initially stimulated by 5 µM ioxynil Na, its incorporation is progressively reduced. This may be best interpreted as being due to a reduced energy supply for RNA synthesis as a result of the concurrent inhibition and uncoupling of electron flow in both chloroplasts and mitochondria. This observation clearly demonstrates the need for extended time-course studies in experiments of this type to distinguish between primary and other mechanisms of herbicide action. Consequently, these findings suggest that atrazine is a potent and specific inhibitor of photosynthetic electron flow, whereas the action of ioxynil Na is a composite of more obvious and subtle subcellular responses, with inhibition of electron flow as only one, albeit major response.

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MINIMISING CEREAL CROP LOSSES FROM SPRAYER WHEELINGS

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ABSTRACT

Cereal crop losses due to wheelings, as a result of the pre-harvest spraying of glyphosate, are determined for both standard tractor wheels (13.6 x 38) and row-crop wheels (8.3 x 44) in tramlined and untramlined crops. The development of wheel deflectors is described as is the effect these deflectors have in reducing crop losses in these situations. Spray tractors without deflectors fitted gave losses of 2-4% of crop yield. Deflectors reduced these losses by up to 78%. Pre-harvest spraying, wheelings, deflectors, glyphosate.

INTRODUCTION

Ever since tractor-mounted spray application of cereal crops began it has been apparent that driving through the crop between sowing and harvest causes uneven ripening of crop which in turn increases the moisture content of the harvested grain, reduces efficiency of threshing and separation by the combine harvester and reduces crop yield.

A more recent development has been pre-harvest spraying some two weeks before harvest for the control of Agropyron repens. This has been shown to be an extremely effective method of control (Richards and Sheppard, 1984) with other benefits such as improved combine harvester performance and reduced moisture content of grain at harvest (Sheppard et al 1984). However, a disadvantage this work highlighted was the significant grain losses from driving through barley crops so near to harvest. These losses were found to be in the region of 2-5% of crop yield.

Row-crop wheels reduce the area over which the crop is flattened, but having a much higher ground pressure than standard wheels, often depress the crop below ground level (particularly apparent in moist soil conditions). Hence less of the flattened crop is likely to be picked up by the combine harvester.

Tramlining the crop helps reduce the amount of flattened crop during early spray applications but when spraying 2 to 3 weeks before harvest the crop has often closed in over the tramlines and will still be flattened by spray tractor wheels. This is perhaps more likely with barley than with wheat.

In order to assess the extent of this problem it was decided to carry out detailed comparisons between standard and row-crop wheels when pre-harvest spraying barley and to construct deflectors for these wheels to see if losses could be reduced.

METHODS AND MATERIALS

Spraying Three sites were selected in 1983 for pre-harvest application of glyphosate. A further two sites were sprayed in 1984. Site and crop details are given in Table 1. One tramlined site was selected in each year as it was considered that the performance of deflectors might be enhanced under these conditions.

Unfortunately the deflectors were not constructed in time for spraying site 1 so this site was restricted to comparing row-crop wheels (8.3 x 44) with conventional tractor wheels (13.6 x 38). At the other five sites these comparisons were made with and without the use of deflectors. At each site the area of the field surrounding the trial was sprayed using deflectors as a way of endurance testing and finding faults in construction. Each treatment was replicated at least 3 times.

Harvest In 1983, crop samples of 50-70 m in length which included sprayer wheeltracks were harvested using a plot combine harvester with grain weighing attachment and a cut of 2.35 m. Adjacent to these strips, untracked crop was also harvested in the same manner. Area of cut was then measured and weights of grain recorded.

From this data it was hoped that the yield loss from driving through the crop with a 12 m boom width of sprayer pre-harvest could be ascertained.

In 1984 a different sampling method was used. Having sprayed the crops, the farmers were left to combine harvest the fields as they normally would after pre-harvest spraying. As soon as was practical after harvest, people who were not directly involved with the trial were asked to count how many heads were present in 1 m centres of wheeltrack, which were still clearly visible. 10 such samples were taken in each wheeltrack at 15 m intervals. To obtain the yield loss represented by this counting, samples of heads were taken from the wheeltracks, dried, and mean weights of grain/head recorded at 15% m.c. Yield samples were taken in the virgin crop at site 5 for comparison. At site 4 the farmer assessed his own yield.

From this data it was possible to determine what the actual

losses attributable to wheelings were, from driving through the crop before harvest.

Design and construction of deflectors The following design criteria for deflectors were determined:

1) Quick and easy to fit to the tractor.

2) Able to cope with crops of various heights and uneven ground.

3) Wide enough to clear wheels but not so wide that they flatten any crop irretrievably.

4) Front deflectors capable of pivoting with wheel.

5) Short enough to avoid sweeping down crop when turning at headlands.

6) Robust enough and so designed that they can part both standing and laid crops.

Front and rear deflectors were constructed in time for 3 sites to be sprayed in 1983 (sites 2 and 3 & 4). At site 2 which appeared relatively flat, the deflectors were set in the mid-height position of the three allowed for in the design as the crop was upright and the ground appeared reasonably flat. However, a mound was discovered which caused both front and rear deflectors on one side of the tractor to be run-over by the tractor wheels. Although this probably would not have occurred, had the driver been familiar with the field, it was considered desirable that this potential hazard be eliminated. A hinge point was therefore introduced on each deflector, to allow them to ride over obstructions that might exceed the change in ground contours allowed for when setting the height for the crop and ground conditions.

The final prototype used in the 1984 trials is shown in Fig. 1.

RESULTS

As shown in Table 2 the sampling method used in 1983 revealed very little as there were such large variations both within and between replications. Indeed yields from adjacent untracked plots varied by as much as 26%. All that can be stated is that visually there was far less crop flattened where deflectors were used than where they were not. This was particularly apparent at site 3, the tramlined crop where no barley at all appeared to be flattened by the row crop wheels with deflectors. Where conventional wheels with deflectors were used the barley was pushed sideways rather than flattened.

The sampling method employed in 1984 confirmed the visual

assessment of the 1983 trials (Fig. 2). Where deflectors were used in the tramlined crop at site 4 there were significant reductions in wheeling loses for both row-crop and conventional wheels of 65% ($P \leq 0.05$) and 57% ($P \leq 0.01$) respectively. Without deflectors row-crop wheels reduced losses by 38% over conventional wheels ($P \leq 0.01$). As might be expected the most significant improvement was found when row crop wheels with deflectors were compared with conventional wheels without; this gave a reduction in losses of 78% ($P < 0.001$).

In the untramlined crop at site 5, again there were significant reductions in wheeling losses for both row-crop and conventional wheels of 28% ($P \leq 0.05$) and 43% ($P \leq 0.001$) respectively. Without deflectors row-crop wheels reduced losses by 28% ($P < 0.01$) over standard wheels. Again the most significant reduction in losses, occurred when row-crop wheels with deflectors were compared with conventional wheels without deflectors giving a reduction in losses of 48% ($P \leq 0.05$).

DISCUSSION

1983 Trials The method of combine harvesting a 2.35 m strip of barley inclusive and exclusive of a spray tractor wheeltrack did not prove accurate enough in determining grain losses due to wheelings which were visually evident. This may have been due to the large variations in yields that were noted in the 1983 harvest as this system had given consistent and significant responses in previous years.

1984 Trials The method of counting heads remaining on the ground in 1 m lengths of spray tractor wheeltrack and relating this to overall crop yield gave results for standard and row crop wheels similar to those found in earlier work and representative of the area of crop flattened (determined by visual assessment).

Despite the irregularity of the tramlines at site 4 in comparison with those at site 3, it was shown that losses could be reduced to 0.8% of overall yield. In a well tramlined field using row-crop wheels with deflectors it is likely that losses could be reduced still further.

As was expected, the deflectors did not perform as well at site 5, the untramlined site, although it was shown that losses could still be reduced on average by 35%.

It is interesting to note that standard wheels with deflectors performed easily as well as row-crop wheels without deflectors in both tramlined and untramlined crops.

Comparing sites 4 and 5 for both standard and row-crop wheels it can be seen that tramlines in themselves do not substantially reduce losses from pre-harvest spraying.

As a result of this work, crop deflectors are now marketed for most types of tractor at a cost of £289.00 for a set of four. Taking into account the average reduction in losses and yields of barley at these sites, it can be seen that deflectors will pay for themselves in less than 30 hectares.

Further developments From these trials it became evident that there were various modifications that would improve the durability and marketability of these deflectors:

1) The hinge point on the front deflector should be moved, behind the centre of the front wheel.

2) A universal fixing bracket would need to be developed to suit all farm tractors, trailed sprayers and other spray vehicles.

3) The front shoe of the deflectors would perform better if made into a full circle rather than being curved upwards.

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Fig. 1

Final Prototype Crop Deflectors

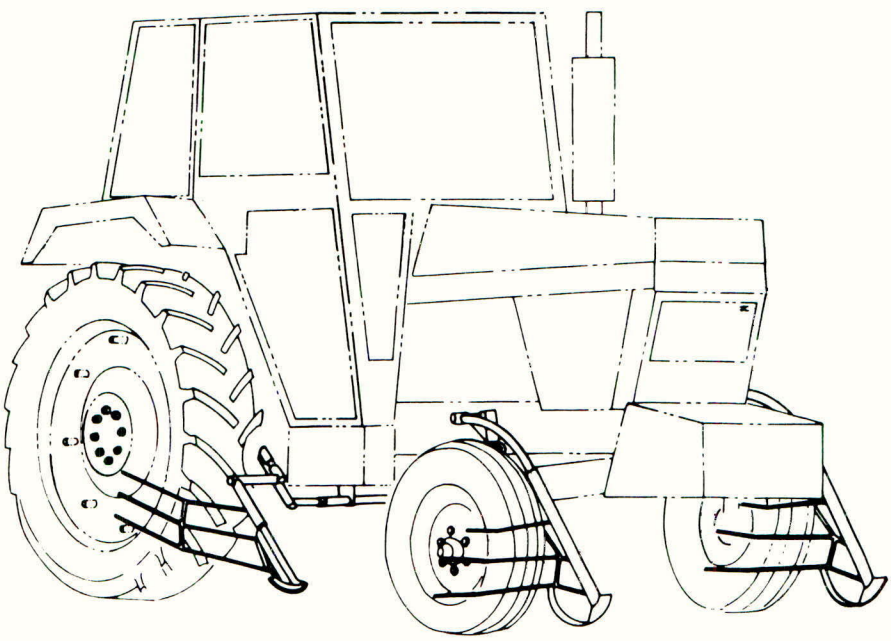
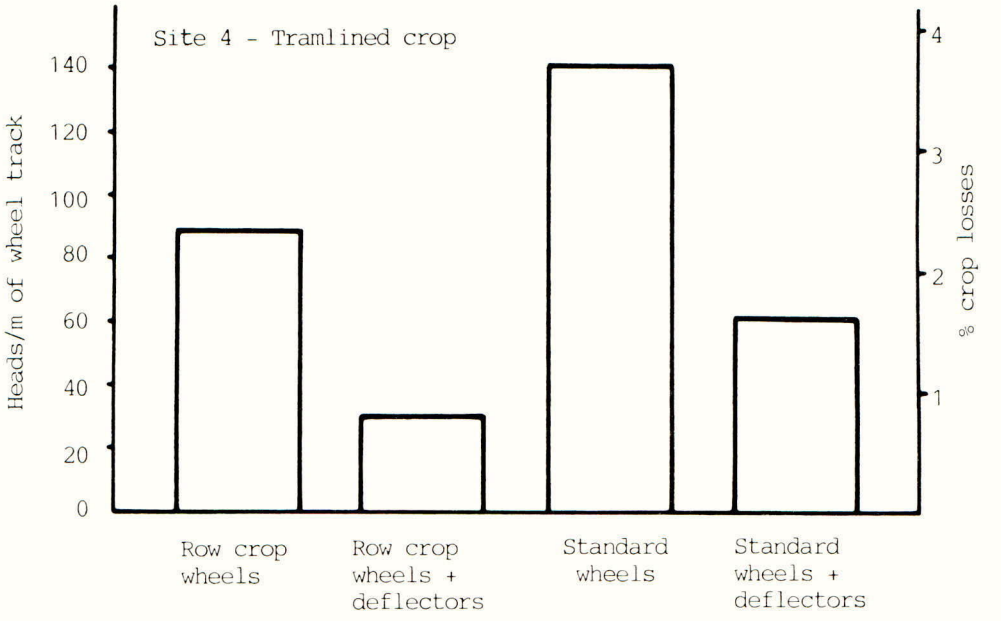
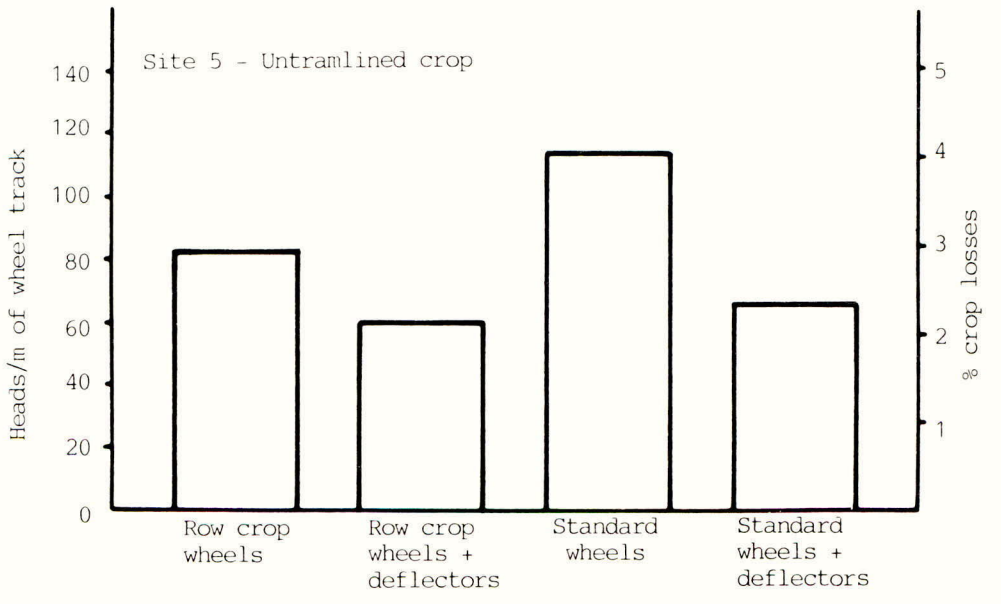


Fig. 2

Barley crop losses from pre-harvest spray wheel tracks





AERIAL PHOTOGRAPHY OF FIELD EXPERIMENTS USING A REMOTELY-PILOTED AIRCRAFT

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ABSTRACT

Low altitude aerial photography of field experiments can be done cheaply, conveniently and safely using a remotely-piloted aircraft. A lightweight radio-controlled model aircraft fitted with a gimbal-mounted 35 mm camera has been used to obtain very detailed vertical photographs of oilseed rape-weed competition experiments. In a simple evaluation exercise, photograph derived point sampling data were found to correlate well with plant biomass data from 1 m^2 samples such that the use of photographs alone would result in a saving of over 75% of the man-hours required for ground sampling. In addition, permanent and complete records of experiments would be obtained. Extension of the technique to include vegetation spectral analysis, and pest and disease monitoring, is discussed.

INTRODUCTION

Field experiment data is time consuming to collect and may be unrepresentative when plant growth is not uniform and only restricted sampling is possible. Aerial photography may be used to overcome these problems, and at the same time provide complete and permanent records of experiments. In particular, it can be used to detect spatial and temporal vegetation patterns, which are not apparent from ground level, and of which the experimenter should be aware. Additionally, it fulfils an important monitoring function when early warning of weed growth, disease or nutrient deficiency is required. However, conventional aerial photography from full-size aircraft has disadvantages when applied to field experimental work. Firstly, it is expensive and, depending on the source and nature of the equipment hired, is unlikely to cost less than £100 per hour and is more likely to cost £500-1000. This expenditure may be justified when an extensive area has to be covered on a one-off basis but not for repeated coverage of single fields or small plots. Secondly, flying time usually must be booked in advance, sometimes weeks or months, making this facility unavailable as and when required. Thirdly, flying regulations and safety considerations preclude low altitude (less than 200 m) flying and hence the large scale photography required.

The problems associated with conventional aerial photography can be overcome by using a remotely-piloted aircraft (RPA) equipped with a 35 mm camera. Such a user-owned system is very cheap to operate, costing little more than the price of film, available whenever required, can be flown slowly and safely at low altitudes, and can be flown by anyone after training. A growing awareness of the applications of aerial photographs

in research, the high cost of conventional aerial photography and the increasing availability of good quality, low priced, lightweight automatic 35 mm cameras have combined to stimulate developments in the use of RPA's in archaeology (Miller 1979), surveying (Tomlins 1983), pollution monitoring (Ellis & Varey 1978) and vegetation mapping (Jonsson et al. 1980). In this paper we describe a purpose built camera carrying model aircraft, its method of operation and results obtained, and discuss the potential of this method for monitoring field experiments.

MATERIALS AND METHODS

Aircraft

The following characteristics were considered essential in the design of a suitable aircraft: a) short take-off and landing, for operating in confined spaces, b) stable and slow flying, c) large enough to be visible at a height of about 300 m but less than 5 Kg total weight to avoid the need for a CAA Exemption Certificate and its associated flying restrictions, e) capable of carrying a camera payload of at least 1 Kg and f) low vibration and noise levels.

Initially a 'Hercules' kit model (Castle Models), marketed for this purpose, was constructed. The camera, a Konica FS 1 fitted with a Konica 40 mm lens, was mounted in a plywood box, lined with sponge rubber to absorb vibration, which could be slotted into the fuselage side without any dismantling of the aircraft. Although encouraging results were obtained with this system, a more advanced one was developed to overcome problems of high flying speed resulting from limited wing area, poor image quality at the wide apertures needed at low light levels, and non-vertical photographs resulting from aircraft roll.

The system currently in use consists of 'Cyclops', a purpose designed aircraft carrying a Contax 137MD 35 mm camera with electric film wind and fitted with a 50 mm f1.7 Zeiss Planar lens. The 2.1 m wingspan aircraft is constructed of balsa and plywood for lightness and covered with heatshrink nylon for added strength. Adjustable rigging wires on wings and tailplane prevent twisting and take some of the strain on these lightweight components. A rectangular cutout in the underside of the fuselage houses the camera. This is mounted on a removable lightweight alloy gimbal. The complete unit is separated from the fuselage by a thin layer of sponge rubber to absorb vibration and attached by rubber bands which also reduce vibration transmission and simplify removal. By effectively mounting the camera outside the fuselage, film loading is quick and does not involve aircraft dismantling. Power is provided by a 10 cc Webra 61F Champion two-stroke glow-plug engine, rigidly mounted, and run on a mixture of methanol and castor oil. Exhaust from the primary silencer is ducted through a length of silicone rubber tubing, a secondary silencer and a further length of tubing to a position rearward of the camera. This arrangement gives a very low noise level and prevents contamination of the camera by the exhaust. The control system consists of a 35 MHz Futaba FP-7FG digital radio providing proportional control of aileron, elevator, rudder and engine plus camera shutter operation. The total weight of this basic system is about 4.5 Kg.

The slow flying speed of this aircraft permits the use of slower shutter speeds or slower film because of reduced ground speeds, and the ailerons improve manoeuvrability. The gimbal compensates for aircraft

roll by keeping the camera horizontal and allows truly vertical photographs to be taken. If oblique pictures are required the camera position on the gimbal can be altered up to 90 degrees from the horizontal. Finally, the superior lens quality allows use of apertures up to f2.8 without significant loss of picture quality.

Photographic technique

The photographic technique can be divided into four stages consisting of pre-flight preparation, take-off, photography and landing. Pre-flight preparation consists of aircraft and camera checks and preparation. Before each flight the aircraft must be checked for correct operation of all controls, airframe integrity and fuel level. Film speed selection is made on the basis of a light meter reading and the picture resolution required. To prevent blurring due to ground speed a minimum shutter speed of 1/500 sec is recommended, so with the aperture priority Contax camera the corresponding aperture is selected on the basis of the prevailing light intensity. Because the lens performs well at wide apertures it is usually possible to use a medium speed, fine grained film such as Ektachrome 100. Focus is set taking into account the aperture and likely minimum camera to subject distance. Take-off must be into wind which can present difficulties in a confined area with obstructions such as trees. However, by restraining the aircraft on the ground until full engine revs are reached, and then releasing with a push, take-off can be achieved within 5 m. The stronger the wind the higher the airspeed and hence the shorter the runway required. Take-off from a smooth surface such as a road is desirable but rough tracks and cultivated fields usually present no serious difficulties. Hand launching by an assistant can be used where the ground is very uneven or the vegetation dense. Although the complete operation, including photography, can be carried out by the pilot alone, the use of two helpers makes for easier operation and more precise positioning of the aircraft in flight.

The flying height required in order to photograph a given area is calculated from the angular field of view of the lens (Table 1). For a 50 mm lens, height is approximately equal to the length of the rectangular field of view. With practice aircraft height can be judged visually or by using a simple sighting scale when the aircraft is directly overhead. For accurate height measurement an altimeter is required. A prototype instrument based around a solid state pressure transducer has been successfully used. Altitude is transmitted back to the pilot and displayed as a continuous digital reading in metres accurate to within $\pm$3%. This instrument is being further developed to reduce its weight and to improve temperature stability.

Having attained the required flying height the aircraft is flown along the centre line of the area to be photographed and towards the pilot who is positioned at the edge of the area. A second person positioned on the opposite edge of the area signals when the aircraft passes overhead. A sequence of overlapping photographs is then taken by the pilot, using a switch on the transmitter, or preferably by a third person using a switch and counter on a flying lead plugged into the transmitter. This latter system is preferable because it allows the pilot to concentrate on correctly positioning the aircraft. If wind direction does not correspond with the required line of flight the aircraft may traverse the area diagonally in which case additional flying height must be allowed to avoid loss of the corners of the area from the photograph. Landing must be into

wind and where an adequately clear landing area is not available the aircraft can be safely set down in the crop.

TABLE 1

Relationship between aircraft height and area photographed with 28 mm and 50 mm focal length lenses

Field of view (m x m)	Area (ha)	Flying height (m) 28 mm	50 mm
25 x 16	0.04	20	35
50 x 33	0.17	39	70
100 x 67	0.67	78	139
250 x 167	4.19	194	347
500 x 335	16.75	389	695

Photograph interpretation

On 26 April 1985 a field experiment to investigate the effect of competition from volunteer barley on the growth and yield of early (August) and late (September) sown oilseed rape was photographed to assess the potential for simply obtaining quantitative data from true colour vertical photographs. The experiment consisted of two rows of twenty four 2 m x 12 m plots spaced 0.5 m apart. A series of overlapping photographs was taken from a height of about 30 m using 100 ASA Ektachrome film. A selection of these, covering the complete experiment, were enlarged, so that individual plants were discernible, and printed. Then a grid of twenty random points was placed over the photograph of each plot and the number of 'hits' on rape and barley recorded. The mathematical relationships between the number of 'hits' and percentage of total biomass attributable to rape and rape dry matter per m^2, measured by ground sampling on 9 May, were calculated. Ground sampling consisted of removal, sorting and weighing of vegetation in a 1 m^2 area in each plot.

RESULTS

Fig. 1 shows the relationships between the percentage of total biomass attributable to rape and photograph score, and between rape dry weight and photograph score for the two sowing dates. For clarity only the means of the three replicates of each treatment are plotted but all statistical analyses were conducted using individual values. In all cases there is a good correlation between field sampling data and photograph score with the regression lines accounting for 73-80% of the variation in the data. In all cases, except the relationship between percentage rape and photograph score for the early sown crop, a slightly closer fit between the two variables is provided by the quadratic equations shown than linear regression equations, although the latter are also highly significant ($P<0.01$). The unexplained variation may be due to errors in the field sampling data, as it was apparent from photographs of some plots that plant growth was not uniform, to the limited number of point quadrats recorded, or to the lack of a uniform relationship between ground cover and plant weight.

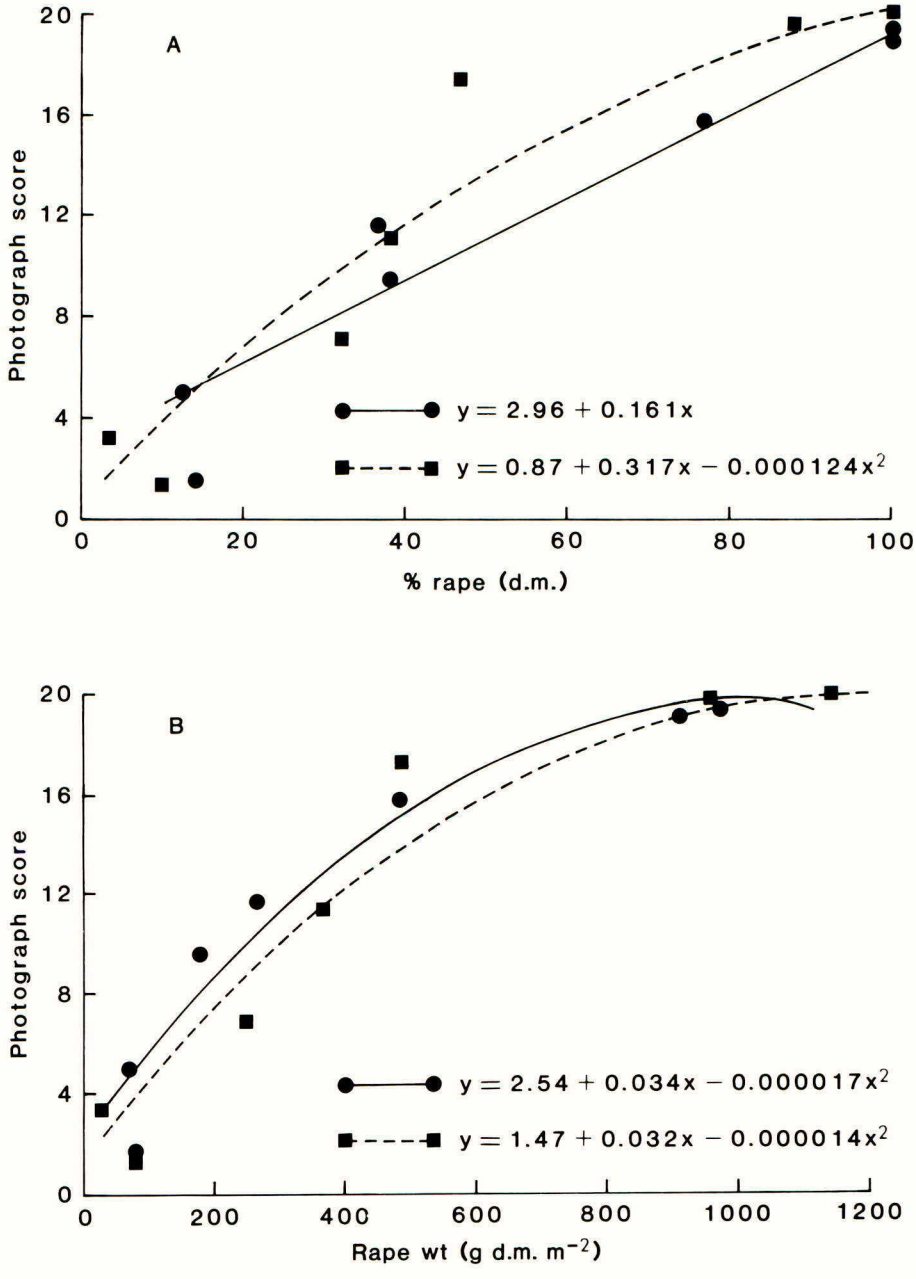

Fig. 1. Relationships between oilseed rape point sampling score and (A) rape as a percentage of total plant dry matter and (B) rape dry matter per unit area for August (●) and September (■) sown rape. For clarity only means of the three replicates of each treatment are shown but best fit lines were derived using separate replicates.

DISCUSSION

This system has produced very detailed aerial photographs at very low cost, but improvements in both aircraft and operating technique are still needed to guarantee good results every time. The main problem associated with the aircraft is vibration which sometimes affects a few frames on a film. Usually it occurs at the low engine speeds required to achieve slow flying speed. Sponge rubber mounting of the engine has given little improvement. The problem may be solved by using a smaller, higher reving engine, assuming that it is powerful enough to achieve a short take-off, or by fitting a four-stroke engine, although this will involve a weight increase. The other problem is the fragile nature of the airframe, in particular the wing, resulting from the need to minimise weight. If damage occurs during a photographic session it is important to be able to carry out repairs at once to avoid wasted time and travel. To this end two identical aircraft have been constructed so that a replacement aircraft is always available. Both control system and camera have been shown to be reasonably robust and to withstand heavy landings. However, aircraft damage is inevitable so spare airframe components which are cheap, simple and quick to construct must be available.

The main operating problem is the achievement of correct aircraft positioning for satisfactory photographic coverage of the study area. This becomes easier as experience is gained but still presents problems when attempting large scale photography of defined areas. Because correct flying height is essential, the prototype altimeter which has been successfully tested is being further developed. To achieve correct timing of the camera operation it may prove beneficial to use a separate radio link for this purpose so that the photographer can take up an appropriate position away from the pilot. The problem of aircraft crabbing when the wind direction is diagonal to the plot may be overcome by swivelling the camera on the gimbal to compensate. The alternatives of flying higher or using a shorter focal length lens are not entirely satisfactory because of loss of detail and edge distortion respectively.

Aerial photography can assist field experimental work in three ways. Firstly, it provides a permanent visual record which can be referred back to once the experiment is finished, and which may be used to detect patterns which may not be apparent from ground observation or sampling. In the example described, systematic sampling was used because random sampling was not practicable, especially in the later growth stages when vegetation was dense. In most cases it appeared from the photographs that the samples were representative of their plots but in a few there was evidence to the contrary. Secondly, aerial photographs can be used to obtain presence/absence data for a plant species as in Fig. 1, or of a pest or disease infestation. This can be done in the field using random quadrats but is more time consuming, can cause plant damage and is possible only when crop height and vigour do not restrict access to the experiment. Once relationships between photograph scores and plant parameters have been defined, field sampling can be supplemented with, or even replaced by, photograph analysis. For example, by taking photographs on frequent occasions field sampling could be reduced and photographs used to derive data by interpolating between occasions on which photograph-sampling relationships have been determined. For the example cited the use of photographs in place of field sampling would give a saving of at least 75% in man-hours.

Thirdly, aerial photography can be used in conjunction with spectral and image analysis techniques to detect pest damage and plant disease, to measure the effects of soil fertility on plant growth, and to estimate plant biomass. The latter involves using infra-red monochrome film and filters to record separately near-red and infra-red reflectance and relating the ratio of the two to leaf area index and hence biomass (Curran 1980). This could be achieved with the existing single camera system by making two flights with different filters and including a reference reflectance source to correct for variations in light quality. Ideally a second camera should be used which would be possible with slight modification to the existing aircraft. This application is being investigated.

This method has considerable potential for monitoring field experiments and for investigating a variety of weed, pest and disease problems. The very detailed photographs obtainable should permit analysis by standard remote sensing digitising and image analysis techniques, allowing detailed information to be collected, stored and analysed more quickly and fully than by field sampling. Where sampling is still necessary, photographs can provide a valuable, permanent visual record. Compared with conventional aerial photography, this method, even with an initial capital outlay of about £800, is very cheap. It also has the advantages of being available on demand and suitable for very low altitude, large scale photography.

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