SESSION 3C

NEW USES OF EXISTING MOLECULES

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POSTERS 3C-1 to 3C-9

THE EFFICACY AND CROP TOLERANCE OF A NEW HERBICIDE FORMULATION CONTAINING TRIASULFURON AND FLUOROGLYCOFEN-ETHYL.

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ABSTRACT

Small plot field trials undertaken in 1988 and 1989 have shown that triasulfuron and fluoroglycofen-ethyl are complementary when used in combination for broadleaved weed control in winter and spring sown cereals. Triasulfuron, essentially systemic in activity, when combined with the contact action of fluoroglycofen-ethyl has provided control of commonly occurring annual broadleaved weeds of cereals. With certain weed species the degree of control was determined by growth stage. Very similar results were obtained from trials located on both organic and mineral soils.

INTRODUCTION

Triasulfuron is a contact and residual herbicide from the sulphonyl urea group, controlling a wide range of broadleaved weeds in cereals. It exhibits a typical sulphonyl urea mode of action, with inhibition of cell division resulting in the control of susceptible species. Control may occur over a period of 3-6 weeks, with less susceptible species such as Veronica spp. showing inhibition and suppression through the whole season (Amrein et al., 1985).

Fluorogylcofen-ethyl discovered by Rohm & Haas is a diphenyl-ether herbicide which are primarily inhibitors of photosynthesis and respiration and which cause a very rapid development of phytotoxic symptoms in susceptible species (Maigrot et al. 1989).

These two compounds would therefore appear to be potentially useful combination partners having complementary modes of action. This paper describes the efficacy and crop safety of A8050, a water dispersible granule containing triasulfuron and fluorogylcofen-ethyl for weed control in cereals from trials over a period of two seasons.

MATERIALS AND METHODS

Triasulfuron and fluorogylcofen-ethyl were formulated as water dispersible granules, A8050, containing respectively 30 and 120g a.i./kg. At the use rate of 0.25 kg FP/ha, 7.5 and 30 g ai/ha of triasulfuron and fluorogylcofen-ethyl are applied. The standards used were either A7916 a 20% water dispersible granule formulation of triasulfuron or an emulsifiable concentrate formulation of ioxynil/bromoxynil (Stellox 380EC).

The trials were conducted throughout the UK by Ciba-Geigy Agrochemicals during the 1987 to 1989 seasons. The sites were located in

commercially grown crops, with trials designed for efficacy evaluation placed in areas of naturally occuring weed populations, whilst areas of low infestation were used to evaluate for the crop tolerance of A8050.

All trials were of a fully randomized complete block design. Three replicates of 3 x 8m plots were used for efficacy evaluation, and four replicates of 3 x 12m plots were used for crop tolerance evaluation.

This paper reports on results obtained from 23 trials designed to determine weed control and 18 trials designed to determine crop selectivity.

All applications were made using a hand-held precision plot sprayer with 6 Lurmark F11002 nozzles, operating at a pressure of 233 kPa and spray volume of 200 l/ha. All applications were made post-emergence in the autumn or spring. Weed control was evaluated throughout the season by a visual assessment of the reduction in plant biomass and also by counts of individual weed species. Crop tolerance was assessed visually with particular attention to necrosis of the leaves and overall vigour of the crop. Yields were obtained using a Claas Compact 25 combine harvester.

RESULTS

TABLE 1. Percent control of broadleaved weeds 28 DAT

Compound Dose (g a.i./ha)	A8050 7.5 + 30	triasulfuron 7.5
Galium aparine	97	89
Stellaria media	98	97
Veronica hederifolia	100	46
Veronica persica	97	55

Visual assessments of the reduction in plant biomass from trials undertaken in 1988, 28 DAT showed that plots treated with triasulfuron alone contained similar plant numbers of the species listed in table 1, to the untreated plot, however, treated plants were showing significant inhibition causing a large reduction of the plant biomass present in these plots. Species known to be highly susceptible to triasulfuron, such as Matricaria spp., Galeopsis tetrahit and Sinapis arvensis had been fully controlled by this time.

The addition of 30 g a.i./ha fluoroglycofen-ethyl to the 7.5 g a.i./ha of triasulfuron in the formulation A8050 also produced a similar degree of growth inhibition. This was, however, accompanied by a severe necrosis of the weed species present apparent initially on leaves fully exposed to the spray droplets. At the assessment 28 DAT, the necrosis was apparent on the stems and leaf margins of lower leaves. This necrosis,combined with the inhibition induced by the triasulfuron component of A8050, resulted in a more rapid and overall greater degree of control, in particular of the species listed in table 1.

TABLE 2. Final percentage weed control of A8050 compared to ioxynil/bromoxynil (90-120 DAT).

Compound	Number of Sites	A8050	ioxynil/bromoxynil
Dose (g a.i./ha)		7.5/30	380/380
Fumaria officinalis	(2)	91	100
Galeopsis tetrahit	(2)	100	100
Galium aparine	(10)	90	80
Geranium dissectum	(1)	93	100
Lamium purpureum	(1)	100	96
Legousia hybrida	(2)	100	95
Matricaria spp.	(4)	100	100
Melandrium album	(3)	87	79
Myosotis arvensis	(4)	99	100
Papaver rhoeas	(6)	99	99
Stellaria media	(12)	95	76
Sonchus arvensis	(2)	71	46
Sinapsis arvensis	(4)	100	95
Veronica hederifolia	(7)	84	90
Veronica persica	(10)	84	83
Viola arvensis	(9)	83	82

The final control of weed species, assessed 90 to 120 DAT, achieved by A8050 in trials undertaken in two seasons is presented in table 2. Complete control of the highly susceptible species; G. tetrahit, L. purpureum, L. hybrida, Matricaria spp. and S. arvensis was recorded. Other species, M. arvensis and P. rhoeas were also controlled to a satisfactory level, however, a number of species including G. aparine, V. hederifolia and V. persica were less well controlled ranging from 83% to 90%.

Further analysis of the results obtained for these species indicated that the size of the weeds at application had a significant effect on the final level of control.

TABLE 3. Effect of weed growth stage at application on weed control by A8050 7.5/30 g a.i./ha, final mean percent control and range.

	Less than 150mm (before branching)			Greater than 150mm (after branching)		
	Number of sites	mean	range	Number of sites	mean	range
Galium aparine	(4)	98	(94-100)	(6)	85	(44-100)
Stellaria media	(3)	96	(87-100)	(9)	94	(71-100)
Veronica hederifolia	(3)	90	(80-100)	(4)	80	(56- 96)
Veronica persica	(6)	97	(88-100)	(4)	62	(0-100)

Table 3 presents weed control data for four species where applications were made to young plants (less than 150mm high or across) or mature plants (greater than 150mm high or across).

Applications to young plants resulted in a mean control figure for the four species of 95%, the corresponding result for applications to mature plants being 80%. For S. media, a species susceptible to triasulfuron, a difference in control of just 2% was found between the two application ranges. However, for the other three species the mean difference ranged between 10% to 35% the largest difference being for V. persica.

TABLE 4. Final percentage weed control by A8050 (7.5/30 g a.i./ha) from trials on organic and mineral soils

	Soil Greater than 10% OM			Soil Less than 10% OM		
	Number of sites	Growth stage at applic.	Final control	Number of sites	Growth stage at applic.	Final control
Galium aparine	3	less than 150mm	91	3	less than 150mm	98
Matricaria spp.	1	4 leaves	99	1	2-6 leaves	100
Papaver rhoeas	1	2 leaves-150mm	100	3	5 leaves-150mm	99
Veronica hederifolia	1	less than 150mm	96	3	2 leaves-150mm	93
Viola arvensis	2	2-6 leaves	96	1	2-6 leaves	93

In addition to examining the impact of growth stage at application on efficacy, trials were undertaken to determine the effect of soil organic matter on weed control. The results are presented in table 4 for species where the growth stage at application was similar in trials on soils with greater than, or less than, 10% organic matter content.

For the five species, the mean control on soils with greater than 10% organic matter was 96.4%; for soils with less than 10% organic matter this was 96.6%, thus demonstrating no observable difference in performance of A8050 on the different soil types. The largest difference occurred with G. aparine, although this was probably due to crop shading of the weeds in one trial.

TABLE 5. Crop safety of A8050 applied at double rate (15/60 g a.i./ha). Mean of maximum percentage damage per site and range and mean percentage yield (relative to the control) and range.

Crop	*Growth stage at application	Visual damage Number of sites	Mean	Range	Yield Number of sites	Mean	Range
W. wheat	12-34	(6)	1	0-9	(3)	101	94-107
W. barley	14-32	(6)	1	0-5	(3)	103	99-107
S. wheat	21-41	(2)	0	0	-		
S. barley	21-41	(2)	1	1-2	-		

* Zadoks et al., (1974)

Crop phytotoxicity

Applications of A8050 were made at twice the anticipated dose rate in both winter and spring cereals. Visual assessments showed that where damage occurred it took the form of small (0.5 - 1.0mm) necrotic lesions where chemical contact had taken place. These lesions were transient and would have been acceptable in commercial use at even the highest level. Yield data obtained from both winter wheat and winter barley showed that there was no significant yield loss (table 5).

DISCUSSION

Triasulfuron/fluoroglycofen-ethyl formulated as a water dispersible granule provided excellent weed control when applied post-emergence.

The two active ingredients formulated in A8050, triasulfuron and fluoroglycofen-ethyl were complementary in activity: the essentially contact nature of the latter was added to the inhibitory effect of triasulfuron providing control of weed species such as G. aparine and Veronica spp, traditionally regarded as being sulphonyl-urea tolerant. A8050 was observed to have a very rapid effect which ensured that yield losses from weed competition were kept to a minimum.

The final level of control of a number of species, in particular V. hederifolia, V. persica and G. aparine was found to depend on their growth stage at application. As these weeds increased in size control become more variable, with high levels of control at some sites and unacceptable levels at others. At the sites with lower final levels of control the initial symptoms of necrosis were high but subsequently regrowth occurred. Although it is probable that weed growth stage was the dominant factor in determining the final control achieved, the observation of some initial, apparently satisfactory activity, followed by regrowth indicates that spray coverage of larger species may be of importance. In the trials reported here the more advanced growth stages were associated with late application timings to crops at GS 31-32. In these situations droplet penetration of the crop canopy would be impeded and complete spray coverage of the weed species difficult to obtain.

The levels of control achieved on organic and mineral soils showed that the level of organic matter in the soil did not affect activity. This lack of effect of organic matter content was expected as, at the application rates employed, neither compound is dependent on root uptake for the control of established weeds. It is also probable that over-wintered crops significantly reduce the amount of applied chemical reaching the soil and the dry conditions prevalent in the spring of 1989 mitigated against significant root uptake.

Annual broadleaved weeds can be controlled by herbicide applications made in the autumn/winter or spring. The time of application should be a function of the optimum application conditions for the herbicide - crop growth stage, soil type and weather conditions. It is often the case however, that crop and weed growth stage limitations for individual herbicides result in application under less than satisfactory conditions with the potential consequence of crop damage or inadequate weed control.

The triasulfuron and fluoroglycofen-ethyl combination is not limited by crop growth stage or soil type. The systemic and contact action should allow use under the cool conditions prevalent in the late winter and early spring. The late spring use of this mixture appears to be dependent on the growth stage of certain weed species, in particular G. aparine and Veronica spp. Preliminary studies initiated during the 1989 season indicate that the addition of reduced rates of mecoprop provides full control of these species at advanced growth stages. Investigations into the effect of droplet size and application volume on late season weed control are also underway.

ACKNOWLEDGEMENTS

Fluoroglycofen-ethyl was supplied by the Rohm & Haas Company Limited.

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THE CONTROL OF ARRHENATHERUM ELATIUS IN CEREALS BY IMAZAMETHABENZ-METHYL*

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ABSTRACT

Arrhenatherum elatius (onion couch or false oat grass) is an arable grass weed of increasing importance, particularly on lighter and more calcareous soils. Infestations can be spread by both seeds and long lived bulbils.

Imazamethabenz-methyl was applied to both small plot replicated and large plot grower trials between 1986 and 1989 and the effects on both the aerial growth and bulbil production determined. Single applications in the spring were more effective than single autumn applications. However, greatest control was achieved by a sequential autumn followed by spring application which gave 96% reduction in flowering heads and 73% reduction in bulbil weight.

Imazamethabenz-methyl was introduced in the U.K. for the control of Avena fatua and Alopecurus myosuroides. Increased usage gave reports of good activity on A. elatius. This paper demonstrates how imazamethabenz-methyl has been used to give effective control of this weed.

INTRODUCTION

The species A. elatius is variable and is capable of existing in two forms. One of these - variety bulbosum, is becoming an increasingly important arable weed particularly on lighter soils and is spread by both seeds and chains of bulbils. It has been shown (Khan, 1987) that seed from non bulbil bearing plants will usually produce non bulbil bearing offspring whereas seed from plants with bulbils produces approximately 70% bulbil bearing offspring and 30% non bulbil offspring. In arable situations selection pressure favours the bulbous form which is generally less competitive in a grazed sward situation.

Control can be approached culturally, chemically or by a combination of these. Cultural control involves the use of short term leys with associated grazing and burial of the bulbils to depth which decreases survival (Khan, 1987). Chemical control to date has been limited to glyphosate or flamprop-M-isopropyl applied towards the end of the growing seasons (Samuel, 1985). The results can be variable (Ayres, 1985 and Birnie, 1983).

* PROPOSED

MATERIALS AND METHODS

Control A. elatius by imazamethabenz-methyl was evaluated in four small plot [2m x 8m] trials with 3 replicates and on 25 large [1 ha] unreplicated grower applied trials. Imazemethabenz-methyl was applied as a suspension concentrate containing 300 g/litres active ingredient as DAGGER* herbicide.

Replicated trials were sprayed using an MDM knapsack sprayer to give 200 l/ha at 2.8 kpa through flat fan 110° nozzles. Unreplicated grower plots were sprayed through standard farm machinery but using the same parameters as for replicated trials. All trials were carried out in either winter cereal or spring barley crops.

In replicated trials control was assessed as a reduction in number of flowering panicles by counts in 0.25m^2 quadrats and by reduction in bulbil weight by removing 50 bulbils from representative parts of each plot, washing to remove adhering soil or root particles and then weighing. In large unreplicated plots only control of flowering panicles was assessed.

Crop plants were assessed throughout the year and any effects monitored.

RESULTS

Replicated Trial Results

Results from 4 replicated trials over 2 years are presented in tables 1 to 3.

TABLE 1. Percentage control of flowering heads from imazamethabenz-methyl treated plots

	Imazamethabenz-methyl autumn/spring					Flamprop-M-isopropyl spring
Application rate kg a.i./ha	1.3/0	1.7/0	1.3/1.3	1.3/1.7	0/1.7	0.7
% control	64(4)	48(2)	89(3)	96(4)	98(3)	98(3)

( ) number of sites
% control assessed as reduction in flowering panicles compared to untreated.
untreated populations had more than 60 panicles /m^2

*Registered Trademark

TABLE 2. Percentage reduction in bulbil weight following applications of imazamethabenz-methyl

	Imazamethabenz-methyl autumn/spring			Flamprop-M-isopropyl spring
Application rate kg ai/ha	1.3/0	1.3/1.7	0/1.7	0.7
% weight reduction	35(3)	73(3)	50(3)	-8(3)

( ) number of sites
% weight reduction of 50 bulbils compared to untreated plots.

TABLE 3. Effect of timing of imazamethabenz-methyl on control of panicles and bulbil production

	Stage of bulbil growth at application					
	1 leaf		pre-tillering*		Post tillering	
Application rate kg a.i./ha autumn/spring	% control of Heads[1]	Bulbils[2]	% control of Heads	Bulbils	% control of Heads	Bulbils
1.3/1.7	96	64	96	73	97	38
0/1.7	89	55	90	50	94	21

* leaves up to 2.5 cm with active growth
1 % control of flowering heads compared to untreated
2 % reduction in weight of 50 bulbils compared to untreated.

The greatest overall control was obtained by a sequential treatment of 1.3 + 1.7kg ai/ha imazamethabenz-methyl and this was effective in controlling both the flowering panicles and reducing the bulbil weight by 73%. Single spring applications were superior to single autumn applications for both panicle and bulbil control (Tables 1 and 2). A single spring application gave only slightly reduced panicle control compared to a sequence but markedly poorer bulbil control. Flamprop-M-isopropyl gave good panicle control but in these trials increased bulbil weight. Repeated use of this material has shown some bulbil control.

Control of flowering heads was not affected by a range of application timings from 1 leaf to tillering, but control of bulbils was greatest from applications made when leaves on the bulbils had reached 2.5cm of active growth but before tillering was initiated (Table 3).

Unreplicated grower trial results

Results from 14 trial plots are given in table 4. No assessment was made of bulbil weight in these trials but visual inspection confirmed that plots treated with imazamethabenz-methyl had smaller bulbils than other plots.

TABLE 4. Percentage Control of flowering panicles following imazamethabenz-methyl treatment in grower trials

	Imazamethabenz-methyl autumn/spring				Flamprop-M-isopropyl spring
Application rate kg a.i./ha	1.3/0	1.7/0	1.3/1.7	0/1.7	0.7
% control	64(7)	47(1)	97(8)	89(15)	97(5)

( ) number of sites

Results obtained from grower trials confirm those given in tables 1 and 2 from replicated trials with a sequential application giving the greatest level of control.

No adverse crop effects were recorded from any site throughout the study.

DISCUSSION

Results from these trials and from monitored commercial usage have established the efficacy of imazamethabenz-methyl for the control of A. elatius. The biology of this species requires that for control to be successful both the flowering panicles and bulbil production need to be controlled. It has been demonstrated that the most effective timing for single herbicide applications is in early spring when the bulbils are actively growing. It has been suggested (Khan, 1987) that this would correspond with tiller initiation and may represent the most vulnerable stage for control of the plant. At this stage chemical would be actively translocated throughout the plant and as bulbils are also initiated at this stage their numbers and size would be reduced. Reduced translocation, particularly to the bulbils and between bulbils on a chain would explain the reduced effectiveness and greater variability of later applications (Birnie, 1983).

Sequential applications of imazamethabenz-methyl gave the greatest overall level of control with the autumn application being made to actively growing bulbils. The autumn application probably reduced tiller production resulting in less vigorous plants in the spring. Imazamethabenz-methyl therefore offers an effective means by which to control this weed.

ACKNOWLEDGEMENTS

The author gratefully acknowledges Alison Short who provided much of the trials data and also John Edmonds for the bulbil weight determination.

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THE CONTROL OF POTATO GROUNDKEEPERS IN CEREAL CROPS

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ABSTRACT

High populations of potato groundkeepers can reduce yields of subsequent crops, act as reservoirs of pests and diseases and cause contamination of seed potato crops. Chemical control can help reduce groundkeeper populations. Maleic hydrazide applied to the ware crop gave between 64 and 77% control of groundkeepers in the following wheat crop although control of progeny emerging in the second wheat crop was poor. In a separate experiment maleic hydrazide was more effective at reducing the emergence of large tubers (>45 mm) than of small tubers (<25 mm) which are more typically returned to the soil as groundkeepers. Also earlier treatment, 5 weeks before defoliation was more effective than treatment at the minimum approved interval of 3 weeks. Of the chemical treatments applied to a following wheat crop, clopyralid was the least effective overall, whereas fluroxypyr and glyphosate gave good haulm control, but less reliable control of daughter tubers.

INTRODUCTION

Lutman (1986) stated that volunteer potatoes (Solanum tuberosum) from groundkeepers are serious weeds in many countries and are a particular problem in the main potato-growing areas of the UK. Tubers left in the ground after mechanical harvesting of the potato crop are the main source of the subsequent volunteer problem. High populations of groundkeepers cause problems to producers of ware and of seed potatoes, both in terms of competition with other crops in the rotation and in terms of the potential carryover of pests and diseases between potato crops. Additionally, the presence of rogue potatoes in seed crops could result in rejection for certification, and contamination problems in ware crops.

A programme of separate but related experiments assessing methods of control of potato groundkeepers was undertaken at three Experimental Husbandry Farms; Arthur Rickwood, High Mowthorpe and Terrington. Preliminary results of the trial at Arthur Rickwood and High Mowthorpe were reported by Bevis and Jewell in 1986. This paper summarizes the completed series.

MATERIALS AND METHODS

Two approaches to control of potato groundkeepers were examined; maleic hydrazide treatment of the mother crop, and herbicide treatment of groundkeepers within the following wheat crop.

Treatment of the mother crop

At Arthur Rickwood maleic hydrazide was applied to replicated plots within a potato crop at 4 kg/ha a.i. at either 20% crop senescence 2 weeks prior to desiccation in 1984 on the variety Maris Piper, or at 10% crop senescence 3 weeks prior to desiccation in 1985, 1986 and 1987 on the variety Kingston. The effect of maleic hydrazide on groundkeeper populations was assessed in the following first and second wheat crops.

At Terrington maleic hydrazide at 4 kg/ha a.i. was applied to the varieties Pentland Crown in 1984, Desiree in 1985 and Romano in 1987 and 1988. Treatment timings were 3 weeks prior to desiccation in 1984, at 5 and 8 weeks before desiccation in 1985 and over a range of 6 dates in 1987 and 1988. Potatoes were harvested from treated and untreated plots and tubers from pre-determined size grades were selected at random and stored over winter in either a ware or chitting store. These tubers were planted in unreplicated rows, at a population of 33,333 tubers/ha, within a potato crop in the following spring to represent groundkeepers. Emergence was assessed and the daughter tubers were harvested and counted.

Chemical treatment of groundkeepers

Herbicide treatments were applied to groundkeepers growing in winter or spring wheat crops at both Arthur Rickwood and High Mowthorpe in 1985, 1986 and 1987, and also in 1988 at Arthur Rickwood. At Arthur Rickwood residual groundkeeper populations arising from the previous potato crop were used. At High Mowthorpe known populations of groundkeepers were established in the spring by planting mini-chitted tubers of less than 35 mm diameter into winter wheat crops in fields which had not grown potatoes for at least 10 years.

At both sites the herbicides used were:- clopyralid at 100 or 150 g/ha a.i. applied at wheat GS 39, fluroxypyr at 300 or 400 g/ha a.i. applied when the flag leaf ligule was just visible, Zadok's growth stage 39, or when emergence of the inflorescence was complete, growth stage 59, and glyphosate at 720 or 1440 g/ha a.i. applied when the wheat grain reached 30% moisture content (MC). Additional treatments at Arthur Rickwood included glyphosate at 720 g/ha a.i. + polyethoxylated tallow amine (PETA) at 800 g/ha a.i., and fluroxypyr at 400 g/ha a.i. at GS 39 followed by glyphosate at 720 g/ha a.i. + PETA at 800 g/ha a.i. at 30% grain moisture content. Not all treatments were tested in all years (Tables 1-4). Clopyralid and fluroxypyr were applied in 280 l/ha water and glyphosate in 210 l/ha water, at Arthur Rickwood, using a tractor-mounted sprayer, to plots ranging from 110 to 229 m^2 in size, with either 3 or 4 replicates. At High Mowthorpe all sprays were applied in 230 l/ha with a hand-held Oxford Precision sprayer. Plot size was 75 m^2 with 5 replicates in each year.

The effect of herbicides on groundkeeper populations was recorded at both sites. At Arthur Rickwood populations were assessed at the time of the glyphosate application for the GS 39 and 58 treatments and immediately

pre-harvest for the glyphosate sprays. At High Mowthorpe groundkeeper numbers were assessed at the time of application and 2 weeks later. In addition the yield and number of daughter tubers were assessed from 10 to 12 plant stations per plot at this site. The viability of the daughter tubers was determined in the following spring when they were chitted and planted into compost in seed trays in a glasshouse. At Arthur Rickwood emergence of the daughter tubers was recorded across the trial area within the second wheat crop.

RESULTS

Effect of maleic hydrazide treatment of the mother crop

Treatment of the mother crop, at Arthur Rickwood, resulted in a consistent reduction of the groundkeeper populations in the following cereal crop, of between 64 and 77% (Table 1). The residual effect in the second cereal crop following potatoes was less apparent, especially in 1987.

TABLE 1. The effect of maleic hydrazide, applied to the mother crop, on the populations of groundkeepers in the following wheat crop (plants/m²). Residual populations in the second cereal crop in parentheses - Arthur Rickwood, 1985-89

Chemical treatment	a/ha a.i.	Year 1985		1986		1987		1988	
(SED)		(0.72)	(0.45)	(1.26)	(0.48)	(0.77)	(0.15)	(0.28)	(0.43)
Untreated		3.5	(3.1)	4.2	(2.2)	2.8	(0.4)	1.1	1.8
Maleic hydrazide	4000	0.8	(2.0)	1.5	(1.9)	0.7	(0.1)	0.3	1.5
df		22	54	20	20	27	27	27	27

The effect of time of maleic hydrazide application on the emergence of harvested and replanted potatoes at Terrington is shown in Table 2. In all comparisons application of maleic hydrazide reduced emergence but response varied with tuber size and application timing. The reduction in tuber viability was greater in the larger than the small tubers. In particular, the control of tubers <25 mm in 1989 was very poor. Results from 1988 and 1989 indicate that application 5 weeks or more before defoliation was more effective than application at the minimum interval of 3 weeks. However, records showed that application 8-10 weeks before defoliation adversely affected the yield of the treated potato crop.

TABLE 2. The effect of maleic hydrazide, applied to the mother crop, on the emergence of replanted daughter tubers (%) - Terrington, 1985-86 and 1988-89

Treatment timing (weeks to defoliation)	Year Tuber size (mm) 1985 40-55	1986 45-55	65-85	1988 35-45	65-75	1989 <25	35-45	65-75
Untreated	100	100	100	100	100	100	97	100
10	-	-	-	10	5	73	20	17
9	-	-	-	2	0	-	-	-
8	-	17	3	-	-	73	17	13
7	-	-	-	-	-	70	10	0
6	-	-	-	3	0	-	-	-
5	-	17	0	10	0	67	20	17
4	-	-	-	-	-	83	37	0
3.5	-	-	-	48	2	-	-	-
3	26	-	-	30	0	80	57	3

The number of tubers produced from the replanted daughter tubers, which had been planted at a seedrate of 33,333 tubers/ha, are shown in Table 3. Responses followed a similar pattern to those for plant emergence. These progeny tubers were then stored in a chitting house and produced normal healthy sprout growth.

TABLE 3. The effect of maleic hydrazide, applied to the mother crop, on the progeny tuber number (000's/ha) of the replanted daughter tubers - Terrington, 1988

Treatment Timing (weeks to defoliation)	Tuber number (000's/ha) Size of planted tubers (mm) 35-45	76-75
Untreated	257	408
10	25	22
9	6	0
6	13	4
5	21	2
3.5	98	4
3	58	1

Effect of chemical treatment of groundkeepers

The effects of chemical treatment on groundkeeper populations in wheat crops are shown in Tables 4 and 5.

TABLE 4. Effect of chemical treatments on overwintered groundkeeper populations (plants/m²). Residual populations in the year following treatment in parenthesis - Arthur Rickwood, 1985-89

Chemical treatment	g/ha a.i.	Cereal GS	Year 1985		1986		1987		1988	
(SED)			(0.72)	(0.45)	(1.26)	(0.48)	(0.77)	(0.15)	(0.28)	(0.43)
Untreated			3.5	(3.1)	4.2	(2.2)	*2.8	(0.4)	*1.1	(1.8)
Clopyralid	100	39	3.5	(3.1)	4.7	(1.4)	-	-	-	-
	150		2.0	(1.7)	4.5	(2.1)	2.8	(0.4)	-	-
Fluroxypyr	300		2.5	(3.0)	4.0	(2.0)	1.4	(0.3)	-	-
	400		0.7	(1.9)	4.7	(2.2)	1.2	(0.3)	-	-
	300	59	0.2	(0.9)	2.2	(2.2)	0.9	(0.4)	0.3	(2.5)
	400		0.7	(0.7)	3.0	(2.2)	0.6	(0.2)	0.1	(1.8)
(SED)							(0.206)		(0.29)	
Untreated							**0.3		**1.1	
Glyphosate	720	30% mc	0.2	(1.0)	2.8	(2.2)	-	-	-	-
	1440	of grain	0.2	(0.6)	2.0	(2.2)	0.05	(0.1)	0.2	(1.6)
Glyphosate + PETA	720) 800)		-	-	1.3	(2.1)	0.06	(0.3)	0.6	(0.9)
Fluroxypyr then glyphosate + PETA	400)) 720) 800)	39 30% mc of grain	-	-	-	-	0	(0.4)	0	(1.4)
df			22	54	20	20	27	27	27	27

* Untreated groundkeeper populations in August against which clopyralid and fluroxypyr treatments were compared.

** Untreated groundkeeper populations pre-harvest against which glyphosate treatments were compared

At Arthur Rickwood the naturally occurring groundkeeper populations were rather variable across the trial site in each year. Results were more consistent at High Mowthorpe where known populations had been planted and data from the three years has been meaned.

Generally clopyralid gave poor control of groundkeeper haulm and had little effect on the vigour of the progeny, although at High Mowthorpe the high rate resulted in low viability of the daughter tubers.

TABLE 5. Mean effect of chemical treatments on groundkeepers two weeks post-treatment (plants/m²), tuber yield (g/plant) and tuber number (tubers/plant) and emergence of progeny (%) - High Mowthorpe 1985-87

Chemical treatment	g/ha a.i.	Cereal GS	Plants /m²	Yield g/plant	Tuber no.* /plant	Emergence of* progeny (%)
(SED)			(0.086)	(0.92)	(0.205)	
Untreated			1.59	10.0	0.99	92
Clopyralid	100	39	1.43	10.5	0.94	80
	150		1.38	10.2	1.04	24
Fluroxypyr	300		1.30	6.3	0.70	75
	400		1.08	5.5	0.54	36
(SED)			(0.271)			
Untreated			1.70	-	-	-
Fluroxypyr	300	59	0.83	5.4	0.68	43
	400		0.79	4.9	0.62	66
(SED)			(0.248)			
Untreated			0.96	-	-	-
Glyphosate	720	30% mc	0.37	10.6	0.94	66
	1440	of grain	0.30	10.6	0.97	30
df			48	16	8	
			24			
			24			

* Results from 1986 and 1987 only.

At both sites fluroxypyr gave moderate control of the groundkeeper haulm. The later timing was more effective and there was little difference between the high and low rate regardless of timing. Control of the progeny was variable; at High Mowthorpe all fluroxypyr treatments reduced the yield and number of daughter tubers and their viability, but those remaining produced normal healthy growth in the following spring. At Arthur Rickwood the survival of the progeny was reduced only in 1985.

Generally glyphosate gave good control of the groundkeeper plants except in 1985 at High Mowthorpe, where late lodging of the wheat crop killed most of the groundkeepers before the sprays were applied. At High Mowthorpe there was no effect on the yield and daughter tuber number of treated groundkeepers as the treatment was applied after these had formed. As with fluroxypyr, viability of the progeny varied from year to year although the full rate of glyphosate was generally most effective in reducing tuber viability. Those plants that did emerge varied from normal and healthy to stunted and malformed. At Arthur Rickwood there was some residual control in 1985 and 1987. Half rate glyphosate + PETA appeared to give similar results to full rate glyphosate. Full rate fluroxypyr followed by half rate glyphosate + PETA gave very good control of groundkeeper haulm in 1987 and 1988 but disappointing control of progeny in the second wheat crop.

DISCUSSION

Competition from subsequent wheat crops tends to limit the number of potato groundkeepers that establish, and also severely limits the number and yield of daughter tubers they produce, compared to potatoes grown in the absence of competition (Lutman, 1977 and 1979, Bevis and Jewell, 1986). However this competition merely limits multiplication of groundkeepers and more deliberate control measures are desirable if the aim is to reduce or eliminate the problem, especially if less competitive crops such as sugar beet or peas are grown.

The use of maleic hydrazide on the mother crop is not a technique available to seed growers but it appears to be of value to ware producers. Although it greatly reduces groundkeeper numbers in the year following treatment, investigations at Terrington suggest that the survivors are able to multiply sufficiently to produce groundkeeper populations in the second year which are almost comparable with the untreated. In addition, where tubers from treated crops were stored and re-planted it appeared that maleic hydrazide did not reliably reduce the emergence of small tubers which, in practice, are most likely to be the source of groundkeeper infestations. Timing of maleic hydrazide application to the mother crop also appears to be important, with the most effective control resulting from application 5 weeks or more before defoliation, compared with the recommended timing of 3 to 5 weeks before.

Control of emerged groundkeepers in subsequent wheat crops with the herbicides available has given extremely variable results. In most seasons groundkeepers are still emerging when cereal crops reach GS 39, currently the latest stage at which fluroxypyr is approved for application. Clopyralid is only approved for use up to GS 32. The later timing of fluroxypyr at GS 59 has generally achieved the most consistent control even though the cereal crop would have been almost completely shading the groundkeepers by this time. The use of fluroxypyr at either rate or timing has generally produced more reliable results than clopyralid. Similar results were reported by Bunn et al., 1986.

In seasons when the emergence of groundkeepers is late or when they are not subjected to stress through either drought or blight attack, they usually retain sufficient actively growing green leaf area for glyphosate to produce useful control of both the foliage and the viability of the daughter tubers. Although this may not be the case in late maturing cereal crops. The half rate of glyphosate applied alone appeared to be less reliable than the full rate, but the addition of PETA tended to improve the level of control to that achieved by the full rate but at less cost. The sequence of fluroxypyr at GS 39 followed by glyphosate pre-harvest gave some encouraging results.

The results obtained in these trials clearly indicate some of the problems involved in carrying out this type of work. Relying on field populations of groundkeepers results in an extremely uneven population on which to test treatments in trial. Where artificial populations of groundkeepers were established, as at High Mowthorpe, the results are easier to interpret and are statistically more accurate, but do not emulate natural field conditions where tubers of varying size and depth emerge more unevenly.

The problems of successfully controlling potato groundkeepers with chemical treatments are highlighted by this work. Lutman (1986) in his review of the biology of this weed states that herbicides should not be relied upon to achieve high levels of control but should be used as part of an integrated programme involving good husbandry of the potato crop and subsequent cereal crops to minimise the problem. These results confirm this view.

ACKNOWLEDGEMENTS

The authors thank the staff at High Mowthorpe, Arthur Rickwood and Terrington EHFs for their assistance.

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USE OF SEQUENCES FOR THE CONTROL OF BROAD LEAVED WEEDS IN MAINCROP POTATOES

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ABSTRACT

The efficacy of metribuzin, bentazone, pendimethalin and pendimethalin plus cyanazine applied alone, in sequence or in a tank mix was compared at three sites, two on mineral soil and one on organic, in 1987 and 1988. All herbicide treatments reduced weed populations, but some caused crop damage. Overall efficacy varied and on the difficult organic site no herbicide treatment was as good as hand weeding. On some sites sequences of herbicides produced a reasonable compromise between effective weed control and crop safety. The variation in performance is discussed and use of sequences evaluated.

INTRODUCTION

Competition from weeds can lead to serious loss of yield in the potato crop (van Heemst, 1985) and their presence can slow or prevent mechanical harvest. The use of herbicides is constrained by the open crop canopy, the dry conditions which often prevail at the time of application and the limited range of materials which can be applied after full crop emergence.

A series of experiments in 1983-86 showed the importance of adequate soil moisture for good weed control from residual herbicides. Metribuzin was more reliable when applied early post-emergence and bentazone was a more reliable and safer post-emergence material than MCPA when used on varieties that are sensitive to metribuzin post-emergence (Orson, 1986).

Organic soils present particular difficulties. Work at Arthur Rickwood Experimental Farm (EHF) has shown that on a soil with 25% organic matter, 1400 weeds/m^2 can be expected to emerge each spring (May, 1984). The activity of many of the residual herbicides is reduced on soils with more than 10% organic matter, leaving a very limited range of materials that can be used effectively and economically. A series of experiments commencing in 1983 at Arthur Rickwood EHF showed that metribuzin and bentazone can be effective, but sometimes cause crop damage. Pendimethalin showed some promise (Jewell and Short, 1986).

The series of experiments reported herein commenced in 1987 with the objectives of obtaining more information on the use of metribuzin, bentazone and pendimethalin and examining the use of sequences.

MATERIALS AND METHODS

The trials were carried out on a range of potato varieties at three

sites with natural weed populations in 1987 and 1988 (Table 1). The herbicides tested were applied with a plot sprayer at 225 l/ha water (or 100 l/ha for the repeat low dose treatments) with either 8001, 8002 or 8003 TeeJets, or F02-80 Lurmark nozzles at 200 or 250 kPa. Plot size varied from 18-44m^2 with 3 replicates, each with two untreated control plots, in a complete randomised block design.

The following herbicides were evaluated, either alone, in sequence or as a tank mix: metribuzin 70% water dispersable granule, bentazone 480g a.i./l a.c., pendimethalin 330g a.i/l EC, cyanazine 500g a.i./l suspension concentrate. The bentazone was always applied in mixture with a 97% mineral oil product ('Actipron').

Weeds were assessed in total and by species on two occasions by counting plants per m^2 and by weed score pre-desiccation at some sites. Visual scores were made for crop vigour at the time of the second assessement and, on most sites, yields were recorded (Tables 2, 3 & 4).

TABLE 1. Experimental sites, soil type, spray timings and dates

Year	Location	Code	Soil type[1]	Variety	Planting date	Pre-ridging a	Pre-crop emergence b	Post-crop emergence c	Repeat low dose[2] d	Repeat low dose[2] e
1987	Terrington EHF Norfolk	TT	ZCL	Romano	5 May	6 May	25 May	8 Jun	5 Jun	10 Jun
1987	Arthur Rickwood EHF, Cambs	AR	LP	M. Piper	28 Apr	6 May	16 May	4 Jun	4 Jun[6]	-
1987	Tunstead, Norfolk	TU	FSL	Estima	24 Apr	-	19 May	10 Jun	19 May[5]	10 Jun[5]
1988	Terrington EHF	TT	ZL	Romano	19 Apr	26 Apr	27 Apr	25 May	20 May[3]	1 Jun[3]
1988	Arthur Rickwood EHF	AR	PL	Estima	26 Apr	5 May	17 May	6 Jun[4]	27 May[5]	6 Jun
1988	Oulton, Norfolk	OU	SZL	Estima	24 Apr	3 May	11 May	25 May	25 May	5 Jun

[1] ADAS soil texture classification 1985
[2] Weeds at cotyledon to 2 true leaves
[3] Bentazone treatment on 25 May and 4 June
[4] Bentazone treatment on 13 Jun
[5] Metribuzin treatment only
[6] Bentazone treatment only

RESULTS

Table 2 shows the control of black bindweed (Fallopia convolvulus), speedwells (Veronica spp.), cleavers (Galium aparine), fat hen (Chenopodium album) and redshank (Polygonum persicaria). Other weeds were present at some of the sites and more information can be obtained from the authors.

TABLE 2. Weed control as a percentage of untreated 1987-88

Treatment	kg/ha a.i.	Timing	black bindweed TT 87	black bindweed AR 87	black bindweed TT 88	black bindweed AR 88	speedwells TT 87	speedwells TT 88	cl.[3] TT 88	fat hen AR 87	fat hen TU 87	fat hen TT 88	fat hen AR 88	redshank AR 87	redshank TU 87
1 metribuzin	1.05	b	84	28	0	0	100	92	0	95	100	100	54	56	100
2 metribuzin	1.05	c	95	-	66	0	98	99	0	-	100	100	100	-	100
3 metribuzin	0.24	d,e	92	-	-	-	100	-	-	-	100	-	-	-	98
4 metribuzin	0.35	d,e	-	-	100	0	-	86	35	-	-	100	89	-	-
5 metribuzin metribuzin	0.70 0.35	a b	84	64	15	18	100	99	0	100	-	100	63	89	-
6 metribuzin metribuzin	0.70 0.35	a c	97	-	78	18	100	92	5	-	100[1]	100	97	-	100[1]
7 metribuzin	0.70	a	86	-	41	0	100	64	0	-	100[1]	100	57	-	100[1]
8 metribuzin	1.05	a	84	59	73	0	100	84	0	85	100[1]	100	74	78	100[1]
9 bentazone	1.44	c	92	90	84	55	91	0	68	98	86	100	97	89	71
10 bentazone	0.72	d,e	86	100[2]	74	0[2]	76	0	70	85[2]	75[2]	75	97	22[2]	84[2]
11 pendimethalin + cyanazine	0.88 0.75	b	-	-	16	64	-	91	16	-	-	100	29	-	-
12 pendimethalin	1.32	b	87	3	-	-	98	-	-	65	-	-	-	56	-
13 metribuzin bentazone	0.70 0.72	b d	94	90	84	27	97	100	80	100	100	100	57	89	98
14 metribuzin bentazone	0.70 0.72	a d	-	-	48	18	-	63	74	-	-	100	89	-	-
15 Hand Weeded															
16 Untreated/m^2			12	32	13	11	12	10	13	33	24	3	35	15	32
SED (v untreated control)+/-			29.1	32.3	3.2	7.2	16.8	3.1	4.1	19.4	14.8	0.6	8.4	62.9	28.6

[1] Timings b and c [2] Timing e only [3] Cleavers

1987 trials

At the Terrington site, weed control was generally good, with the sequences similar to the equivalent larger single doses. Generally, the earlier metribuzin was applied, the poorer the control, and the higher the dose of bentazone used, the better the control. Pendimethalin produced similar control to the earlier timings of metribuzin. The effects of herbicides on crop vigour were small and variable although the pendimethalin treatment lead to transient distortion of the leaves.

At the Arthur Rickwood site, there were no significant differences

TABLE 3. Crop vigour and ware yield.

Treatment	kg/ha a.i.	Timing	Crop Vigour (0-9)[1] TT 87	AR 87	TT 88	AR 88	Ware Yield (t/ha) TT 87	AR 87	TT 88	AR 88	OU 88
1 metribuzin	1.05	b	5.0	5.3	9.0	5.3	47.2	46.6	42.5	27.4	59.3
2 metribuzin	1.05	c	4.7	-	9.0	2.0	45.5	-	45.2	27.1	53.3
3 metribuzin	0.24	d,e	4.7	-	-	-	46.0	-	-	-	-
4 metribuzin	0.35	d,e	-	-	6.3	3.7	-	-	41.8	27.5	53.1
5 metribuzin	0.70	a	5.3	5.7	8.3	6.7	48.8	43.3	41.0	30.1	56.2
metribuzin	0.35	b									
6 metribuzin	0.70	a	4.7	-	7.0	5.0	41.2	-	41.2	29.3	53.2
metribuzin	0.35	c									
7 metribuzin	0.70	a	5.3	-	8.4	6.3	49.1	-	40.1	29.9	61.7
8 metribuzin	1.05	a	5.0	6.0	6.3	7.0	51.7	46.1	40.9	30.1	57.4
9 bentazone	1.44	c	5.3	4.0	9.0	3.7	51.8	43.4	48.4	30.2	54.4
10 bentazone	0.72	d,e	4.7	5.3	7.7	4.7	49.4	44.8	47.8	31.5	49.3
11 pendimethalin + cyanazine	0.88 0.75	b	-	-	9.0	5.0	-	-	39.7	31.3	56.4
12 pendimethalin	1.32	b	4.7	5.7	-	-	45.9	43.0	-	-	-
13 metribuzin	0.70	b	5.0	4.0	9.0	5.7	50.6	43.7	43.2	31.6	54.2
bentazone							0.72				
14 metribuzin	0.70	a	-	-	7.7	5.3	-	-	45.8	29.3	57.8
bentazone	0.72	d									
15 Hand Weeded			-	5.7	-	7.7	-	46.8	-	35.8	-
16 Untreated			5.3	6.0	8.7	7.2	47.9	42.9	39.2	28.9	55.5
SED +/-			-				3.81	3.03			2.91
LSD (p=0.05) (v untreated)			-	0.4	1.1	1.2	NS	NS	4.2	5.6	NS

[1] A score of 9 indicates high vigour

between treatments except pendimethalin which was the least effective and particularily weak against redshank. Metribuzin used alone was weak against black bindweed and mayweed (Matricaria spp.). Incorporation appeared to improve its control of black bindweed, but reduced the control of mayweed. Pre-ridging followed by pre-emergence application gave a good compromise. Bentazone gave good control of black bindweed and mayweed, but was weak on annual meadow grass (Poa annua). The combination of metribuzin and bentazone in treatment 13 gave good control. Bentazone caused transient crop scorch which appeared worse where the crop had previously been treated with metribuzin. The hand weeded treatment gave the highest yield, but there were no other significant differences.

At the Tunstead site metribuzin worked consistently well. Bentazone alone gave a lower level of control, and was weak on redshank. The sequences of metribuzin and bentazone gave a reasonable level of weed control, but no better than metribuzin alone. No effect on crop vigour was recorded. Due to patchy waterlogging, this site was not taken to yield.

TABLE 4. % total weed control and weed vigour pre-desiccation

Treatment	kg/ha a.i.	Timing	% control total weeds TT 87	AR 87	TU 87	TT 88	AR 88	OU 88	weed vigour (0-9)[1] TT 88	AR 88
1 metribuzin	1.05	b	97	70	100	17	60	60	7.0	5.0
2 metribuzin	1.05	c	98	-	100	34	76	100	6.0	2.3
3 metribuzin	0.24	d,e	98	-	91	-	-	-	-	-
4 metribuzin	0.35	d,e	-	-	-	41	66	60	3.7	4.3
5 metribuzin	0.70	a	95	82	-	16	68	80	6.3	4.3
metribuzin	0.35	b								
6 metribuzin	0.70	a	99	-	100	35	80	100	5.0	4.0
metribuzin	0.35	c								
7 metribuzin	0.70	a	97	-	98	4	43	100	5.3	4.7
8 metribuzin	1.05	a	98	70	-	26	9	90	5.3	4.3
9 bentazone	1.44	c	49	74	80	29	9	0	3.3	5.3
10 bentazone	0.72	d,e	13	58	82	18	18	40	3.0	5.7
11 pendimethalin +	0.88	b	-	-	-	0	51	60	7.0	6.0
cyanazine	0.75									
12 pendimethalin	1.32	b	95	23	-	-	-	-	-	-
13 metribuzin	0.70	b	98	84	98	46	53	80	3.7	3.7
bentazone	0.72	d								
14 metribuzin	0.70	a	-	-	-	39	35	100	3.7	4.0
bentazone	0.72	d								
Untreated/m^2			85	202	68	76.6	197	0.5	7.3	7.0
SED (v untreated control)+/-			14.5	17.4	13.2	11.1	37.7	26.0	1.47	0.88

[1] A score of 9 indicates high vigour

1988 trials

At the Terrington site, good control of black bindweed was obtained from some metribuzin and bentazone treatments, but pendimethalin plus cyanazine had little effect. Only those treatments which included bentazone post-emergence had any effect on cleavers. Bentazone alone did not have any effect on speedwells. The untreated crop gave the lowest yield.

At the Arthur Rickwood site, bentazone used alone produced poor overall control because it had no effect on annual meadow grass. The good control from pendimethalin plus cyanazine was not sustained until harvest. The sequential treatments gave good season long weed control. The high rate of metribuzin post-emergence produced the best weed control, but had the most effect on crop vigour. The hand weeding treatment gave the highest yield, but this trial was affected by both blackleg (Erwinia spp.) and stem canker (Rizoctonia solani) and this affected the yield results.

The Oulton site had few weeds and numbers were too low to assess treatment effects. There were no significant differences in yield.

DISCUSSION

These trials show considerable variation in herbicide performance. Overall, it appeared that metribuzin was more effective in giving long term weed control at later timings and at the post-emergence timing it was very effective. The sequence of pre-emergence metribuzin followed by post-emergence bentazone gave a good level of control of the weeds reported in table 2, but its total weed control was only marginally better than pre-emergence metribuzin and not as good as post-emergence metribuzin. Both post-emergence metribuzin and bentazone adversely affected crop vigour, the former especially where the variety was known to be susceptible. Pendimethalin, with or without cyanazine, produced modest or poor overall weed control, with the exception of one site, and very poor control of individual weeds on some sites.

In both years, the hand weeded treatment at Arthur Rickwood EHF produced the highest yield. On the mineral soil sites, the best yields were obtained where good weed control was obtained without a reduction in crop vigour.

The sensitivity of some potato varieties to metribuzin restricts its use to pre-emergence only with an apparent reduction in efficacy. The availability of bentazone for post-emergence use gives greater flexibility, but its use at the full rate, whilst being effective, can sometimes lead to crop damage. The sequence of pre-emergence metribuzin followed by bentazone would appear to offer a compromise between efficacy and crop damage for metribuzin sensitive cultivars.

ACKNOWLEDGEMENTS

The authors would like to thank their colleagues for carrying out the trials and supplying data. The willing co-operation of the farmers at the Oulton and Tunstead sites is also gratefully acknowledged.

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USE OF SILICONE ADJUVANTS TO INCREASE ACTIVITY AND RAINFASTNESS OF ACIFLUOROFEN

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ABSTRACT

Previous work identified silicone surfactants that provide increased efficacy and rainfastness for the herbicides acifluorofen, glyphosate, and bentazone. The study reported in this paper was undertaken to determine the effect of one of these silicone surfactants on the dosage rate of acifluorofen required for weed control.

The effectiveness of acifluorofen on velvetleaf (Abutilon theophrasti) in the glasshouse was a function of the adjuvant used, adjuvant application rate, and acifluorofen application rate. The silicone surfactant ('Dow Corning® Q2-5309') greatly enhanced the activity of low application rates of the acifluorofen and provided rainfastness to the herbicide. Enhancement was observed with common lambsquarters (Chenopodium album), ivyleaf morningglory (Ipomoea hederacea), and to a lesser extent with barnyardgrass (Echinochloa crus-galli).

INTRODUCTION

Previous work (Jansen, 1973; Zabkiewicz et al., 1987) has shown that certain silicone surfactants enhance the efficacy of foliar applied herbicides. In more recent work (Roggenbuck et al., 1988; Fields et al., 1988; Roggenbuck et al., 1989), it was shown that specific silicone surfactants enhance the efficacy of acifluorofen, glyphosate, and bentazone and also provide rainfastness to velvetleaf. Thus, the silicone surfactants insure that these water-soluble herbicides maintain their efficacy in the event that rainfall occurs shortly after herbicide application.

This present study was undertaken to determine if the use of a polyalkyleneoxide silicone copolymer surfactant ('Dow Corning Q2-5309') would allow a reduction in the herbicide application rate while maintaining weed control. A second objective was to determine the weed species specificity of the enhancement effect provided by the silicone surfactant.

Velvetleaf (Abutilon theophrasti) was selected for the application rate study because it is a particularly troublesome weed in the midwestern United States. Acifluorofen as 'Blazer 2L' was chosen to expand upon previous studies. Soybean (cv. Corsoy '79) was included in the study to assess the effect of the silicone surfactant on soybean herbicide injury.

To determine the specificity of the silicone surfactants, common lambsquarters (Chenopodium album), ivyleaf morningglory (Ipomoea hederacea), and barnyardgrass (Echinochloa crus-galli) were selected to represent a range of weed species.

MATERIALS AND METHODS

Velvetleaf seeds were placed in 0.946 l plastic cups filled with 'Baccto' professional potting mix. The cups were placed in a glasshouse at 25°C (±2°C) with 18 h day and 6 h night with supplemental lighting to provide 1200 $\mu E \cdot m^{-2} \cdot sec^{-1}$. After emergence, the plants were thinned to one plant per cup and watered as needed. Herbicide treatments were made after 21 days when the velvetleaf plants were at the 5-leaf stage and 13 cm tall. The soybean plants were treated when they were 18 days old and 15 cm tall.

Acifluorofen as 'Blazer 2L' was applied at rates ranging from 0 to 1.12 kg a.i./ha, alone and in combination with either crop oil concentrate ('Herbimax') at 2.34 l/ha, or silicone surfactant ('Dow Corning® Q2-5309') at 0.58, 0.88, and 1.17 l/ha. The spray volume was 234 l/ha and the application pressure was 173 kPa. The sprayer used was a link-belt sprayer equipped with a 'Tee-Jet 8001-E' nozzle.

To evaluate rainfastness, the plants received 2.54 cm simulated rainfall in a 5 minute period 15 minutes after the herbicide application. The plants were evaluated visually for injury 7 days after herbicide application. The data presented are the means of two experiments with four replications each.

In the second study, common lambsquarters, ivyleaf morningglory, and barnyardgrass were grown as described above. They were sprayed at the age of 20 days, 15 days, and 8 days respectively. Herbicide was applied at rates ranging from 0 to 0.22 kg/ha. The silicone surfactant was added to the herbicide at only the 1.17 l/ha rate. Simulated rainfall and visual injury ratings were handled as in the first study.

RESULTS

Acifluorofen provided velvetleaf control at only 10-20% of the recommended label rate when the silicone surfactant was used as an adjuvant at a rate > 0.66 l/ha (Table 1 and Fig. 1). Velvetleaf control was achieved at 0.11 kg/ha acifluorofen with the silicone surfactant while 1.12 kg/ha acifluorofen was required using crop oil concentrate. Velvetleaf control was not achieved when no adjuvant was used.

Rainfastness was obtained when the silicone surfactant was used with acifluorofen (Table 1 and Fig. 2). Simulated rainfall 15 minutes after herbicide application resulted in minimal loss of efficacy of the acifluorofen when the silicone surfactant was present. By contrast, the herbicide and crop oil concentrate combination lost most of its efficacy with the rainfall treatment.

TABLE 1. Acifluorofen Injury To Velvetleaf And Soybeans 7 Days After Treatment

Adjuvant Rate l/ha	Acifluorofen Rate Kg a. i./ha	Velvetleaf % Injury No Rain	Velvetleaf % Injury + Rain	Soybean % Injury No Rain	Soybean % Injury + Rain
None	0	0	0	0	0
	0.11	5	0	8	3
	0.22	8	4	10	5
	0.34	13	7	18	9
	0.45	23	9	24	11
	0.56	31	10	36	18
	1.12	40	24	44	20
Crop oil concentrate (2.34 l/ha)	0	0	0	0	0
	0.11	33	14	42	25
	0.22	54	24	44	28
	0.34	62	28	68	32
	0.45	60	30	74	35
	0.56	72	32	77	40
	1.12	78	43	88	43
Dow Corning Q2-5309 (0.58 l/ha)	0	0	0	0	0
	0.11	77	65	40	33
	0.22	82	82	59	46
	0.34	88	83	70	52
	0.45	90	87	75	57
	0.56	87	87	75	55
	1.12	83	73	88	57
Dow Corning Q2-5309 (0.88 l/ha)	0	0	0	3	0
	0.11	86	75	44	32
	0.22	88	83	58	57
	0.34	87	82	70	63
	0.45	87	89	73	67
	0.56	90	84	79	63
	1.12	89	84	89	71
Dow Corning Q2-5309 (1.17 l/ha)	0	0	0	8	0
	0.11	83	77	45	35
	0.22	83	82	58	56
	0.34	85	85	73	64
	0.45	88	81	82	68
	0.56	89	83	84	69
	1.12	89	83	89	70

The silicone surfactant was effective at 0.88 l/ha, a rate lower than usually recommended for conventional adjuvants. The silicone surfactant was slightly less effective at 0.66 l/ha. Previous studies (Roggenbuck et al., 1988) have shown that the silicone surfactant becomes progressively less effective at lower rates, with rainfastness falling off fairly rapidly as the rate is decreased.

The acifluorofen and silicone surfactant combinations caused soybean damage comparable to the acifluorofen and crop oil concentrate combination. The damage observed in this glasshouse study was more severe than would be expected in field-grown soybean.

Control of common lambsquarters and ivyleaf morningglory was achieved at extremely low rates of acifluorofen whether silicone surfactant or crop oil concentrate were used (Table 2). Injury to either species was greater than 85% at 0.06 kg/ha acifluorofen, but rainfastness was obtained only with the silicone surfactant.

Control of barnyardgrass was obtained only when acifluorofen rates greater than 0.22 kg/ha were applied. Similar results were observed with the silicone surfactant and crop oil concentrate, but rainfastness was not obtained with either adjuvant.

TABLE 2. Acifluorofen Injury To Common Lambsquarters, Ivyleaf Morningglory, and Barnyardgrass 7 days After Treatment

		Common Lambsquarters		Ivyleaf morningglory		barnyardgrass	
	Treatment	- rain	+ rain	- rain	+ rain	- rain	+ rain
	Acifluorofen						
1	(0.03 Kg a.i./ha)	13	5	23	5	10	0
2	+ crop oil concentrate	53	25	73	10	10	10
3	+ Q2-5309	80	53	100	30	18	13
	Acifluorofen						
4	(0.06 Kg a.i./ha)	25	5	33	11	10	0
5	+ crop oil concentrate	85	33	98	20	15	10
6	+ Q2-5309	90	58	100	85	38	20
	Acifluorofen						
7	(0.11 Kg a.i./ha)	33	5	55	20	23	15
8	+ crop oil concentrate	95	33	98	33	43	15
9	+ Q2-5309	90	78	100	95	48	23
	Acifluorofen						
10	(0.22 Kg a.i./ha)	45	13	60	20	30	13
11	+ crop oil concentrate	93	55	100	33	80	38
12	+ Q2-5309	100	85	100	95	80	38
13	Control	0	0	0	0	0	0

Figure 1. Percentage Velvetleaf Control as a Function of Adjuvant and Acifluorofen Rate in the Absence of Rainfall

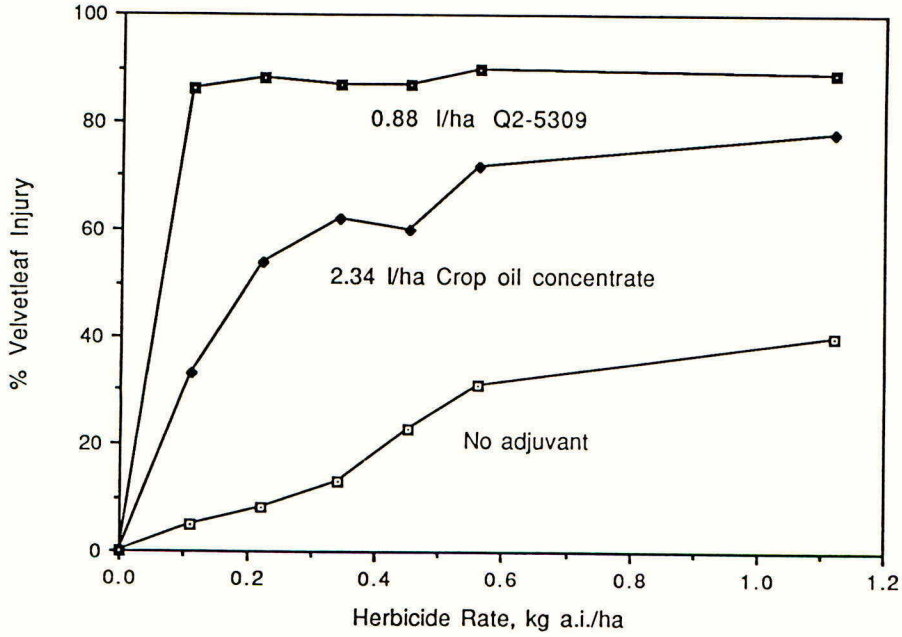
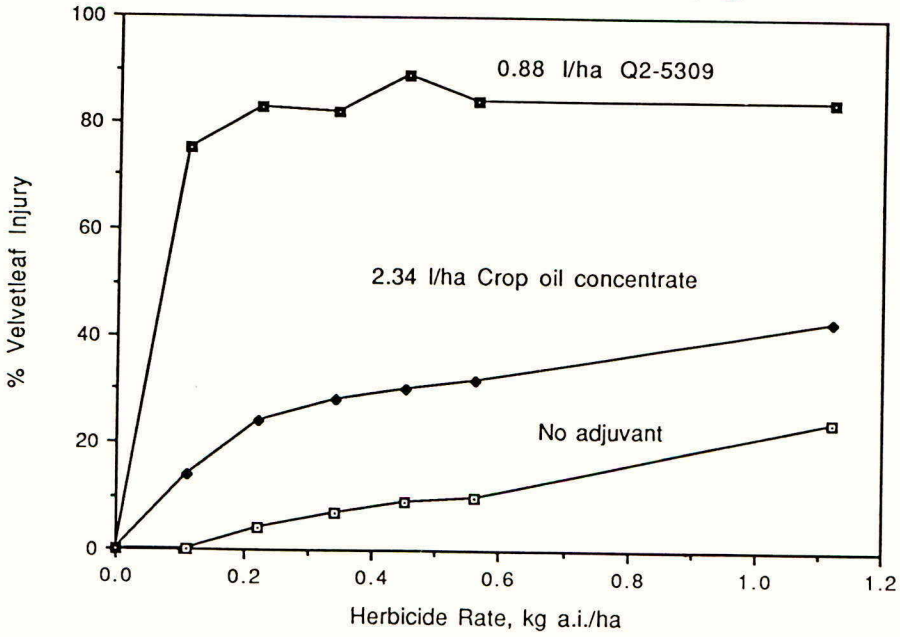


Figure 2. Percentage Velvetleaf Control as a Function of Adjuvant and Acifluorofen Rate with 2.54 cm Simulated Rainfall



DISCUSSION

This study demonstrated that velvetleaf control can be obtained at acifluorofen dosage rates well below label recommendation rates when a silicone surfactant is used as an adjuvant. Rainfastness is also achieved. Increased efficacy and rainfastness are also obtained with ivyleaf morningglory and lambsquarters, but these effects are less than with velvetleaf. The silicone surfactant provides no greater enhancement compared with conventional crop oil concentrate when tested with acifluorofen on barnyardgrass. Injury to all of the plants was greater than would be expected of field grown plants; field studies should now be undertaken to confirm the results of this study.

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RATIONAL APPROACHES TO SELECTION OF SURFACTANTS FOR OPTIMISING UPTAKE OF FOLIAGE-APPLIED AGROCHEMICALS

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ABSTRACT

A systematic investigation of the foliar uptake promoting properties of a series of fatty alcohol polyoxyethylene surfactants has demonstrated some important relationships between their structure and concentration, the physicochemical properties of the penetrating molecules and the plant species treated. From such data it is possible to construct a basic model for foliar uptake activation which could have predictive value in pesticide formulation design as well as in the development of new uses for existing agrochemicals.

INTRODUCTION

The exact mechanisms by which surfactant adjuvants enhance the foliar uptake and biological activity of agrochemicals are poorly understood (recent review, Holloway & Stock, 1989). This problem has inevitably led to a mainly empirical approach to optimising performance with many potential activator surfactants being screened for their effect when added to formulations of any new compound. In an attempt to provide some basic scientific guidelines for this important aspect of pesticide formulation design we are making a systematic investigation of the major physicochemical factors that influence the activation of foliar uptake of compounds by nonionic surfactants, the adjuvant class most widely used as pesticide activators (McWhorter,1985; Hellsten,1987; Mulqueen,1989). Such factors will include the properties of the compound being taken up, the structure and concentration of the surfactant added to the formulation and the nature of the plant species being treated (Jansen et al., 1961; Smith et al., 1966).

This paper describes an experimental protocol for evaluating surfactant performance on foliar uptake enhancement and presents some results obtained for one commercial series of fatty alcohol polyoxyethylene surfactants with a number of neutral radiolabelled candidate compounds. Such experiments have created the possibility of modelling in order to predict the surfactant requirements needed to improve and optimise the foliar uptake of compounds with particular physicochemical properties.

MATERIALS AND METHODS

Plants

Two water repellent plant species, wheat (Triticum aestivum cv. Minarette) and oil-seed rape (Brassica napus cv. Rafal), and two more wettable, less waxy species, field bean (Vicia faba cv. Maris Bead) and chickweed (Stellaria media), were used for investigation. All plants were grown from seeds in pots under the following controlled environment (CE) conditions: 16 h photoperiod, 425 μmol m^{-2} s^{-1}; light 20°C, r.h. 74 - 81%; dark 15°C, r.h. 88 - 93%. Formulations of test compounds were applied to 3 - 4 week-old plants.

Test compounds

Five neutral ^{14}C-labelled compounds covering a range of physicochemical properties were evaluated in uptake activation studies. They were 3-O-methyl-α-D-glucose (log octanol-water partition coefficient (P) ca -3), phenylurea (log P 0.8), pirimicarb [2-dimethylamino-5,6-dimethylpyridin-4-yl dimethylcarbamate] (log P 1.6), cyanazine [2-(4-chloro-6-ethylamino-1,3,5-triazin-2-ylamino)-2-methylpropionitrile] (log P 2.1) and WL110547 [an experimental tetrazole (Kerr & Whitaker, 1987)] (log P 3.6). Candidate compounds were formulated as 0.05% wt/V solutions in acetone-water (1 : 1).

Surfactants

Four C_{13}/C_{14} fatty alcohol ethoxylates (Marlipal 34 series, Hüls, Marl, FRG) were used containing an average of 6, 11, 15 and 20 moles of ethylene oxide (E) per mole of alcohol, respectively. The hydrophobic moeity for the series is an approximately equal mixture of the two alcohols (A) and is comprised of ca equal proportions of normal and methyl branched homologues. The physical properties of the products ranged from liquids (AE6) to waxy solids (AE20) at room temperature. Each surfactant was added to a solution of test compound to give concentrations of 0.02, 0.1 and 0.5% wt/V.

Experimental design

The activation screening procedure for each test compound and plant species involved the application of 12 different treatment solutions (four surfactants each added at three different concentrations) and one control which contained no added surfactant. In this way the influence of surfactant structure and concentration, and the interaction between these two variables, on the uptake of a compound could be determined precisely. Uptake enhancement was assessed 1 d after application and at one other time interval dependent on the rate of uptake of radiolabel from the test compound. A 4 x 3 factorial statistical analysis was used for each screening experiment. Uptake/recovery values and any interactions observed between the main treatment effects were analysed with a Genstat 5 programme.

Foliar applications

Droplets (10 x 0.2 μl, ca 10,000 dpm test compound) were applied to the central region on adaxial surfaces of individual leaves with a microapplicator (Holloway,1986). The entire procedure was carried out inside the CE room and all applications were replicated four times.

Assessment of foliar uptake

Surface deposits of radioactive material were recovered from treated areas of leaves by cellulose acetate stripping and quantified by liquid scintillation counting (LSC) (Silcox & Holloway, 1986). Any radiolabel that had penetrated into treated plants was subsequently determined by combustion-LSC analysis (Harvey OX400 Biological Oxidizer) of the excised cellulose acetate-stripped treated area and, when necessary, of the excised distal and proximal portions of the treated leaves to monitor movement. Uptake or recovery is expressed as a percentage of the radioactive dose originally applied to the leaves.

RESULTS

The factorial experimental design of our investigations allowed results to be plotted in the form of response surfaces. Examples of the surfaces obtained for ^{14}C-methylglucose on wheat and ^{14}C-WL110547 on field bean 1 d after application are shown in Figs 1 and 2, respectively. Surface recovery of radiolabel was used to assess efficiency of activation; troughs in a response surface indicate surfactant structure-concentration combinations with good foliar uptake promoting properties.

Influence of properties of compounds

The physicochemical properties of the compounds tested had most influence on the slope of the response surface in the plane of the x-axis (surfactant E content). At one extreme was the highly water soluble methylglucose, whose uptake was enhanced most by A surfactants of high E content (Fig. 1). At the other extreme was the lipophilic WL110547 the uptake of which was promoted best by low E content A surfactants (Fig. 2). Surfactant structure, however, had much less influence on uptake enhancement of the other three compounds which had log P values intermediate between those of methylglucose and the tetrazole.

Influence of surfactant concentration

Foliar uptake of candidate compounds could be increased significantly by raising the surfactant concentration in the formulation. For ^{14}C-methylglucose (Fig. 1) and ^{14}C-WL110547 (Fig. 2) the effect was linear ($p = 0.001$). However, for other compounds, e.g. phenylurea, it was necessary to exceed a threshold surfactant concentration before there was substantial uptake enhancement. Interactions were also observed in some cases between surfactant structure and concentration. Thus, for ^{14}C-WL110547 there was a strong linear x linear interaction ($p = 0.001$), the preference for low E surfactants for optimal activation becoming more apparent as surfactant concentration was increased.

Influence of plant species

The overall dimensions of the response surface for a particular compound varied considerably according to the plant species treated. Although the vertical component of the surface (percentage recovery/ uptake) was the most strongly influenced, significant effects were also observed on the angle of slope on the other two axes. The waxy species studied were generally more permeable than the less waxy ones investigated, but differences in activation responses between species could not be readily correlated with their cuticular wax content.

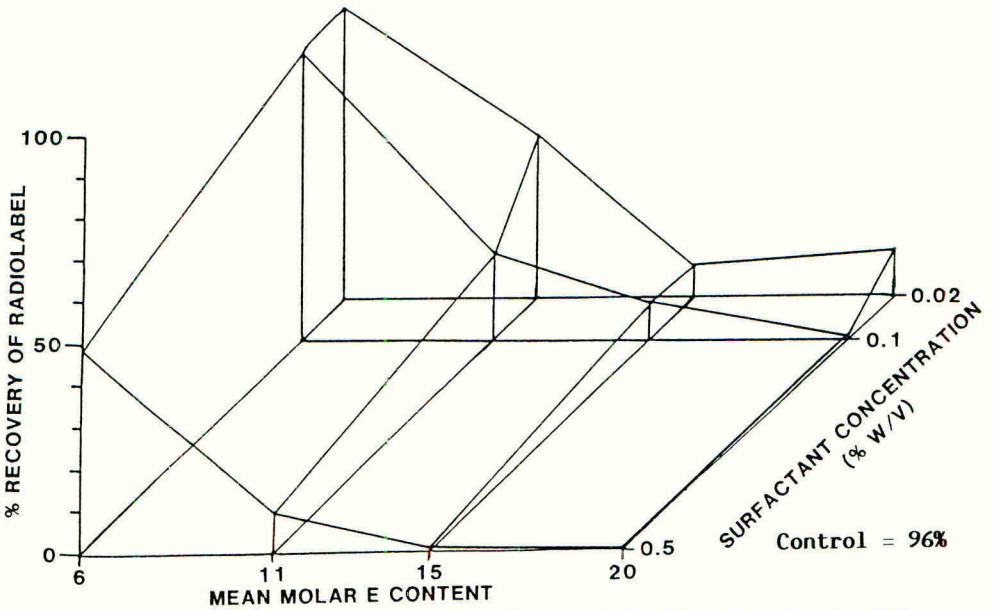

FIG. 1. Response surface for wheat leaves 1 d after treatment with formulations of ^{14}C-methylglucose containing AE6, 11, 15 and 20.

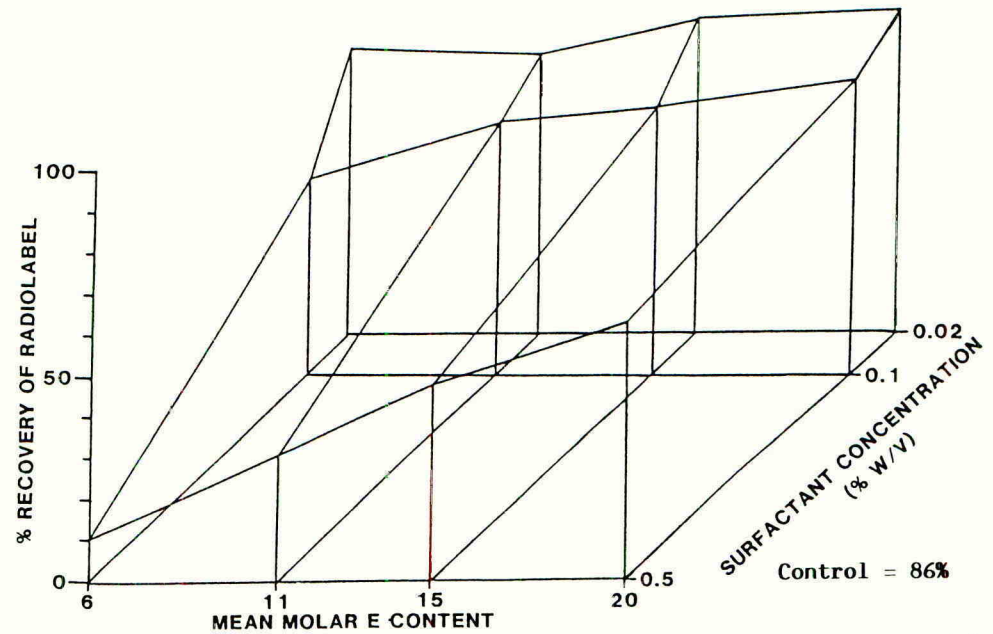

FIG. 2. Response surface for field bean leaves 1 d after treatment with formulations of ^{14}C-WL110547 containing AE6, 11, 15 and 20.

DISCUSSION

Results reported here show the value of adopting a comprehensive screening approach to the evaluation of surfactant effects on the foliar uptake activation of compounds. The complex nature of the interactions that result in uptake enhancement are well illustrated by our data on CE grown plants; further studies on the mechanisms of activation are currently being carried out with radiolabelled surfactants.

Although only a limited number of compounds, surfactants and plant species were examined in the present work, some foundations of a predictive model for surfactant-induced uptake activation can be deduced. The basic model is a response surface similar to that used in our screening experiments, the x-axis describing the physicochemical properties of surfactants in terms of their E content, the y-axis the magnitude of uptake of the compounds and the z-axis the physicochemical properties of the compounds expressed in terms of their log P values. From the data available for C_{13}/C_{14} alcohol surfactants such a model would predict a shift in the requirements for uptake enhancement from high E content for optimal uptake of low log P compounds to low E content for high log P compounds. However, for compounds of intermediate lipophilicity there would be a plateau in the response surface at a critical log P, signifying the lack of a structural requirement for optimal surfactant activation. It would appear that this value is around 1-2 but it can vary according to plant species. Surfactant concentration is the major factor governing the height of the model surface relative to the uptake axis but there is also an important influence of plant species. Although the lack of performance of structurally unsuitable surfactants can often be compensated for by increasing their concentration in a formulation this information is not indicated by our model. However, undesirable phytotoxicity may limit their use in practice. It should also be noted that the model proposed is concerned solely with uptake phenomena; it is possible that enhanced uptake of a compound may not always be translated into an improvement in its biological efficacy. In addition compromises in surfactant selection may be necessary to counteract deficiencies in retention and coverage when formulations are sprayed onto plants.

A greater understanding of relationships between the physicochemical properties of surfactants and those of pesticidal active ingredients in promoting foliar uptake of the latter will not only simplify protocols for optimising formulation performance, but may also lead to improvements in the margins of selectivity, especially for herbicides. The need to extend the range of uses for existing agrochemicals is particularly relevant in the light of current concerns about environmental safety and the more rigorous registration procedures which are increasing development costs for new compounds. This goal could well be achieved with the aid of surfactant adjuvants and a more rational approach to formulation design.

ACKNOWLEDGEMENTS

David Stock is grateful to Shell Research Limited for financial support through a postgraduate studentship. We thank Mrs. J. Hynam for producing the typescript in record time.

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A GLOBAL EVALUATION OF "NEW" HERBICIDE ACTIVITY, 1984 - 1988, ITS CHANGING DYNAMICS AND A LOOK AT ITS FUTURE DIRECTION

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ABSTRACT

Herbicides have been the leading class of Agrochemicals since the late 1960's, in both the monetary value spent on their use by the world's farming industry, and in the number and range of new herbicide compounds Agrochemical research brings into public development. There are questions about the future vitality of "new" herbicide technology due to the maturing state of herbicide usage in the developed countries of the world. This paper reviews "new" herbicide activity with the conclusion that herbicides, will continue their leading role in crop protection, but there may be disparity between the future satisfaction level of some of the major world herbicide markets.

INTRODUCTION

Since the early 1960's, investment in herbicide technology has had the fastest growth rate of any of the disciplines of modern day crop protection (Woodburn *et al.*, 1988). Propelled by the demand of large unexploited world markets and a wide range of novel activity, herbicides have led all other crop protection technology in three significant areas; firstly, the total monetary value spent on their use, secondly, the number of new compounds brought to development and commercialization, and thirdly, in the breadth of numbers of unique new herbicide activity (Hopkins, 1989).

How vital is "new" herbicide technology with a sluggish economic climate for crop protection in developed countries, with most of the world's herbicide markets fully mature, and patents expiring on an increasing number of the current dominant commercial herbicides? Will herbicides continue to maintain their global Agrochemical leadership in the next decade and beyond?

One of the most reliable indicators of the vitality of any technology is the measurement of the continuing expenditures in basic research for the discovery and development of new products. This paper provides a global audit of the world's Agrochemical industries efforts to bring "new" compounds to the market with a particular focus on "new" herbicide activity. This data may answer some of the above questions and at the same time provide some insight to the on going dynamics and future direction of herbicides.

LEVEL OF DISCOVERY OF ALL CLASSES OF "NEW" COMPOUNDS 1984 - 1988

A global audit of "new" crop protection compounds discovered and brought to public testing during the period 1984 - 1988 indicates that herbicides are still the leading class of pesticides being researched by the 75 or so Agrochemical companies and research institutions known to be expending resources for the discovery of "new" products. A count of the industry's five year output including, compounds that became commercial products, those dropped or put on hold, and those still in development, yields a yearly average of 265 "new" compounds in some phase of public testing (Hopkins, 1989).

Of this total, 38% were herbicides, 30% were insecticides, 21% were fungicides, and 11% were all others. This data correlates very closely with the global economic value of each class of use. The 1988 world Agrochemical pesticide market was just over 20 billion dollars (Woodburn *et al.*, 1988), with 44% ($8,925 million) spent on herbicides, 30% ($6,075 million) on insecticides, 21%

($4,200 million) on fungicides, and 6% ($1,250 million) on plant growth regulators, nematicides, and fumigants. This data confirms that in monetary value and in number of "new" compounds, herbicide technology, at least for the present, is maintaining its Agrochemical leadership role.

A GLOBAL PROFILE OF "NEW" HERBICIDE ACTIVITY 1984 - 1988

In the period 1984 - 1988 a total of 170 new herbicide compounds, not including new combinations of existing molecules, have been in some phase of public testing on a global basis (Hopkins, 1989). A more detailed look at the eventual fate of the 170 "new" herbicide compounds, provides the following information:

- 21% of all the compounds in development reached commercial status, yielding 36 "new" herbicide products (an average of about 7 new herbicide products per year).
- The average time for the above 36 compounds to become commercial products was 7.3 years. This was on the average almost a year less than the time taken for "new" insecticides and fungicides to become commercial products over the same period.
- 39% of all compounds in development were dropped or put on hold, yielding 66 casualties (an average of about 13 "new" herbicide compounds dropped each year).
- 40% of all compounds were still in some phase of public development at the end of 1988, yielding 68 hopeful "new" herbicide products for the future (an average of almost 14 "new" herbicide compounds coming from discovery research and entering public testing annually).

Another important criterion to measure the vitality of any class of crop protection activity is the breadth of novel "new" activity entering the development pathway, as compared with activity that is a redundancy or an extension of existing technology. In the 1960's and 1970's, announcements of the discovery of novel "new" herbicide activity was not uncommon. In this period, it appeared finding novel herbicide activity was easier than finding unique activity in insecticides or fungicides. However, with most major herbicide markets reaching maturity and becoming very competitive, and with growth rates leveling off, is there enough motivation for the industry to invest the higher level of resources needed to continue the discovery of unique "new" herbicides at the same level as in the past?

An overview of all known "new" herbicide compounds and products that have been in some phase of development in the past five years and have advanced to a point to have their structure made public (Hisada *et al*, 1986; Thomson, 1989; Worthing, 1987; Shibuya, 1988; Hopkins, 1989) yields some information regarding this question. A profile of the 105 "new" herbicide compounds in development over the past five years, that meet the above criteria indicates:

- approximately 71% of all "new" herbicide compound activity can be classed as redundancies or extensions of existing technology
- approximately 29% of all "new" herbicide compound activity can be classed as unique or semi-unique new technology
- in the same time span, the estimated ratios of redundant to unique insecticide and fungicide compound activity was 79% : 21% and 82% : 18% respectively.

In the authors opinion, examples of some of the more significant novel "new" herbicide activity, with the names of the companies that discovered them, and the approximate date they were first made public (Thomson, 1989, Worthing, 1987) are:

- Cougar, (Diflufenican), Rhone-Poulenc, 1980
- Scepter, (Imazaquin), American Cyanamid, 1981

- Flexidor, (Isoxaben), Elanco, 1982
- Command, (2-(2-Chlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one), FMC, 1982
- Facet, (Quinclorac), BASF, 1984
- Grasp, (Tralkoxydim), ICI, 1985

Some of the more significant redundant "new" herbicide activity (authors opinion) and an audit of number of "new" compounds that were in some stage of development in this period include:

- phenoxy-propanoate type post-emergent grass herbicides-16 new leads
- sulfonylurea type pre- and post-emergent grass and broad leaf herbicides-15 new leads
- anilide type pre-emergent grass herbicides-7 new leads
- diphenyl ether type post-emergent broad leaf herbicides-4 new leads

Of the 170 "new herbicide compounds: 6% were biological in origin, while 94% were chemical (Hopkins, 1989), indicating, that at least for the decade, chemical activity will continue to dominate "new" herbicide technology.

What future market direction does this data suggest for "new" herbicide technology? A look at the leading herbicide markets and the numbers of "new" herbicide compounds being directed at each may provide some insight.

FUTURE GLOBAL HERBICIDE MARKETS AND THEIR ESTIMATED SHARE OF "NEW"HERBICIDE COMPOUNDS AND PRODUCT ACTIVITY

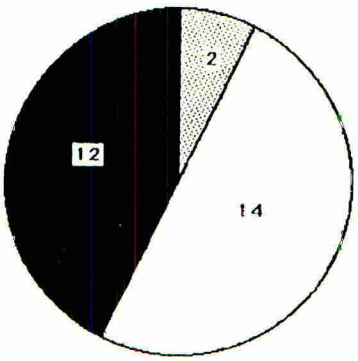
A review of all "new" herbicide compound activity and the markets they appear to be directed toward for the period 1984-1988, provides a market profile for the future and some indication of which may have the greater number of new product alternatives compared to those which could be left unsatisfied. Table 1 shows the top seven global markets for herbicides, in current dollar value, as of 1988, and a projection of their value in 1995, based on an estimated annual growth rate of 1.9% predicted for global herbicide usage in this period (Woodburn *et al.*, 1988).

TABLE 1. The top seven global herbicide markets, their estimated value in 1988, and projected value in 1995.

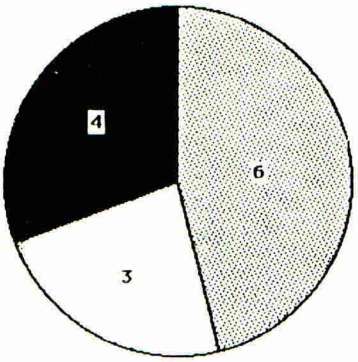
Market	Estimated Value 1988 ($ millions)	Estimated Value 1995 ($millions)
1. Maize	$1,640	$1,835
2. Fruit and vegetables	1,450	1,625
3. Soybeans	1,440	1,610
4. Wheat	1,250	1,400
5. Rice	875	980
6. Sugar beets	475	530
7. Cotton	420	470

Matching the 170 "new" herbicide compounds that were in some phase of public development between 1984 and 1988 with the primary crop(s) this new activity appear to be directed toward, is one way of looking at the priority these markets may share when, and if, they become commercial products. Figure 1 details the estimated dollar value of these markets in 1995, and the approximate number of "new" herbicide compounds (and their current status), directed at each (Hopkins, 1989).

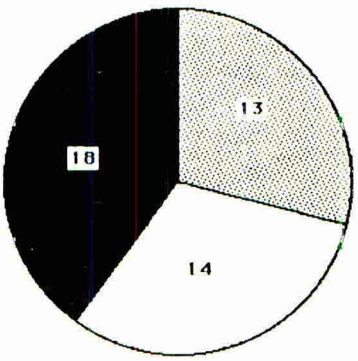
FIGURE 1. Estimated value of the top seven global herbicide markets in 1995 and the approximate number of new compounds that are or were directed at each.



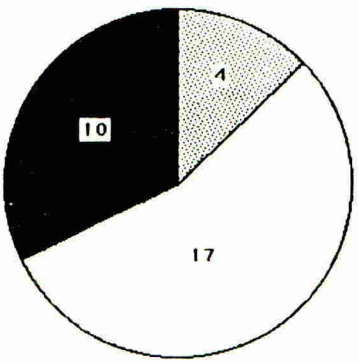
Maize Herbicides
1995 $ Value - $1,835 million



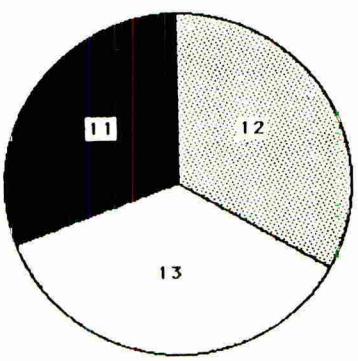
Fruit & Vegetable Herbicides
1995 $ Value - $1,625 million



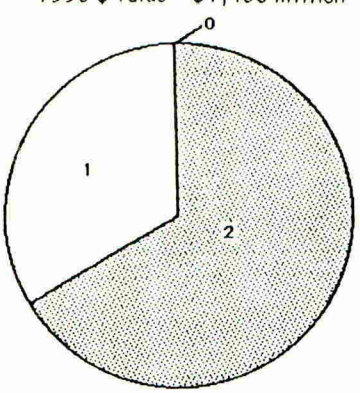
Soybean Herbicides
1995 $ Value - $1,610 million



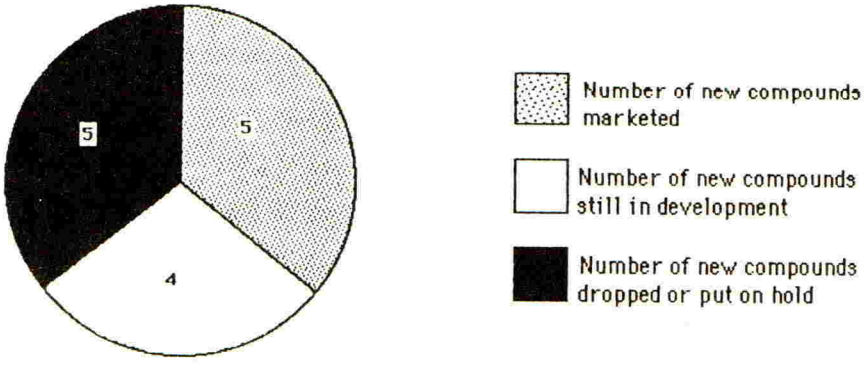
Wheat Herbicides
1995 $ Value - $1,400 million



Rice Herbicides
1995 $ Value - $980 million



Sugar Beet Herbicides
1995 $ Value - $530 million



Cotton Herbicides
1995 $ Value - $470 million

A more detailed look at the type of herbicide activity being directed toward two of these markets (rice and wheat) shows a potential future disparity between the grass weed segments versus the broadleaf weed segments. In rice, of the 36 "new" herbicide leads, 73% were broad leaf activity and 27% grass, in wheat, of the 31 "new" herbicide leads, 75% were for broad leaf control and 25% grass.

CONCLUSIONS

Even in the face of a maturing global market, herbicide technology appears to be able to maintain its leading role in crop protection through the next decade, both in terms of market value and number of "new" compounds discovered and brought to public development. It also appears that there are continuing opportunities to discover novel herbicide activity, as well as expand the several unique compound discoveries that have emerged in the past five years.

For the future, "new" herbicide activity will continue to be dominated by chemicals, rather than biologicals. However, the number of "new" biological leads appears to be increasing as some of the world's markets are finding a need for specialized products to deal with weed problems not managed by standard herbicide practices.

A look at where "new" herbicide compound activity is being directed, indicates two of the world's major markets (rice and soybeans) are receiving a high level of "new" compound priority; three are receiving a moderate level (maize, wheat, and cotton), and two are receiving a relative low level (fruits and vegetables and sugar beets). And within the rice and wheat markets, broad leaf activity appears to be receiving four times the priority of grass weed activity. This could denote some areas of low satisfaction for the latter markets in the next decade and beyond.

The global herbicide industry has made excellent progress in meeting the ever changing needs of the world's farming industry, while at the same time, keeping a concern for its environmental impact by developing safer products and decreasing levels of chemical application. At present, "new" herbicide technology appears to be upholding these standards.

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POSSIBILITY OF CLOMAZONE USE FOR WEED CONTROL IN POTATOES IN POLAND

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ABSTRACT

Small plot experiments and commercial scale trials on weed control in potatoes with clomazone (2-(2-chlorophenyl)methyl-4, 4-dimethyl-3-isoxazolidinone) were carried out in Poland during 1986 to 1989. When clomazone was applied pre-emergence to potatoes in small plot trials at 0.24 kg ai/ha extensive crop damage resulted. However, no crop damage was observed when it was applied in the same trials at 10 days after planting. In commercial-scale trials, clomazone applied at 10 days after planting, either alone or at a reduced rate in tank mix with linuron or metabromuron, similarly caused no crop damage. In all trials situations, clomazone gave good brood-leaf weed control.

INTRODUCTION

In Poland, pre-emergence application of herbicides is the most common method of weed control in the potato crop. Herbicides provide good control of many weed species, when applied to soil with sufficient moisture content.

Clomazone from FMC Corporation is a novel herbicide for the potato crop. The commercial product is an emulsifiable concentrate marketed under the name 'Command 4 EC'.

This paper reports the initial studies on the efficacy of clomazone for weed control in potatoes.

MATERIALS AND METHODS

Clomazone was tested in field experiments at Bonin in Northern Poland, during the years 1986-1989 and in commercial trials located on 12 sites during the years 986-1989.

In the small plot trials (33m2) clomazone was applied at two timings either 10 days after planting or 15 days later which was approximately 5 days before emergence of the potato shoots (pre-emergence). In the large scale trials , application was made only 10 days after planting. Commercial trials were of 0.5-2.0 ha in large fields.

The formulation used in the trials was a 480 g/l EC. When used alone in the small plot trials, clomazone was applied at 0.24 kg a.i./ha while in the case of tank mixes in the commercial trials, the dose of clomazone was reduced to 0.098 kg a.i./ha.

The other herbicides in the tank mixes were linuron (0.5 kg a.i./ha) or metobromuron (1.0 kg a.i./ha). A spray volume of 300-400 l/ha was utilized in both the small plot experiments and the commercial trials. Weed control was assessed through weed counts on 2 x 1m^2 quadrats in the small scale trials and by visual assessments in the commercial trials. Crop tolerance assessments were made using the EWRC scale where 1 = no damage, 9 = total crop destruction.

RESULTS

The quality of weed control resulting from the two application timings of clomazone was similar (Table 1). The small plot trials confirmed the efficacy of clomazone against the following broadleaved weeds : Chenopodium album, Thlaspi arvense, Stellaria media, Galinsoga parviflora, Anthemis spp., Galeopsis tetrahit. Clomazone was less effective against Veronica spp., Viola arvensis, Equisetum arvense, Elymus repens and Polygonum spp.

TABLE 1. Efficacy of weed control with clomazone (0.24 kg a.i./ha) in small plot trials (Bonin, mean for the years 1986-1989)

Weed species	Timing of application	
	10 days after planting	pre-emergence
Anthemis spp.	xx	xxx
G. parviflora	xxx	xxx
S. arvensis	xxx	xxx
T. arvense	xxx	xxx
S. media	xxx	xxx
G. tetrahit	xx	xxx
C. album	xxx	xxx
Polygonum spp.	x	xx
Veronica spp.	x	x
V. arvensis	x	x
E. arvense	0	0
E. repens	0	0

Efficiency : xxx - 95-9) %; xx - 90-94 %; x - 75-89 %; 0 - 0-74%

Tank mixes of clomazone at reduced rates with linuron (0.5 kg ai/ha) or metobromuron (1.0 kg ai/ha) showed an efficiency of weed control similar to that of clomazone alone. (Table 2).

TABLE 2. Efficacy of weed control with clomazone applied alone or in tank mixes (mean for the 12 sites in 1988/1989).

Weed species	Mean % of weed control Clomazone alone (0.24 kg ai/ha)	Clomazone tank mix (0.098 kg ai/ha)
Chenopodium album	96.4	95.2
Thlaspi arvense	97.3	98.1
Stellaria media	95.2	96.8
Equisetum arvense	10.2	8.38
Fumaria officianalis	96.7	94.7
Tripleurospermum inodorum	89.5	81.7
Viola arvensis	75.4	74.9

TABLE 3. Influence of date of clomazone application on yield and crop injury to 3 potato varieties in small plot trials (Bonin, mean for the years 1986-1988)

Treatment	Dose kg ai/ha	Potato varieties Injury (1)			Yield Index (2)		
		San	Uran	Narew	San	Uran	Narew
clomazone 10 days after planting	0.24	1-2	1	1-2	108	108	112
clomazone pre-emergence	0.24	5-7	3-4	5-6	106	111	111
prometryn pre-emergence	2.00	1	1	1	100	100	100

(1) Injury according to EWRC scale where 1=no damage, 9=total crop destruction
(2) Yield index expressed as % of prometryn

Clomazone applied alone at 0.24 kg ai/ha before crop emergence severly injured the potato plants (up to 5-7, EWRC scale) although did not result in any decrease in yield (Table 3). In commercial trials when clomazone was applied 10 days after potato planting, either alone or with other herbicides, injury to the crop was not observed.

DISCUSSION

Four years of small plot experiments have shown that clomazone at the rate of 0.24 kg ai/ha is an effective herbicide for broadleaf weed control in potato crops. There was a high incidence of plant injury following pre-emergence application of clomazone, but potato yields were not significantly reduced. Therefore, as in the commercial trials, clomazone should be applied 10 days after planting. The mechanism of selectivity of clomazone may be based on mechanical separation of potato plant from the chemical

In the year after application of clomazone, injury to the following cereal crop was observed where overlap spraying had occured.

The use of clomazone for weed control in early potato varieties requires further investigation in commercial trials. Additional research effort should be directed towards understanding the relationship between plant injury and crop yield.

THE INFLUENCE OF SOME POST-EMERGENCE HERBICIDES ON THE INCIDENCE OF BLACKLEG AND SOFT ROT IN THE POTATO

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ABSTRACT

The study examines the influence of some post-emergence herbicides on blackleg and soft rot in the potato. In three years of field trials post-emergence herbicides bentazone, metribuzin, quizalofop-ethyl, fluazifop-P and haloxyfop were applied. Most herbicides tended to reduce blackleg incidence and severity of potato tuber soft rot, but only metribuzin significantly reduced numbers of rotted tubers under conducive conditions.

INTRODUCTION

According to our previous investigations (Lewosz and Kowanski, 1986, Lewosz 1987) some herbicides, especially metribuzin applied before the emergence of potato plants, have a tendency to reduce potato blackleg disease and tuber latent infection. This study examined the influence of five post-emergence herbicides on the incidence of blackleg, soft rot and bacterial latent infection.

MATERIALS AND METHODS

In field experiments at Bonin, Northern Poland, in the years 1986-1988, bentazone (Basagran), metribuzin (Sencor 70 WP), quizalofop-ethyl (Targa 10 EC), fluazifop-P (Fusilade S) and haloxyfop (Gallant 125 EE) were tested. In small plot trials with five potato cultivars, treatments were randomized within four replicated blocks. All herbicides were applied after potato emergence. Mechanical cultivation was the control treatment. During the growing season several observations were taken on blackleg incidence. Tuber samples from four plants were randomly taken from each plot. The samples were scored two months after lifting for the incidence of soft rot. Latent bacterial infection was checked in 50 tubers per plot. Tubers were incubated at high humidity in closed plastic bags at 20 to 22°C and the proportion of soft rotting tubers was calculated after five days incubation.

RESULTS

Analysis of variance showed significant differences between cultivars in the incidence of blackleg, soft rot and latent tuber infection. However, the influence of the herbicides on the diseases within cultivars was similar. The herbicides tended to reduce the incidence of blackleg in comparison to mechanical cultivation.

Two months after lifting, the severity of soft rot in stored tubers

was low. In the control 2.2% tubers showed symptoms of soft rot, in herbicide treatments the average was 0.8% tubers infected but the differences between herbicides were not significant. After incubation under conducive conditions 25% tubers were rotting in the control, in the herbicide treated - 22.1%. Only in the metribuzin treatment was the severity of rotting less than in mechanical cultivation (Table 1).

TABLE 1. Influence of herbicides on the incidence of blackleg and soft rot in potato

Treatment	% of plants with black-leg symptoms	% of tubers with soft rot	
		in sample	under conducive conditions
Untreated	1.1	2.2	24.9
fluazifop-P	0.8	0.9	21.4
haloxyfop	0.4	0.2	27.3
quizalofop-ethyl	1.5	0.3	28.2
metribuzin	0.8	0.9	17.6*
bentazone	0.7	1.7	19.6
metribuzin + bentazone	0.6	0.8	18.4

* significantly different from the control

DISCUSSION

Earlier a study on the influence of pre-emergence herbicides indicated that mechanical cultivation in potatoes increased the incidence of blackleg and soft rot. It has been confirmed also that the use of metribuzin decreases the severity of potato bacterial diseases.

In the laboratory tests (Lewosz 1987) there was no direct inhibition of the growth of Erwinia carotovora ssp. atroseptica. It may be suggested that metribuzin either increases the resistance of potato plant and tuber or changes the composition of soil microflora, thus creating conditions unfavourable for the development of soft rotting bacteria.

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SESSION 3D

HERBICIDE RESIDUES IN SOIL

SESSION ORGANISER MR G. C. WHITE

POSTERS 3D-1 to 3D-5

USE OF BIOASSAYS TO CHARACTERIZE THE RISK OF INJURY TO FOLLOW CROPS BY SULFONYLUREA HERBICIDES

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ABSTRACT

After the introduction of the low-dose sulfonylurea herbicides, the need arose for a sensitive, reliable bioassay to monitor soil residues and to predict the risk of injury to follow crops. Traditional analytical methods were of limited value because they could not detect concentrations still herbicidally active in soil to certain extremely sensitive crops. We describe in detail a lentil root bioassay method and how it was used to characterize the risk of injury to follow crops in the field. Data for 11 crops grown after chlorsulfuron treatments made to wheat show that the bioassay conservatively predicts the risk of carryover injury. Sampling must be carefully controlled to ensure that data can be properly interpreted and contamination must be avoided. Bioassays inherently offer an advantage over chemical assays because they directly reflect plant-available herbicide in the soil and do not require an extraction step. Data must be carefully interpreted to ensure proper estimation of risk to follow crops, but are a valuable tool for investigating farmer's complaints.

INTRODUCTION

After the introduction of the first sulfonylurea herbicide 'Glean®' chlorsulfuron in 1983, use rates of many new herbicides have decreased. Although absolute use rates have decreased, relative activity to sensitive follow crops has increased (Beyer *et al.*, 1987). The large difference in sensitivity among crops and weeds has led to problems in some areas with injury to certain subsequent crops.

Because the sulfonylureas were a new class of herbicides and the capability to analyze them using traditional instrument-based analytical methods was strained by their low use rates, a sensitive bioassay was developed to detect chlorsulfuron and metsulfuron methyl in soil. This bioassay serves two purposes: 1) to monitor the dissipation of sulfonylureas in the field, and 2) to predict follow crop injury. Recent advances in analytical technology (Peter *et al.*, 1989), however, have changed the status of bioassays as the most sensitive analytical method.

Herbicide concentrations alone may not allow prediction of herbicide phytotoxicity in the soil (Hance, 1987). Bioassays serve a useful purpose because they give an actual measure of the plant susceptibility. Bioassays are relatively simple to perform, but results are sometimes difficult to interpret. Predicting herbicidal activity in the field using data from soil samples is confounded by soil spatial variability (Hance, 1987), which results in a nonuniform distribution of herbicide residues in the plane across the field and also with depth.

We report the procedures to sample soils, details of a laboratory bioassay, and how the results were interpreted to predict risk of follow crop injury.

SOIL SAMPLING PROCEDURES

Because of the spatial variability of soil properties, soil sampling exerts a major influence on predicting carryover risk to field-grown crops by herbicides. Predictions are only as good as the information obtained from the soil samples. If soil sampling occurs in areas of a field that are lower in herbicide residues than the rest of the field, the prediction of injury risk will be underestimated. To overcome this limitation, an adequate number of samples must be taken and sampling guided by identifiable characteristics within the field. Areas such as headlands, eroded knolls, chalky, saline, alkaline, wet or permanently shaded areas, and northern slopes should be purposefully sampled and considered as high-risk areas.

Farmer's fields must be mapped when sampled to assist in interpreting and correlating bioassay results with field results. Injury ratings from the crop surrounding the sample should be included for complaint fields. We recommend taking two samples from different parts of the field that represent each major type of topography, soil type, and other higher risk area. This procedure increases the confidence in test results. As a general rule a minimum of six samples per field should be taken. With small research plots, no map is needed, however, the soil variability should be considered.

Use a sampling tool which takes samples of uniform diameter. We use a bulb planter because it is easily available. A plunger (e.g , a no. 10 rubber stopper attached to the end of a broom handle) is used to push out the soil from the cylinder. However, some soils (very dry or clayey) are difficult to sample and must be sampled with a sturdier tool. In that case, select a sampling tool that is easily cleaned and maintained. Risk of cross contamination between samples increases in wet soil.

For sulfonylureas sample the 0-15 cm depth. Under conditions where leaching potential is high, an additional depth may be required, i.e., 15-30 cm. Take the 0-15 cm sample first, then carefully enlarge the hole with a shovel before taking the 15-30 cm sample.

Always take control samples first, collecting a minimum of two of the same soil type as the treated field and from an area known not to have been treated recently with any herbicide or known to have herbicide concentrations that will not cause injury to crops. Because other pesticides may interfere with the bioassay, every attempt should be made to obtain a herbicide residue-free control soil sample. When sampling research plots, always begin with the lowest rate of a compound and work up to the highest rate. Remove all soil from the sampling tool between samples. Between fields or different compounds, clean the tool with chlorine bleach (5% sodium hypochlorite).

Because of the high level of activity of sulfonylureas, handle and store sample bags and other sampling equipment and ship samples in a way that eliminates any possibility of herbicide contamination. Since chlorsulfuron is active at concentrations below 1 ppb, very small amounts can contaminate and ruim a bioassay. Do not store bioassay sampling supplies in the same room where any herbicide is stored or handled. Keep samples in a cool place out of direct sunlight while in transit from the field. If samples cannot be shipped within 24 hours of collection, they are stored in a freezer until they can be shipped.

Samples do not need to be frozen or shipped in dry ice, if they arrive within two days of shipment.

BIOASSAY PROTOCOL

Bioassay soil samples are received and stored at 4° C until assayed. The soil from one sample bag is placed into a 800 ml plastic pot. Ten lentil (*Lens culinaris* L. 'Chilean') seeds are placed evenly on the soil surface, lightly tamped into the soil, and covered with approximately one-half centimeter of soil. A 10 gram subsample of soil is measured for pH.

The pots are placed into cabinets which maintain the rooting zone at a constant temperature of 18° C. The cabinets are themselves located within a growth room where the day temperature is maintained at 24° C and reduced to 18° C at night. During the observed 16-hour photoperiod, a minimum light intensity of 400 $\mu E/M^2/sec$ is provided.

The samples are watered as needed on a daily basis for a standard 21 day test period. Seven days after emergence, the plants are thinned to six plants per pot. On the 21st day the roots are extracted from the soil and excess soil is carefully washed off with water.

The roots are evaluated by comparing the growth of the secondary and tertiary roots of the treated samples to that of the untreated samples. Length, number, and abnormal appearance of the roots are characteristics incorporated into the injury rating. Injury is expressed as a percentage of the untreated sample.

MATERIALS AND METHODS

Sampling techniques outlined above were used for small plot studies with alfalfa (*Medicago sativa* L. 'Ladak 65'). Test sites were located in Great Falls, Power, Fort Benton, and Creston, Montana with soil pH ranging from 7.04 to 8.10, and soil textures were a sandy loam (2,3% OM), , sandy loam (2,3% OM), clay loam (1.7% OM), and a clay (4.7% OM), respectively. Either 8.75 or 17.5 g a.i./ha of chlorsulfuron were applied 23 or 43 months prior to planting to alfalfa between 29 April and 1 May 1986. Injury to alfalfa foliage was visually evaluated 82 to 92 days after planting. None of the 43-month plots showed injury. Soil samples were taken on the planting date and sent to our laboratory to be analyzed by the bioassay procedure.

Results of field evaluations were collected from the Pacific Northwest and northern Great Plains of the US for 11 crops (235 observations) grown 12-36 months after chlorsulfuron treatments. The crops included alfalfa, spring barley (*Hordeum vulgare* L.), clover (*Trifolium spp.*), flax (*Linium usitatissiumum* L.), lentils (*Lens culinaris* Medic.), peas (*Pisum sativum* L. *var. Arvense* Poir.), potato (*Solanum tuberosum* L.), safflower (*Carthamus tinctorius* L.), sorghum (*Sorghum bicolor* (L.) Moench), sugarbeet (*Beta vulgaris* L.), and sunflower (*Helianthus annuus* L.). Soil samples were collected and bioassayed with lentils.

A wheat field in Tekoa, Washington, treated in April, 1985 with 17.5 g a.i./ha of 'Glean®', was planted to lentils in March, 1986 and sampled according to the protocol above. Lentil roots from plants adjacent to sampling points were dug up and evaluated.

The distribution of injury within a field (Fig. 2) illustrates the spatial variability of herbicide injury found in a field in Washington and is typical of other fields. Bioassay injury was highest in the hilltops (samples 5 & 6), where the

TABLE 1. Prediction of Risk of Injury for 11 Crops by Chlorsulfuron.

ACTUAL CROP INJURY	PREDICTED RISK		
	NONE	LOW	HIGH
	% of observations		
HIGH (>25%)	0	4	56
LOW (>10-25%)	1	25	24
NONE (0-10%)	99	71	20
TOTAL	100	100	100

TABLE 2. Distribution of Lentil Root Injury by Residual Chlorsulfuron in a Field in Tekoa, Washington.

SAMPLE NUMBER	LENTIL ROOT INJURY		TOPOGRAPHY
	BIOASSAY %	FIELD %	
1	20	0	LOW AREA, HEADLAND
2	50	0	LOW AREA, HEADLAND
3	0	10	RIDGE
4	0	10	RIDGE
5	50	25	HILL TOP
6	35	25	HILL TOP
7	10	10	MID-RIDGE
8	20	10	MID-RIDGE
9	10	0	LOW AREA
10	10	0	LOW AREA
11	0	10	MID-RIDGE
12	0	10	MID-RIDGE
13	10	0	LOW AREA
14	10	0	LOW AREA

soil remained drier and the herbicide did not degrade as quickly as in other parts of the field (Table 2). Field-grown lentil root injury was less at this location than that in the bioassay. Bioassay injury was also observed in samples 1 & 2, from a low area and headland. Herbicide treatments commonly overlap in headlands and may have occurred here. Although lentil root injury was seen in the bioassay samples, field-grown lentils did not show injury at this sampling location.

RESULTS AND DISCUSSION

To use bioassays to predict risk of injury to follow crops, bioassay injury results from soil samples must be correlated with field-grown crop injury. An example of this is shown for alfalfa grown in Montana (Figure 1). We considered 0-9% alfalfa injury as 'none', 10-24% alfalfa injury as 'low', and 25% or greater alfalfa injury as 'high'. The 'low' crop injury category corresponded to marginally acceptable injury and the 'high' crop injury catetory corresponded to unacceptable injury. Residual levels of chlorsulfuron causing lentil root injury of up to 30% could be tolerated with no apparent injury to alfalfa. Chlorsulfuron residue causing 40% lentil root injury resulted in 20% alfalfa injury. At lower levels of injury, the lentil root bioassay conducted in a growth chamber was more sensitive than alfalfa grown in the field. This was the case for the other crops used in this study.

At higher levels of injury this relationship did not hold. Greater alfalfa injury was observed in the field when lentil root injury exceeded 60%. By setting the risk threshhold for unacceptable crop injury at a relatively low level (25%), we can prevent an underestimation of field crop injury risk by a bioassay species.

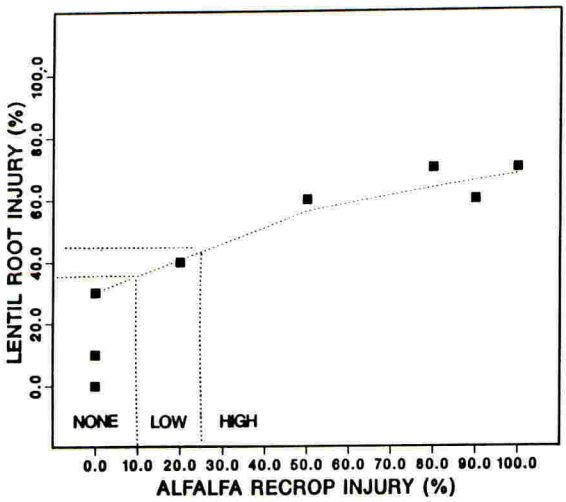

FIGURE 1. Correlation of Bioassay Injury with Field-Grown Alfalfa Injury from Residual Chlorsulfuron.

For a variety of other crops, the bioassay overestimated the risk of injury in the field (Table 1). When no injury risk was predicted by the bioassay, only 1% of the plots showed low injury, and none showed high injury. When low injury risk was predicted by the bioassay, 71% of the plots showed no injury, 25% showed low injury, and 4% showed high injury. When high injury risk was predicted by the bioassay, 20% of the plots showed no injury, 24% of the plots showed low injury, and 56% of the plots showed high injury. This illustrates the conservative nature of the predictions made with the bioassay, a desired characteristic when these data are used to predict the risk of follow crop injury.

CONCLUSIONS

If bioassay results are correlated with field results and spatial variability is accounted for in sampling, bioassays can be effectively used to predict follow crop injury. We have found the lentil root bioassay to be a sensitive indicator of injury risk to field-grown crops with a comfortable safety margin. This is especially valuable when investigating field complaints, where sampling often follows the appearance of crop injury.

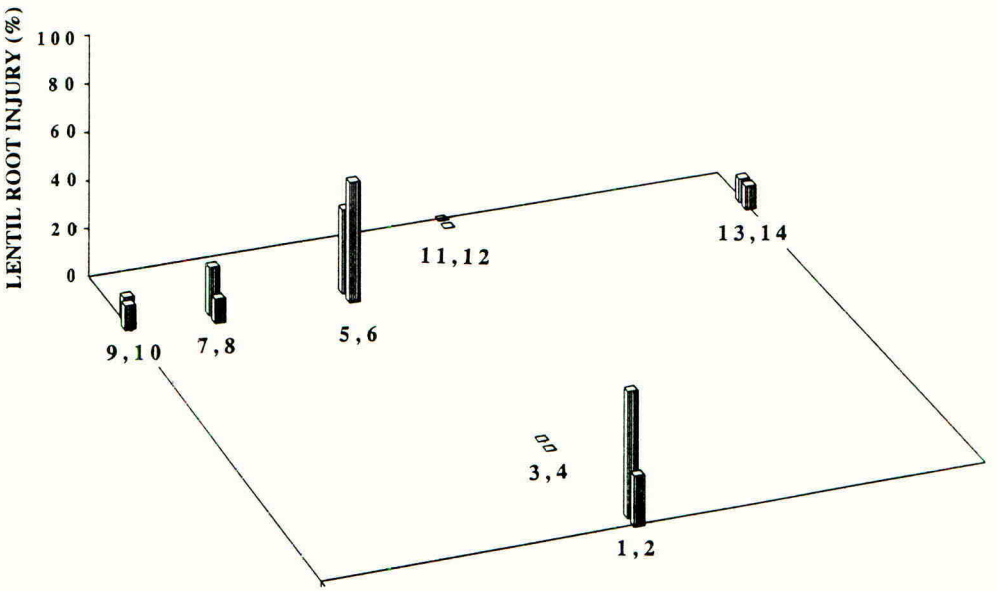

FIGURE 2. Distribution of Bioassay Injury across a Field in Tekoa, Washington.

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IMAZAQUIN RESIDUE AND PERSISTENCE IN A HUMID TROPICAL SOIL

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ABSTRACT

The study evaluated two bioassay techniques (Emergence and Seedling growth) using five tropical crop species for the detection of imazaquin (2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-3-quinoline carboxylic acid) residue in a humid tropical soil. The persistence and downward movement of imazaquin was, thereafter, monitored in the early and late cropping seasons of the humid tropics using the standard curve developed from the bioassay.

Results showed that the five crops used in the emergence bioassay were not sufficiently sensitive for the detection of imazaquin residue in the soil. On the other hand, in the seedling growth bioassay, sorghum (Sorghum bicolor L) and corn (Zea mays L.) were most correlated to imazaquin residue in the soil, their shoot length and dry weight were the parameters with the least $GR_{(50)}$ values. Using the standard curve derived from the corn shoot bioassay, imazaquin at a field rate of 150g a.i./ha was observed to persist for only 8 weeks after treatment (WAT) in the late season and 10 WAT in the early season implying that susceptible crops like corn and sorghum can thereafter be safely planted.

INTRODUCTION

Imazaquin is a relatively new herbicide registered for the control of weeds in soyabean, however, research at IITA, Nigeria (Poku and Akobundu 1985), has confirmed its potential in Nigeria for the control of weeds in Soybean and Cowpea. Being relatively new, its persistence has not received much attention. Information on the persistence of imazaquin in soil is of importance, especially to a grower applying imazaquin to Soybean or Cowpea with plans to rotate the following season to corn. Information on the lateral and downward leaching of imazaquin is also important in order to know the potential for ground water contamination. Because imazaquin is relatively new, methods for the detection of its residue in the soil have not been standardised.

This paper reports the results of bioassay studies for the detection of imazaquin residue and its persistence in a tropical soil. Details of the individual experiments are being published in other journals.

MATERIALS AND METHODS

Bioassay

Two bioassay experiments (Emergence and Seedling growth) were conducted in the screenhouse of the Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife between January and October, 1987.

In the emergence bioassay, six imazaquin concentrations corresponding to 25, 50, 75, 100, 150 and 300 a.i./ha were used in addition to a control. Each pot had a diameter of 28 cm, a depth of 24 cm and a surface area of 0.062 m^2. The pots were filled with sterilized field soil, with a pH of 6.1 and organic matter content of 1.11%, an alfisol of the Oba series (Harpsted, 1973). Five tropical test crops, rice (Oryza sativa Var. 9732), Okra (Abelmoscus escalentus var. sekona), sorghum (Sorghum bicolor L. Var. SRN 484), tomato (Lycopersicum esculentum L. Var. 32A IFE II) and corn (Zea mays L. Var. Western Yellow) were pre-germinated in wooden trays filled with sawdust. Five germinated seeds of each crop, with a radicle length of 1 - 3 mm, were selected and planted in each pot to a depth of 2.5 cm and covered lightly with soil prior to herbicide applicaton. The amount of herbicide needed for each rate was calculated and applied with an atomizer. In the case of the control, only 500 ml of water was added to the pot. Each pot was watered every other day with 500 ml of water. Emergence counts were carried out every other day from planting until the time when 100% emergence was obtained in the control. This was on the average, 6 days after planting for rice, 3 days for sorghum and maize, 5 days for okra and 7 days for tomato. In the Seedling Growth Bioassay, pre-germinated seeds of the five crops used in the emergence bioassay were allowed to grow to a 2-leaf stage for the seedling growth bioassay. Five seedlings of each crop were transplanted into a plastic pot of the same dimension as the emergence bioassay pots prior to herbicide application. Four replications per crop were used for the same herbicide concentrations as in the emergence bioassay and similar cultural operations were carried out. Each bioassay was repeated.

The data collected, included shoot length, root length and dry weight, were subjected to analysis of variance, stepwise multiple regression and correlation. The data presented is the reduction in growth of each parameter as a percentage of the corresponding control. The transformation equation used is as given (Groves and Forster, 1985). Dose-response curves based on percent growth reductions and LOG_{10} of imazaquin concentrations were plotted for all the parameters evaluated for each crop using the quadratic equations. Values of GR_{50} (amount of herbicide required to produce 50% growth reduction as compared with the control) were determined from the dose-response curves. Co-efficient of determination (R^2) was used to identify the plant part that correlated most with the residue level, while the GR_{50} values were used to identify the most sensitive plant species. A stepwise multiple regression of the growth reduction in shoot length and shoot dry weight of maize and sorghum, and the LOG_{10} of imazaquin concentration was used to establish standard curves for a quantitative determination and/or prediction of imazaquin residue in the humid tropical soil.

Persistence

This experiment was conducted at the Teaching and Research Farm of Obafemi Awolowo University, Ile-Ife in the late (September - December), and early (April - July) cropping seasons of 1987 and 1988 respectively. The weather conditions at the farm (Lat. 7°28'N, Long. 4°33'E and altitude 244M) during the periods of the experiment in both seasons were typical of the pattern in the tropical rain forest belt of the humid tropics. The experimental plot had been in fallow for the previous eight years; the land was cleared, ploughed twice and harrowed with a tractor mounted disc plough or harrow. The soil, an alfisol, had similar characteristics to that used for the bioassay study. In both seasons, the experiment was on a 15 m x 37 m plot containing three replicates of 15 m x 11 m each and 2 m strip separated each replicate. Each replicate was further divided into four plots of 11 m x 3 m each. Three rates of imazaquin, viz: 100, 150 and 300 g a.i./ha and a control were randomized among the four plots of each replicate. The experiment in the early season of 1988 was on an adjacent but similar plot. In each trial, the rates of imazaquin were sprayed on the 11 m x 3 m strip in each replicate using a portable pressurized sprayer previously calibrated to deliver 200 litres of spray solution per ha at a pressure of 2 - 3 kg/cm^2. The spraying was done on 20th September 1987 and 10th April 1988 for the late and early season trials respectively. Soil samples were collected from a randomly selected area of 1 m x 1 m on each of 11 m x 3 m herbicide treated strips and the control in the three replicates for a total of 36 samples on each sampling date. Each soil sample was taken with a hand trowel to a depth of 0 - 7.5 cm in the first 1 m x 1 m plot and 7.5 - 15 cm in the second plot and 15 - 22.5 cm in the third plot. The soil samples from each depth were thoroughly mixed separately and put in cellophane bags for the screenhouse bioassay. In the screenhouse, each bag was transferred into plastic pots measuring 5 cm diameter and 7 cm depth.

For the screenhouse bioasssay, corn (Zea mays L. Var. Western Yellow) seeds were sown at 100 seeds per wooden tray, measuring 45 cm x 35 cm x 7.5 cm filled with steam pasteurized greenhouse soil. Watering was done regularly every other day for six days, a period found by an earlier bioassay study to be sufficient to produce suitable corn seedlings for the bioassay. Subsequent corn seedlings were produced for each bioassay by seeding corn six days before a set of soil samples were collected. Two uniform corn seedlings were then transplanted into each pot containing herbicide treated soil samples and the control. Shoot length of the bioassay seedlings was determined at the end of two weeks, a period determined from the consistent previous bioassay. The experiment was a split-split plot design with a randomized complete block arrangement. The data collected were analysed statistically and the means were compared using the least significant difference (LSD) test at the 5% level of probability (Steel and Torrie, 1980). Data on shoot length and shoot dry weight were expressed as per cent of the control. These values were used to determine the residue level of imazaquin, from a standard curve developed from the earlier bioassay experiment conducted with the soil of the experimental soil (Fig. 1). Stepwise multiple regressions were carried out between the residue level and the time of sampling for each sampling depth. Disappearance and mobility curves obtained by corn shoot length for the late and early season trials were plotted.

RESULTS AND DISCUSSION

Bioassay

There was no consistent progressive emergence inhibition in any of the crops tested at the different rates of imazaquin. Thus, emergence inhibition cannot be a good indicator of imazaquin residue in this tropical soil. The use of crop emergence as a bioassay for the detection of herbicide residue in the soil has not been commonly reported (Brattain et al., 1982, Nyfeller et al., 1982). However, crop emergence inhibition could serve as a quick and reliable bioassay for detecting the presence of imazaquin in the soil if a sensitive crop is ever identified.

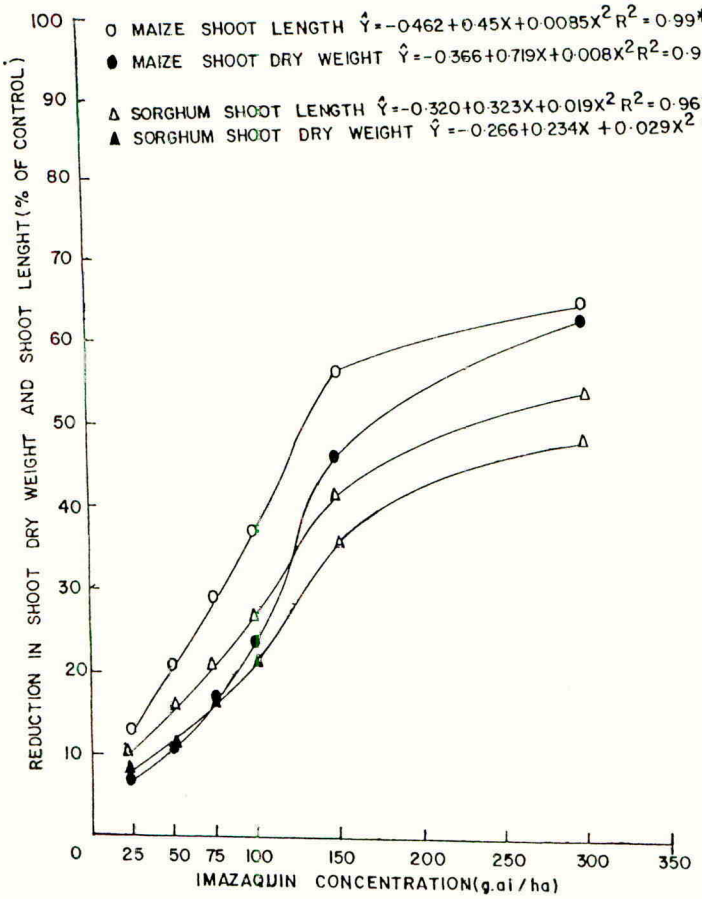

fig.1 REDUCTION OF MAIZE AND SORGHUM SHOOT DRY WEIGHT AND SHOOT LENGTH IN RESPONSE TO DIFFERENT CONCENTRATIONS OF IMAZAQUIN.

**Significant at 1% level of probability
*Significant at 5% level of probability

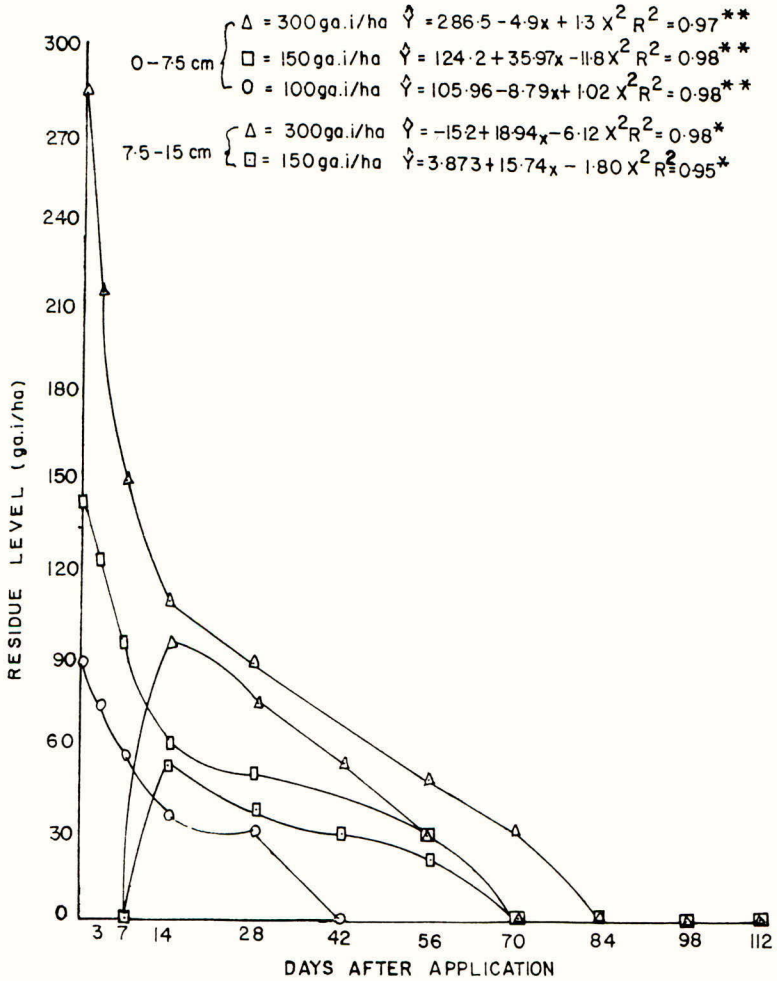

fig. 2 **DISAPPEARANCE AND DOWNWARD MOVEMENT OF IMAZAQUIN IN A TROPICAL ALFISOL IN THE LATE CROPPING SEASON.**

**Significant at 1% level of probability

*Significant at 5% level of probability

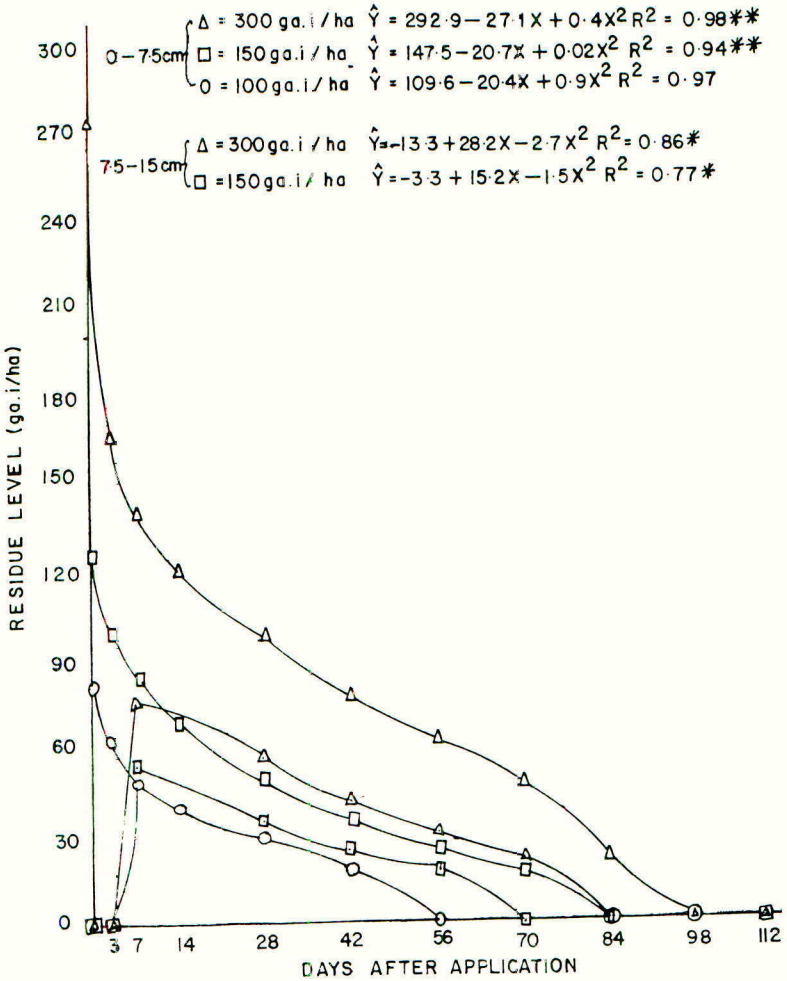

fig. 3 DISAPPEARANCE AND DOWNWARD MOVEMENT OF IMAZAQUIN IN A TROPICAL ALFISOL IN THE EARLY CROPPING SEASON.

**Significant at 1% level of probability

*Significant at 5% level of probability

The results of the seedling growth bioassay showed that the $GR_{(50)}$ for the shoot length in both maize and sorghum was 150 g a.i./ha, while the $GR_{(50)}$ for root length and shoot dry weight in maize were 165 g a.i./ha and 185 g a.i./ha respectively. The $GR_{(50)}$ for the other crops and parameters were generally higher or above the range of concentrations of imazaquin tested. Thus the shoot length in maize and sorghum, and shoot dry weight in maize are considered the most sensitive parameters for the detection of imazaquin residue in this tropical soil. Renner and Meggitt, (1987) and Basham et al., (1987) have similarly confirmed the suitability of corn for the detection of imazaquin residue in soils.

The best fit standard curves observed from the stepwise multiple regression of the reduction in shoot length and dry weight of maize and sorghum on the different concentrations of imazaquin are presented in Figure 1. The curves are similar to the typical general dose-response curves in herbicide studies (Akobundu et al., 1975, Finney, 1947). These curves can be used to predict quantitatively or determine the residue level of imazaquin on a tropical soil of similar properties to that used in this study.

Persistence

Imazaquin at the recommended rate of 150 g a.i./ha persisted for eight weeks after application (WAT) in the late season but 10 WAT in the early season in the top 0 - 7.5 cm of the soil (Figures 2 and 3). This indicates that effective weed control following imazaquin application in the tropical environment can be expected beyond the critical growth period of most tropical arable crops. Sensitive crops can safely follow, if planted ten weeks after imazaquin application. Utulu et al., (1986); Akinyemiju et al., (1986) have similarly reported the short persistence of several herbicides in the humid tropics. In this study, persistence of imazaquin was shorter by two weeks in the late season probably due to the differences in climatic factors of the two seasons. Doubling the rate of application prolonged the persistence of imazaquin in both seasons suggesting that great caution must be taken in the application of imazaquin in order to avoid any carry over problems. However, the persistence at both recommended and double the recommended rates, and in the two seasons in this study was still 10 - 12 weeks shorter than that reported for imazaquin at similar rates by Basham et al., (1987) on three Arkansas soils in USA.

Imazaquin at the 100 g a.i./ha rate did not reach to the 7.5 cm - 15 cm soil depth and in addition, irrespective of rate of application no residue was detected at the 15 cm - 22.5 cm soil depth indicating that contamination of ground water is not likely to occur due to the application of imazaquin for weed control on this tropical soil.

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REFINEMENT OF TERRESTRIAL MICROCOSMS FOR EVALUATING FATE AND EFFECTS OF CHEMICALS

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ABSTRACT

A cost-effective terrestrial microcosm approach is presented for evaluating potential ecosystem effects of agricultural and other chemicals. Intact soil cores stored in the greenhouse under controlled conditions were used to test two ecotoxicologically well-known substances, Lindane (80% τ-HCH) and Na-Pentachlorophenol (PCP) at two concentration levels. The distribution of Lindane in different subsystems of the model-ecosystem and the microbial biomass were chosen as parameters to describe the effects of the test materials during an exposure period of 12 weeks. At the end of the test period, about 13% of the total Lindane applied was found in the top soil layer, 0.3% in plants, and about 0.03% in leachate. The bioaccumulation factor for plant to soil increased with increasing exposure concentrations of Lindane. The microbial biomass was not affected by either of the two test substances after 12 weeks, although the substrate induced soil respiration was enhanced when PCP treated soil was exposed to laboratory conditions for 6 days.

INTRODUCTION

National legislation and International agreements are the driving forces for the development of laboratory and field tests to reduce hazardous effects when releasing agricultural and industrial chemicals to the environment. Laboratory test methods usually include fairly simple test systems which are reproducible, but not representative of ecosystems. On the other hand, field tests do represent ecosystems, but their results are difficult to reproduce, costly and time consuming.

An alternative approach that attempts to represent complex ecosystem-level processes cost effectively in the laboratory is the use of microcosms, or laboratory model ecosystems. A microcosm has been defined as a small, controlled laboratory system that closely mimics the processes and interactions of a larger ecosystem (Gillett and Witt, 1979). Both terrestrial and aquatic microcosms of various size and complexity have been used to study the fate and effects of pesticides and other complex compounds (Metcalf, 1977; Cole et al., 1976; Gillett and Gile, 1976).

Battelle's terrestrial microcosm program was started at Columbus in 1978 by using fly ash as test material (Van Voris et al., 1982; Tolle et al., 1983). This research was extended to include evaluations of the fate and effects of scrubber sludge (Van Voris et al., 1984) and phosphorus smokes (Tolle et al., 1988). Recently, the terrestrial microcosm has been employed in evaluating the fate of soil-applied genetically engineered microorganisms (GEMs) (Bentjen et al., 1989). Depending on the type of ecosystem the microcosm was attempting to mimic, it contained natural grassland vegetation or was seeded with a mixture of plant species that were capable

of field-representative growth in the confines of the small surface area of the microcosm. The parameters monitored included nutrient loss in leachate, biomass yield, element uptake in plants, soil microorganism respiration, and analysis for GEMs in leachate, soil, and plants (Van Voris et al., 1982; Tolle et al., 1983, 1985, 1988, 1989; Bentjen et al., 1989).

Battelle Frankfurt recently has refined the terrestrial microcosm approach by improving methods used to store soil core microcosms in the greenhouse and by expanding monitoring endpoints to include mesofauna and photosynthetic capacity (Knacker et al., 1989).

The objective of this report is to show some data of a study performed at Battelle Frankfurt on the fate and effects of Na-Pentachlorophenol and Lindane when applied to the terrestrial soil-core microcosm. The ecotoxicologically well-known test materials were selected in order to compare results described in this study with previously published laboratory studies. Possible effects on the model ecosystems were determined by measuring the photosynthetic capacity of single leaves from plants grown naturally on the soil core, plant biomass yield, leaf area growth, nutrient content of the soil and leachate, soil respiration, microbial biomass, mesofauna, and the distribution of the test material in different subsystems of the microcosms. In this report, however, first results will be shown on the fate of Lindane in the microcosm and on the effects of both test materials on soil respiration. Further results will be presented elsewhere.

MATERIALS AND METHODS

Design and extraction of terrestrial microcosms

The terrestrial microcosms (length: 60 cm; diameter: 18 cm) were extracted from a brown soil of a natural grassland (Arrhenatheretum elatoris association) without effecting the layering of the soil by using a specially fabricated, steel driving tube and a hydraulic ram. The steel driving tube was designed so that the high density polyethylene (HDPE) tube fitted inside and rested on the top lip of the stainless steel cutting edge. The hydraulic ram was used to slowly push the driving tube into the ground and to remove it from the ground using chains attached to handles on the driving tube.

The soil cores encased in the HDPE tubes were transported to the greenhouse and placed in temperature controlled wooden containers. The microcosms rested on a chemically inert gauze in a porcelain funnel that was connected to a leachate collection bottle. Microcosm maintenance involved checking plants for signs of stress, watering plants, collecting leachate, recording greenhouse temperature, light, and humidity, harvesting plants, and insect control.

After an adaptation period of four weeks 0.86 mg (Lindane L) and 4.28 mg (Lindane H) Nexit Stark containing 80% γ-HCH (Celamerck GmbH, D-6507 Ingelheim) and 21.4 mg (PCP L) and 106.9 mg (PCP H) Na-Pentachlorophenol (Fluka AG, CH-9470 Buchs) were applied. The values given are the amounts of test material applied per microcosm; eight microcosms were used for each of the four treatments and the control.

Residue analysis of Lindane

To measure the content of Lindane in leachate, plant and soil samples were taken from each of the eight microcosms per treatment and combined to either two or four samples. The soil samples measured before Lindane application were collected

in the field from the edge of the holes where the soil cores had been taken out.

Lindane was extracted from plant material, the top soil layer (20 cm), and leachate by using dichloromethane. Plant extracts were purified by using silicagel, while the other extracts were analyzed without a purification step on a gas chromatograph-masspectrometer (GC 5890 A/5970 Mass Selective Detector, Hewlett Packard) according to a modified method described in DEV (1984). The recoveries were 25% for plant material, 115% for leachate, and 95% for soil. Each value shown is an average of either two or four measurements.

Soil respiration and microbial biomass

To estimate the microbial biomass and to determine the relative amount of the bacterial and fungal biomass in the soil treated with Lindane and PCP the substrate-induced respiration (SIR) method was used (Anderson and Domsch, 1973; 1978). Soil samples were taken from the top layer (20 cm), passed through a 2 mm sieve and stored at 4–6 °C. To calculate the microbial biomass, the initial CO_2 production which was released by the soil samples in response to glucose-induced respiration was measured. The soil samples measured before test substance application were collected in the field from the edge of the holes where the soil cores had been taken out. Each value shown is an average of four measurments; each measurement is based on a sample collected from two microcosms.

Additionally, the glucose-amended respiration of the soil was determined under laboratory conditions. These soil samples were taken from the top layer (20 cm) of untreated microcosms and exposed to Lindane (L: 5.4, H: 26.8 mg/kg) and PCP (L: 107, H: 535 mg/kg) at levels which corresponded to 50% of the test material amount applied to the soil cores. The samples were placed on petri dishes in a 2 cm deep layer and stored at 22° C in the dark. Values shown are averages of three samples per treatment.

RESULTS AND DISCUSSION

The results of the residue analysis of Lindane are shown in Table 1 and Fig. 1. The absolute amount of Lindane found after 12 weeks was about 13% of the total amount applied assuming that the average weight of the 20 cm soil-core top layer was 5.1 kg. This value is rather low when considering that the degradation time (DT)-50 is expected to be 15–53 weeks and the bulk of Lindane should be adsorbed by the upper 4 cm of the top soil layer (DFG, 1982). However, to collect leachate, additional water was added to the microcosms which may have caused some transport of Lindane to other soil layers and produced temporary anaerobic conditions which accelerate the degradation of Lindane (DFG, 1982). Lindane is not entirely immobile in the soil since low amounts of it were detected in the leachate. The amount of Lindane measured in the leachate during the test period of 12 weeks was about 0.03% of the total for both treatment levels.

The bioaccumulation factor for plant to soil was calculated as being 1.38 and 1.87 for Lindane,L and Lindane,H, respectively. These values do not confirm results obtained (Bacci and Gaggi, 1986), who assume that τ-HCH is not taken up by roots. Adding together the amounts of Lindane detected after 6 and 12 weeks about 0.3% of the total was found in plants in each of the two treatment levels during the exposure period.

TABLE 1. Content of Lindane (µg/kg) in the leachate, plant and soil over a 12 week exposure period.

Treatment	Lindane (µg/kg)				
	0	4	6	8	12
Leachate					
Control	0	0	-	0	0
Lindane L	0	0.32	-	0.35	0.14
Lindane H	0	2.88	-	1.01	0.47
Plant					
Control	0	-	0	-	0
Lindane L	0	-	71.6	-	22.0
Lindane H	0	-	449.2	-	180.0
Soil					
Control	0	-	-	-	0
Lindane L	0	-	-	-	16.9
Lindane H	0	-	-	-	96.5

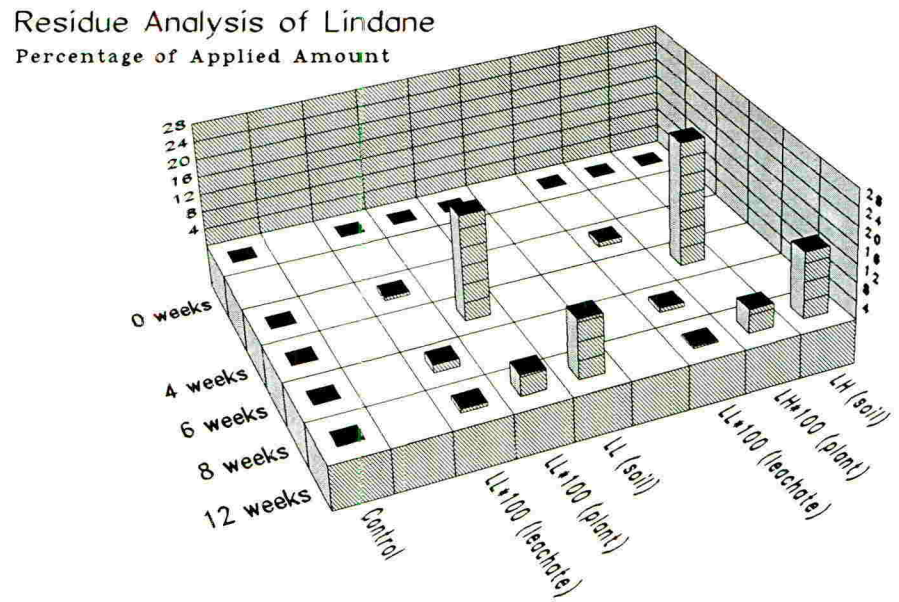

FIG. 1. The relative amount of Lindane in the leachate, plant and soil. Note in plant and leachate the amount increased by factor of 100.

The data shown in Table 2 demonstrate that there is no statistically significant difference in soil microbial biomass between control and treatments after a exposure period of 12 weeks. The amount of microbial biomass measured was within the range of 12.8 – 210.7 mg/100 g determined for several soils in central and northern Europe including grasslands on brown soils (Domsch et al., 1979). The ratio of bacterial to fungal respiration was about 25% to 75% before the application of the test materials and was not changed at the end of the test period.

TABLE 2. Microbial biomass of soil core microcosms over a 12 week exposure period.

Treatment	Microbial Biomass (mg/100 g soil)			
	0 week	S.D.	12 week	S.D.
Control	51.5	± 5.3	69.6	± 9.0
Lindane L	55.2	± 8.8	74.0	± 11.4
Lindane H	53.4	± 3.8	63.2	± 16.6
PCP L	59.0	± 3.6	73.6	± 11.6
PCP H	60.6	± 3.5	67.6	± 10.8

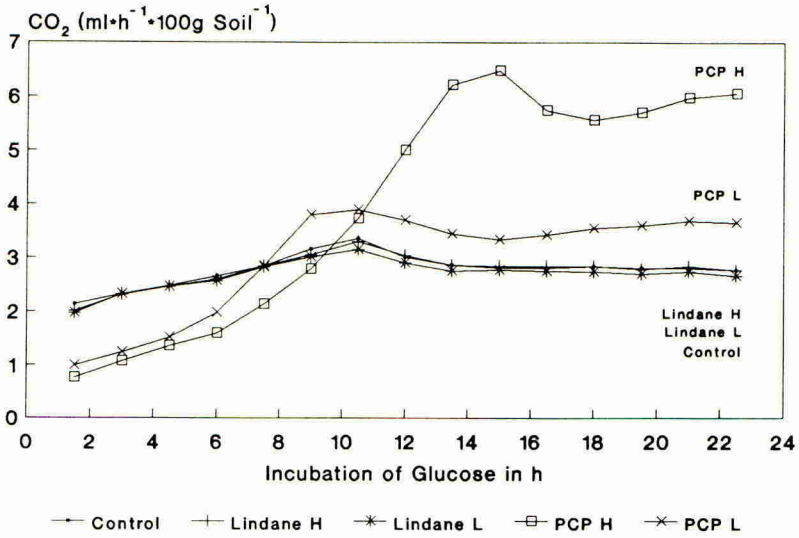

FIG. 2. Glucose-induced respiration of the soil exposed to Lindane and Na-Pentachlorophenol under laboratory conditions for 6 days.

Fig. 2 exhibits the glucose-amended respiration of soil from the same location used for the microcosm study, which was exposed to the test materials under laboratory conditions for 6 days. At the beginning of the measurement, the respiration of the PCP treated soil was decreased whereas after about 8 h it was significantly increased compared to the control. Lindane-treated soils did not show effects compared to the controls. A maximum of CO_2 production was found for Lindane-treated soils and the control after about 10 h, while the maximum for PCP,L and PCP,H was reached after 8 and 12 h, respectively. Applying the SIR method for short term soil respiration measurements, it was shown (Vonk and Barug, 1987) that PCP effects occurred at concentrations 100 times higher than those used in this study (2-10 ppm). On the other hand, increased numbers of bacterial cells in glycine-percolated soils were found after the exposure to 10-200 ppm PCP for 10 days (Sato, 1987).

CONCLUSION

The data shown in this report together with the soil-core microcosm development and field validation studies performed at Battelle Columbus demonstrate that the terrestrial microcosm is a useful tool for evaluating ecosystem-level effects of chemicals. This has been taken into consideration by the recent publication of two protocols for conducting microcosm tests. The U.S. EPA has published a proposed rule in which Battelle's terrestrial microcosm is used to develop data on the toxicity and fate of chemical substances and mixtures under the Toxic Substances Control Act (U.S. EPA, 1987). In addition, the American Society for Testing and Materials (ASTM) published Battelle's microcosm protocol to test for the environmental fate, ecological effects, and environmental transport of chemicals that may enter terrestrial ecosystems (ASTM, 1988).

Nevertheless, the terrestrial microcosm approach can be further refined by tests to find out which parameter(s) should be selected as being representative for the different hierarchical ecosystem levels and to validate these refinement test conclusions by comparison with field data.

ACKNOWLEDGEMENT

Part of the work performed at Battelle Frankfurt was financially supported by the Umwelt-Bundesamt (UBA), Berlin.

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APPLICATION OF SUPERCRITICAL FLUID EXTRACTION AND SUPERCRITICAL FLUID CHROMATOGRAPHY TO RESIDUE ANALYSIS

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ABSTRACT

Supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) using carbon dioxide can be used to advantage for extraction and cleanup of pesticide residues from agricultural products. SFE can be used for the direct extraction of products, or can be applied to the cleanup of crude extracts obtained by conventional solvent extraction of representative-sized samples. SFE can be coupled on-line with GC analysis and/or SFC analysis using various selective detection options, including mass spectrometry, flame photometric, and nitrogen/phosphorus-selective thermionic detectors. Battelle has designed an automated SFE-GC cleanup and analysis system based on this technology to lower total analysis costs.

INTRODUCTION

Under supercritical conditions, carbon dioxide is a rather nonpolar fluid that serves as an excellent solvent for many nonpolar and moderately polar pesticides. However, it is a poor solvent for highly polar compounds, such as carbohydrates and proteinaceous material that comprise the bulk of non-oily agricultural crops or highly polar interferences that may be co-extracted in conventional residue analyses. Consequently, supercritical carbon dioxide is of interest as a selective solvent in residue analysis work to provide selectivity in extraction or to provide selectivity in chromatographic cleanup or analysis.

Because of the mild conditions required to maintain carbon dioxide in a supercritical state, a temperature above 31.1°C and a pressure above 72.8 bar, the equipment required to work with supercritical carbon dioxide is relatively simple. The equipment is now widely available from a number of manufacturers and is in the same price range as commonly used GC and HPLC equipment.

SUPERCRITICAL FLUID CHROMATOGRAPHY

SFC is generally performed under rather mild temperature conditions, 40 to 150°C, and, therefore, is suitable for many thermally unstable compounds that can not be determined by GC. SFC is also for suitable nonvolatile compounds because elution in SFC, unlike that in GC, does not depend upon volatility. Admittedly, these advantages over GC can also be achieved using HPLC; however, SFC provides a distinct advantage over HPLC in that SFC can be interfaced with highly sensitive or selective

ionization detectors, such as FID, FPD, and NPD, as well as with UV, MS, and FT-IR detectors. Many moderately polar pesticides that normally need to be derivatized prior to GC analysis can be determined by SFC without derivatization. Two examples of such compounds are the herbicides, 2,4-DB and dinoseb. SFC-FID chromatograms obtained from the analysis of 20 ng of underivatized materials are given in Figures 1 and 2, respectively. This work was performed using a Lee Scientific SFC system with a 15 m x 0.10 mm I.D. methyl silicone fused silica capillary column pressure programmed from 80 to 300 bar at a temperature of 75°C.

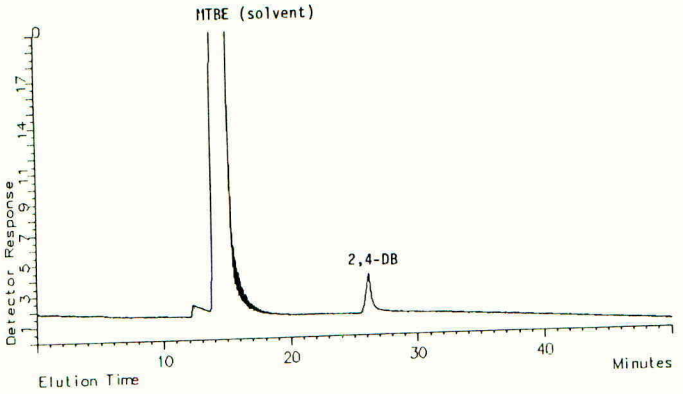

Figure 1. SFC-FID Chromatogram of 2,4-DB

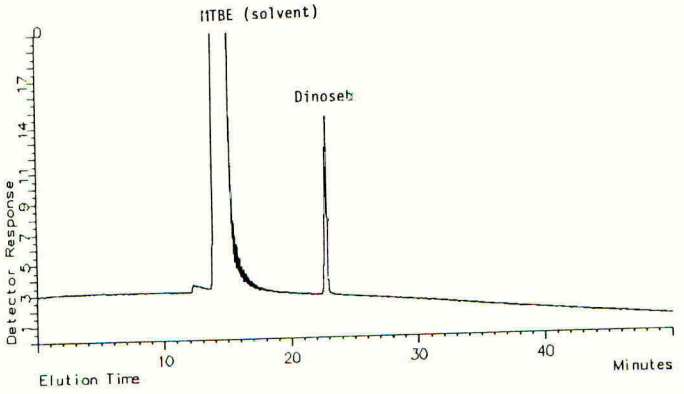

Figure 2. SFC-FIC Chromatogram of Dinoseb

SUPERCRITICAL FLUID EXTRACTION

One of the important considerations involved in obtaining reliable data in residue analysis work is that of obtaining a subsample for analysis that is representative of the total sample of interest. For vegetable crops, 100 g to several kilograms of material may be required to provide a representative sample. Usually, the total sample collected is chopped and blended; a 5 to 20-g subsample is taken for extraction; and a small portion of the extract after cleanup, generally less than one percent of the extract representing 0.01 to 0.1 g of sample, is introduced into a chromatographic system for the final analysis.

Three main approaches were considered for addressing the problem of obtaining a representative sample for on-line SFE-GC or SFE-SFC applied to crop analysis. One approach involves packing a 5 to 10-g subsample into an extraction cell and splitting the effluent by a factor of 100 to 10,000 ahead of the GC or SFC analysis. This approach requires a relatively large extraction cell, e.g., 22 mm I.D. x 50 mm, and a pumping system capable of providing a flow rate of up to 50 mL/min. The consumption of extraction solvent would be quite high, and the reproducibility of the split ratio would be a problem in quantitative work. Wet samples would either need to be dried ahead of time or blended with an adsorbent to soak up the water and give a powdery solid that the extraction fluid can pass through.

A second approach requires homogenizing the sample in such a way that a 0.01-g subsample taken for SFE is representative of the total sample. This approach may be suitable for dry products that have already been processed to produce a powder or flour, but would not be suitable for coarse wet crop materials without additional sample preparation. Wet crop materials could be homogenized and dried, or homogenized and mixed with an adsorbent such as diatomaceous earth to produce a powdery material to aliquot for SFE. Such sample preparation steps would take a considerable amount of manual effort and add significantly to analysis costs.

A third approach uses a simple conventional solvent procedure for extracting a 10 to 100 g representative sample, such as homogenization in acetone, and uses an aliquot of the extract that represents 0.01 to 0.1 g of sample for subsequent SFE. The SFE serves as a cleanup process rather than as the initial extraction process, and is used to selectively extract analytes from a solvent residue deposited on a cleanup column. This latter approach was selected for our work because it is the simplest one to implement and is much more amenable to automation.

The design of the system assembled for implementing this approach for automated SFE cleanup followed by GC analysis is given in Figure 3. The system is comprised of a Micromiretics autosampler, a sample loop (2, 10, or 50 μL), an extractor column (10 cm x 1.0 mm I.D. packed with a 5-μm ODS) in the oven of a Varian 3700 gas chromatograph, two Valco switching valves, two Valco multiposition valves, an interface, and a Lee Scientific Model 501 SFC system that includes an SFC pumping system, GC column oven containing a 30 m x 0.25-mm I.D. DB-5 column with a film thickness of 0.25 μm, and NPD and FID detectors. The valves and other components of

the system are controlled by Valco air actuators, controllers, and serial valve interface.

The valve positions used in each of the five steps of an automated SFE-GC analysis using the system are given in Table 1. In Step 1, the autosampler fills the sample loop with the crop solvent extract. In Step 2, pressure-controlled gaseous CO_2 is used to transfer the sample to the extractor column at a slightly elevated temperature and reduced pressure to permit the solvent to be volatilized and vented while the analytes of interest are retained on the column. The column is then pressurized to accomplish an SFE cleanup in which early eluting interferences are vented in Step 3, and the analytes of interest are transferred to a GC column in Step 4 via an interface that includes a pressure-reducing restrictor and provision for cryotrapping the analytes. GC analysis is performed in Step 5. During the analysis, the extractor column is exhaustively extracted with supercritical CO_2 in a backflush mode to prepare for the next sample. Except for the initial homogenization/solvent extraction process and autosampler loading, the entire operation is automated, and one person can process 10-30 samples in a day.

Table 1. Valve Positions for Automated SFE-GC Analysis

Analysis Step	Position of Given Valve			
	V-1	V-2	V-3	V-4
1	A	A	A	A
2	B	A	B	A
3	B	B	C	A
4	A	B	A	B
5	A	C	D	A

The above analysis procedure can be modified for the determination of nonvolatile or thermally unstable analytes by interfacing the SFE to SFC instead of GC. The analytes of interest would still be transferred via a pressure-reducing restrictor to concentrate the analytes at the head of the SFC column to minimize band broadening.

CONCLUSIONS

SFE is a very promising technique that can be incorporated into residue analysis procedures to make them more amenable to automation. SFC using selective ionization detectors or mass spectrometry can be applied to the determination of a wide variety of pesticides without derivatization, including many nonvolatile or thermally-labile compounds.

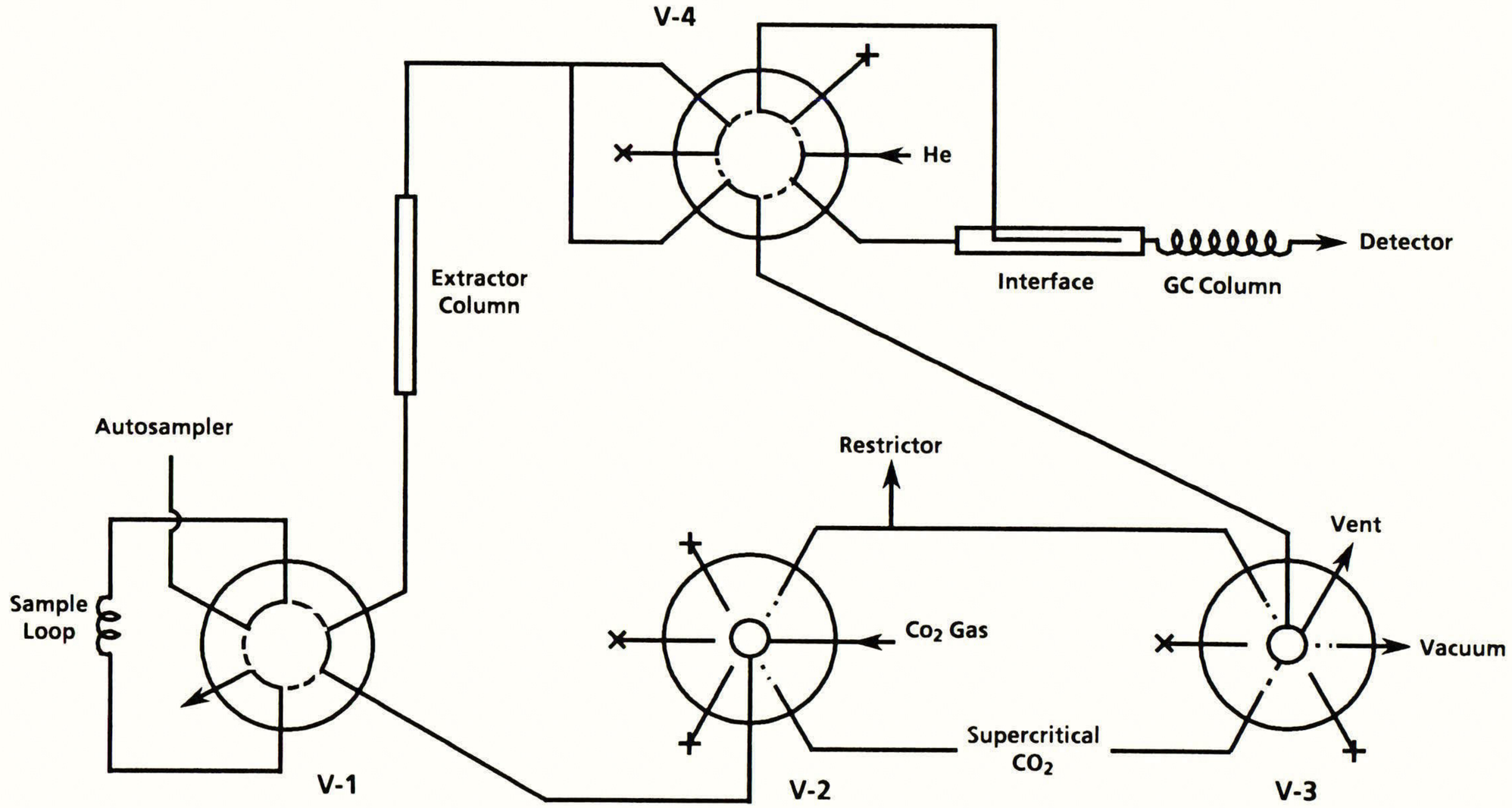

Figure 3. Valving System for Automated SFE-GC Analysis (solid lines in valves indicate Position A; dotted lines indicate Positions B, C, or D)

LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY OF PESTICIDES IN AGRICULTURAL MATRICES: TECHNOLOGY ASSESSMENT

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ABSTRACT

Identification and quantification of molecules in complex agrochemical matrices routinely involve extensive sample preparation. Many compounds are not suitable for gas chromatography due to high polarity or thermal lability. Methods have been developed using liquid chromatography/mass spectrometry(LC/MS) which combine on going chromatographic separation with the ability to analyze underivatized, nonvolatile, highly polar compounds in agrochemical matrices.

INTRODUCTION

Pesticide and herbicide classes typically used in agriculture are carbamates, methyl urea, chlorinated carboxylic acid and oximes. Increased presence in the environment, accompanied by formation of metabolites and possibly toxic degradation products have resulted in a greater need to separate and identify them. The currently available analytical methods for determination of the four classes of pesticides are not extensive. The use of gas chromatography (GC) for analysis is unacceptable because of the thermal lability of the compounds. Compound separation and quantification is achieved by high-performance liquid chromatography (HPLC) coupled to detectors that exhibit limited sensitivity and specificity, both of which are needed for analysis of compounds in complex agricultural matrices.

The coupling of HPLC and mass spectrometry (LC/MS) provides separation and detection of thermally labile compounds in complex matrices. One specific LC/MS technique, thermospray LC/MS (TSP LC/MS) is ideal for the analysis of pesticides in aqueous mobile phases which are pumped directly into the mass spectrometer. The ionization of thermally unstable compounds is soft (little fragmentation of the primary ions) and provides molecular weight information. Numerous envirnomental and clinical samples have been analyzed by TSP LC/MS (Blakely et al., 1980, 1983; Liberato et al. 1983; Voyksner et al., 1984; Bellar et al., 1988).

MATERIALS AND METHODS

The sulfonylurea herbicides chlorsulfuron, metsulfuron methyl and DPX-A7887 were obtained from Du Pont Company, Wilmington, De. The hydrocortisone was purchased from Sigma Chemical Company, St. Louis, Mo.

The ammonium acetate, 99 % pure, gold label was purchased from Aldrich Chemical Company, Inc., Milwaukee, WI. HPLC grade acetic acid was purchased from J.T. Baker Inc., Phillipsberg, NJ. All solvents were high purity chromatography grade (Burdick and Jackson Labs, Muskegon, MI). The deionized water was supplied by Millipore Milli-RO system and filtered with a Millipore filtering apparatus (Millipore Corporation, Bedford, MA) prior to use.

Samples were analyzed on a Finnigan model 4500 quadrupole mass spectrometer linked to a Finnigan Incos data system. A Model TS 106G Vestec Thermospray LC/MS interface was coupled to the mass spectrometer. HPLC separations were performed on a 4.6 mm o.d. x 25 cm Zorbax ODS column with a mobile-phase of methanol/ammonium acetate or acetonitrile/water/acetic acid at a flow rate of 1.0 ml/min.

Sulfonylurea herbicide standards were added to soil (5g) and water (500 ml) samples and extracted with methylene chloride. Under a gentle stream of nitrogen, the extract was evaporated to dryness and redissolved in 50 μl of mobile-phase for TSP LC/MS analysis.

RESULTS AND DISCUSSION

TSP LC/MS instrument paramenters are very important in optimizing the sensitivity of the method for each type of compound. The vaporizer temperature of the TSP LC/MS probe is dependent on the solvent (mobile-phase) and the compound. Generally, a higher vaporizer and jet temperature, the stronger the total ion signal. An optimum vaporizer temperature for sulfonylurea was determined. The optimal source block temperature was also determined.

The ionization that occurs during thermospray LC/MS is very sensitive to changes in temperature. As the HPLC effluent is superheated to a nebulized state it will leave the vaporizer nozzle as a fine mist. Temperatures below an optimum setting will cause the HPLC effluent to spray into the TSP LC/MS source as a liquid instead of a nebulized mist and cause insufficient desolvation. If temperatures are higher than optimum a the formation of a gas stream will occur and an unsuitable state for ion formation will exist. In both cases, the ion formation depends on the energy of the nebulized droplets and the amount of desolvation that occurs. A change in temperature (± 20 °C) will effect the signal levels significantly. The signal response of the analyte and the solvent are both dependent on temperature. Thus, a convenient method of optimizing the TSP interface temperatures is to use solvent ions rather than sample ions during inital tuning of the system for maximum sensitivity.

Ammonia chemical ionization (CI), which produces protonation and ammonium addition, resembles thermospray ionization in which ammonium acetate is used as a volatile buffer in the mobile phase. The primary ions observed are the $[M + H]^+$ ions and the $[M + NH_4]^+$ adduct ions. The ionization process is soft and very few fragments are produced (Blakley et al., 1980; Voyksner et al., 1984).

The use of TSP LC/MS for the analysis of herbicides and pesticides has

shown very good sensitivity and selectivity with detection limits of 5 to 100 ng for full scan spectra (Voyksner et al., 1984). The application of TSP LC/MS analysis to agricultural matrices is expanding and the utility of the detection method is becoming more apparent.

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