TABLE 5

The effect of pre-emergence SC 0574 on mean yield (t/ha) of winter cereals in 'weed-free' trials, France 1985-1987

Treatment (kg ai/ha)	Winter barley			Winter Wheat	
	1985	1986	1987	1985	1986
Untreated	5.97	5.71	7.17	7.44	6.07
SC 0574 4.0	6.13	5.78	7.44	7.30	6.10
SC 0574 8.0	6.04	5.70	7.24	7.12	6.10
No trials	5	9	3	5	12
SED	0.11	0.10	0.23	0.18	0.07
CV (%)	2.91	3.88	3.80	3.97	2.80

TABLE 6

The effect of pre- and early post-emergence SC 0574 on mean yield (t/ha) of winter cereals in 'weed-free' trials, UK 1986

Treatment (kg ai/ha)	Timing	Winter barley	Winter wheat
Untreated		7.66	7.62
SC 0574 4.0	Pre-em	7.64	7.64
SC 0574 8.0	Pre-em	7.57	7.54
SC 0574 4.0	Post-em	7.68	7.52
SC 0574 8.0	Post-em	7.35	7.72
No trials		5	2
SED		0.15	0.17
CV (%)		3.23	2.28

TABLE 7

The effect of pre-emergence SC 0574 on mean yield (t/ha) of winter cereals in West Germany compared to pre-emergence standards

Treatment (kg ai/ha)	Winter barley		Winter wheat		Winter rye	
	1985	1986	1985	1986	1985	1986
Untreated	5.06	5.12	6.19	5.57	4.61	5.03
SC 0574 4.0	6.31	5.99	7.20	6.61	4.41	5.10
Standard	6.17*	5.95*	7.39**	6.24°	4.61**	5.10°°
No trials	7	14	6	11	4	5

Standards, kg ai/ha, were * pendimethalin, 2.0; ** methabenzthiazuron, 2.8; ° methabenzthiazuron, 2.8 (5 trials) or chlortoluron 2.1 (6 trials); °° methabenzthiazuron, 2.8 (3 trials) or pendimethalin, 1.5 (2 trials).

When applied pre-emergence SC 0574 showed excellent selectivity in all crops drilled at normal depth into conventionally prepared seedbeds on all soil types, confirming the findings of Glasgow et al (1987). Post-emergence applications also showed excellent selectivity in wheat treated at all stages from emergence to tillering but sometimes caused transient leaf scorch and vigour reduction in barley. There was no indication of varietal sensitivity to SC 0574.

The effect of SC 0574 on grain yield in trials with low weed infestations is presented in Tables 5 and 6. Under these conditions SC 0574 did not significantly ($p = 0.05$) affect the overall yield of wheat or barley in any season. In weedy trials in West Germany (Table 7) SC 0574 increased yield, particularly where A.myosuroides and G.aparine were present. These increases were often greater than with the standards where G.aparine was the dominant species. For example, in winter barley SC 0574 increased yield by 1.13 t/ha (a 32% increase) and 1.36 t/ha (a 21% yield increase) in two trials where G.aparine comprised 60% and 40% respectively of the total weed population. Yield increases by the standard in the same trials were 0.88 t/ha and 0.87 t/ha.

DISCUSSION

Post-emergence herbicides currently recommended for the control of G.aparine in winter cereals are mostly used in spring and tend to be effective only when the weather is warm (Orson 1985). Residual products applied in the autumn, though effective against many species, have given rather variable and often poor control of G.aparine (Bradford & Smith 1982, Orson 1984). Lovegrove et al (1985) related this variability to soil type finding that residual herbicides were less effective on heavy soils. The trials reported in this paper have demonstrated that SC 0574 applied pre- or early post-emergence in the autumn gives consistently high levels of residual control of G.aparine. SC 0574 was effective on heavy soils (where G.aparine was more widespread) as well as light soils, in wet or dry autumns and when temperatures were low.

In a review, Makepeace (1982) concluded that the control of broad-leaved weeds had generally benefited yield but with no difference in the yield increase between autumn applications of herbicides compared to spring. However, Wilson et al (1985) suggested that autumn or winter control of heavy infestations of competitive broad-leaved species in addition to the early control of competitive grasses such as A.myosuroides was desirable. This contention is supported by results from these trials where SC 0574 increased yield substantially where high populations of G.aparine and A.myosuroides were controlled.

SC 0574 was effective against a range of broad-leaved and grass weed species germinating in the autumn. Broader spectrum control was obtained by using mixtures of SC 0574 and other herbicides. SC 0574 is thus an alternative residual herbicide offering flexible application timing with the benefit of reliable control of G.aparine. Reliable weed control combined with a high degree of crop safety offers the potential for maximising the yield benefit achieved by weed control in winter cereals.

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A NOVEL SALT/ESTER COFORMULATION CONTAINING BENAZOLIN FOR BROAD-LEAVED WEED CONTROL IN CEREALS

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ABSTRACT

CR 17607 is a new post-emergence herbicide developed for cost effective wide spectrum broad-leaved weed control in cereals. It is a solubilised concentrate combining benazolin and mecoprop salts with ioxynil and bromoxynil esters (22.2 + 413.0 + 27.8 + 55.6 g a.i./l).

In extensive United Kingdom trials in 1985 and 1986, CR 17607 at 4.5 l/ha gave quick, and highly effective control of major overwintered broad-leaved weeds of winter cereals including cleavers (Galium aparine), common chickweed (Stellaria media) mayweeds (Matricaria spp., Anthemis spp. and Chamomilla spp.), and speedwells (Veronica spp.). CR 17607 has a wide application window and was well tolerated over a range of crop stages from GS 12 to GS 31 inclusive.

Efficacy and crop safety compared favourably with standard treatments based on hydroxybenzonitrile esters + mecoprop or fluroxypyr, and metsulfuron-methyl tank mixed with mecoprop. These trials results were confirmed in the first season's commercial usage of CR 17607 (JAGUAR*) in 1987.

INTRODUCTION

In the late 1970's the introduction of products based on hydroxybenzonitriles and mecoprop or dichlorprop esters set a new standard of broad-leaved weed control in cereals. These have now been largely phased out because of the risk of vapour drift damage to susceptible neighbouring crops from the mecoprop and dichlorprop ester components.

To overcome this problem products based on hydroxybenzonitrile esters were introduced for use either alone or in tank mixture with mecoprop salt. One such product, containing benazolin + ioxynil + bromoxynil esters (ASSET+) was launched in 1982 particularly for spring use against tough overwintered weeds (Orson and Marshall 1985).

However, a ready for use coformulation was clearly desirable, and the difficult problem of preparing a biologically active and stable mixture based on these constituents and mecoprop salt was eventually resolved by

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the use of a special formulation system. The field performance of CR 17607, and that of appropriate standard treatments was compared in extensive trials undertaken in spring and winter cereals. This paper examines the response of tough winter hardened weeds in autumn sown cereals to late winter and spring treatments of CR 17607 during 1985 and 1986.

MATERIALS AND METHODS

CR 17607, a solubilised concentrate formulation of benazolin salt + ioxynil ester + bromoxynil ester + mecoprop salt (22.2 + 27.8 + 55.6 + 413 g a.i./l) was tested in trials in 1985 and 1986 at 4.5 l/ha, covering the major varieties of autumn sown wheat, barley and oats.

In 64 small plot efficacy trials, distributed throughout the United Kingdom, the performance of CR 17607 was compared with two commercial standard treatments:

A a benazolin + ioxynil + bromoyxnil ester product (50 + 62.5 + 125 g a.i./l) tank mixed with mecoprop salt (570 g a.i./l) at 2.0 + 3.25 l/ha

B an ioxynil + bromoxynil esters + mecoprop salt product (56 + 56 + 448 g a.i./l) at 4.5 l/ha.

In 18 trials double doses were also tested.

In 27 trials a further standard treatment was included:

C an ioxynil + bromoxynil + fluroxypyr ester product (100 + 100 + 90 g a.i./l) at 2.0 or 2.5 l/ha.

In 1986 a comparison was also made in nine trials with:

D metsulfuron-methyl 200 g a.i./kg tank mixed with mecoprop (570 g a.i./l) at 0.03 kg + 4.2 l/ha.

Crop yield was recorded in 12 weed free trials in 1985 and 1986, six in each year, for CR 17607 at 4.5 and 9.0 l/ha. Standard treatment A at 2.0 + 3.25 and 4.0 + 6.5 l/ha was included in both seasons and standard treatment B at 4.5 and 9.0 l/ha in 1985.

Applications in the efficacy trials were made in the spring at crop stages ranging from GS 12 to GS 32 to weeds which were frequently tough, having survived winter conditions. In later sprayed trials, weeds were often large i.e. up to 40cms high or 15cm diameter. In the yield trials applications were made at crop stages GS 30, GS 31 and GS 32.

Plot sizes were 7 to 10 x 2m in small plot efficacy trials and 21 x 3m in yield trials with three and six replicates respectively. Treatments were applied by pressurised knapsack sprayer fitted with TeeJet 8001 or 11002 nozzles in 200-220 l/ha of water at 210 to 280 kPa pressure to deliver a BCPC medium quality spray.

Assessment of crop safety in winter wheat and barley, (scorch or vigour loss) and weed control (cover and number) was made visually on a percentage scale. Yield trials were harvested by Claas or Massey Ferguson combines adapted for small plot work. Grain yields were statistically analysed and hectolitre weights recorded.

RESULTS

Efficacy

The results from both years' trials were similar. Initial weed response to CR 17607 at 4.5 l/ha was very satisfactory and fully comparable to standard treatment A (benazolin + ioxynil + bromoxynil esters + mecoprop tank mixture) and the two hydroxybenzonitrile based products, standard treatments B and C (Table 1). CR 17607 was however, considerably faster acting than metsulfuron-methyl + mecoprop, standard treatment D.

Final control (10 weeks after treatment) of common chickweed, mayweeds, cleavers and common poppy, the most competitive yield depressing weeds in cereals (Wilson 1984) together with that of speedwells, dead-nettles (Lamium spp.), polygonums (Polygonum aviculare, Bilderdykia convolvulus), and many other important species was excellent and at least equal to the standards (Table 2). Indeed, CR 17607 outperformed metsulfuron-methyl + mecoprop, particularly on cleavers and speedwells. Field pansy (Viola arvensis), a much less competitive but perceptibly important weed was well suppressed by CR 17607. None of the standards were more effective.

Crop Safety

CR 17607 was well tolerated by the crops. Crop effects were restricted to slight scorch which disappeared within 2-3 weeks. Scorch was far more apparent for standard treatment B (Table 5). In the absence of weed there was no adverse effect on yield for CR 17607, even at 9.0 l/ha up to and including GS 31. However, some reduction in yield followed treatment at GS 32 (Tables 3 and 4).

TABLE 1

Efficacy trials 1985 & 1986 : percentage weed control (1-2 week assessment)

Weed	CR 17607		Standard Treatments							
			A		B		C		D	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Stellaria media	47-87	61 (18)	45-89	65 (18)	38-88	64 (18)	52-65	57 (6)	33-50	41 (4)
Matricaria spp.*	65-100	86 (10)	68-100	84 (10)	55-100	82 (10)	68-91	78 (3)	43-50	47 (2)
Galium aparine	50-100	78 (10)	40-97	75 (10)	47-99	78 (10)	49-61	55 (2)		47 (1)
Veronica persica	40-100	78 (9)	35-100	74 (9)	42-100	77 (9)	55-56	55 (2)		18 (1)
Veronica hederifolia	57-85	74 (5)	61-88	78 (5)	58-85	70 (5)	45-49	47 (2)		20 (1)
Viola arvensis	20-87	51 (13)	17-90	47 (13)	17-83	45 (13)	17-58	45 (5)	8-42	24 (2)
Papaver rhoeas		95 (1)		97 (1)		97 (1)				
Fumaria officinalis	63-88	75 (2)	72-78	75 (2)	68 75	71 (2)	53-70	62 (2)		57 (1)
Polygonum aviculare	75-95	85 (4)	75-90	76 (4)	63-100	76 (4)		72 (1)		45 (1)
Bilderdykia convolvulus	69-79	75 (4)	75-88	76 (4)	63-76	72 (4)	75-83	79 (2)		39 (1)
Sinapis arvensis	83-88	85 (2)	82-88	85 (2)	79-86	82 (2)	75-100	83 (2)		59 (1)
Lamium purpureum		88 (1)		92 (1)		87 (1)				
Aphanes arvensis		45 (1)		40 (1)		50 (1)				
Overall		72 (46)		71 (46)		72 (46)		68 (9)		42 (9)

( ) = Number of trials
* includes Anthemis spp. and Chamomilla spp.

TABLE 2

Efficacy trials 1985 & 1986 : percentage weed control (10 week assessment)

Weed	CR 17607		Standard Treatments							
			A		B		C		D	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Stellaria media	90-100	99 (35)	90-100	99 (35)	83-100	99 (35)	98-100	99 (15)	94-100	99 (5)
Matricaria spp.*	90-100	98 (20)	88-100	99 (20)	87-100	99 (20)	78-100	95 (12)		100 (2)
Galium aparine	91-100	98 (19)	90-100	99 (19)	93-100	99 (19)		100 (4)	3-80	42 (3)
Veronica persica	93-100	99 (15)	93-100	99 (15)	93-100	99 (15)		100 (4)	3-80	42 (2)
Veronica hederifolia	88-100	98 (12)	89-100	99 (12)	75-100	98 (12)		100 (2)	78-99	89 (2)
Viola arvensis	10-100	81 (26)	10-100	78 (26)	10-100	82 (26)	10-100	77 (8)	75-99	91 (4)
Papaver rhoeas	93-98	94 (3)	93-100	97 (3)	91-100	97 (4)	93-98	96 (2)		
Lamium purpureum	98-100	99 (4)	98-100	99 (4)	99-100	99 (4)		100 (2)		100 (1)
Myosotis arvensis		97 (2)	97-98	97 (2)	93-98	95 (2)	98-100	99 (2)		
Aphanes arvensis	84-100	93 (4)	64-100	86 (4)	87-100	94 (4)		64 (1)		
Fumaria officinalis		100 (3)		100 (3)		100 (3)				
Polygonum aviculare		93 (1)		100 (1)		100 (1)		98 (1)		
Bilderdykia convolvulus	97-100	99 (4)	99-100	99 (4)	99-100	99 (4)		100 (1)		100 (1)
Legousia hybrida		100 (2)	98-99	98 (2)		100 (2)				96 (1)
Sinapis arvensis		100 (1)		100 (1)		100 (1)				
Chenopodium album		91 (1)		92 (1)		96 (1)		95 (1)		
Capsella bursa-pastoris		100 (1)		100 (1)		100 (1)		100 (1)		
Senecio vulgaris		100 (1)		100 (1)		100 (1)		100 (1)		
Overall		95 (64)		95 (64)		96 (64)		93 (18)		85 (9)

( ) = Number of trials
* includes Anthemis spp. and Chamomilla spp.

TABLE 3

Weed free yield trials 1985 & 1986 : yields as percentage of untreated, winter barley

Treatment l/ha	Growth stage, year and trial number											
	GS 31						GS 32					
	1985			1986			1985			1986		
	1	2	3	1	2	3	1	2	3	1	2	3
CR 17607 4.5	101.0	93.2	99.2	98.3	103.0	101.3	99.9	100.9	99.4	96.7	100.7	98.4
CR 17607 9.0	107.7	92.4	98.8	99.6	106.4	102.8	102.5	96.0	93.4	94.2*	96.7	95.1
Standard treatment A 2.0+3.25	103.9	100.3	96.5	96.9	103.0	102.2	99.8	94.0	98.0	96.7	99.7	98.0
Standard treatment A 4.0+6.5	97.0	91.5	95.0	101.1	103.1	98.5	94.0	91.2	92.5	92.4*	97.6	98.5
Standard treatment B 4.5	105.5	95.6	100.0				95.0	94.3	102.4			
Standard treatment B 9.0	97.7	90.3	96.0				93.1	90.7	91.0*			
LSD p = 0.05*	NS	NS	NS	NS	NS	NS	NS	NS	7.1	4.2	NS	NS
CV	3.0	3.5	2.6	1.5	3.1	2.9	3.0	3.5	2.6	1.5	3.1	2.9
Untreated t/ha	4.6	3.7	6.8	5.3	4.3	4.4	4.6	3.7	6.8	5.3	4.3	4.4

TABLE 4

Weed free yield trials 1985 & 1986 : yields as percentage of untreated, winter wheat

Treatment l/ha	Growth stage, year and trial number									
	GS 31						GS 32			
	1985			1986	1985			1986		
	1	2	3	1	1	2	3	1	2	3
CR 17607 4.5	97.6	98.1	99.3	97.0	98.2	101.6	102.4	100.6	104.3	95.7*
CR 17607 9.0	101.9	96.4	99.5	99.7	96.5	98.8	101.0	96.0	98.9	93.6*
Standard treatment A 2.0+3.25	98.1	97.6	100.1	101.1	99.4	100.6	101.1	98.0	102.5	97.3
Standard treatment A 4.0+6.5	100.5	96.5	99.3	98.9	96.2	96.4	99.4	96.6	99.5	93.1*
Standard treatment B 4.5	100.1	96.3	99.4		95.9	100.4	99.0			
Standard treatment B 9.0	98.7	93.1*	98.7		95.0	99.1	98.7			
LSD p = 0.05*	NS	3.6	NS	NS	NS	NS	NS	NS	NS	3.1
CV	1.6	1.3	1.1	1.5	1.6	1.3	1.1	1.5	4.9	1.1
Untreated t/ha	5.2	5.5	7.6	6.0	5.2	5.5	7.6	6.0	7.0	8.0

TABLE 5

Crop safety, application GS 12-31 : percentage scorch, maximum effect

Treatment	l/ha	Maximum effect Winter barley		Winter wheat	
CR 17607	4.5	6	(14)	3	(9)
Standard treatment A	2.0 + 3.25	4	(13)	3	(11)
Standard treatment B	4.5	11	(24)	8	(18)

( ) = double doses

DISCUSSION

Two seasons' replicated trials with CR 17607 at 4.5 l/ha have demonstrated its excellent and consistently high standard of efficacy against the major broad-leaved weeds of winter cereals under a wide range of conditions. Similarly effective results were achieved in over 200 grower trials.

This special salt/ester formulation combines the benefits of a quick weed response to hydroxybenzonitrile esters with the sustained activity of benazolin and mecoprop to give cost effective season long control of a wide spectrum of weeds. The use of mecoprop salt eliminates any risk of vapour drift to neighbouring susceptible crops. CR 17607 achieved a high standard of crop safety over a wide application window from GS 12 to GS 31 inclusive.

Although not reported in this paper CR 17607 has also been successfully developed for use in spring cereals at the reduced dose of 3.0 l/ha and the trials results in both winter and spring cereals have been borne out by a successful season of commercial usage in 1987.

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WEED CONTROL IN WINTER CEREALS WITH DPX-L5300 IN MEDITERRANEAN COUNTRIES

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ABSTRACT

DPX-L5300, a new herbicide from Du Pont, has been tested in cereals in the Mediterranean countries, during the last five years. It has demonstrated a high activity at very low rates (5 to 20 g a.i./ha) in a wide spectrum of broad leaved weeds. Although DPX-L5300 can be applied to cereals from 2/3 leaves to stem elongation, the best results have been obtained at the mid-tillering stage of the cereal, when all weeds have already germinated and are in active growth. High temperatures and soil moisture enhance DPX-L5300 activity. In dry situations, the addition of a wetting agent increases its effect. A good selectivity has been observed in all the most important varieties of wheat and barley cultivated in Mediterranean countries. Tank mixtures with the most frequent grass herbicides have been tested and no interference has been noticed to either broad leaved weeds or grasses. DPX-L5300 did not affect the normal rotational crops sowed after cereals.

INTRODUCTION

The control of broad leaved weeds in the Mediterranean countries has been adequate with the phenoxy herbicides alone or in mixtures. However the phenoxy herbicides have limitations :

1 - The late application time of the phenoxy herbicides allows early weed competition particularly the uptake of nutrients and soil water.

2 - The late time of application does not allow tank-mixing with many of the herbicides used for grass weed control and consequently two applications are required for complete weed control.

3 - The volatility of many herbicides causes a big risk for the neighbouring crops, particularly from aerial application.

The chemical and physical properties, toxicology, mode of action, selectivity, environmental fate, and the first field test results for DPX-L5300 were well described by Ferguson et al (1985).

During 1985 and 1986 a wide programme of field trials was carried out to determine efficacy, dose rate, persistence, residual effects and crop safety under a wide range of field conditions.

The main objectives of the current series of trials with DPX-L5300 were :

1 - To establish the lowest effective use rate required to control the most important weed species ;

2 - To determine the specific rate or mixture required to control the main weed species ;

3 - To check the compatibility of DPX-L5300 with other grass and broad leaved herbicides ;

4 - To check the selectivity of DPX-L5300 in most of the main varieties of wheat and barley used in this region;

5 - To verify whether the addition of a wetting agent could improve the performance of DPX-L5300;

6 - To check the persistence of the compound in the soil ;

7 - To establish the optimum time of application.

METHODS AND MATERIALS

Trials were conducted in the main cereal areas of Spain and Italy and included many climatic and soil conditions and both irrigated and non irrigated fields.

A 75 % dry flowable formulation of DPX-L5300 was used in all trials. The standard compounds used for comparison were 2,4 D isobutylic ester or mixtures of 2,4 D + MCPA in tests with susceptible weeds to DPX-L5300. In tests including Galium aparine, Veronica hederaefolia and V. persica, mecoprop, bromoxynil and ioxynil mixtures were used.

Applications were made with knapsack motor sprayers or hand-held sprayers using an inert gas propellant. Spraying pressure was about 200 KPa and application volumes varied between 200 and 500 l/ha. Plots were between 2 m x 5 m and 4 m x 10 m replicated 3 or 4 times. Evaluations were made considering the population density reduction compared with untreated plots on a 0-100 % scale. Evaluations were made at least two times, at 10 to 15 DAT and at 60 to 80 DAT. Evaluations of Avena spp. were made by counting the number of ears per m^2 and calculating the percentage reduction relative to untreated plots.

The times of application varied between cereal growth stages Zadoks 12 and 37. A visual assesment on a 0 - 100 scale was made in order to determine crop damage, 0 = no damage and 100 = total crop destruction.

RESULTS

The results show that DPX-L5300 is a broad spectrum herbicide, effective at very low rates. Table 1 shows the weed control in Spain at 7.5 and 10 g a.i./ha versus 2,4 D at 500 g a.i./ha. Some species tolerant to 2,4 D such as Stellaria media, Lamium amplexicaule, Lithospermum arvense, Spergularia rubra and Spergula arvensis were controlled at 7.5 g a.i./ha DPX-L5300.

TABLE 1
DPX-L 5300 FIELD TEST RESULTS IN SPAIN 1985/1986/1987
EVALUATION 50 - 60 DAT
% WEED CONTROL - SUSCEPTIBLE SPECIES TO DPX-L5300

WEED SPECIES	Number of Tests	Rate of DPX-L5300 g a.i./ha 7.5	10	2,4D ester 500 g a.i./ha
Papaver Rhoeas	19	88.46	98.03	83.31
Stellaria media	7	94.00	100.00	0.00
Anthemis spp.	5	95.50	95.50	80.29
Silene spp.	4	100.00	100.00	58.50
Sinapis arvensis	4	91.00	95.45	97.23
Lamium amplexicaule	3	70.50	83.75	7.50
Anagallis arvensis	1	93.00	97.13	75.19
Carlina corimbosa	1	97.00	99.38	100.00
Capsella bursa pastoris	1	75.00	91.25	93.25
Diplotaxis erucoides	1	71.25	93.75	93.75
Lithospermum arvense	1	-	87.50	0.00
Spergularia rubra	1	-	98.75	0.00
Spergula arvensis	1	-	97.50	31.60
Vaccaria pyramidata	1	-	88.80	89.50
Chenopodium album	1	91.25	-	98.75

TABLE 2
DPX-L5300 FIELD TEST RESULTS IN ITALY 1986/1987
EVALUATION 50 - 60 DAT
% WEED CONTROL - SUSCEPTIBLE SPECIES TO DPX-L5300

WEED SPECIES	Number of Tests	Rate of DPX-L5300 g a.i./ha 5	7.5	10	2,4D ester 550 g a.i./ha	2,4D+MCPA amine salts 250 + 310 g a.i./ha
Stellaria media	12	97	98	99	-	64
Papaver Rhoeas	9	94	94	95	94	-
Capsella bursa pastoris	5	90	93	98	-	80
Ranunculus arvensis	5	92	95	96	90	90
Bifora radians	4	96	97	97	37	-
Cardamine hirsuta	2	100	100	100	-	100
Myagrum perfoliatum	2	92	96	99	-	34
Matricaria chamomilla	2	100	100	100	82	-
Ranunculus sardous	2	91	96	100	-	85
Sinapis arvensis	2	87	93	94	100	-
Rhynchosinapis cheiranthos	2	98	100	100	100	-
Viola tricolor	2	77	92	95	-	98
Calepina irregularis	1	100	100	100	100	-
Geranium dissectum	1	90	90	95	85	-
Rapistrum rugosum	1	100	100	100	100	-
Thlaspi arvense	1	100	100	100	100	-
Vicia sativa	1	95	98	98	70	-
Viola arvensis	1	82	90	95	82	-

In Italy, (Table 2), the same level of efficacy was confirmed at lower rates of DPX-L5300. Over 90 % control of almost all species tested was found following the application of DPX-L5300 at 5 g a.i./ha; only Viola tricolor, V. arvensis and Sinapis arvensis needed 7.5 g a.i./ha DPX-L5300 to achieve greater than 90 % control.

The addition of a wetting agent (Table 3) dramatically increased the efficacy of DPX-L5300 particularly in the warm areas. 58.5 % and 55.5 % control of the important weeds Chrysanthemum segetum and Fumaria officinalis respectively were achieved at relatively high rates (18.75 g a.i./ha of DPX-L5300). The addition of a surfactant (alkylphenol polyglycol ether) at 0.02 % increased the control of these weeds to 94.0 and 94.2 % respectively.

TABLE 3

DPX-L5300 FIELD TESTS RESULTS IN SPAIN 1986/1987
INFLUENCE OF SURFACTANT. DPX-L5300 18.75 g a.i./ha.
EVALUATION 50 - 60 DAT -
% OF WEED CONTROL

WEED SPECIES	Number of Tests	DPX-L5300 alone	DPX-L5300 Surfactant 0.02 %
Chrysanthemum segetum	3	58.5	94
Fumaria officinalis	8	55.5	94.2

Tank mixtures were tested in order to improve the performance of DPX-L5300 in weeds considered tolerant such as Galium aparine, Veronica hederaefolia and V. persica. In Italy (Table 4) a mixture of 10 g a.i./ha of DPX-L5300 with either 1000 g a.i./ha of mecoprop or 87.5 + 87.5 g a.i./ha of ioxynil and bromoxynil increased the control of G. aparine from 54 % to 85 and 83 % respectively. On V. persica and V. hederaefolia an improvement in control from 40 % for DPX-L5300 alone to 85 and 95 % for DPX-L5300 + Surfactant respectively.

TABLE 4

DPX-L5300 TANK MIXTURES FOR SPECIFIC WEEDS
% OF WEED CONTROL - ITALY
EVALUATION 50 - 60 DAT

PRODUCT	Rate g a.i./ha	Weed Species Galium aparine	Veronica persica	Veronica hederaefolia
DPX-L5300	10	54 (4)	76 (6)	40(12)
DPX-L5300 + mecoprop	10 + 1000	85 (3)	-	85 (3)
DPX-L5300 + ioxynil + bromoxynil	10 + 87.5 + 87.5	83 (4)	100 (2)	95 (2)

( ) = Number of Tests.

In Spain three mixtures were tested of 15 g a.i./ha DPX-L5300 in combination with :

1 - Mecoprop at 1800 g a.i./ha.

2 - Ioxynil + bromoxynil at 150 g a.i./ha each.

3 - Cyanazine at 250 g a.i./ha.

On G. aparine the mixtures with mecoprop and with ioxynil + bromoxynil increased control from 37 to 97 % and on V. hederaefolia, the three mixtures improved control from 0 % to 96, 91 and 98 % respectively.

In one experiment in southern Spain poor control of Raphanus raphanistrum was achieved due probably to the short persistence of DPX-L5300. In this area, this species emerges early in the season and grows quickly, so that by the time DPX-L5300 is applied, even at early cereal growth stages, weed plants are at rosette stage with a deep pivotal root. In this situation the quantity of chemical absorbed by the plants is only enough to control weed growth for about 2 to 3 months after which regrowth occurred. In this case improved control was achieved by sequential applications. 95 % control obtained with two applications of 7.5 g a.i./ha at GS13 followed by GS30.

Two series of tests were carried out to test the compatibility of DPX-L5300 with other compounds, mainly grass herbicides : one with isoproturon and another with diclofop methyl, difenzoquat and flamprop isopropyl in order to check possible interferences in their efficacy on Lolium rigidum and Avena spp. Up to date, no antagonism has been observed with any of the mixtures examined (Table 5).

All varieties of wheat and barley tolerated double rates of DPX-L5300 (30 - 32 g a.i./ha). Nevertheless the addition of surfactant caused temporary yellowing in some cases. This was particularly noticeable in Durum wheat varieties.

All crops subsequently planted in the trial sites were evaluated to check the residual effect of DPX-L5300 and specific rotational tests were carried out with DPX-L5300 at 20 and 40 g a.i./ha. Crops were drilled as follows :

- 230 DAT Corn, Sunflower and Sorghum;

- 250 DAT Wheat, Barley, Oats, Broad Beans, Vetch, Lupine and Lentil.

- 270 DAT Beans, Broad Beans, Turnips, Vetch, Oil seed rape and Peas;

- 399 DAT Cotton, Corn, Chickpea, Melon, Sorghum and Potatoes;

- 450 DAT Sunflower, Chickpea, Corn, Sorghum and Beans.

All these crops grew normally and no symptoms of phytotoxicity were evident.

TABLE 5

DPX-L5300 COMPATIBILITY FIELD TESTS WITH GRASS HERBICIDES - SPAIN - 1987.
% WEED CONTROL - EVALUATION 80 - 90 DAT.

PRODUCTS	Rates in g a.i./ha	SPECIES Avena ludoviciana	Avena macrocarpa	Lolium rigidum	Alopecurus myosuroides
DPX-L5300 + Diclofop methyl	11.25+900	64.5(2)	88.8(4)		
Diclofop methyl	900	57.5(2)	92.8(4)		
DPX-L5300 + Difenzoquat	11.25+1000	66.5(2)	96.3(4)		
Difenzoquat	1000	62.5(2)	95.6(4)		
DPX-L5300 + Flamprop isopropyl	11.25+600	96.7(2)	99.5(4)		
Flamprop isopropyl	600	99 (2)	100(4)		
DOX-L5300 + Isoproturon	11.25+1 500	-	-	91.9(6)	84.5(2)
Isoproturon	1500	-	-	85.5(6)	86.7(2)

( ) = Number of Tests.

DISCUSSION

DPX-L5300 (5 - 10 g a.i./ha) applied in winter and spring at early growth stages of broad leaved weeds demonstrated a wide range of efficacy on the main weeds present in the cereal fields examined, including Papaver Rhoeas, Sinapis arvensis, Stellaria media, Anthemis spp., Lamium amplexicaule, Capsella bursa pastoris, Ranunculus arvensis.

The addition of a surfactant enhanced the activity of the compound particularly in warm climates such as those in southern Spain and Italy. This effect was particularly noticeable on Fumaria officinalis and Chrysanthemum segetum. The control of specific weeds such as Veronica hederaefolia, V. persica and Galium aparine was improved by mixtures of DPX-L5300 with mecoprop, ioxynil, bromoxynil or cyanazine.

Since the application time for DPX-L5300 is similar to many of the products used in grass control and no indication of incompatibility using the full recommended rates of these compounds were found, tank mixtures may be used in order to save one application.

Since DPX-L5300 is not volatile, it will have less propensity to move from treated areas to sensitive crops.

DPX-L5300 was selective in most of the varieties of wheat and barley examined. When a surfactant was used to increase product efficacy, some temporary yellowing was recorded, but the crop recovered in 10 to 20 days.

DPX-L5300 rapidly dissipates under mild climates giving complete flexibility in the use of the normal rotational crops grown in Mediterranean countries.

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THE CONTROL OF CIRSIUM ARVENSE (CREEPING THISTLE) BY SULFONYL UREA HERBICIDES AND A COMPARISON OF METHODS OF ASSESSING EFFICACY

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ABSTRACT

The aim of this study was to assess the efficacy of the sulfonyl urea herbicides DPX-L5300 and metsulfuron-methyl on Cirsium arvense, and to develop and examine the usefulness of a technique to assess root viability of treated plants in the season when herbicides were applied.

There were four field trials of randomised block design. Two were in winter wheat and two were on land which had not been disturbed for two years. Eight weeks after spraying, samples of C. arvense were removed from each plot. The shoot material was counted and weighed. The root material was planted in compost and after three weeks, assessments of shoot regrowth were made.

Mecoprop, MCPA, glyphosate and clopyralid proved more effective than sulfonyl ureas in controlling C. arvense. The root viability test gave an indication of herbicidal activity.

INTRODUCTION

Cirsium arvense (creeping thistle) is a perennial weed of both grassland and arable crops. It is strongly competitive and is reported to have an allelopathic effect on crops (Wilson, 1981). At present, control of C. arvense is difficult and expensive, being largely based on glyphosate or clopyralid. The two sulfonyl urea herbicides DPX-L5300 (Ferguson, 1985) and metsulfuron-methyl offer the possibility of cheaper control.

The aim of the trials was to assess the efficacy of metsulfuron-methyl and DPX-L5300, with and without surfactant, compared with chemicals which are currently used to control C. arvense. The treatments were evaluated by field assessments and, since control of below ground material is essential, by the viability of the roots.

Turner and Cussans (1981) reported a technique for assessing the effect of glyphosate on Elymus repens (common couch) in field experiments. Samples of rhizome were divided into single node lengths, planted in compost and the amount of regrowth measured. Since the roots of C. arvense produce buds and new shoots (Hamdoun, 1972) the adoption of this technique could be used as an alternative to measuring the growth in treated areas in the year after spray application.

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MATERIALS AND METHODS

Four field trials were sprayed during May to August 1986. Two of these trials were in winter wheat and two were on land which had not been disturbed for at least two years. Natural populations of C. arvense were used. A randomised block design was used with three or four replicates.

The trials were sprayed with a knapsack plot sprayer fitted with Lurmark F03-110 nozzles at 2.5 bar pressure. A volume equivalent to 300 l/ha was used. Before spraying the trials, permanent quadrats totalling $1m^2$ were marked out in each plot. Six-eight weeks after application of the spray, the area of the permanent quadrats was dug, including one in each untreated plot. Work by Sagar & Rawson (1964), and preliminary digs at two of the sites suggested 20 cm was a reasonable sampling depth to obtain most of the root material. Both shoot and root material were collected from the quadrats. The shoots from each sample were counted and weighed. The root material was divided into tap root and lateral roots and cut into fragments 40-60 mm long. Each sample was planted in a wooden fruit tray on 20 mm of Levingtons potting compost and covered with a further 20 mm. The trays were placed randomly on flat ground in the open and watered as necessary to keep them moist. After three weeks the proportion of root fragments which had produced shoots were counted.

Additionally, the trials on undisturbed land were assessed the year after treatment, either by shoot numbers (Cambridge) or a percentage of control score (March).

TABLE 1

Site Details

	Winter Wheat		Undisturbed Land	
	Norfolk	Essex	Cambridge	March
Spray dates	6/5/86	19/5/86	2/6/86	12/6/86
	11/6/86	29/5/86	16/6/86	-
	12/8/86	-	-	-
Sampling dates				
1st Spray	3/7/86	26/6/86	15/7/86	24/7/86
2nd Spray	3/8/86	17/7/86	5/8/86	-
3rd Spray	19/8/86	-	-	-
1987 assessments	-	-	26/6/87	27/7/87

Winter wheat trials

The main purpose of this work was to assess the efficacy of DPX-L5300 (methyl 2-[3-(4-methoxy-6-methyl-1,3,5,-triazin-2-yl)-3-methylureidosulphonyl] benzoate) and metsulfuron-methyl. They were applied at two timings; second node detectable stage (GS 32) and full flag leaf emergence (GS 39) of the crop. Growth stage 32 may have corresponded to a normal commercial application to control broad-leaved weeds in the spring. C. arvense was at the rosette, vegetative stage with many shoots still emerging. At the second timing, the maximum number of shoots had emerged and the C. arvense was at flower bud emergence. This was beyond

the time when effective control of many other weeds could be expected. At each of these timings, metsulfuron-methyl and DPX-L5300 were applied with and without a non-ionic surfactant. They were compared with mecoprop salt and clopyralid at GS 32 and clopyralid at GS 39. Pre-harvest glyphosate applied at a bulk grain moisture content of 30% was included as a treatment in one trial. The rates of the chemicals used were standard label recommendations (Table 2). There were two unsprayed plots per block.

TABLE 2

Treatments - winter wheat trials

Treatment	Rate/ha	GS of crop
mecoprop salt	2.85 kg a.i.	32
metsulfuron - methyl	6 g a.i.	32, 39
metsulfuron - methyl + non-ionic wetter*	6 g a.i.	32, 39
DPX-L5300	22.5 g a.i.	32, 39
DPX-L5300 + non-ionic wetter*	22.5 g a.i.	32, 39
clopyralid	75 g a.i.	32, 39
glyphosate salt	1.44 kg a.e.	pre-harvest

* Agral at 250 ml per 1000 l of water.

Undisturbed land trials

DPX-L5300 and MCPA were applied at full flower bud emergence. Clopyralid was applied at early flower bud emergence at the Cambridge site and full flower bud emergence at the March site. DPX-L5300 was applied with and without a non-ionic surfactant (Table 3).

TABLE 3

Treatments - undisturbed land trials

Treatment	Rate/ha
clopyralid	200 g a.i.
MCPA - salt	1.7 kg a.i.
DPX-L5300	15 g a.i.
DPX-L5300 + non-ionic wetter	15 g a.i.
DPX-L5300	30 g a.i.
DPX-L5300 + non-ionic wetter	30 g a.i.

RESULTS

The results are given in Tables 4-10. Overall, control was disappointing. There was a large variability between the trials. The poorest control was recorded at the two sites with the highest and most vigorous populations of C. arvense (Norfolk and March).

Control of shoot number was generally poor (Tables 4 and 5). In the winter wheat trials, treatment at GS 32 was more effective than treatment at GS 39. In the undisturbed land trials, clopyralid was most effective at the Cambridge site, where it was applied before the thistles reached full flower bud emergence. The recommendations for clopyralid are for application at the rosette stage of C. arvense. This was not achieved in the trials on undisturbed land or for the later timing in the winter wheat trials. The sulfonyl ureas gave poor control in undisturbed land trials, stunting but not killing the plants. The addition of surfactant did not produce a consistent effect. Mecoprop and clopyralid in the wheat trials and clopyralid and MCPA in the undisturbed land trials gave the best control, particularly in the Cambridge and Essex trials where the C. arvense was not so dense and vigorous. All the treatments (except glyphosate) prevented thistledown production.

TABLE 4

Change in shoot number between spraying and assessment expressed as proportion of shoot number at spray application in winter wheat trials (standard errors in brackets)

Treatment and timing	Norfolk	Essex
GS 32		
mecoprop	0.48 (± 0.06)	0.58 (± 0.19)
metsulfuron - methyl	1.37 (± 0.47)	0.62 (± 0.16)
metsulfuron - methyl + non-ionic wetter	0.79 (± 0.20)	0.96 (± 0.47)
DPX-L5300	1.28 (± 0.16)	0.99 (± 0.22)
DPX-L5300 + non-ionic wetter	0.69 (± 0.21)	0.57 (± 0.15)
clopyralid	1.18 (± 0.38)	0.56 (± 0.15)
control	1.09 (± 0.26)	0.96 (± 0.34)
GS 39		
metsulfuron-methyl	1.18 (± 0.09)	0.70 (± 0.16)
metsulfuron - methyl + non-ionic wetter	0.97 (± 0.24)	1.26 (± 0.34)
DPX-L5300	0.62 (± 0.13)	1.18 (± 0.13)
DPX-L5300 + non-ionic wetter	1.30 (± 0.06)	0.89 (± 0.12)
clopyralid	0.92 (± 0.06)	0.95 (± 0.03)
untreated control	1.22 (± 0.09)	0.56 (± 0.12)

Control of C. arvense fresh weight (Tables 6 and 7) was more satisfactory than the control of shoot numbers but showed a similar trend. In terms of control of regrowth from the roots (Tables 8 and 9) early timing again proved most effective in the winter wheat trials. The sulfonyl ureas were significantly less effective at preventing regrowth than clopyralid, mecoprop or MCPA. Metsulfuron-methyl and DPX-L5300 gave similar levels of regrowth and there was no consistent advantage in adding

surfactant to either. Shoots emerging from clopyralid treated roots showed symptoms of the herbicide six weeks later. Glyphosate treated roots showed very little regrowth.

TABLE 5

Change in shoot number between spraying and assessment expressed as proportion of shoot number at spray application in undisturbed land trials (standard errors in brackets)

Treatment	March	Cambridge
clopyralid	1.10 (± 0.084)	0.56 (± 0.099)
MCPA	0.95 (± 0.257)	0.60 (± 0.12)
DPX-L5300 15 g ai/ha	1.4 (± 0.171)	1.91 (± 0.04)
DPX-L5300 15 g ai/ha + non-ionic wetter	1.32 (± 0.276)	1.16 (± 0.058)
DPX-L5300 30 g ai/ha	1.28 (± 0.519)	1.18 (± 0.20)
DPX-L5300 30 g ai/ha + non-ionic wetter	1.43 (± 0.05)	1.92 (± 0.29)
untreated control	1.48 (± 0.114)	1.56 (± 0.18)

TABLE 6

Percentage control of shoot fresh weight/m^2 in winter wheat trials (standard errors in brackets).

Treatment and timing	Norfolk	Essex
GS 32		
mecoprop	89.8 (± 4.6)	74.7 (± 15.1)
metsulfuron- methyl	55.0 (± 19.7)	61.3 (± 14.7)
metsulfuron - methyl + non-ionic wetter	93.9 (± 1.1)	70.3 (± 13.1)
DPX-L5300	44.5 (± 18.1)	44.8 (± 18.5)
DPX-L5300 + non-ionic wetter	67.4 (± 22.8)	58.5 (± 17.5)
clopyralid	49.5 (± 16.7)	60.1 (± 9.1)
GS 39		
metsulfuron - methyl	27.1 (± 10.3)	16.6 (± 9.6)
metsulfuron - methyl + non-ionic wetter	25.2 (± 20.7)	78.2 (± 14.1)
DPX-L5300	29.7 (± 22.8)	60.2 (± 21.7)
DPX-L5300 + non-ionic wetter	41.2 (± 20.6)	33.0 (± 22.5)
clopyralid	32.9 (± 23.1)	57.4 (± 25.7)

TABLE 7

Percentage control of shoot fresh weight/m^2 in undisturbed land trials (standard errors in brackets).

Treatment	March	Cambridge
clopyralid	52.3 (± 13.5)	50.3 (± 27.6)
MCPA	39.9 (± 19.2)	64.4 (± 23.4)
DPX-L5300 15 g ai/ha	9.2 (± 4.3)	38.2 (± 21.6)
DPX-L5300 15 g ai/ha + non-ionic wetter	1.9 (± 1.9)	36.7 (± 6.7)
DPX-L5300 30 g ai/ha	42.6 (± 5.2)	32.9 (± 23.3)
DPX-L5300 30 g ai/ha + non-ionic wetter	16.8 (± 8.6)	54.3 (± 13.2)

TABLE 8

Percentage control of regrowth of root pieces in winter wheat trials (standard errors in brackets).

Treatment and timing	Norfolk	Essex
GS 32		
mecoprop	88.4 (± 11.6)	58.8 (± 18.4)
metsulfuron - methyl	36.1 (± 21.9)	47.4 (± 25.1)
metsulfuron - methyl + non-ionic wetter	33.3 (± 28.9)	50.0 (± 28.9)
DPX-L5300	13.6 (± 13.6)	74.0 (± 24.7)
DPX-L5300 + non-ionic wetter	19.3 (± 11.7)	25.0 (± 25.0)
clopyralid	83.8 (± 5.4)	59.6 (± 21.2)
GS 39		
metsulfuron - methyl	24.5 (± 21.7)	66.6 (± 28.9)
metsulfuron - methyl + non-ionic wetter	26.7 (± 13.9)	28.2 (± 14.8)
DPX-L5300	0 (± 0)	52.9 (± 25.1)
DPX-L5300 + non-ionic wetter	10.5 (± 7.1)	43.6 (± 25.6)
clopyralid	43.8 (± 20.1)	53.2 (± 29.0)
Pre-harvest glyphosate	97.7 (± 2.3)	-

DISCUSSION

The sulfonyl ureas were expected to give good control over both shoot material and regrowth from the roots. It is possible that warm and dry weather in June and early July created conditions of water stress. During such times, little growth would occur and the sulfonyl ureas could be immobilised before they were phytotoxic.

TABLE 9

Percentage control of regrowth of root pieces in undisturbed land trials (standard errors in brackets).

Treatment	March	Cambridge
clopyralid	55.7 (± 28.9)	98.9 (± 1.1)
MCPA	73.6 (± 13.3)	67.7 (± 19.5)
DPX-L5300 15 g ai/ha	0.0 (± 0.0)	25.3 (± 25.3)
DPX-L5300 15 g ai/ha + non-ionic wetter	8.5 (± 8.5)	27.3 (± 13.9)
DPX-L5300 30 g ai/ha	3.7 (± 3.7)	24.3 (± 24.3)
DPX-L5300 50 g ai/ha + non-ionic wetter	0.0 (± 0.0)	43.1 (± 29.7)

TABLE 10

1987 assessments of shoot regrowth in undisturbed land trials (percentage control)

Treatment	March	Cambridge
clopyralid	49%	89%
MCPA	40%	72%
DPX-L5300 15 g ai/ha	1%	27%
DPX-L5300 (15 g/ha) + non-ionic wetter	0%	4%
DPX-L5300 30 g ai/ha	0%	27%
DPX-L5300 (30 g/ha) + non-ionic wetter	19%	47%

Almost all the results show high standard errors. This is probably due to the difficulty experienced in positioning plots in a natural and uneven population of a perennial weed. Although care was taken in siting the trials, it was difficult to judge the density of root material from shoots present. Two of the trials had unusually large and established populations which were atypical of those normally found in cereals. There was also a large variability in the results of the root viability test.

Root collection and planting is time consuming and labour intensive. This method gave an indication of the herbicidal effect on the shallower roots and these results compared favourably with shoot assessments made in 1986 and 1987 and shoot fresh weight assessments made in 1986. However, the heavy workload involved in this method is unlikely to make it a practical alternative to assessing shoot material in the season after treatment.

In conclusion, the work indicates that mecoprop at GS 32 and the pre-harvest application of glyphosate provide the best control of C. arvense in cereals, and clopyralid at or before bud emergence and MCPA give better control than DPX-L5300 on undisturbed land. The root viability test gave a useful indication of herbicidal activity on the subterranean region of the plant.

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SESSION 5

HERBICIDE RESISTANCE IN CROPS AND WEEDS

CHAIRMAN MR R. J. MAKEPEACE

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GENETICALLY-ENGINEERED HERBICIDE TOLERANCE - TECHNICAL AND COMMERCIAL CONSIDERATIONS

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ABSTRACT

Gene transfer and cell selection techniques have been used successfully to create crop plants tolerant to several major herbicides. By the early 1990s, the first modified crops will be marketed - by the year 2000, this new technology will begin to exert a dramatic influence on weed control practices and on the introduction of new herbicide products. The decision to commercialize herbicide-tolerant plants will depend on the performance, cost and environmental acceptability of the herbicide. The nature of the resistance mechanism and the accessibility to commercial germplasm represent other key factors in the decision-making process. Ultimately, herbicide-tolerant crops will accelerate the trend towards commercializing fewer, more effective, less costly and environmentally more acceptable weed control products.

INTRODUCTION

The rapid progress that has been made in the development of gene transfer systems for higher plants has surprised even the most optimistic of researchers in the field. Today, nearly two dozen species of crop plants including vegetables, cotton, oilseed rape, alfalfa and sunflower can be routinely manipulated using available Agrobacterium tumefaciens or free DNA transformation systems (Fraley et al. 1986). Within the next 2-3 years it is likely that all major crop species will be accessible to improvement using this technology. In addition to the tremendous progress that has been made in the transformation of plants, equally dramatic progress has been made in the identification of single gene agronomic traits which confer insect (Fischhoff et al. 1987, Vaeck et al. 1987), viral disease (Abel et al. 1986, Tumer et al. 1987) and herbicide tolerance when expressed in transgenic plants.

Advances have been particularly dramatic in the engineering of selective herbicide tolerance, because existing knowledge of herbicide mode-of-action and metabolism has permitted rapid identification of key target genes. It is quite clear now that within the period of the next 5-10 years, commercial level, selective tolerance mechanisms will be available for major existing herbicides as well as for newly-introduced products. The availability of selective crop resistance to many of today's potent, broad-spectrum herbicides will exert a dramatic effect on weed control practices as well as on the introduction of new chemical products. In this paper, we will focus on the technical aspects of engineering herbicide tolerance as well as on other factors which will influence the commercialization and adoption of this new technology.

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ENGINEERING OF SELECTIVE HERBICIDE TOLERANCE

The biochemical basis for the mode-of-action of several herbicides has now been elucidated through physiological, biochemical and genetic studies. This research has facilitated the identification and molecular cloning of genes encoding herbicide-sensitive and insensitive target proteins from both microbes and higher plants. The enzymes which detoxify herbicides have also been studied and genes encoding these proteins have been cloned from a variety of sources. The herbicides for which tolerance has been achieved are discussed in detail below:

Glyphosate tolerance

The shikimate pathway enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), involved in aromatic amino-acid biosynthesis has been identified to be the specific target of this herbicide in bacteria (Steinrucken & Amrhein 1980). Subsequent studies have shown that glyphosate also inhibits this enzyme in higher plants (Mousdale & Coggins 1984, Rubin et al. 1984, Nafziger et al. 1984).

The first attempts to engineer glyphosate tolerance in transgenic plants have taken advantage of a bacterial gene encoding a glyphosate-tolerant EPSPS. A mutant gene encoding a resistant enzyme was isolated from S. typhimurium and was shown to contain a single base pair change resulting in a proline to serine amino-acid substitution at position 101 of the protein (Comai et al. 1983, Stalker et al. 1985). Chimeric genes were constructed in which the mutant EPSPS coding sequence was driven by either the octopine synthase promoter or the mannopine synthase promoter (Comai et al. 1985). The chimeric genes were introduced into tobacco cells using Agrobacterium vectors and plants were regenerated from the transformed cells. The transformed plants carrying the chimeric genes were two to three times more tolerant to glyphosate than the control plants. Recently, the chimeric EPSPS gene has also been introduced into transgenic tomato plants (Fillatti et al. 1987). The transgenic tomato plants expressing the gene were tolerant to glyphosate at a rate of 0.84 kg a.i./ha. The plants, however, were reduced in growth after spraying relative to unsprayed control plants. Calgene Inc. expects to commercialize glyphosate-tolerant crops in the early 1990s.

In a second set of experiments, Monsanto researchers (Shah et al. 1986) utilized high-level expression of a plant EPSPS gene to engineer glyphosate-tolerant plants. A full-length cDNA clone for EPSPS was isolated from a glyphosate-tolerant suspension cell line of Petunia hybrida. The amino-acid sequence predicted from the nucleotide sequence indicated that the enzyme was synthesized as a precursor polypeptide with an amino-terminal 'transit peptide' sequence of 72 amino-acids. The transit peptide is responsible for post-translational targeting of the precursor enzyme to the chloroplast (della-Cioppa et al. 1986). The wild-type petunia EPSPS cDNA was placed under control of the promoter for the 35S transcript of cauliflower mosaic virus (CaMV) and the resulting chimeric gene was transferred to a binary vector. Introduction of the chimeric EPSPS gene into petinia cells led to the growth of callus at concentrations of glyphosate sufficient to inhibit completely the proliferation of wild-type callus. Transformed petunia plants were regenerated and these plants were tolerant to application of formulated glyphosate at 0.9 kg a.i./ha, approximately four times the quantity necessary to kill the control plants. However the growth of these

plants was reduced relative to unsprayed controls.

More recently, experiments have been carried out with a mutant EPSPS gene which encodes an enzyme that is 1000-fold less sensitive to glyphosate inhibition than the wild-type petunia enzyme. Transformed tobacco plants expressing the glyphosate-resistant EPSPS gene were found to be significantly more tolerant to glyphosate than plants over-expressing the wild-type EPSPS gene. Tobacco plants expressing the mutant gene displayed no visible injury when sprayed with 0.9 kg a.i./ha of glyphosate; the treated plants flowered normally and set seed at levels identical to unsprayed controls.

Phosphinothricin tolerance

Phosphinothricin is a potent competitive inhibitor of glutamine synthetase (GS) from E. coli and higher plants (Bayer et al. 1972, Leason et al. 1982, Colanduoni & Villafranca 1986, Manderscheid & Wild 1986). Inhibition of GS by phosphinothricin causes a rapid accumulation of ammonia which is toxic to plant cells (Tachibana et al 1986). Initial attempts to confer tolerance to this herbicide in plants focussed on the isolation of a resistance gene from herbicide-resistant plant cells. Donn et al. (1984) selected alfalfa suspension cell lines that were 20 to 100-fold more tolerant to the herbicide than the wild-type cells; GS activity was elevated 3 to 7-fold in the tolerant cell lines. Increased enzyme synthesis was apparently sufficient to overcome the toxic effects of the inhibitor. To date, there is evidence by these researchers that expression of the GS gene confers low-level tolerance to phosphinothricin in transgenic plants. Since the plant GS complements the glnA defect in E. coli (DasSarma et al. 1986), it may be possible to select for resistant forms of plant GS in E. coli to increase tolerance levels.

A second approach to confer tolerance to phosphinothricin in transgenic plants has been recently described which involves expressing an enzyme that detoxifies the herbicide (De Block, personal communication). A gene that encodes the enzyme phosphinothricin acetyl transferase in S. hygroscopicus has been cloned and characterized. This enzyme acetylates the free amino group of phosphinothricin and protects the bacterium from the autotoxic effects of bialaphos. The gene was placed under the control of the cauliflower mosaic virus 35S promoter and introduced into tobacco cells; the resulting calli were resistant to high levels of the herbicide (500 mg/l). A number of plants were regenerated from the transformed calli and sprayed with 4 to 10 times the dose of herbicide required effectively to kill the control plants. All transgenic plants assayed were completely resistant to the herbicide. The expression of the detoxifying enzyme at a level as low as 0.001% of the total extractable protein was sufficient to protect plants against field level applications of the herbicide. The expression of this gene in transgenic tomato, oilseed rape and potato plants also conferred complete resistance to the herbicide. There are no data available on the fate of the acetylated metabolite in transgenic plants.

Sulfonylurea tolerance

The biochemical site of action of sulfonylurea herbicides has been recently elucidated. LaRossa & Schloss (1984) first showed that sulfometuron-methyl inhibited the growth of certain strains of E. coli and S. typhimurium. The sulfometuron-methyl inhibition of bacterial growth could be reversed by the inclusion of branched-chain amino-acids in the

culture medium. It was subsequently determined that the enzyme acetolactate synthase (ALS) which is required for the synthesis of leucine, isoleucine, and valine was the target of sulfometuron-methyl in S. typhimurium. Analogous studies have also been carried out in higher plants by selection for sulfonylurea-tolerant mutants of haploid tobacco cells (Chaleff & Ray 1984). The diploid tobacco plants which were regenerated from the mutant cells retained the herbicide-resistant phenotype under field test conditions. It was established by genetic crosses that the herbicide-resistant phenotype was due to a single dominant or semi-dominant nuclear mutation at one of two unlinked loci which co-segregated with herbicide-resistant ALS activity. Sulfonylurea-tolerant mutants of Arabidopsis thaliana have also been isolated by screening for growth of seedlings in the presence of the herbicide (Haughn & Somerville 1986). The tolerance was due to a single dominant nuclear mutation at the locus designated csr. Recently sulfonylurea-tolerant soybean mutants have also been isolated (Chaleff, personal communication). Like the glyphosate target enzyme EPSPS, ALS is a nuclear-encoded chloroplast-localized enzyme in higher plants (Chaleff & Ray 1984, Jones et al. 1985). The genes encoding the wild-type and mutant ALS have been isolated from tobacco and Arabidopsis and their nucleotide sequences have been determined (Mazur, personal communication). The amino-acid sequences deduced from the nucleotide sequences predict the presence of a presumptive chloroplast transit peptide at the amino-terminal ends of these two polypeptides. Recently, transgenic tobacco plants containing the chimeric ALS genes have been shown to demonstrate high level tolerance to sulfonylurea herbicides. A program is in progress with Northrup King to evaluate and commercialize sulfonylurea tolerance in tobacco.

Imidazolinone tolerance

The mode of action of these herbicides is similar to that of sulfonylurea herbicides in that they interfere with the biosynthesis of the branched-chain amino-acids valine, leucine, and isoleucine and also inhibit the enzyme ALS (Shaner et al. 1984). Maize cell cultures that are tolerant of imidazolinones have been developed (Anderson & Georgeson 1986) and several mutant maize cell lines having greater than 100-fold tolerance have been isolated. Some of these cell lines have been characterized as having altered ALS activity. Plants were regenerated from one of these cell lines and the tolerance was shown to be inherited as a single dominant nuclear gene. Plants homozygous for the resistance gene were 300-fold more tolerant to a number of imidazolinone herbicides. Field studies have found no detrimental effect of the mutant gene on the growth and development of maize plants. A back-crossing program is in progress with Pioneer to introduce this gene into commercial germplasm - the first tolerant corn seed could be marketed in the early 1990s.

Atrazine tolerance

Atrazine and other triazine herbicides interfere with photosynthetic electron transport by interacting with the 32 kd chloroplast protein (Gardner 1981, Erickson et al. 1984). Back-crossing of atrazine tolerance from related wild species has been carried out successfully, but many of the atrazine-tolerant commercial lines fail to perform agronomically at levels comparable to non-tolerant lines (Souza Machado 1982). Although attempts are underway to develop chloroplast transformation systems using mutant 32 kd genes as selectable markers, there are currently no data available supporting the success of this approach. Similarly, attempts have been made

to deliver the 32 kd protein from the cytosol to chloroplasts using chloroplast transit peptide sequences; however little or no tolerance appears to have been derived from this method.

It is well known that atrazine-tolerant plants such as corn contain elevated levels of glutathione-S-transferase (GST) (Shimabukuro et al. 1971, Guddewar & Dauterman 1979). GSTs are 27,000-32,000 kd proteins which catalyze the conjugation of glutathione with a large number of hydrophobic, electrophilic compounds including atrazine (Mozer et al. 1983). In corn, GSTs are encoded by a small multigene family (Shah et al. 1986). Recently researchers at Ciba-Geigy have reported introducing an atrazine-metabolizing GST gene into transgenic tobacco plants (Helmer 1986). The plants demonstrate increased tolerance to atrazine; however, Ciba-Geigy has indicated it is only pursuing atrazine tolerance as a model research project in plant biology.

Bromoxynil tolerance

The herbicide bromoxynil is another known inhibitor of photosynthetic electron transport (Friend & Olsson 1967). Calgene scientists (Stalker & McBride 1987) have recently reported the isolation of a bacterium, Klebsiella ozaene, which contains a plasmid encoding a nitrilase enzyme specific for the hydrolysis of bromoxynil. The products of the reaction appear to be 3,5-dibromo-4-hydroxybenzoic acid and ammonia - these comprise some of the same metabolites as found in naturally-resistant grass species. It is not clear if the same enzyme also metabolizes ioxynil, a related herbicide. A 2.6 kb DNA fragment was identified which when cloned into E. coli conferred bromoxynil degradation capability. It has recently been reported that expression of the nitrilase gene in transgenic tobacco and tomato plants confers tolerance to bromoxynil.

COMMERCIALIZATION OF HERBICIDE-TOLERANT CROP PLANTS

Agrochemical company perspective

The research and development necessary to generate herbicide-tolerant plants has in most cases evolved from studies to understand herbicide mode-of-action and metabolism. Such programs, which have been carried out by most major companies, have primarily been aimed at designing new herbicides or generating information to support registration. The rapid development of gene transfer and cell selection techniques has now provided a new option and decision point for agrochemical companies - whether or not to pursue selective resistance mechanisms for new and existing products. In many ways this decision follows the same rationale as that used to justify traditional safener or antidote programs, to pursue crop selectivity through chemical synthesis, or to extend product range through package mix combinations. The key question is whether the particular chemistry has the performance and cost benefits and the environmental compatibility to be competitive against other existing and developmental herbicides in a given cropping and weed control situation. In addition to normal environmental impact considerations such as toxicity, soil leaching, ground-water contamination, metabolism, resistant weeds, etc., engineering herbicide tolerance in plants raises several additional issues (Hauptli et al. 1985). These include the potential for transferring herbicide tolerance to weed species and the introduction of undesirable agronomic characteristics associated with the tolerance trait itself. The mechanism for evaluating these impacts is in place within the framework of the USDA, EPA and FDA registration processes.

Given the vulnerability that agrochemical companies face in introducing and maintaining herbicide products, there is little doubt that all possible environmental impacts of engineered tolerance will be thoroughly evaluated prior to commercialization.

While the cost of registering an existing herbicide for use on tolerant crops will be substantially less than for discovery and development of a new product for that crop, many other factors enter into the decision-making process. Since it will take 4-7 years to introduce a tolerance gene into a significant percentage of the germplasm for a given crop, it is critical to understand thoroughly the economic and performance characteristics of the tolerant crop-herbicide combination compared to other competitive products. Such an effort is certainly not justified unless the tolerant crop-herbicide combination solves an uncontrolled weed problem or brings additional value-added benefits (reduced weed control cost or greater environmental acceptability) to the cropping situation. Given the lengthy time-lines for introduction of tolerant crops, another factor will be patent life and post-patent pricing strategies for a particular herbicide.

The cost of registering a herbicide on tolerant crops will depend largely on the particular crop, herbicide and resistance mechanism employed. For example, the cost of registering an established product on an engineered non-food crop would be minimal since the EPA has no residue metabolism requirements for these types of crops. However to extend the registration to a food crop could cost in the neighbourhood of $250,000-500,000. This would assume that all other existing data for that crop are deemed adequate to support the new uses. Registration in new crops or crop groupings or in new geographic areas could trigger additional studies on environmental fate, metabolism, etc. which could run into several millions of dollars.

The availability of selective crop tolerance to broad-spectrum herbicides such as glyphosate, sulfonylureas, imidazolinones and phosphinothricin promises to exert a dramatic influence on weed control practices. Such technology will accelerate a maturing herbicide industry's trend towards commercializing fewer, but more effective, less costly and environmentally more acceptable products. As the methods for gene transfer and for trait incorporation into commercial germplasm become routine, the engineering of selective herbicide tolerance will become an accepted and essential strategy for development of weed control systems.

Seed company perspective

The commercialization vehicle for engineered tolerant crop plants will be seed companies which are either owned by or are collaborating with agrochemical companies to introduce the tolerance genes into commercial lines. Where transformations or cell culture selections can be performed directly with commercial germplasm, such programs will involve routine agronomic evaluation and scale-up. If the herbicide tolerance genes are in an unacceptable genetic background, the breeding effort to back-cross herbicide tolerance into commercial lines will be extensive. For some crops (trees, vines, etc.) the long time-frames for back-crossing would make this strategy unattractive.

Seed companies have historically selected lines that demonstrate the highest level of tolerance to widely-used commercial herbicides in a given crop, but there has traditionally been little interest in working

exclusively with a particular company's product. Rather, the intent has been to make sure that newly-released lines are compatible with leading weed control products on the market. With the ability to engineer selective resistance to particular herbicides which may be more effective, less expensive or environmentally more acceptable than existing products, there is greater interest by seed companies in obtaining and introducing tolerance genes into their commercial germplasm. Herbicide tolerance provides a unique 'branding' for a company's germplasm and may provide a marketable value-added advantage in particular cropping situations. In some countries, patents on engineered herbicide tolerance genes, vectors or plants may provide a form of protection for other agronomic traits in a given cultivar. In such situations, the seed company's interest in branding their own germplasm and capturing some of the value-added benefits from the agrochemical's novel performance would be counter to the chemical company's desire to maximize value by introducing herbicide tolerance in all major sources of commercial germplasm.

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BREEDING HERBICIDE-TOLERANT CULTIVARS - A CANADIAN EXPERIENCE

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ABSTRACT

The breeding and physiological research programs which led to the development of triazine-tolerant cultivars of brassica crops in Canada are described. The agronomic advantages and drawbacks of the cultivars produced to date are discussed, together with their impact on crop rotational practice and their effect on weed control strategies. Spin-off benefits to other areas of basic and applied research, to extension services and to education are outlined. However the major benefit has been the opportunity to design and test through to commercial cropping the techniques required to utilise genetics and plant breeding to solve intractable weed control problems.

INTRODUCTION

The development of herbicide-tolerant cultivars in Canada occurred as an after-thought, following the initial detection and chemical control of chloro-triazine resistance in broadleaved weeds like Chenopodium album (Bandeen & McLaren 1976) and Brassica rapa (Maltais & Bouchard 1978) in maize. Basic research to unravel the resistance phenomenon led to the discovery of the mechanism of resistance located at the chloroplast level (Radosevich 1977, Souza Machado et al. 1977), the cytoplasmic inheritance of this trait (Souza Machado et al. 1978) and the potential to transfer triazine tolerance into economic crops like canola, rutabaga (swede) and cole crops (Souza Machado et al. 1980). The trait was transferred to cultivated spring rapeseed Brassica napus (Beversdorf et al. 1979) and broccoli B. oleracea (Ayotte et al. 1987).

The transfer of chloro-triazine resistance into Brassica crops was justified, because of the absence of weed control recommendations involving registered herbicides at the pre-emergence and post-emergence stage of cultivation. The presence of cruciferous weeds like Thlaspi arvense and Sinapis arvensis (formerly Brassica kaber) in canola, the latter crop having been specifically bred for very low erucic and glucosinolate content, would lead to poor harvest seed grades due to adulteration by T. arvense and S. arvensis if these weeds were not controlled during the growing phase. The first chloro-triazine-resistant canola cultivar to be released was OAC Triton (Beversdorf & Hume 1984) followed by Tribute in 1986 and OAC Triumph in 1987.

BREEDING OBJECTIVES

With canola the objectives were to breed a chloro-triazine-resistant cultivar with as high a yield as was possible and of a quality to meet existing standards for canola, namely less than 5% erucic acid and less than 30 micromoles of glucosinolates per gram of oil-free meal. The cultivar was intended for areas with serious infestations of cruciferous weeds, which was one of the limiting factors restricting the expansion of the canola hectarage.

The breeding program with rutabaga was aimed at producing a 'Laurentian' type of cultivar with chloro-triazine resistance, purple top, globe shape and yellow flesh. The objectives with the cole group of B. oleracea crops was initially to produce a stable genotype with 2n=18 chromosomes of the CC genome and a chloro-triazine-resistant cytoplasm.

RESOURCES FOR RESEARCH

The initial grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to investigate the phenomenon of triazine tolerance and its economic potential amounted to about $92,000 Cdn. These were followed by assistance towards a breeding program to develop commercial cultivars, also from NSERC in the range of $200,000 Cdn. Subsequent support came from the Canola Council of Canada, Canada Packers Inc. and the New Crop Development Fund of Agriculture Canada. The breeding program has also been supported financially by the Ontario Ministry of Agriculture and Food (OMAF). Management and quality studies were also included in these assisted programs. Support for the spring canola breeding program up until the 1984 release of OAC Triton was about $300,000 Cdn. Funding to support the rutabaga and cole crops program was mainly from NSERC amounting to $140,000 Cdn. with assistance from provincial sources including OMAF.

THE GROWERS' SCENARIO

Initial problems with canola were that OAC Triton lodged badly and recorded poor yields. Emergence was slower in the spring than with triazine-susceptible cultivars and therefore the crop was more prone to damage by flea beetle (Phyllotreta spp.). The oilseed crushers did not like the fact that it was about 2% lower in oil content than comparable triazine-susceptible cultivars. Early test crushing experience with OAC Triton indicated that there was a heat-stable red pigment which was difficult to remove. In Western Canada OAC Triton was too late in maturity for much of the main canola-growing area and the harvested seed was often high in chlorophyll content. Attempts have been made to overcome these problems by breeding. The recent cultivar Tribute has earlier maturity and lower seed chlorophyll and OAC Triumph has better lodging resistance, about 1% higher oil content and slightly higher yields.

Better chloro-triazine-resistant cultivars continue to appear in the registration trials, some of which have been bred by a private plant breeding company. Following the release of OAC Triton in 1984, the area planted in Ontario increased to about 15,000 has in 1986. However, low canola prices in 1987 reduced the area planted. In Ontario OAC Triton occupies about 50% of the total spring canola area. It has been grown in fields where closely-related cruciferous weeds, which were not controlled by pre-plant incorporated trifluralin, were subsequently controlled by pre-emergence and post-emergence applications of triazines. The use of OAC Triton has also helped growers to diversify out of maize, where there were high atrazine residues in the soil and to permit the control of quackgrass or common couch (Elymus repens) with atrazine, while growing a rotational crop of canola. In Western Canada, the chloro-triazine-resistant cultivars OAC Triton and Tribute have constituted only about 3% of the total hectarage, or about 80,000 ha. These cultivars were grown in locations where weeds were difficult or expensive to control by other means.

Grower problems with rutabaga and the chloro-triazine-resistant line CTR/Laur mainly involved seed distribution, after the disbanding of the Rutabaga Growers Marketing Board which was originally to handle the seed sales. Trials at Research Stations indicated that when CTR/Laur and the chloro-triazine-susceptible cultivar Laurentian were planted early in May, no significant differences were noted between the two. However, delayed plantings up to late June resulted in significant depression of yield as compared to Laurentian. Susceptibility to Turnip Mosaic Virus was more severe in CTR/Laur at the later planting dates. Emergence and seedling development were initially slower with CTR/Laur than Laurentian, but if planted early in May, no yield differences were noted between CTR/Laur and Laurentian. Control of cruciferous weeds was possible within and between the rows, using cyanazine as a pre-emergence treatment.

SPIN-OFFS FOR RESEARCH

The research program has resulted in a considerable number of side benefits to basic and applied research. Collaboration with Dr C. Arntzen in the USA during the initial stages of the physiology research at Guelph (Souza Machado et al. 1978) had led to numerous refereed publications on photosynthesis involving chlorophyll fluorescence and chloroplast membrane alterations (Arntzen et al. 1979, Steinbach et al. 1981, McIntosh & Hirschberg 1983). The role of organelle involvement in cytoplasmic inheritance has been studied using chloro-triazine-resistant and susceptible biotypes of B. campestris and Amaranthus spp. (Vaughn 1985). Other areas of basic research involve protoplast fusion to combine the chloro-triazine resistance trait with mitochondrial male sterility, its use as a genetic marker and the creation and testing of chloro-triazine-resistant spring canola hybrids (Grant & Beversdorf 1985), as well as anther and microspore culture methods for producing doubled haploids.

Spring canola was not grown prior to the release of OAC Triton, therefore a whole testing program had to be developed to obtain data to support registration for this and other cultivars. Extensive trials in Eastern and Western Canada involving herbicide testing and registration have been completed, as well as research on the reasons for the depressed yields and photosynthetic rates of the chloro-triazine-resistant canola lines. Field trials have also been carried out to determine the extent to which they could become a volunteer weed problem. Several graduate students were involved in the breeding program at the Masterate and Doctorate level and many are now working in plant breeding professionally.

At the applied extension level, rapid techniques to detect chloro-triazine resistance or susceptibility in weeds have been worked out based on measuring chlorophyll fluorescence (Ali & Souza Machado 1981, Ahrens et al. 1981). This technique has helped extension and chemical company personnel to solve field problems associated with weed escapes or faulty herbicide application. Bioassays using chloro-triazine-resistant and susceptible seeds can be used to monitor atrazine or urea herbicide residues in top-soil used for gardens. Teaching experiments in plant physiology and weed science to undergraduates have involved these biotypes to demonstrate the Hill reaction with isolated chloroplasts and the role of photosynthetic inhibitors and pre-emergence and post-emergence herbicides.

CONCLUSIONS

The breeding potential of transferring chloro-triazine resistance into economic crops is a goal that has yet to be fully achieved, particularly with respect to yield levels. An encouraging aspect so far with the canola and rutabaga programs has been the stability of resistance maintained over several generations. Although it has not proven to be the panacea to all our cropping problems, it has served to test a novel approach to weed control, involving use of genetic and plant breeding techniques to adapt crops to existing inexpensive registered herbicides and has encouraged rotation of field crops to cope with volunteer crop problems, rather than sustain a monoculture system of cultivation.

The development of new selective chemical compounds like the DuPont product DPX A7881 to control wild mustard in Brassica crops, the re-registration toxicology requirements of cyanazine because of the Industrial Biotest Laboratory issue, and increasing pressure on atrazine registration because of alleged well-water contamination, make the future status of chloro-triazine-resistant crop cultivars unclear. However, the research and development work in this area to date has proven to be challenging, productive and an experience that has benefitted agricultural and plant science.

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APPEARANCE OF SINGLE AND MULTI-GROUP HERBICIDE RESISTANCES AND STRATEGIES FOR THEIR PREVENTION

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ABSTRACT

The first resistances to appear were to single herbicide types. Sequential multiple resistances have evolved to photosystem II herbicides, and broad multiple resistance among oxidant-generating herbicides (paraquat, nitrodiphenylethers, PSII-inhibitors), as well as to herbicides degraded by monoxygenases (diclofop-type diphenylethers, and some phenylurea, dinitroaniline and sulfonylurea herbicides). The necessary strategies to delay and allay these resistances include herbicide rotations, mixtures, increased use of less persistent compounds and synergists that lower selection pressure. In the cases of triazine and dinitroaniline resistances, herbicide rotations have proven to be better ploys than the early models had predicted. Resistance has not evolved anywhere where these strategies have been used, either fortuitously or by design.

INTRODUCTION

Resistance to herbicides has evolved throughout North America and Europe, in spots in the Middle and Far East, Australia and New Zealand. There is less propensity to publish new cases so that the extent of resistance becomes harder to ascertain. There has been a high level of complacency, as in almost all cases there have been suitable alternative herbicides to control the resistant weeds. Resistance has evolved very rapidly to some of the replacements. The cost of alternatives may be quite large; in certain cases, ten-fold greater (Ammon & Irla 1984). The most widespread resistances are to s-triazine herbicides but others have been appearing. The multiple resistances, i.e. resistances to herbicides of different chemistries or modes of action, are especially worriesome. One need only look at similar multi-resistances in insecticides to realize the dire implications. Still, there are simple strategies available to preclude, delay or alleviate the evolution of resistant populations. Except for a few understandable and explainable exceptions, all cases of resistance have occurred where there was repeated mono-culture/mono-herbicide use. A possibility exists that *Avena fatua* may evolve the same mechanism that cereals use for differential tolerance. Resistance has not appeared where strategies of rotation of herbicides have been used, despite predictions by earlier models (Gressel & Segel 1982) based on the data then available. The use of mixtures and the single annual applications of non-persistent herbicides have historically proven to be very effective strategies to prevent or delay resistance. The reasons why synergists should delay the appearance of resistance in certain weeds are discussed. Often, it will only be possible to design strategies after the first cases of resistance appear and genetics and the modes of action studies are performed. It is then necessary to overcome the emotional-geographic barrier which says that "what happened there won't happen here", and to apply preventative strategies.

THE EVOLUTION AND SPREAD OF RESISTANCES

Photosystem II inhibiting herbicides

Resistance has appeared world-wide to the s-triazine herbicides atrazine and simazine in about 50 weed species. In almost all cases these highly persistent herbicides were used alone, repeatedly for 5-10 years, in maize, orchards or along rights-of-way. Except in a recent case with *Abutilon theophrasti* (Andersen & Gronwald 1987), triazine resistance is maternally inherited, and has been traced to a single amino-acid transversion coded by a chloroplast gene which controls herbicide binding to its site of inhibition (e.g. Gressel 1985). Other sites are mutable in algae resistant to photosystem II herbicides, giving varying resistances to phenylurea and uracil type herbicides and the same may be true in weeds. There are many plastids per cell, each with DNA. The generation of oxygen radicals by the triazine-susceptible plastids would be phytotoxic, even if there were some resistant plastids. For this reason it is supposed that: (a) plants bearing recessive plastid mutant traits would be exceedingly rare - so rare that the frequency can only be guessed as being lower than 10^{-20}; (b) there is probably some stage of a plant's life cycle where there are fewer copies of plastid DNA per cell, or there is some sort of segregation, allowing plastids bearing recessive mutations to become predominant. If not, such mutant phenotypes would be even more rare. Peculiar leaf mosaic patterns in triazine-resistant *Solanum nigrum* plants were genetically traced to a a nucleus-coded gene which greatly enhanced the rate of chloroplast mutations (Arntzen & Duesing 1983). This "plastome mutator" gene may have increased mutations allowing triazine-resistant populations to evolve, despite the infinitesmally low natural frequency of the gene. The presence of other chloroplast mutations would cause the mutator gene to be bred out of resistant populations after repeated herbicide selection.

Sequential appearances of PSII herbicide resistance

Atrazine-resistant weeds are usually resistant to all s-and as-triazines, some phenylureas (but not diuron) and some uracils. They have usually remained sensitive to pyridazinone-type herbicides as well as to pyridate. A case of atrazine/chloridazon (pyrazon) co-resistance in *Chenopodium album* has been reported in fields with a crop rotation of maize (with atrazine) and sugar-beet with chloridazon (Solymosi *et al.* 1986). It should be presumed that each mutation was an independent event and the frequency of each different resistant chloroplast biotype should have been the same. Thus, if it took eight years to obtain populations of triazine-resistant biotypes, it should take another eight to obtain resistance to each of the PSII herbicides used as a replacement. Triazine resistance became a fact throughout the Hungarian monoculture maize growing areas within 8-12 years of use after maize and atrazine were co-introduced. Resistance to PSII herbicides that previously controlled atrazine-resistant *Chenopodium album* evolved, not in the expected 8-12 years but in 2-3 years after each was introduced. Thus, there are recently evolved *C. album* biotypes that are atrazine-resistant, atrazine and choridazon-resistant, chloridazon-resistant, atrazine and pyridate-resistant, and atrazine, chloridazon and pyridate-resistant (Solymosi *et al.* 1986 and Solymosi pers. comm.). In all cases resistance seems to be at the chloroplast level, as with atrazine resistance. The plastome mutator gene frequency should be much higher in triazine-resistant populations than in wild-type populations. This higher frequency of the mutator genes would facilitate a much more rapid sequential evolution of secondary and tertiary resistances to PSII herbicides, and has obvious implications in designing strategies to prevent resistance (Gressel 1986).

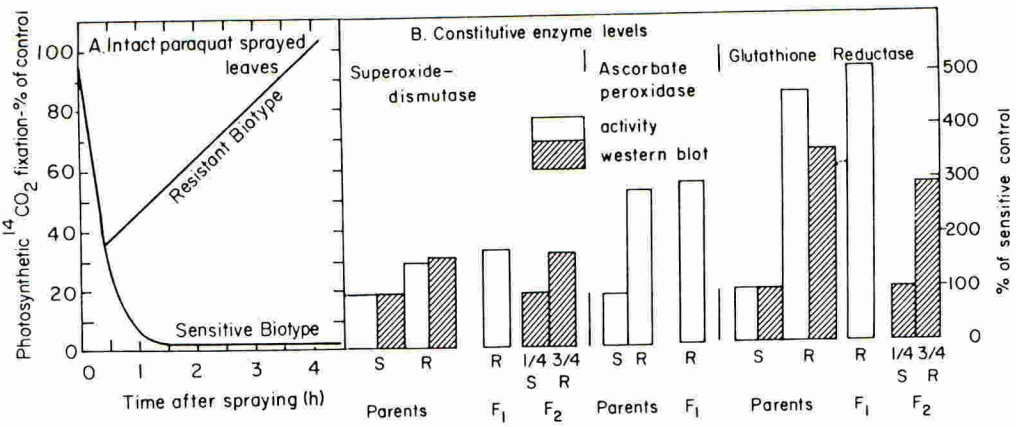

Fig. 1. Paraquat effects in resistant *Conyza bonariensis*. A, The transient effects on chloroplasts. B, The levels of Halliwell-Asada active-oxygen detoxification pathway in the chloroplasts. A, Resistant and susceptible plants of *Conyza bonariensis* were sprayed with 0.1 mM paraquat and photosynthesis was measured as an estimation of paraquat affecting chloroplasts. Source: Redrawn from Shaaltiel & Gressel (1987b). B, Enzyme levels without paraquat treatment in resistant and susceptible *Conyza bonariensis* and their crosses. The F_1 plants were all resistant. Source: Collated and drawn from data in Shaaltiel & Gressel (1986) and Shaaltiel (1987).

Oxidant-generating herbicides

Three *Conyza* spp. have evolved paraquat resistance in Egypt, Japan and Hungary (Gressel *et al.* 1982, Tanaka *et al.* 1982, Pölös *et al.* 1987). *Poa annua* and *Lolium perenne* in the UK (cf. Gressel *et al.* 1982), *Epilobium ciliatum* in Belgium (Bulcke *et al.* 1985) and *Hordeum glaucum* in Australia (Powles, 1986) have also evolved paraquat resistance. These cases seem inconsistent with predictions that resistance would appear only to persistent herbicides exerting season long selection pressure (Gressel & Segel 1982). However, the persistence of the herbicide was replaced by that of growers who persisted in applying paraquat 4-10 times per season for 6-14 years. In at least two cases, there is a modicum of cross-tolerance to the oxidant-generating herbicide such as atrazine in *C. canadensis* (Pölös *et al.* 1987), and atrazine, acifluorfen and SO_2 in *C. bonariensis* (Shaaltiel 1987).

We have studied paraquat resistance in *C. bonariensis*. Paraquat rapidly penetrates into leaves and chloroplasts of the resistant leaves, transiently inhibiting photosynthesis in both *C. bonariensis* (Fig. 1A) and *C. canadensis* (Pölös *et al.* 1987). Resistance is due to a single dominant gene that pleiotropically controls elevated plastid levels of at least three enzymes that participate in the detoxification of the active oxygen generated by paraquat: superoxide-dismutase, ascorbate-reductase and glutathione-reductase (Fig. 1B). The oxygen-detoxifying enzymes probably preserve the plants from death while paraquat is dissipated or sequestered, a process which can be visualized only much later (cf. Fuerst *et al.* 1985). It is not clear if this is the sole mode of paraquat resistance that has evolved. The levels of the oxygen-detoxifying enzymes have not been measured in intact chloroplasts of other resistant (vs. susceptible) biotypes that have evolved.

Resistance to tubulin-binding dinitroaniline herbicides

Eleusine indica has evolved resistance to dinitroaniline herbicides. In all cases trifluralin was used as the sole herbicide in some U.S. monoculture cotton for more than 6 years (Mudge *et al.* 1984). This resistance has been traced to a modification in the tubulin of the resistant biotype which precludes herbicide binding (Vaughn 1986). Thus, there is no herbicide-inhibition of microtubule formation. This is analogous with resistance to benzimidazole fungicides by fungal pathogens. It would be quite informative to know the mode of inheritance of this resistance.

Resistance to herbicides degraded by monoxygenases

Wheat is naturally resistant to a large number of herbicides because it is capable of degrading them using monoxygenases (=mixed function oxidases = cytochrome P_{450}s) (e.g. Sweetser *et al.* 1982, Brown *et al.* 1987). This property of wheat is especially useful as some key weeds such as *Avena fatua*, *Alopecurus myosuroides* and *Lolium* spp. are normally incapable of degrading the same herbicides, allowing differential control. The recent widespread multi-resistances may be the unwanted swallows of spring. These include *Lolium rigidum* resistant to diclofop-methyl (and related diphenylethers) and sulfonylureas (Heap & Knight 1986), and *Alopecurus myosuroides* to chlortoluron, sulfonylureas, diclofop-methyl and pendimethalin (Moss & Cussans 1987). This is probably due to the evolution of greater monoxygenase activity (Cole & Owens 1987, Kemp & Caseley 1987). The spread of such multi-resistances, could abrogate most grass-selective wheat herbicides. It is imperative to ascertain the genetics and molecular biology of this resistance. As discussed below, the strategies are different for preventing single-gene resistances (usually controlling binding), and for polygenic or gene-dose resistances. *A. fatua* already possesses low levels of wheat type degrading enzymes but the activity is too low to allow resistance (e.g. Shimabukuro *et al.* 1987). Is it sufficient to modify an *Avena* control gene to obtain resistance? Would resistance be polygenic requiring combined gene doses or must there be gene amplifications as with insecticide resistance (e.g. Mouches *et al.* 1986)? Different modes of resistance can evolve to the same herbicide. Sulfonylurea resistance selection has led to tobacco (Chaleff & Ray 1984) and *Arabidopsis thaliana* (Haughn & Somerville 1986) with modified acetolactate synthase, the target enzyme of the herbicide. Chlorsulfuron-resistant alfalfa (lucerne) (Stannard *et al.* 1987) and soybean (Sebastian & Chaleff 1987) were also selected, and they have some other (unknown) mechanism of resistance. Are the latter cases due to monoxygenases, and are there multiple cross-resistances? Will plants that have a multiple resistance, including to sulfonylurea herbicides, also be resistant to the imidazolinone and to the new triazolo-pyrimidine herbicides that inhibit acetolactate-synthase (Shaner *et al.* 1984, Berwick *et al.* 1987)? It will also be interesting to know more about the mode(s) of resistance and further cross-resistances in *Stellaria media* that evolved resistance to mecoprop and other phenoxy herbicides (Lutman & Lovegrove 1985).

STRATEGIES TO DELAY AND CONTAIN RESISTANCE

Two major factors control the duration it takes for resistant populations to evolve: the selection pressure and the initial frequency of resistance genes. The buffering effect of the soil seed reservoir and the fitness of the resistant biotypes can considerably delay the evolution of resistance. The problem of

frequency of resistant individuals is paramount in deriving strategies: there are differences in genetics and frequencies that require diametrically different strategies. For single major-gene resistances, the initial frequency is usually a fixed number; it does not change with herbicide application rate (selection pressure). The frequency can vary considerably with application rate, especially when there is polygenic inheritance or gene amplification responsible for resistance. In the latter cases, if we treat with a low dose of herbicide, we may obtain populations with resistance due to any of the separate effects of any number of genes which together will be at a high frequency. We might also select for a duplication of any one of these genes. If the dose is later raised, we will rapidly get further resistance, due either to a combination of the pre-enriched polygenes or to further gene duplications. Had high dose been used from the beginning, resistance would have been much delayed because of the much greater rarity of individuals with two or more of the polygenes or with multiple gene amplifications. In these gene dose cases, high initial doses are clearly called for from the time the herbicide is released. The "Catch 22" situation for single genes is that the use of the lowest herbicide rates will lower the selection pressure for target site mutations for resistance, considerably delaying resistance. Thus, we rarely know how to delay resistance, until resistance occurs for the first time, can be studied, and strategies outlined. Another "Catch 22" situation - whenever a new resistance has appeared and has been confirmed, it has either been discounted as being a rare instance (true), and not being indicative of future evolution (untrue). Such instances have often been ignored or concealed. The necessary research effort has usually been initiated 4-6 years after the first confirmed reports... too late to delay or prevent co-evolution elsewhere. Spreading resistance by seed or pollen for distances of more than a few hundred metres is usually rare in agronomic situations, although vehicular spread is probably the norm along rights-of-way. Thus, most cases of resistance are due to separate instances of parallel evolution through selection.

Most of the studied significant cases of resistance seem to be traced to single genes (but we have no genetics for the dinitroaniline or monoxygenase resistances). We will thus assume in the following sections that a major ploy to delay resistance will be to decrease selection pressure by using lower herbicide rates or less persistent herbicides.

Thresholds/windows/persistence

Lowering the selection pressure allows more susceptible individuals to remain alive. Their seeds dilute the seed produced by any resistant individuals in the population. The susceptible individuals are usually more fit than the resistant ones, i.e. they have a greater reproductive output per plant. There are a variety of ways to lower selection pressure without losing effective weed control. The use of thresholds for deciding when to treat with herbicides is discussed in Sessions 9 and 10 of this conference. As innocuous levels of weeds are left, treatments are applied later, and not always over the whole field, selection pressure becomes greatly reduced.

Herbicides of the phenoxy type gave adequate economic weed control, but because of their low persistence, they allowed later non-competitive weed germination of susceptible seeds. This low selection pressure is probably a major reason why there are hardly any reported resistance problems from 2,4-D or MCPA use. If *s*-triazines with less persistence had been used in maize, resistance would probably have never evolved. New wheat herbicides (e.g. chlorsulfuron) have an effective persistence of 2 years or more. It is logical to predict that some weed species will rapidly evolve resistance to this phenomenal group of herbicides as well as to the similarly-acting imidazolinones and triazolo-pyrimidines.

Mixtures and synergists

Mixtures can and have prevented the appearance of resistance. Chloroacetamides such as alachlor and metalachlor have long been mixed with atrazine, mainly because they are superior to atrazine alone for grass control. They control *Amaranthus* and *Chenopodium* spp., which are also controlled by the lowest atrazine doses. The use of chloroacetamides allows using less atrazine, decreasing the selection pressure. It also requires that resistant *Amaranthus* and *Chenopodium* spp. would have to evolve co-resistance to chloroacetamides. If there is a gene for chloroacetamide resistance, it would have to be in the same plant as that having atrazine resistance. The frequency of doubly-resistant individuals would be the compounded frequencies of atrazine and chloroacetamide resistances, a very low number.

Herbicide synergists allow the use of lower herbicide doses. Tridiphane is an example of such a compound. It prevents grasses from metabolizing atrazine (Lamoureux & Rusness 1986), allowing grass control at lower atrazine rates (Ezra *et al.* 1985). These low rates are still sufficient to control broad-leaved plants... but with lower selection pressure. Tridiphane may not delay grasses from evolving triazine resistance but it could delay resistance in the broad-leaved species. The latter evolved triazine resistance much before the grasses. Grasses would have to co-evolve tridiphane and atrazine resistance.

We have recently shown that paraquat resistance (Fig. 1) can be suppressed by adding chelators which remove the copper or zinc from superoxide dismutase or the copper from ascorbate peroxidase (Shaaltiel & Gressel 1987a, Shaaltiel 1987). These allow the use of much lower paraquat rates to control some of the weeds which were harder to kill with this herbicide. The use of lower rates (lower selection pressure) should delay the evolution of other modes of paraquat resistance as well as suppress this type of resistance.

The multiple-resistance due to elevated monoxygenases can be suppressed by aminobenzotriazole (Kemp & Casely 1987) and presumably by more plant-compatible monoxygenase inhibitors such as tetcyclasis (Cole & Owens 1987). These compounds not only suppress the weeds' monoxygenases, they suppress those of wheat (Cabanne *et al.* 1985). It is unclear how these synergists could be used in wheat/weed selective systems, and it is thus not yet apparent how they will be useable in agriculture. One can try to predict their effect on the evolution of resistance when the uses are known. In summary, mixtures will probably delay resistance in weeds that are targets to both components... especially if the selection pressures are lowered. Synergists will delay resistance in the weeds which were not the direct reason for their use, again by lowering selection pressure.

Herbicide rotation

It was initially modelled that if it would take ten years for resistance to occur to a given herbicide used in mono-herbicide culture, it would take 15 years if that herbicide were used in 2 out of 3 years and 20 years if the herbicide were used every other year (Gressel & Segel 1982). There are large areas in the U.S. corn-belt with 15-20 such treatments with atrazine in maize in rotation, without the appearance of triazine resistance. Triazine resistance has repeatedly occurred on smaller total acreages worldwide after 7-12 consecutive usages in monoculture. It is clear that rotation in this case has proven to be a far better ploy in preventing resistance than the models predicted, for reasons

recently understood. Most triazine-resistant weeds that have evolved are very non-competitive with the wild types of the same species. This is best expressed when there is no triazine present, i.e. in the rotational years. Early measurements of this lack of fitness, while showing 2-5 times greater fitness of the wild-type (Conard & Radosevich 1979) were probably underestimates. Fitness was not measured throughout the life cycle (including to overwintering, tolerance to late frosts, early germination and the critical period of establishment), nor were measurements made at field seeding densities. In addition there is now evidence that cultivation and many herbicides such as ioxynil (Thiel & Boger 1984), DNOC (Lehoczki *et al.* 1984), and 2,4-D (Salhoff & Martin 1986) harm the resistant biotypes more than the wild type. Similarly, dinitroaniline-resistant *Eleusine* is supersensitive to some tubulin inhibitors (Vaughn *et al.* 1987). Thus, the totality of events during the rotational years when triazines are not used has the effect of pushing the triazine-enriched frequency of resistant types back towards their initial frequencies. These factors are being considered in newer models which show that herbicides like triazines and dinitroanilines can have very long usefulness when used in rotation.

Another aspect of these newer models is that predictions can be made about what might happen when resistance appears and practices perforce change. Unfortunately the agricultural ecologists have not followed the decrease in frequency of resistant individuals after triazine usage was stopped. It was predicted that the frequency of resistance would decay to *ca* 1% of the population in 6-7 years (Conard & Radosevich 1979). Our present model suggests that the decay will be much quicker and that the low levels of resistant material will allow triazines to be used in rotations a few years after resistant populations appeared.

The trifluralin-resistant *E. indica* is also unfit (Mudge *et al.* 1984), which probably explains why this resistance has occurred only in mono-herbicide mono-culture despite widespread dinitroaniline use. Still, this situation may not be universal. There have been no indications that the paraquat-resistant weeds are much less fit (our group's unpub. results). Crop plants selected for non-binding of sulfonylurea (Chaleff & Ray 1984) and imidazolinone (Anderson 1986) herbicides at the level of the target enzyme do not apparently lack much fitness. In such cases rotation may at best delay resistance for only the time the rotation is in place. At worst, because of the persistence of levels killing some weed species during the rotational year, selection pressures will be continued throughout this period. These are factors which must be measured.

CONCLUDING REMARKS

Learning from the mishaps of history is the best way to prevent the evolution of resistance. It is impossible to integrate in advance all knowledge about a new herbicide to predict when or if resistance will occur. The more information on the physiology, biochemistry and genetics of a compound's action and selectivity is known, the more can be predicted. When the first resistances appear in the lab or in the field, further, more knowledgeable predictions can be made. When there is widespread resistance, we can learn much on prevention and containment by studying resistance *and* by analyzing why resistance did not occur elsewhere. The worst thing we can do, whether as producers or users of herbicides, is to ignore confirmed resistance. There are many different strategies that can be applied to delay or prevent resistance.

These strategies may reduce the current levels of usage of that herbicide, dismaying both farmer and chemical-manufacturer. Still, such strategies will keep the herbicide in useage for longer, compensating for the initial loss of use or sales. It is this longer-term benefit that must be realised.

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IMPLICATIONS OF HERBICIDE-TOLERANT CULTIVARS AND HERBICIDE-RESISTANT WEEDS FOR WEED CONTROL MANAGEMENT

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ABSTRACT

This paper examines the rationale behind research which endeavours to produce herbicide-tolerant cultivars. The value and limitations of herbicide-tolerant cultivars are described for triazine-tolerant oilseed rape in Canada and imidazolinone-tolerant maize in the USA. Consideration is given to the possible problems which might arise in releasing herbicide-tolerant crops with respect to their agronomic performance, the risk of spreading the tolerance trait to related weeds, cross-resistance to other herbicides and volunteer crop control. The background to herbicide-resistant weeds is examined together with the principles of herbicide-resistant weed management. The competitive effects, biological fitness and long term consequences of herbicide-resistant weeds are reviewed. Suggestions are made as to areas for future research which would contribute to our understanding of the consequences of introducing herbicide-tolerant cultivars and the development of herbicide-resistant weeds.

INTRODUCTION

Herbicides may be regarded as essential tools in successful crop production. Increasing scrutiny is being placed on environmental, management and economic aspects of herbicide use therefore it is important not to forget the intrinsic value of these chemicals for weed control. The property of selectivity of action in a herbicide is often fundamentally desirable in the development of a compound. Selectivity is seldom absolute. The terms herbicide-tolerance and herbicide-resistance exemplify the variation in response to a herbicide that may occur. Tolerance may be described as a relatively minor or gradual difference in intraspecific variability (LeBaron & Gressel 1982a), whereas resistance is defined as a decreased response in a population of animal or plant species to a pesticide or control agent as a result of their application (Anonymous 1965). Thus, a resistant weed is one that survives and grows normally at the usual effective dose of a herbicide (LeBaron & Gressel 1982a).

The purpose of this paper is to consider the applied aspects of the development of herbicide-tolerant cultivars and herbicide-resistant weeds. The background, value and limitations of herbicide-tolerant cultivars will be reviewed together with the management of herbicide-resistant weeds.

GENERAL CONSIDERATIONS : HERBICIDE - TOLERANT CULTIVARS

The development of herbicide-tolerant cultivars can be pursued via three principal methods; (1) classical plant breeding, (2) tissue culture and (3) gene manipulation. Individual techniques are reviewed by Chaleff (1986) and Gressel (1987a). In each procedure the object is to produce herbicide-tolerance de novo in a crop species previously susceptible to the target herbicide. From the herbicide manufacturer's point of view herbicide-tolerant cultivars may provide an extension to the existing market and prolong the market share for herbicides that are off patent. Furthermore, the impending withdrawal from use of a range of hitherto valuable herbicides on the basis of environmental or toxicological grounds could leave some important gaps in the choice of chemical weed control. The production of herbicide-tolerant cultivars could help to alleviate any inadequacies in weed control. Current liaison between chemical manufacturers and seeds companies provides an adequate testimony to the idea that farmers may soon be offered an integrated crop production package including the compatible cultivar-herbicide combination. Commercial interest in such specialist crops is perhaps unlikely to provide speedy or significantly profitable returns in the short term.

The situations where herbicide-tolerant cultivars could receive consideration are varied. Generally, however, one would wish to see the use of a broad-spectrum, environmentally safe, low cost herbicide where herbicide tolerance had been specifically incorporated. Therefore, a poorly competitive crop where the existing expensive herbicides do not give enough crop safety or a sufficient weed control spectrum would stand to benefit from the introduction of a herbicide-tolerant cultivar. Crop rotation restrictions may be imposed due to the carry-over of herbicides in soil. Such cropping restrictions can be reduced where a crop has tolerance to the residual herbicide. More specialised situations where herbicide-tolerant cultivars might be used for weed control include companion crops (Faulkner 1982) and clean seed production (Faulkner 1976).

The engineering of herbicide-tolerant cultivars does raise important issues as far as potential problems are concerned. Will herbicide use increase ? While pesticide inputs are designed to be reduced with disease and pest-resistant cultivars, this situation is unlikely to arise with respect to herbicide-tolerant cultivars. Will herbicide-tolerant cultivars pose problems as volunteer weeds ? Is it possible that cross resistance will spread to related weed species and be extended to include herbicides which have a similar mode of action ? These questions and others can perhaps be addressed by taking practical observations of the use of herbicide-tolerant cultivars outside Europe.

CASE STUDIES : HERBICIDE-TOLERANT CULTIVARS

Triazine-tolerant oilseed rape

Triazine tolerance as a trait was obtained from a weed biotype of B.campestris and transferred by Beversdorf et al. (1980) to both Polish rape (B. campestris) and Argentine oilseed rape (B.napus). In the species described triazine tolerance is a trait which shows maternal inheritance

(Souza Machado 1982) based on genes coded for by the chloroplast genome. The important point about understanding the nature of inheritance is that a cytoplasmically-controlled trait of this type cannot be inherited via the pollen (Tilney-Bassett 1975). Thus the spread of triazine tolerance or resistance from a cultivated resistant oilseed rape plant to closely related weeds would not be a major threat (Souza Machado 1982).

Spring sown oilseed rape (canola) is grown annually on 2.8 million ha in Canada, making this the most important oilseed crop. Farmers are encouraged to produce canola since it fits well with rotational practices and provides a sound financial return. There are two principal restrictions to the adoption of canola production. First, in regions where continuous maize production occurs, the residues of triazine herbicides in soil preclude canola production due to the crop's sensitivity to triazine carryover. Second, the widespread infestations (400,000 ha) of wild mustard (B. kaber) and stinkweed (Thlapsi arvense) (Grant & Beversdorf 1985) cannot be controlled by trifluralin, the most popular herbicide used for the control of green foxtail (Setaria viridis) and a range of broad leaved weeds. The competitive effects of stinkweed and wild mustard are severe, reducing yield by up to 25 %. In addition the weed seeds contaminate the grain, reducing the oil quality.

The introduction of the triazine-tolerant canola (TTC) cultivar OAC Triton has provided an attractive option to farmers who wish to grow canola in triazine-burdened land or where stinkweed and wild mustard are problems. The herbicides cyanazine (post-emergence) or metribuzin (pre or post-emergence) provide the selective broad spectrum weed control together with Cruciferous weed control in TTC. Unexpectedly, one of the dramatic improvements in weed control which followed the introduction of TTC in Eastern Canada took place in the maize crop. In the move away from continuous maize growing the previously uncontrolled wild prosso millet (Panicum dichotomiflorum) could now be effectively controlled in TTC using the graminicide sethoxydim. This compound also gave important control of wild oat (Avena fatua) and Setaria species. Volunteer TTC does not present a weed problem since the phenoxyacetic acid-based compounds used in the following wheat crop provide the necessary control.

The incorporation of the triazine tolerance trait causes significant reductions in several areas of agronomic importance. OAC Triton shows poor early seedling vigour, lower oil yield and oil content together with delayed maturity. The triazine-tolerant genotype also reduces the competitive fitness of the crop compared to near-isogenic lines of triazine-susceptible genotypes (Gressel and Ben-Sinai 1985). Triazine tolerance has been found to result from a gene mutation. A chloroplast membrane-located polypeptide changes in one amino acid (serine to glycine) (Hirschberg and McIntosh 1983). This mutation causes various chemical and structural changes in the chloroplasts of tolerant plants (Fuerst et al. 1986), thus reducing the affinity for triazine binding at the site of action and thereby promoting triazine tolerance. Associated with these changes are slower CO_2 fixation rates in the resistant biotypes (Ahrens & Stoller 1983, Holt et al. 1981). This physiological disability is reflected in the agronomic performance of present cultivars of TTC. Grant & Beversdorf (1985) used the heterosis from F1 hybrid forms of B. napus to examine the feasibility of improving the yield and quality of a TTC genotype Atr-Regent. Hybrids produced yields which were significantly

greater than Atr-Regent but in most cases the hybrids were intermediate to the parents in the economically important traits.

The adoption of TTC cultivars by Canadian farmers is presently restricted by the agronomic performance of the cultivars. Faulkner (1982) suggested that unless the weed problems of the crop are exceptionally severe, it would be pointless to breed tolerant cultivars unless they were as good as existing cultivars in other characters. The order of importance of TTC can be judged by the fact that in 1986 3.2 % of the canola area grown in Western Canada (84,000 ha) was sown to OAC Triton. Recently the future of TTC has been questioned with the forthcoming introduction of a selective sulfonylurea herbicide, DPX A7881 for the control of stinkweed and wild mustard in canola.

Imidazolinone-tolerant maize

The imidazolinones are a new class of herbicide which include the non-selective compound imazapyr (Shaner & Anderson 1985) and the compounds imazaquin and imazethapyr, providing broad- spectrum weed control in leguminous crops (Peoples et al. 1985). The biochemical mechanism of action of the imidazolinones is quite specific (Shaner et al. 1984), they kill plants by inhibiting amino acid biosynthesis via an interference with the enzyme acetohydroxyacid synthase (AHAS) (Shaner et al. 1984, Shaner & Anderson 1985). Imidazolinone-tolerant maize cell lines have been selected in cell culture and regenerated into whole plants which possess imidazolinone tolerance (Shaner & Anderson 1985). The tolerance is due to an altered AHAS enzyme and the trait is believed to be inherited as a single dominant gene. Recently, Anderson & Georgeson (1985) have shown that imidazolinone-resistant maize cells also showed cross-resistance to sulfonylurea herbicides. This is not unexpected since the primary mechanism of action of both herbicide families is similar (Shaner et al. 1984).

The development of imidazolinone-tolerant maize cultivars could provide farmers with the option to use a new generation herbicide which may be environmentally more desirable than the existing triazines. Broad spectrum weed control could be particularly valuable with respect to the troublesome graminaceous weeds, shatter cane (Sorghum bicolor) and wild proso millet (Panicum dichotomiflorum). Indeed, farmers could usefully integrate imidazolinones with the existing range of herbicides used in the traditional maize-soyabean crop rotation. Volunteer imidazolinone-tolerant maize plants could be controlled selectively in soyabeans using graminicides such as sethoxydim or fluazifop-butyl. Rather than restrict these crops to only a few herbicides, imidazolinone-tolerant maize cultivars would allow an extra choice of herbicide. It will become apparent in later discussions that it is desirable to have access to a wide range of herbicides to minimise the risk of developing herbicide-resistant weed species. Future adoption of herbicide-tolerant cultivars may again depend upon the agronomic performance of the cultivars available. To date, there is no evidence to suggest that imidazolinone-tolerant maize lines will be at any yield disadvantage over their imidazolinone-susceptible counterparts (Shaner 1987, Personal communication).

FUTURE CONSIDERATIONS

Present knowledge suggests that herbicide-tolerant cultivars will not cause a major revolution in cropping practices, rather they will fulfil a specialist niche where particularly troublesome weeds cannot be controlled by existing herbicides. In Europe it would seem imprudent to invest in herbicide-tolerant cultivars for compounds whose long-term futures are in doubt on the basis of their environmental fate or toxicology eg. triazines or bipyridilliums. Similarly, it must be acertained that the volunteers from herbicide-tolerant cultivars could be easily controlled in mixed cropping situations. The cost of glyphosate will probably preclude the introduction of crop tolerance to this herbicide from all but high value specialist crops. Sulfonylureas and imidazolinones are herbicides which may be in the greatest demand in pursuits to generate herbicide-tolerant cultivars. These herbicides combine a specific mechanism of action with low mammalian toxicity and weed control efficacy. Increased use of the imidazolinones and sulfonylureas in general crop production would be undesirable since due to their similar mode of action , both herbicide families would exert a heavy selection pressure on a weed flora which is likely to produce resistant biotypes.

The introduction of herbicide-tolerant cultivars may be accompanied by a risk of spreading this trait to closely-related weed species and the development of cross-resistance to more than one herbicide. The potential for inter and intraspecific transfer of herbicide tolerance should be evaluated with the same thoroughness which biological control agents receive with respect to host specificity. Presently, research on this topic is notably scarce. In the genus Avena it is possible to produce fertile progeny from inter-specific crosses between cultivated oats, Avena sativa L. and the wild oat Avena fatua (Luby & Stuthman 1983). Therefore the value of achieving selective wild oat control in the diclofop-methyl tolerant oat cultivar, Savena 1 (Warkentin et al. 1987) must be carefully considered. The diclofop-methyl tolerance trait in Savena 1 appears to be inherited by two genes in a simple Mendelian manner and can be incorporated into oat cultivars by a series of backcrosses. However, the risk of passing the diclofop-methyl tolerant genes into wild oat populations would make commercial exploitation of this trait unacceptable. By contrast, the triazine tolerance found in Setaria viridis has been shown to be of potential value in inter specific crosses with Setaria italica, foxtail millet, (Darmency & Pernes 1985). Weed control in foxtail millet is presently difficult to achieve and if satisfactory agronomic performance of a triazine-tolerant cultivar could be produced, this would be very useful.

LeBaron & Gressel (1982b) recognised that cross-resistance to different members of the triazine family could be expected due to their similarity of mode of action. However, evidence of cross-resistance in plants from members of different herbicide families has not been documented. A comprehensive study by Fuerst et al. (1986) showed that triazine-resistant biotypes of smooth pigweed (Amaranthus hybridus), common lambsquarters (Chenopodium album), common groundsel (Senecio vulgaris) and canola (Brassica napus) showed resistance to atrazine, bromacil and pyrazon. The triazine-tolerant biotype showed a greater sensitivity to dinoseb. Similarly, triazine- resistant biotypes of awned canary-grass (Phalaris paradoxa) and black-grass (Alopecurus myosuroides) showed

resistance to methabenzthiazuron, a urea herbicide, and also increased tolerance to diclofop-methyl (Rubin et al. 1985). The competitiveness and the biological fitness of the resistant biotype of P. paradoxa was not disadvantaged over the susceptible biotype, indeed emergence rate, plant height and main spike weight were greatest in the resistant biotype. Herbicide cross resistance was also found in a population of annual ryegrass, Lolium rigidum resistant to diclofop-methyl. The resistance was extended to fluazifop-butyl, chlorsulfuron, metsulfuron-methyl and CGA 82725 (Heap & Knight, 1986).

These studies should alert researchers to the need to examine the specificity of the herbicide tolerance trait which has been included in the new cultivar. Crops can pose serious problems as volunteer weeds and it is important to establish the spectrum of activity of a wide range of herbicides on the herbicide-tolerant cultivar before it is released. In the case of increased sensitivity to a herbicide compared to the normal herbicide-susceptible cultivars, this herbicide could provide a useful means of volunteer control. The corollary is that where the herbicide-tolerant cultivar is found to show decreased sensitivity to a herbicide which is non selective in the herbicide-susceptible cultivars, this herbicide may also be used for selective weed control in the herbicide-tolerant cultivar.

GENERAL CONSIDERATIONS : HERBICIDE-RESISTANT WEEDS

The title herbicide-resistant weeds is immediately alarmist. The implications of herbicide-resistant weeds similar to herbicide-tolerant cultivars require explanation. To achieve this one can review historically the discovery, distribution and practical consequences arising from the occurrence of herbicide-resistant weeds in North America (Bandeen et al. 1982) and outside North America (Gressel et al. 1982). In summary, herbicide- resistant weeds have been recorded in monoculture situations where one persistent herbicide has been applied annually to provide weed control. Cross-resistance of weeds to unrelated groups of herbicides may also appear as a consequence of the initial resistant population (Gressel 1986).

CONSEQUENCES : HERBICIDE-RESISTANT WEEDS

In Europe, a wide range of species are reported to show herbicide resistance. These include Senecio vulgaris, Amaranthus retroflexus, Solanum nigrum, Poa annua, Chenopodium album and Alopecurus myosuroides. Herbicide-resistant weeds must be correctly identified before corrective action can be taken. Immediately this may present a problem, since the number of weed scientists who would have the expertise and the facilities to diagnose a herbicide-resistant weed biotype may be very limited.

Parochetti et al. (1982) described the specific alternative control methods available for herbicide-resistant weeds. The paucity of documented case histories in Europe was noted. However, these authors suggested some general principles. Essentially the following criteria might be examined

in selecting a control programme for herbicide-resistant weeds:

(1) Containment or voluntary quarantine of the location where the resistant weeds are found, to prevent the spread of the weed to other locations.
(2) The use of alternative or additional herbicides; in both cases the mechanism of action should be different to that of the original herbicide.
(3) Alteration of the existing cropping system especially if a monoculture can be avoided.

These principles have been used to varying degrees of success in the control of herbicide-resistant weeds. Ammon (1984) reported that in Switzerland atrazine-resistant plants of Chenopodium album were observed in the sixth year of continuous maize cultivation in the early 1970's. A crop rotation including maize (2 years), cereals (2 years) and sugar beet (1 year) was introduced. The herbicides used included atrazine plus contact foliar herbicides, phenoxy compounds and soil-applied herbicides. However, these measures did not appear to limit the increase of atrazine-resistant biotypes of C. album. Also in Switzerland, Beuret & Neury (1983) found that triazine-resistant weed species were present in maize, vineyards, fruit crops and non-crop areas. While a change towards the use of substituted urea herbicides gave satisfactory control of the triazine-resistant weeds, the risk of building up a new form of resistance was emphasised.

In the event that herbicide-resistant weeds cannot be controlled, competition with the crop will follow. There may be however, a significant difference between the competitive abilities of the resistant and susceptible biotypes. Putwain et al. (1984) showed that in the presence of interspecific competition, triazine-susceptible Senecio vulgaris was at a substantial advantage in terms of ecological fitness over the triazine-resistant biotype. Under non-competitive conditions Holt (1983) reported greater dry matter production and plant height in the triazine-susceptible biotype of S. vulgaris than in the resistant biotype. In competitive plantings with resistant and susceptible biotypes, the susceptible biotype had a greater biomass production of stems, roots, leaves and reproductive structures than predicted from non-competitive studies, while the resistant biotype had less. The physiological basis for the reduced growth vigour associated with the triazine-resistant genotype has been discussed previously.

The population dynamics of the triazine-resistant and susceptible biotypes of S. vulgaris have been examined in conjunction with observations of the phenology (Putwain et al. 1982). The long-term objective of the study was to formulate a model which could be used to predict the equilibrium of resistant and susceptible genotypes. Putwain et al. (1982) concluded that research should be initiated to determine the ecological fitness of resistant and susceptible biotypes in a crop environment, the comparative longevity and dynamics of seeds of these genotypes in soil and the impact of other environmental factors.

CONCLUSIONS

History has shown that weeds respond to the annual application of persistent, broad spectrum herbicides by developing herbicide-resistant biotypes. It is therefore naive to expect that herbicides can continue to be used simply as a means of providing efficient, cost-effective weed control. Instead, the longer term effects of herbicides on the abundance and diversity of the weed flora should receive consideration. Presently, one may attempt to model or predict the influence of herbicides on the development of resistant biotypes. Such a model may consider : (1) herbicide effects on selection pressure, (2) herbicide persistence and (3) herbicide effects on the seed bank for herbicide-resistant biotypes (Gressel & Segel 1982). Research is needed to provide data from field experiments upon which one can test the validity of such models and therefore assess their value as forecasting tools.

In the meantime there is clearly merit in making farmers and growers aware of the herbicide-resistant weed situation. Informed advice should also be given on the most satisfactory ways in which to implement weed control programmes which encompass the use of herbicide rotations, mixtures, increased use of less persistent compounds (Gressel 1987b) and appropriate cultural control and crop rotation practices. Basic research should continue in both crop and weed species to determine the mechanism of action of herbicides, the inheritance of herbicide tolerance, the ecological and environmental consequences of deliberately engineered herbicide-tolerant cultivars and the accidental spread of herbicide-resistant weed biotypes. It is only by improved understanding in these areas that we can hope to manage herbicide-resistant cultivars and herbicide-resistant weeds. Careful consideration should be given to local appraisal of the legislative, rotational, environmental, volunteer crop and resistant weed implications of herbicide-tolerant cultivars. The challenge is available now for weed scientists, crop breeders and agronomists to exchange expertise and collaborate in the research efforts required for the continued safe use of herbicides.

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SESSION 6

HERBICIDE BEHAVIOUR IN SOIL: I

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INTERACTIONS OF PESTICIDES WITH THE SOIL MICROBIAL BIOMASS

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ABSTRACT

The present status of measuring the side-effects of pesticides on the soil microflora is evaluated. It is concluded that there are drawbacks arising partly from the methods themselves, but mainly from the variability inherent in soil.

Some alternative approaches, emphasising soil biomass measurements are discussed. These offer considerable potential as sensitive indicators of response to stress but still suffer from the problems associated with the use of soil.

INTRODUCTION

Modern agriculture is dependent upon the use of biologically active chemicals to maintain yields and product quality. This has resulted in the general perception that the environment may be at risk through exposure to these chemicals. Consequently, there has been much research to try to quantify and evaluate the degree of risk involved. The soil microflora is well recognized as being essential in cycling organic matter and plant nutrients. It is not surprising, therefore, that much attention has focussed on interactions between the microflora and pesticides. Herbicides have been examined in particular detail as they are used, and so enter soil, in much greater quantities than any other pesticide. Recent development of compounds with very high, broad-spectrum, activity has heightened awareness of the possibility of affecting the soil microflora. The assertion that such compounds are environmentally safer than chemicals with lower activity because 'so much less chemical is used' does not stand critical examination. Environmental risk accrues from the product of dose and activity and is not solely dose related.

As a result of this awareness of hazard, many registration authorities have requirements which oblige companies to provide data demonstrating lack of critical effects of their products on the soil microflora. Most of these requirements, certainly in Europe, are based on the recommendations published by Greaves *et al.* (1980) and subsequently revised by Somerville *et al.* (1986). These recommendations refer primarily to assessing effects on soil respiration and ammonification and nitrification. These generally are accepted as the most appropriate means of detecting pesticide-induced stresses on the soil microflora which are presently available.

This acceptance of their status does not imply that they are intrinsically good methods. Indeed, most researchers will readily agree that they have grave shortcomings. It is disturbing that, despite their limitations, these methods have been advocated for at least thirty years with little change. More disturbing is the lack of serious contenders for their place as generally recommended methods of detecting perturbation in the soil microflora. This despite the great efforts by microbial ecologists to devise means of analysing the composition and behaviour of soil microbial populations. It also contrasts, markedly, with the advances being made in

agricultural microbial biotechnology, dependent as that is on a full and detailed understanding of how micro-organisms behave in different environments.

MICROBIAL INTERACTIONS WITH HERBICIDES

The literature presents thousands of papers reporting effects of herbicides and other pesticides on different aspects of the composition and activity of the soil microbial biomass. The accumulated data purports to show that different herbicides can and do affect the soil microflora in many ways, some being interpreted as harmful. It is clear, however, that the situation is extremely confused. Although many papers cannot be compared, through lack of essential information or choice of inadequate techniques, it is still inescapable that many herbicides cause widely differing effects, when examined in very similar experiments by different investigators. This is exemplified by data published by Anderson (1987) and produced in a ring test, by five laboratories, of the effect of mercuric chloride on carbon mineralization in soil (Table 1).

TABLE 1

Ring test by 5 laboratories (A-E) to examine inhibition of carbon mineralization in a sandy loam soil by mercuric chloride. (Data from Anderson, 1987)

Time (days)	Mineralization (% Control)					Mean ± SD	% Deviation from mean
	A	B	C	D	E		
1	96	93	77	92	98	91±8	8
7	63	42	21	57	80	53±22	42
14	55	28	15	40	58	39±18	46
28	45	29	7	19	38	28±15	53

These data show clearly that each laboratory was producing significantly different results, especially as incubations progressed. Anderson (1987) has also demonstrated that repeat experiments in the same laboratory can be quite reproducible, thus emphasising the small differences in technique between laboratories as a major source of variation in results.

Obviously, there are many other reasons why data from different experiments are not comparable. It is not my purpose to review these but, taken as a whole, the observed variability does lead to the conclusion that, at least with the currently recommended techniques, it is not possible to determine unequivocally if a herbicide will harm the soil microflora.

This conclusion, which has been drawn by some researchers (including the author) for many years, is confirmed by the recent decision of the Dutch authorities (van Doorn, 1987) to follow the U.S. Environmental Protection Agency and withdraw their requirement for soil microflora data, except for data on soil nitrification for soil-incorporated chemicals. Their view is that "nowhere is it spelt out precisely what is relevant information on the side-effects on soil microflora and how this information should be weighed when judging a pesticide's admissibility".

NEW APPROACHES

In view of the acknowledged flaws in the recommended methods of evaluating pesticide-induced stresses on the soil microflora, are there alternative approaches which might be adopted? Greaves (1987) has outlined some possibilities, though these simple approaches have not met with general approval. These include an automated miniaturized toxicity test (Cooper *et al.*, 1978), using large numbers (hundreds) of pure cultures of micro-organisms isolated from soil. The technique has been used for more than seventy herbicides and the results, generally showing no untoward toxicity, even at doses ten times higher than that expected in soil, confirmed by conventional side-effect testing. Greaves (1987) advocated this as a Tier 1 test which could preclude the need for expensive further tests for many chemicals which show no inherent toxicity.

If the view that the only acceptable way of assessing side-effects is to work with soil itself prevails, despite the problems of variability and lack of reproducibility, the available alternative methods are few and, as yet, relatively untried. Determination of the response of the microbial biomass itself seems most hopeful, though there is the inevitable disagreement as to choice of exact method.

The favoured method of measuring soil microbial biomass is that developed by Jenkinson and Powlson (1976) and based on fumigation or some variant of it (e.g. Lynch and Panting, 1980). Anderson and Domsch (1978) developed a physiological or respiratory method which may apply to a wider range of soils and conditions (e.g. pH and organic matter content) than that of Jenkinson and Powlson (1976). Sparling (1981) advocates the use of micro-calorimetry. A range of further approaches has been developed, all claiming some advantages. Thus, ATP determintion (Oades and Jenkinson, 1979; Jenkinson *et al.*, 1979) can give good correlation with biomass and can give early indications of change in microbial function. Following this work, Brookes *et al.* (1983) and Brookes *et al.* (1987) have used measures of the adenylate energy charge in soil to indicate the metabolically active biomass. Van de Werf and Verstraete (1987) have monitored, in a continuous respirometer, the oxygen uptake of soils. The data obtained is used to derive simultaneous estimates of biomass and a number of microbial growth kinetic parameters. Their results led them to conclude that soil biomass is a sufficiently sensitive indicator of stress in the soil to identify it as a parameter with potential value for microbial ecologists.

A particularly interesting approach is that developed by Killham (1985), following the earlier finding, by Killham and Firestone (1984), that soil micro-organisms divert more energy from growth into maintenance of cell integrity as stress increases. This leads to the expectation that an increasing proportion of C-uptake will be respired as stress increases. Thus, changes in the ratio of respired C to biomass C should be a good indicator of response to pesticide-induced stress. Indeed, Killham's (1985) data confirm this possibility, showing that the ratio was a sensitive indicator of stress from simulated acid rain containing heavy metals.

Killham (1985) points out that marked discrepancy between the effects of environmental stress on the ratio and on respiration raises "fundamental questions concerning our future approach to assessing the impact of environmental stresses and concerning interpretation of previous studies which relied solely on respiration "..... as determinants of stress-induced microbial changes". In some instances, growth of the microbial biomass

could be stopped entirely while respiration data might show no adverse impact of the environmental stress.

I have no doubt that this approach of Killham (1985) has great potential as a means of detecting pesticide side-effects. It remains to evaluate it with a range of chemicals and I hope this can be done as a matter of urgency.

APPLICATION OF BIOMASS MEASUREMENTS

Aside from the disagreements with regard to detailed choice of method, it is clear that all the methods suffer from common serious drawbacks, at least in the context of their application to determining pesticide side-effects in soil. These, essentially, mainly stem from the nature of soil itself. Soil is a living, dynamic system which is frequently likened to animal or plant tissue in attempts to present its complexity of function. As such, it responds not only to the pesticide under test, but also to all the stresses placed upon it during preparation for the test. Of these, soil storage is perhaps the foremost. Anderson (1987) and van de Werf and Verstraete (1987) have highlighted the loss of biomass occurring during soil storage (Table 2). So long as adequate experimental controls are included,

TABLE 2

Changes in microbial biomass during storage of a parabrown soil at different temperatures. (Data from Anderson, 1987)

Time (days)	Biomass (mg microbial C kg^{-1} dry soil) at 2'C	17'C	27'C
0	296	296	296
7	308	280	248
28	272	228	168
70 (% Loss)	244(18)	188(36)	112(62)

this loss of biomass should not affect the evaluation of the effect of pesticide treatment. However, significant (up to 50%) losses can also occur (Table 3) during the incubation of unamended soils (Anderson, 1987).

TABLE 3

Changes in microbial biomass of a fresh parabrown soil during incubation. (Data from Anderson, 1987)

Time	Biomass (mg microbial C kg^{-1} dry soil)	
(days)	Unamended soil	Soil + Glucose
0	390	390
7	380	516
28	316	527
70	190	630

They are accompanied by changes in the quality of the surviving microflora which may result in more or less susceptibility to the pesticide than would

occur in the field.

The lesson to be learned is clear. For any method to be fully useful for measuring microbial parameters in soil, particular care must be taken to ensure that the soil used is as representative of that in the field as possible. Preferably, it should be freshly collected but, if storage cannot be avoided, this should be at 2-4'C for a maximum of three months and drying should be avoided without limiting gas exchange. Even if these conditions are satisfied, all methods will still only give data which is comparative within a single experiment, though, with careful experimentation, comparisons between experiments in one laboratory may be acceptable. Other comparisons may not be valid owing to the high inherent variability in soils and their microbial components (Cook and Greaves, 1987).

CONCLUSIONS

There is little doubt that currently recommended methods of assessing interactions of pesticides with the soil microbial biomass are seriously disadvantaged. Despite this, there is a clear need to undertake some testing of chemicals before they are released into the environment, if only to allay public demands that some protective measures are taken.

Although relatively little advance has been made in our knowledge of how to test effectively for hazardous side-effects, recent work has indicated promising lines of research. In particular, analysis of microbial biomass in terms of its metabolic efficienty seems to be potentially most rewarding. Unfortunately, none of the newer methods has yet been fully evaluated with pesticides and this must be rectified urgently. Recent trends in research funding, both in the U.K. and elsehwere, have resulted in a significant decline in research in this area. It is a matter of concern that this is so at a time when there is a marked tendency for the agrochemical industry to produce chemicals of considerably greater potency, albeit to be used at lower doses, than previously. The lack of any known significant side-effects on the soil microflora, in practice, cannot be taken as evidence that effects will never occur. Indeed, it could be argued, boosted by increasing chemical potency, that we have been lucky and side-effects are imminent. If this risk is to be avoided, we must maintain research to ensure that valid, effective methods of predicting risks to the microflora from pesticides are made available.

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THE INFLUENCE OF THE HERBICIDE TRIFLURALIN, ALONE AND IN THE PRESENCE OF SIMULATED ACID RAIN, ON THE ALGAE AND CYANOBACTERIA OF A SANDY LOAM SOIL

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ABSTRACT

Field plots were established in a sandy loam soil. The plots were treated with trifluralin to give concentrations of 0, 1 and 2 mg/kg in the top 1 cm of soil. One week later, and weekly throughout the growing season, plots at each herbicide level were treated with simulated acid rain at pH 4.5 and 3.5 respectively. The response of the soil algae and Cyanobacteria was monitored for 12 months using a slide colonization technique. The diatom Hantzschia and the Cyanobacterium Oscillatoria formed greater than 95% of the flora colonizing slides from control plots. Growth of Hantzschia was inhibited by both 1 and 2 mg/kg trifluralin, particularly when these treatments were followed by application of simulated acid rain. Inhibition was noted for up to 12 months after herbicide treatment. It is suggested that the acid may have facilitated the action of trifluralin on the algal cells by weakening cell membranes. It may also have affected degradation and adsorption characteristics of the herbicide. Oscillatoria was less sensitive to the trifluralin and simulated acid rain. This may be attributable to its clumped growth form and to the presence of mucilaginous sheaths covering its filaments. The data suggest a potential threat to populations of soil algae and Cyanobacteria in areas in which acid rain falls on soils containing trifluralin residues.

INTRODUCTION

The soil algae and Cyanobacteria have recently begun to be recognized for their role in the maintenance of soil fertility (Fogg et al., 1973, Pipe & Shubert, 1984). Concomitantly, the potential threat to soil fertility through interference with the activities of these organisms by agricultural practices such as herbicide use has been examined (McCann & Cullimore, 1979). Much of this work has been conducted under in vitro conditions (Pipe & Shubert, 1984). This is unfortunate, since the complex behaviour of herbicides in the soil renders prediction of field responses from in vitro studies virtually impossible.

In the present paper, a field study on the influence of the herbicide trifluralin (2,6-dinitro-NN-dipropyl-4-trifluoromethylaniline) on the algae and Cyanobacteria of a sandy loam soil is described. In addition to the application of trifluralin, the soil was also treated with simulated acid rain, in an attempt to elucidate any combined effect of these agents. The response of the algae and Cyanobacteria was determined by

means of an implanted slide technique (Pipe & Cullimore, 1980), in which microscope slides are placed vertically in the soil and become colonized by the microorganisms therein. On removal of the slides from the soil, the colonizing organisms can be examined microscopically, identified and enumerated.

MATERIALS AND METHODS

Establishment of field plots

Field plots (20 x 20 cm) were established in a sandy loam soil (clay 19%, sand 63%, silt 18%, organic matter 12%, water-holding capacity 53%, pH 7.5) shortly after spring thaw. Microscope slides, back-to-back in pairs, were implanted vertically into each plot (3 pairs per plot) such that the top 1 cm of each pair remained above the soil surface.

Application of trifluralin

Plots were treated with trifluralin in the middle of June (3 weeks after slide implantation), to give concentrations of 0, 1 and 2 mg/kg in the top 1 cm of soil (9 plots per treatment level). The herbicide was applied in a water carrier using a spray bottle with the nozzle adjusted to deliver a fine spray evenly over the surface of each plot. This was followed by incorporation by careful mixing of the soil to a depth of 1 cm, thereby approximating the application method recommended for trifluralin (Anderson, 1977).

Application of simulated acid rain

One week after trifluralin application, and weekly throughout the growing season, 3 plots at each herbicide level were treated with simulated acid rain at pH 4.5 and 3.5 respectively. The treatment solution consisted of de-ionized water containing (mg/l): SO_4^{2-}, 2.1; NO_3^-, 0.95; Cl^-, 0.29; NH_4^+, 0.48; Na^+, 0.34; K^+, 0.25; Ca^{2+}, 1.15; Mg^{2+}, 0.19 (average ionic composition of natural rainfall in the study area). Sulphuric acid was added to yield the specified pH values. The remaining 3 plots at each herbicide treatment level received the above solution adjusted to the average pH of natural rainfall in the area (5.6). All treatments were conducted using a spray bottle with the nozzle adjusted to deliver a fine spray evenly over the surface of each plot. The volume of treatment solution (200 ml per plot) was sufficient to moisten dry soil to water-holding capacity to a depth of 1 cm. Total natural rainfall during the simulated acid rain treatment period was 22 cm.

Recovery and examination of slides

Microscope slides were removed from the plots 2, 3 and 12 months after treatment with trifluralin (1 pair of slides from each plot on each occasion). They were prepared for microscopic examination as described by Pipe & Cullimore (1980), using molten 2% water agar to form a coating over the soil particles and microorganisms. The extent of colonization of the slides by algae and Cyanobacteria was determined by identifying and counting all cells observed on the part of each slide corresponding to the top 1 cm of the soil. Mean values were calculated for each separate treatment (6 slides per treatment). Significance of the data was determined by means of the Mann-Whitney test (Snedecor & Cochran, 1967).

Determination of soil pH

Soil samples were collected for the determination of pH each week immediately before treatment of plots with simulated acid rain, and at the conclusion of the experiment, 12 months after herbicide treatment. These samples were taken from a set of 27 plots set up and treated in exactly the same way as were those containing implanted slides. By using separate plots for pH determination, interference with the soil in the implanted slide plots was avoided. The soil pH was determined by the method of Pramer & Schmidt (1964).

RESULTS

A variety of algae and Cyanobacteria were observed colonizing the slides following their recovery from the soil plots. These were: the diatoms Hantzschia, Navicula and Pinnularia; the green algae Chlorella, Chlorococcum, Hormidium and Spongiochloris; and the Cyanobacteria Anabaena and Oscillatoria. Attention in this paper will be devoted to Hantzschia and Oscillatoria, which together formed greater than 95% of the flora colonizing slides from control plots. Hantzschia occurred consistently on all slides from control plots, and Oscillatoria on all except those recovered 2 months after herbicide treatment. Data presented for both of these organisms are therefore limited to the 3 and 12 months after herbicide treatment slide recovery times.

Colonization of slides by Hantzschia and Oscillatoria is shown in Fig. 1. Data represent the numbers of cells of each organism observed on the part of the slides corresponding to the top 1 cm of the soil, and are the means of values for 6 replicate slides at each treatment level. Treatment levels at which colonization was significantly different from that on control slides (slides from plots receiving no trifluralin and no simulated acid rain) are shown in Table 1.

Concerning data for Hantzschia in unacidified soil, the trifluralin appeared to have reduced colonization of slides recovered both 3 and 12 months after herbicide treatment (Fig. 1). However, the apparent reduction was significant only on slides recovered after 12 months (Table 1). In plots sprayed with simulated acid rain, significant reductions were observed at 3 and 12 months (pH 4.5) and at 3 months (pH 3.5) after trifluralin application (Table 1). In most cases, colonization of slides removed from soils which were acidified but not treated with herbicide was not significantly different from colonization of control slides (Table 1).

For Oscillatoria in unacidified soil, some stimulation of colonization was apparent for slides recovered from plots 3 months after treatment with 1 mg/kg trifluralin (Fig. 1). However, this was not significant (Table 1). There was likewise no significant difference between colonization at 1 mg/kg trifluralin and that in control plots after 12 months. For slides recovered from unacidified plots treated with 2 mg/kg trifluralin, a significant decrease in colonization over that on control slides was observed at both 3 and 12 months after

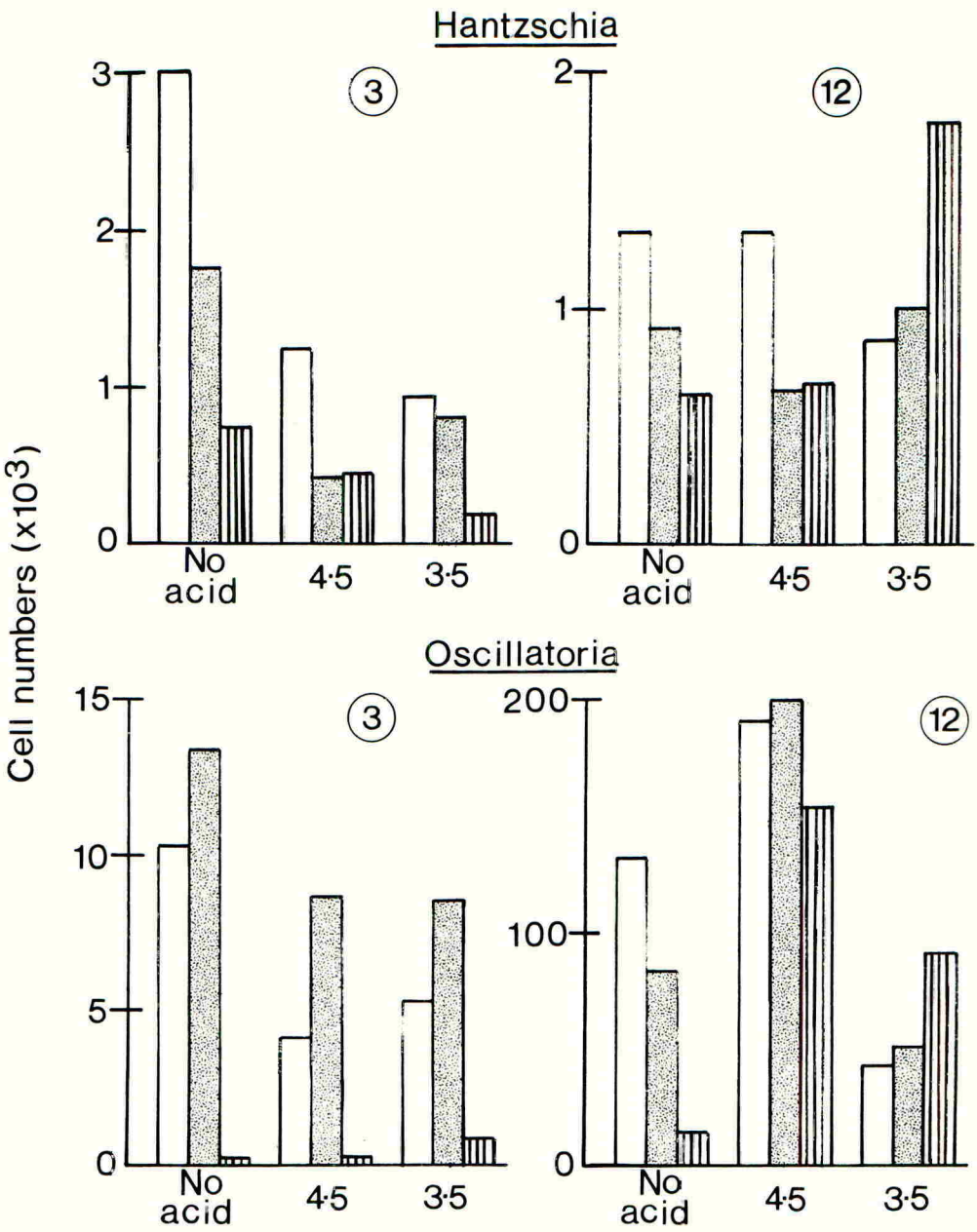

Fig. 1. Number of cells of Hantzschia and Oscillatoria counted on slides recovered from plots 3 and 12 months after treatment with trifluralin (months indicated inside circles). Bars represent data for trifluralin concentrations as follows: □ 0 mg/kg; ▨ 1 mg/kg; ▥ 2 mg/kg. The numbers 4.5 and 3.5 indicate the pH of the simulated acid rain treatments.

TABLE 1

Statistical analysis of colonization at each treatment level as compared with that on control[a] slides

Treatment level		Colonizing organism			
Trifluralin (mg/kg)	Simulated acid rain (pH)	Hantzschia		Oscillatoria	
		3	12	3	12
1	None	NSD	*	NSD	NSD
2	None	NSD	**	*	*
0	4.5	NSD	NSD	NSD	NSD
1	4.5	*	**	NSD	NSD
2	4.5	*	*	*	NSD
0	3.5	NSD	*	NSD	NSD
1	3.5	*	NSD	NSD	NSD
2	3.5	*	NSD	*	NSD

[a]No trifluralin, no simulated acid rain.
The numbers 3 and 12 refer to months after treatment with trifluralin.
NSD - No significant difference.
* - Significantly different from control data at 0.05 level.
** - Significantly different from control data at 0.01 level.

herbicide application (Table 1). In plots sprayed with simulated acid rain, the only treatments which produced data significantly reduced from that for control slides were both of the 2 mg/kg trifluralin treatments after 3 months (Table 1).

DISCUSSION

It is assumed in the following discussion that colonization of implanted slides is indicative of growth of the colonizing organisms in the soil. Accordingly, it is evident that while the growth of the diatom Hantzschia was unaffected 3 months after treatment with trifluralin in unacidified soil, inhibition had occurred by 12 months after treatment. This indicates a somewhat delayed response to the herbicide treatment. It is generally believed that the persistence of trifluralin in soils is 6 months or less (Anderson, 1977), depending, of course, on soil conditions. The herbicide is known to be degraded by a variety of different mechanisms and pathways (Probst et al., 1975). It is possible that in the present study, degradation products forming between the 3 and 12 month sampling times may have been more harmful to Hantzschia than

was the parent compound. In support of this statement, the dealkylation known to occur in the degradation of trifluralin can result in an increase in biological activity (Hill, 1978). Metabolites resulting from dealkylation are, however, thought to be short-lived in the soil (Cripps & Roberts, 1978).

In soils sprayed with simulated acid rain following trifluralin treatment, inhibition of Hantzschia was more pronounced than that noted above, and significant reduction of growth was observed 3 months after herbicide treatment. Growth of the diatom in plots treated with acid alone (no trifluralin) was unaffected. It therefore appears that while the acid at the pH levels and application rates employed was in itself harmless to Hantzschia, it may have enhanced the susceptibility of the alga to trifluralin in the soil. This was observed less clearly 12 months after herbicide treatment. In order to interpret these data, it is necessary to consider how the acid might affect firstly the mode of action of trifluralin on Hantzschia cells, and secondly the behaviour of the herbicide in the soil.

Addressing the first of these points, sulphuric acid is a contact herbicide, weakening and disorganizing cellular membranes (Anderson, 1977). Trifluralin is a mitotic inhibitor (Probst et al., 1975) and can adversely affect development of cell membranes and cell walls during mitosis (Anderson, 1977). The deleterious effect of herbicide-induced impairment of development of cell membranes and walls may therefore have been enhanced by the contact effect of the acid, hastening the weakening and disorganization of these structures.

Concerning the possible effect of the acid on the behaviour of trifluralin in the soil, this may have included the following phenomena. The acid may have affected herbicide degradation, accelerating the formation of metabolites which might be more toxic than the parent compound to Hantzschia. Soil acidity has been observed to enhance photodecomposition of trifluralin by nitro group reduction (Probst et al., 1975), but according to Hill (1978), the loss of the nitro group usually results in loss, not increase, of biological activity. The acid may also have affected the extent of adsorption of the trifluralin to the soil. Trifluralin is known to become quite strongly adsorbed to soil, the extent of which is affected by a variety of factors including pH (Anderson, 1977). It is possible that the acid treatments reduced the extent of adsorption of the trifluralin, rendering it more available for uptake by, and more harmful to, the Hantzschia cells.

In concluding the discussion of the apparent acid-enhanced action of trifluralin on Hantzschia, it should be mentioned that long-term alteration in soil pH was not observed at any time during the study. Measurements made each week immediately before acid treatment, and at the conclusion of the experiment, showed soil pH in all plots to be at its normal level.

The growth of Oscillatoria in the soil was less affected by the treatments than was that of Hantzschia; under no circumstances was trifluralin at 1 mg/kg observed to affect the Cyano-

bacterium. This apparent greater resistance of Oscillatoria to the herbicide and the acid may have a morphological explanation. The Oscillatoria was present on the slides in massive clumps of trichomes, rather than as scattered cells as was the case for Hantzschia. It is reasonable to assume similar growth habits of the organisms in the soil itself. The cells in the centre of large clumps of Oscillatoria may therefore be physically protected against chemicals such as trifluralin and sulphuric acid applied to the soil. This protection may be further enhanced by the presence of mucilaginous sheaths covering the Oscillatoria filaments.

Throughout this discussion, the assumption has been made that colonization of slides reflects the growth of colonizing organisms in the soil itself, implying that any reduction of colonization is indicative of mortality of the organisms. The possibility that mortality may not necessarily be indicated should be entertained. The treatments used in the study may instead have affected the ability of the organisms to colonize the glass slides. For example, motility may have been impaired, preventing the organisms from moving towards the slides prior to colonization. Such possible sub-lethal physiological effects are as much a cause for attention as are direct lethal effects (Pipe & Cullimore, 1984).

It is evident from the data presented in this paper that, at field application rates, trifluralin can exert a significant effect on members of the soil algal and cyanobacterial flora for up to 12 months after application. Should acid rain fall on soils containing trifluralin, the effect on these organisms can be expected to be even more pronounced. Such an impairment of the normal growth of algae and Cyanobacteria may seriously impede their participation in the maintenance of soil fertility.

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ACCELERATED BIODEGRADATION OF PESTICIDES IN SOIL AND ITS EFFECT ON PESTICIDE EFFICACY

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ABSTRACT

The biodegradation processes associated with a given pesticide may be influenced by the rate and frequency of its application, the cropping system, and the presence of other pesticides applied either simultaneously or sequentially, in addition to the chemical and physical characteristics of both the soil and the chemical itself, and the prevailing environmental conditions. The interactions of these factors may result in either accelerated or reduced rates of biodegradation thereby affecting efficacy. Frequent, repeated applications of many pesticides are known to result in accelerated rates of biodegradation of these pesticides in soils. Some pesticides may act as inducers of microbial enzymes which degrade other pesticides, even though they themselves are not necessarily substrates for the microbial enzymes induced. Other pesticides may inhibit the biodegradation of some pesticides in soil. Recognition and an understanding of these interactions is important if we are to preserve these chemicals as important agricultural tools.

INTRODUCTION

The accelerated biodegradation of some pesticides in soil and the accompanying loss of pest control is becoming an increasingly important problem for the farmer, the agricultural scientist, consultant and advisor, and the agrichemical industry. With the development of synthetic fertilizers and pesticides, it became feasible to grow many crops continuously rather than in rotation with one another. The production of fewer crops continuously offered a sufficiently large economic advantage that many farmers readily adopted the associated pest control strategies and began using the same chemicals in the same fields almost habitually. By early to mid 1970's extension agents began receiving performance complaints about several of the pesticides being used more regularly. Although it may be common to have some performance complaints with most heavily used pesticides, complaints became more consistent with some products (Tollefson 1986; Rahman et al. 1979). In the U.S. many of these initial problems evolved around the use of methylcarbamate insecticides and thiocarbamate herbicides in continuous corn.

The development of microbial populations capable of rapidly degrading sequential applications of pesticides had been demonstrated under laboratory conditions for several pesticides: phenoxyalkanoates (Audus, 1951); chlorpropham (Kaufman & Kearney 1965); dalapon (Kaufman 1964); and several phenylamides (Kaufman & Blake 1973). The occurrence of this phenomenon under field conditions has also been described. Hurle and Rademacher (1970) compared the dissipation of DNOC and 2,4-D in soil treated for the first time and soil from field plots treated annually over a period of 12 years. 2,4-D dissipation was more rapid in previously treated soil than in soil treated for the first time, whereas pretreatment had no effect on the

rate of EPTC dissipation from soil. Similar promotions have been obtained with 2,4-D (Kirkland & Fryer 1966, 1972; Newman & Thomas 1949; Aly & Faust, 1964; Fryer & Kirkland 1970), endothall (Horowitz 1966), but not with simazine or linuron (Fryer & Kirkland 1970). The time for MCPA applications to reach the limit of detection was reduced from 3 weeks after three previous applications to 4 days after 10 previous applications.

The actual significance of this phenomenon in soil would be of minor consequence to pesticides which are primarily active as foliar or aerial contact chemicals. Pesticides which are primarily active in soil or through root absorption from soil, however, could expect to have limited effectiveness. Research efforts in my laboratory have been directed at examining the degradation of thiocarbamate, methylcarbamate, phenylamide and organophosphate pesticides in their respective problem and nonproblem soils, and to ascertain what effect, if any, these problem soils may have on the persistence and performance of structurally related pesticides. "Problem" and "nonproblem" soils used in these investigations are defined as: (a) Problem soil, soil in which the chemical applied failed to control the target pest; and (b) Nonproblem soil, an identical soil type with an identical cropping history, but without any known use of any chemical, or an identical soil type from an untreated border area (fence row) adjacent to a problem field.

MATERIALS AND METHODS

We examined the degradation of ^{14}C-ethyl-and ^{14}C-propyl-EPTC in three pairs of problem-nonproblem soils, the degradation of ^{14}C-carbonyl carbofuran in five pairs of problem-nonproblem soils, and the degradation of ^{14}C-ethyl- and ^{14}C-methylthio terbufos in five pairs of problem-nonproblem soils. All soils were obtained from our Midwestern corn producing areas where most of these problems have appeared. All soils were received in a fresh moist condition from field locations, and were immediately sieved through a 2 mm No. 10 U.S. Standard sieve prior to storage in polyethylene bags at 5°C. The persistence and degradation of the appropriate ^{14}C-labeled pesticide and structurally related ^{14}C-herbicides, insecticides, and fungicides were then examined in each soil. All soil metabolism experiments were performed by incubating ^{14}C-pesticide treated soils in soil biometer flasks. At periodic intervals treated soils were sacrificed, extracted and analyzed for ^{14}C content and product distribution.

RESULTS

The results of investigations with ^{14}C-ethyl-EPTC in EPTC problem and nonproblem soils are shown in Figure 1. Degradation of EPTC with evolution of $^{14}CO_2$ from the ethyl moiety occurred far more rapidly in EPTC problem soils than in nonproblem soils. Soil sterilization by either autoclaving or gamma irradiation drastically reduces the rate of EPTC degradation. Similar results have been described by others with EPTC (Obrigawitch et al. 1982a,b, 1983; Wilson 1984), butylate (Skipper et al. 1986; Obrigawitch et al. 1983; Wilson 1984), and vernolate (Wilson 1984). Degradation of carbofuran with evolution of $^{14}CO_2$ from the carbonyl position occurred far more rapidly in carbofuran problem soils than in nonproblem soils (Figure 2). Felsot et al. (1981), Harris et al. (1984), and Read (1986) also demonstrated an accelerated degradation of carbofuran in soils having a history of carbofuran use. The inhibition of degradation of these chemicals by antibiotics added to the soil, or by soil

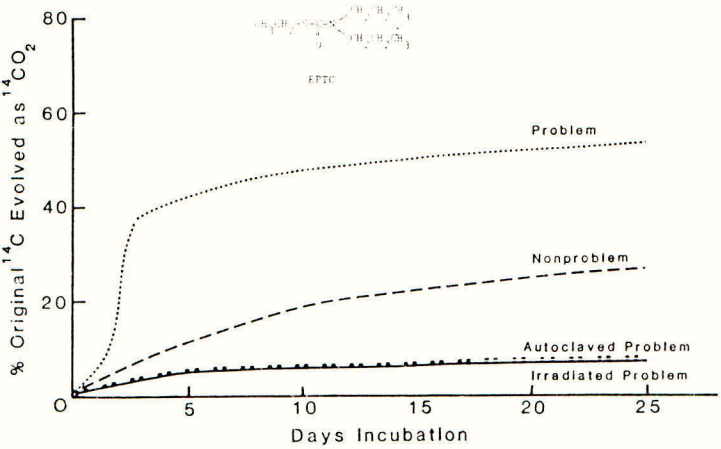

Fig. 1. Degradation of ^{14}C-ethyl-EPTC in Eradicane problem (sterile and nonsterile) and nonproblem soils.

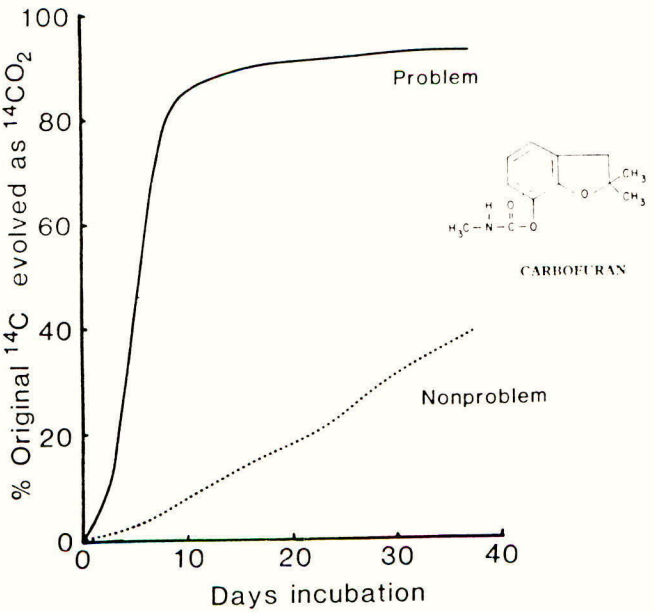

Fig. 2. The degradation of ^{14}C-carbonyl-carbofuran in carbofuran problem and nonproblem soils.

sterilization (autoclaving or gamma irradiation) confirmed the importance of an active microbial population in the degradation of these chemicals.

Soil microorganisms capable of metabolizing either EPTC or carbofuran have been isolated from problem soils and characterized. Tam et al. (1987) described the isolation of an Arthrobacter strain which metabolized EPTC as a sole source of carbon. Venkateswarlu and Sethunathan (1985) isolated Pseudomonas cepacia and a Nocardia sp. from flooded alluvial soil amended with carbofuran which metabolized carbofuran fairly rapidly in mineral salts medium or soil extract agar supplemented with yeast extract. Karns et al. (1986) isolated an Achromobacter species capable of rapidly utilizing carbofuran as a sole source of nitrogen. Degradation of EPTC was mediated by a 50.5 MDA plasmid in the Arthrobacter cells (Tam et al. 1987). The loss of this plasmid resulted irreversibly in mutants unable to degrade EPTC. Whether or not the carbofuran degradation capacity of Achromobacter is associated with a plasmid chromosome has not yet been determined (Karns et al. 1986). The role of plasmids in microbial degradation of certain organophosphates (Serdar et al. 1982) and chlorinated compounds such as benzoates and phenoxyalkanoates is also known (Tomasek et al. 1986). It has been suggested (Waid 1972) that the ability of adapted populations to persist for many months in the presumed absence of the substrate must result from the conservation of a very effective genetic mechanism within the soil microflora even though it has no apparent survival value among individual cultures in the absence of an appropriate substrate. Several review articles on degradative plasmids and their molecular nature and mode of evolution are available (Farrell & Chakrabarty 1979; Chakrabarty 1976).

The correlation of laboratory results as shown here to field performance and efficacy problems of thiocarbamate herbicides and carbofuran insecticide has been demonstrated by numerous investigators (Skipper et al. 1986; Obrigawitch et al. 1982a,b, 1983; Wilson 1984; Lee et al. 1984; Kaufman & Edwards 1983; Kaufman et al. 1985; Nelsot et al. 1981; Read 1986) and others. Similar observations have been also described with other pesticides: the insecticides aldicarb (Read, 1987), parathion (Sethunathan 1973), and diazinon (Sethunathan & Pathak 1971); the fungicides DCNA (Groves & Chough 1970), benomyl and carbendazim (Yarden et al. 1985), and iprodione (Entwistle 1983); and the herbicides 2,4-D, dalapon, chlorpropham, propham, TCA, pronamide, napropamide, bensulide, alachlor and diethatyl (Gray & Joo 1985), endothall (Horowitz 1966), amitrole (Riepma 1962) and diphenamid (Katan et al. 1984), and the nematicide ethoprop (Rohde et al. 1980).

DISCUSSION

There are several aspects of the phenomenon of accelerated biodegradation which should be given serious consideration. First of all, the phenomenon is a natural process which, in fact, was first described in relationship to pesticides nearly four decades ago by Audus (1951) in his research on the biodegradation of the herbicide 2,4-D. His work, and the work of many subsequent investigators clearly demonstrated that once a microbial population adapted to degradation of a foreign molecule, in this case a pesticide, subsequent applications would be degraded much more rapidly. Much of the initial lag period involves the adaptation process and the development of a population level, or metabolic rate sufficient to detect a significant loss of the pesticide. Once the process has been established subsequent applications will be degraded without the lag period

which occurred with the first application. A more surprising aspect of this phenomenon is the persistence of the effect from one growing season to another. While plant pathogenic organisms are known to survive for several years on plant debris or alternate hosts, the persistence of a pesticide degrading population much beyond one year was not considered likely. Results of numerous investigators (Fryer & Kirkland 1970; Kirkland & Fryer 1972; Newman et al. 1952; Torstensson et al. 1975) have revealed otherwise, however. As new information is developed on the existence and persistence of pesticide degrading plasmids, we will gain new insights into the persistence of these important biological factors in soil.

A second important part in the characterization of the accelerated degradation of pesticides involves the presence of adequate pest pressure. In some of our early investigations we compared the degradation of selected insecticides in soils from fields where a consecutive use pattern had been established but no efficacy problems were being observed, with their degradation in soils from fields where efficacy problems had been observed. The same rapid rate of degradation was observed in both soil types. Further investigation ultimately led us to the understanding that in the absence of adequate pest pressure a farmer may not recognize that the chemical applied is no longer able to be as effective as it was initially.

A third important consideration in the development of a problem soil for an individual chemical is the effect of that microbial population on the biodegradation and persistence of other structurally related chemicals used thereafter. Ample information exists which clearly demonstrates that isolated microorganisms capable of degrading one pesticide are frequently capable of degrading other similar pesticides either more or less efficiently (Audus 1960; Engelhardt et al. 1971, 1973; Blake & Kaufman 1975; Kaufman & Blake 1973; Kaufman 1977; Kaufman et al. 1985). The mechanisms whereby this occurs are discussed in more detail elsewhere (Kaufman et al. 1985; Roeth 1986) The implications of such observations are clear and must be considered when attempts are made to reestablish pest control in soils where accelerated biodegradation of one or more chemicals has been observed. To date, cross degradation or cross enhancement has been detected in the field within specific chemical classes, i.e., thiocarbamates (Roeth 1986), methylcarbamates (Harris et al. 1984), etc. Read (1983), however, reported a cross enhancement occurred between the methylcarbamate insecticide carbofuran and the organophosphate insecticide fensulfothion. Other cross enhancements have been observed in the laboratory with soils taken from problem field sites (Kaufman et al. 1985).

Developing suitable methods for (a) regulating the rate of pesticide biodegradation in soil, (b) preventing the development of problem soils, and (c) controlling or eradicating microbial populations in problem soils once they have developed is essential for maintenance of adequate pest control with soil-applied chemicals. At present essentially no information is available on how, once a problem field has developed, it can be converted back to a nonproblem field for the chemical involved. Although soil sterilization might be effective in high-cash-return crops, it would not be economically feasible for most field crop soils. Thus, new technology is needed on how to reclaim problem fields. Rotation of chemicals has shown some degree of success in preventing problem field development, but the long range effectiveness of this preventive measure is not known. Crop rotation has been tentatively examined but is only

successful when the pesticide complex used on one crop is suitably different from that used on the rotational crop, or the rotation sufficiently interferes with the life cycle of the pest. Several microbe or enzyme inhibitors appear effective for extending soil persistence (Kaufman et al 1985; Obrigawitch et al. 1982a; Roeth 1986), but the technology involved needs further development.

The practical significance of these observations is of considerable importance to agriculture. Successful pest control depends upon maximum performance of our pest control chemicals. The deliberate combination of certain pesticides, or addition of microbial or enzyme inhibitors to pesticide formulations, or the rotation of certain crops and pesticides shows considerable promise for the purpose of controlled persistence of biodegradable pesticides.

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DECOMPOSITION OF EPTC BY SOIL MICROBES IN TWO SOILS

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ABSTRACT

The decomposition of the herbicide EPTC by soil microbes was investigated in two soil types with and without previous EPTC and EPTC degrader microbe exposure. We isolated a bacterial strain grown on EPTC as sole carbon and energy source. The loam soil (I) without previous EPTC exposure was divided into subsamples which were I.a. autoclaved, I.b. control, I.c. treated with 6 kg/ha EPTC two times, I.d. autoclaved and inoculated with the EPTC degrader strain (HE2), I.e. inoculated with strain HE2, I.f. inoculated with 10% of 16 years previously EPTC exposed soil (II). The other soil was a brown forest soil with 16 years previous EPTC exposure (II). The decomposition of EPTC was faster in all fresh soil samples than in the autoclaved control. The fastest decompositions were in I.c.d.e. samples (5-7 days), in the II. soil it was 10 days, while in I.b.f. samples it was 14 days. The decomposition of EPTC in I.b. and II. soils was nearly equal. For this reason the decomposition did not accelerate in I.f. samples although expected, while the decomposition of EPTC accelerated in I.c.d.e. samples as expected.

INTRODUCTION

The accelerated decomposition of the herbicide EPTC (s-ethyl N, N-dipropylthiocarbamate) with the antidote R-25788 (N, N-diallyl-2, 2-dichloroacetamide) which was used for controlling grass weeds in maize fields was reported from New Zealand maize fields where EPTC exposure (Rahman et al., 1979). Studying this phenomenon, it was reported that soil microorganisms were the major reason for the lack of weed control (Gray, 1979; Rahman et al., 1981) and it was shown that some, but not all soils could be conditioned by repeated applications of EPTC but that there were many other factors which determined whether a soil would become conditioned, besides repeated application (Capper, 1982).

Several chemicals were tested by Stauffer Chem. Co. to improve the efficacy of weed control obtained with EPTC + R-25788 when applied to conditioned and normal soils. One chemical, R-33865 (O, O-diethyl-O-phenyl-phosphorothioate) was particularly effective in restoring the herbicidal activity of EPTC (i.e. acting as a herbicide extender).

The objectives of the work reported in this paper were:

a to isolate microbes which are able to grow on EPTC as sole C and energy source

b to determine the period of EPTC decomposition in soil samples with and without previous EPTC and EPTC degrader bacterial strain exposure

c to determine the effect of R-33865 on EPTC degraders

MATERIALS AND METHODS

Soils

I. Loam soil (Borsodszirak), with no previous EPTC exposure was used for greenhouse experiments. This soil (50 kg) was treated with 6 kg/ha EPTC in May and August 1986.

II. Brown forest soils (Putnok) with 16 years previous EPTC exposure, which was taken in November 1986, and was used as the EPTC degrader donor.

The soils were stored at 4°C in paper bags for 6 months.

For the list of soil samples, see abstract.

EPTC handlings

The samples were passed through a 2 mm sieve, and gas chromatography did not detect any residual EPTC from field application. EPTC was applied at 50 mg/l to two sub-samples (200 g, oven-dry basis) of each soils according to Lee et al. (1984). The moisture of soils was 30%. The 500 ml flasks of samples were plugged with cotton wool and incubated in the dark at 25°C for 16 days. At 2-3 day intervals , 10 g soil samples were removed aseptically to determine EPTC levels using gas chromatography.

Isolation and selection of EPTC degraders

EPTC degraders were isolated from the I.b.c. and II. soils. Diluted samples (10^{-1} -10^{-7}) were made and 0.1 ml of the dilutions was plated onto basal salt agar + 400 mg/l EPTC medium (BSAEM). The BSAM was prepared from: NH_4NO_3, 0.5g; KH_2PO_4, 0.4g; K_2HPO_4, 1.6G. NaCl, 0.1g; $MgSO_4 \cdot 7H_2O$, 0.2g; $CaCl_2 \cdot 2H_2O$, 0.5mg; $CuSO_4 \cdot 5H_2O$, 0.5mg; $FeSO_4 \cdot 7H_2O$, 0.5mg; $ZnCl_2$, 0.5mg; agar 20g; dist. water, 1000 ml. All types of strains were transferred from countable plates to BSAEM tubes after 10-day incubations at 25°C. The strains were purified on nutrient agar plates and were maintained on BSAEM.

One hundred and twenty-one isolates were investigated for their ability to grow on EPTC as their only C and energy source in 100 ml BS+250 mg/l EPTC (BSEM) by liquid shake culture carried out in triplicate. The EPTC was dissolved into the water by shaking aseptically in Stohman flasks. The samples were inoculated with 1 ml of 1-9 x 10^9 i/ml of suspensions of strains grown on nutrient medium.

The growth of strains was controlled photometrically measuring the turbidity of the initial (1-9 x 10^7) and the final state (after 14 days) at 530 nm.

By this method we found only one bacterial strain which was able to grow on EPTC. This strain was isolated from loam I.c. soil (two previous EPTC exposures). this was marked as strain HE2. The identification of HE2 strain is in progress.

Investigation of EPTC degrader strain

The decomposition of EPTC was investigated by pure culture of HE2 strain. The 200 ml of BSEM (in duplicate) was inoculated with 2.3 x 10^7 i/ml HE2, and was completed with 0.1; 1; 10; 100 mg/l of R-33865, when we investigated the effect of this extender. The flasks were shaken two times per day for two mins and were incubated at 25°C for 33 days. Then, 10 ml of samples were anlaysed for EPTC by gas chromatography for 2-3 days.

GLC determination of EPTC

The EPTC in 10 g soil samples was extracted into 25 ml of petroleum ether (70°C BP) acetone reagent (3:2 v/v) according to Lee et al. (1984).

The petroleum ether extract (1.5 µl) was injected into a CHROM-4 gas chromatograph containing a glass column (2.4m x 3mm) packed with Chromosorb W (80/100 mesh) coated with 10% SE-30. The oven temperature was 180°C and the injector temperature was 220°C.

A standard curve of the peak height of EPTC was prepared for EPTC concentrations of 0.25-50 mg/l in petroleum ether. Results were expressed as percentage EPTC recovered from the soil relative to day 0.

RESULTS

Figures 1 and 2 show the fate of EPTC added to soil samples. The loss of EPTC in the autoclaved soil sample was 55% over 15 days because of volatilization.

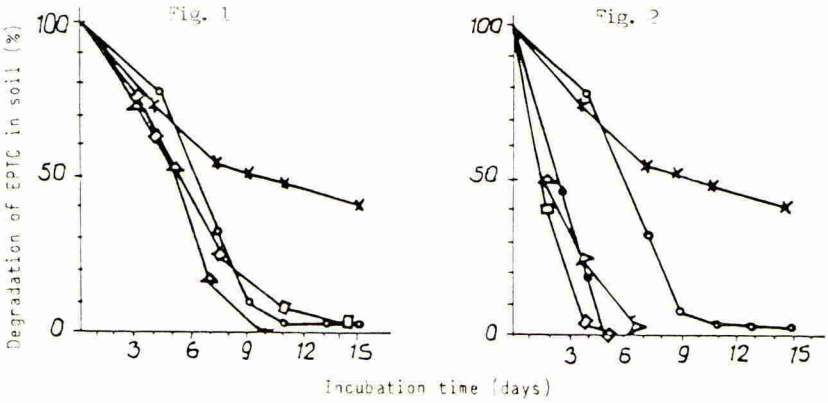

Fig. 1.-2. The degradation of EPTC in soil. X, autoclaved control: O, no previous EPTC exposure; ●, two times previous EPTC exposure; □, autoclaved I.b. soil inoculated with HE2 (2.3x10^7 i/ml); Δ, I.b. soil inoculated with HE2 (2.3x10^7 i/ml); ■, 90% I.b. soil + 10% II. soil; ▲, 16 years previous EPTC exposure (II).

The EPTC was degraded over 14 days in the I.b. (no previous EPTC exposure) and I.f. (I.b. inoculated with 10% of II. soil). The pattern of EPTC loss began as a lag phase of 5 days. This was followed by very rapid decomposition to 9th day and the residual amount of EPTC (4 and 9%) disappeared after 14th day. For the II. soil, the pattern of EPTC decomposition was similar to I.b. and f. samples but was slightly faster and there was not such a long residual decomposition after the 9th day. The EPTC decomposition in I.c.d.e. samples was rapid over 5-7 days. We did not measure the lag phase. There was a two-day difference in the disappearance of EPTC from the I.d. and I.e., which might be explained by the antibiotic sensitivity of HE2 strain.

The HE2 strain was sensitive to: ampicillin, carbenicillin, chloramphenicol, erythromycin, gentamycin, kanamycin, neomycin, penicillin, polymyxin B, streptomycin, tobramycin; and resistent to nalidixic acid.

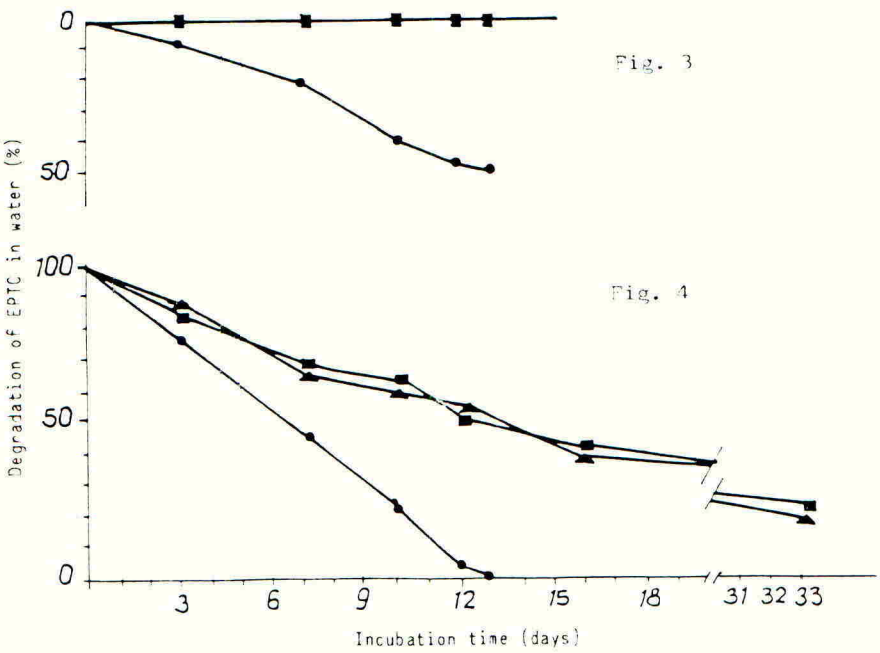

Fig. 3-4. The degradation of EPTC in 250 mg/l EPTC solution without (Fig.3) and with (Fig.4) the EPTC volatilization. ■ , sterile control BSEM; ● , BSEM inoculated with HE2 (2.9×10^7 i/ml); ▲ , BSEM + HE2 + 0.1 mg/l R-33865.

Figures 3 and 4 show the pattern of decomposition of EPTC by HE2 (2.3×10^7 i/ml). The loss of EPTC in sterile control was 50% over 15 days, and 88% over 33 days because of volatilization. In the HE2 inoculated samples the decomposition pattern show a lag phase of 5-6 days, and it is followed by a rapid decomposition to the 13th day when

the EPTC disappeared fron the solution. We measured total inhibitory effect of R-33865 at 0.1; 1; 10 and 100 mg/ concentration on the HE2 strain, and the results of these extender tests were similar to the sterile control.

DISCUSSION

The data obtained during the above described investigation corroborate those reports which showed that the accelerated decomposition of EPTC in soils with previous EPTC exposure was caused by microbes and the activity of these microbes on EPTC inhibited using chemical R-33876. Our results were affected by EPTC volitilization. The losses of EPTC by volitilization were 55% from the soil and 50% from the BSEM over 14 days.

The decomposition of EPTC was faster in all soil samples than in sterile control. In the samples of I.b. (no previous EPTC exposure), I.f. (90% I.b. + 10% II.) and II. (16 years previous EPTC exposure), the decomposition of EPTC was more or less similar, although in the II. soil, it was complete over 10 days. In the case of I.f. soil, faster decomposition was expected by adding 10% of II. soil as a starter microbial inoculum of EPTC degraders, but faster decomposition did not occur because the decomposition of EPTC in the II. soil was unexpectedly similar to the I.b. sample. In the case of soil previously exposed to two EPTC applications (I.c.) and the HE2 inoculated soil samples (I.d.e.), the EPTC decomposition accelerated and the EPTC disappeared over 5-7 days from these soils, without a demonstrable lag phase. For the I.e. sample, the decomposition was slower than I.d. which accounted for the sensitivity of the HE2 strain to antibiotics, and why the HE2 strain was inhibited alightly by the other soil microbes.

The sensitivity of HE2 strain to R-33865 was investigated in BSEM. The growth of HE2 in the control (without R-33865) was good and the pattern of EPTC loss began as a lag phase of 5-6 days. After the 6th day the decomposition accelerated up to the 13th day, when the EPTC disappeared from the medium. The decomposition time and pattern of EPTC in BSEM was similar to the I.b. soil sample.

The activity of the HE2 strain in soil I.d.e. and in BSEM on EPTC was different which was affected by the different EPTC concentration (50 mg/kg and 250 mg/l) and the presence of the other carbon and energy sources in the soil which made the activity of HE2 easier on EPTC according to Lee (1984) who showed that many microbial strains were able to metabolise the 14C EPTC in the presence of glucose.

The chemical R-33865 inhibited the growth of the strain HE2 at 0.1; 1; 10 and 100 mg.1 concentrations when BSEM was with it. The amount of EPTC decreased by volitilization and the patterns of EPTC losses were similar to the sterile control. These results were affected by the high EPTC concentration and the lack of other carbon sources from the BSEM. The presence of other carbon sources decreased the inhibitory effect of R-33865 on the soil microbes which was slight even at 10 mg/l concentration (Nagy et al., 1986).