SESSION 4A

WEED CONTROL IN FRUIT: CROP TOLERANCE AND HERBICIDE EFFICIENCY

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EVALUATING THE TOLERANCE OF FRUIT CROPS TO HERBICIDES: PROBLEMS AND PROGRESS

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INTRODUCTION

The number of herbicide recommendations in fruit crops in the UK has increased steadily for over 20 years. Recommendations have mainly been based on small scale field testing supplemented by user trials. This method has generally proved satisfactory, but in spite of the number of treatments available more are still needed to enable all weeds to be controlled chemically from the time of planting.

The main constraints on developing new treatments are:-

1) the small potential market for the product

2) the high risk factor

3) the relatively high cost of field testing.

This cost arises from a number of factors. The large plant to plant variability makes large trials advisable if growth and yield differences are to be detected. The variability in crop response to treatments increases trial numbers; testing in different soil and weather conditions is essential and applications may be needed at most times of the year. Effects on crop yield and quality are expensive to assess. Treated and untreated plots must be maintained weed free for the duration of the trial to get a true measure of effects; even small amounts of weed can reduce growth. Long-term effects in fruit crops can be important; some effects may not be seen until the year after treatment. Cultural methods also vary and may affect response (Clay, 1980d). With strawberries the crop may be planted most months of the year, on ridges or level soil, and grown as spaced plants or matted rows. Some crops will be covered by polythene tunnels.

The major problem of variable response to soil-acting herbicides has been demonstrated in experiments at the Weed Research Organization (WRO). The dose of simazine normally applied in spring (1-2 kg/ha) is usually too damaging to be used on strawberries in the UK but in experiments it only caused damage in 8 out of 12 applications over 4 years (Clay, 1978a, b). In apples when simazine (7 kg/ha) and metribuzin (4 kg/ha) were applied to newly-planted or 1 year old trees in spring each year from 1975 to 1979, damage only occurred in the final year (Table 1). This indicates how misleading conclusions can be from field testing in some years. The danger of misinterpreting results can be reduced by including a 'damaging' standard to show if conditions were conducive to damage. These results also illustrate the dangers of advising growers to try out new treatments with soil-acting herbicides for themselves.

In view of these problems it is not surprising if chemical industry is reluctant to develop new treatments for use in fruit crops. Official bodies have played a major role in the UK (see previous conferences) but now have reduced resources for such work and these need to be put to the best use. Methods of determining tolerance that reduce the amount of field testing have been developed at WRO. This development has taken account of the many factors governing herbicide tolerance in fruit. The relative importance of these is reviewed in this paper and the WRO testing methods outlined. The requirements and prospects for greater efficiency in testing in the future are also discussed.

Table 1

The effect of four herbicides applied in spring 1979 to apples 1 year after planting

Herbicide	Methazole	Metribuzin	Oxyfluorfen	Simazine
Dose (kg/ha)	9	4	10	7
	% untreated			
Score of plant condition[+] 29 June	100	29	100	74
Girth[o] 26 November	97	-[x]	94	79

[+] % untreated, 0 = plant dead, 100 = plant healthy, [x] 50% trees dead
[o]Girth, 15 cm above union, untreated value 107 mm

FACTORS AFFECTING TOLERANCE

Soil-acting herbicides

The selectivity of soil-acting herbicides in fruit is regarded as largely due to depth protection - most of the absorbing roots occurring below the herbicide containing layer (Fryer and Makepeace, 1977).

Soil factors are responsible for keeping the bulk of herbicide near the soil surface. Adsorption on certain soil constituents, particularly organic matter, and absorption between soil particles can limit downward movement so that commonly used herbicides such as simazine and lenacil are often not moved in any quantity below 5 cm depth. The influence of soil factors is reflected in the different doses recommended for different soil textural classes (Fryer and Makepeace, 1978) though this disguises large differences in adsorptivity within these classes (Harris and Sheets, 1965). Thus, in the field, crop roots may never encounter significant quantitites of the herbicide.

Climatic factors also affect tolerance. Downward movement of herbicide in soil is caused by water movement from rainfall or irrigation, but rainfall in the UK is unpredictable in amount and intensity; this is a major problem in herbicide evaluation. Abnormal rainfall probably accounted for damage to the apples in 1979 as shown in Table 1, since the soil was wet at spraying and 120 mm of rain fell in the following two months increasing downward movement of herbicides. Conditions were much drier in the previous four years when no damage occurred. Excess rain or irrigation can also result in greater uptake and damage. Differences in transpiration rate after spraying may also be a factor contributing to variable response. Shone and Wood (1972) showed that simazine uptake in blackcurrants was directly related to transpiration rate. The varying doses of herbicide needed to damage strawberries in sand culture in successive years (Clay, 1980a) may indicate the important effect of transpiration rate and growing conditions on tolerance.

Another soil factor that can contribute to variable response is soil pH. Strawberries have been more damaged by simazine in alkaline soils in Canada (Leefe, 1968) and in Kent (Buttfield, J. 1979, private communication). Experiments in solution culture failed to reproduce this effect (Pinho, 1979), suggesting that the greater availability of simazine in alkaline soils may be responsible for the damage in the field.

The inherent tolerance of fruit plants may be an important factor in selectivity with some treatments. Whereas with simazine on strawberries tolerance

is low (Oorschot and Haker, 1964), woody plants probably immobilise and detoxify a proportion of simazine entering by the roots. Shone and Wood (1972) treated roots of blackcurrants in solution culture with radioactive simazine and found the herbicide was present in all parts of the plant without showing damage. Thus, inherent tolerance may be important with some crop/herbicide combinations.

The information reviewed indicates the considerable variation in response to soil-acting herbicides and underlines the difficulty of field evaluation. It suggests that excluding major sources of variation such as soil and rainfall from a tolerance evaluation system should lead to improved efficiency.

Foliage-acting herbicides

There is less variation in tolerance to herbicides of this type compared with soil-acting herbicides. Field evaluation has however indicated problem areas such as weather at spraying, season of application, persistence of herbicides in plants and the dangers of root damage and uptake. Phenmedipham applied in high temperatures is known to result in greater leaf damage to strawberries. Glyphosate damages shoots of fruit plants sprayed in the growing season, but translocation into the rest of the plant is only significant with small plants or on large plants from late summer and autumn applications (Davison, 1975; Atkinson *et al.*, 1978a). Earlier treatments of shoots or suckers appears safe although damage to root systems has been reported (Staalduine, 1979). Glyphosate applied in summer and autumn can persist in bushes and trees and cause damage the following year (Davison, 1975; Stinchcombe and Stott, 1978; Atkinson *et al.*, 1980). Similar effects have been observed with 2,4-D on apples cv. Bramley and pears (Clay and Ivens, 1968). In general the phenoxy alkanoic herbicides have not caused damage as directed sprays in tree fruit except where shoots are accidentally sprayed (Clay and Ivens, 1968) or where volatile formulations are applied in hot weather. In strawberries, tolerance to 2,4-D is influenced by growth stage; applications during the flower initiation period can be particularly damaging (Davison and Bailey, 1978).

Root uptake of herbicides whose main activity is through the leaves can occur. 2,4-D is known to be potentially damaging to pears; damage can recur in the season after treatment (Davison and Clay, 1970). Other herbicides such as mecoprop may inhibit root growth in trees but not produce symptoms in the foliage (Atkinson *et al.*, 1978b). All these circumstances in which damage can occur need to be considered in the evaluation of new foliage-acting herbicides for fruit.

Non-damaging and stimulatory effects

There are numerous reports of such effects from the use of soil-acting herbicides in fruit. Chlorthiamid and dichlobenil often induce leaf margin chlorosis on tree and bush fruit and similar symptoms are seen with simazine on stone fruit but there is no evidence that this is linked with growth or yield reduction. Thus herbicide symptoms are not synonymous with damage. Growth stimulation has been reported with triazine herbicides used on fruit trees (Ries, 1977). In experiments on strawberries ethofumesate caused leaf distortion but leaf weight was increased (Clay, 1982). Pendimethalin applied in spring has resulted in both leaf stunting effects and increased fruit yield or earliness (Clay, 1978b) but the reasons for this effect on fruiting are unknown. The significance of such effects can only be found by field experiments but tests on container-grown plants can often give early indication of their occurrence.

EVALUATION USING CONTAINER-GROWN PLANTS

The objectives of the work at WRO were to develop methods for assessing tolerance of new herbicides and of new crop cultivars and to study some of the plant and herbicide factors affecting responses. The methods were to meet the following

criteria:-

1) be suitable for testing herbicides with different modes of entry and activity

2) provide a complete range of response, independent of weather

3) eliminate effects of soil adsorption on availability to roots

4) provide information on the relative importance of root and shoot entry

5) increase the number of treatments that could be tested compared with the same input on field testing.

The methods developed have been described previously (Clay and Davison, 1978; Clay, 1980a, b). They use whole plants and consist of two types of test to asses herbicide activity via foliage and roots separately. All plants are kept outdoors; during the treatment period rain is kept off using a large transparent mobile cover.

Test for activity via roots

This method was developed from one used at Long Ashton Research Station (Caseley, 1964; Luckwill and Caseley, 1966). Plants are grown in 25 cm pots in silica sand and watered with Hewitt's nutrient solution. During the treatment period pots are stood in large foil saucers to prevent herbicide leaching. Herbicide is applied in 500 ml nutrient solution to the sand surface. Four or five doses of each herbicide are normally used. Plants are assessed for damage by visual scores of plant condition and quantitative measurements. Scoring by the same observer for any one experiment has generally given reliable results and is often more informative than measurements (Clay and Davison, 1978; Clay, 1982). In short term experiments measurements may not record such features as leaf chlorosis and distortion which are important in assessing tolerance (Clay, 1982). In tests with new herbicides on fruit plants standard herbicides such as simazine, lenacil or propyzamide are included as reference compounds for which there is much information on field tolerance in the UK. ED values (doses causing 20 or 50% growth inhibition) can be calculated using a computer program. To compare results from different treatments and experiments a number of indices have been used (Clay, 1980a). The tolerance index (TI = ED value test herbicide divided by ED value standard herbicide) indicates relative tolerance in sand. The dose response index (RI = ED 50/ED 20 value) indicates the slope of the dose/response curve and gives information on the possible effects of overdosing or the likelihood of sub-lethal effects. The speed of action index (SI = time for herbicide to cause maximum damage) is based on regular scoring of damage. Effects on root morphology are observed by washing off sand at harvest and effects on root and shoot growth can be compared.

Tests for foliage-activity

Strawberries and blackcurrants have been used, grown in containers of soil or soil based composts. Little difference was found in herbicide response with strawberries grown in different composts or fertilizer levels. Plants are brought indoors for spraying using a laboratory sprayer but taken out on the same day. Spray is kept off the soil surface by polystyrene granules or other material which is removed after spraying. Sprays can be directed onto the base of small blackcurrant bushes. By use of the pot sprayer treatments can be applied when planned and application is largely independent of outdoor weather.

The usefulness of these methods for indicating tolerance or toxicity of herbicides in the field has been illustrated for several crops. With apples a similar relative tolerance to four herbicides was shown in a sand culture test as in the field (Table 2, 1). With strawberries relative tolerance of 8 herbicides in field tests corresponded well with that in earlier pot tests at WRO (Clay, 1978a, b; Clay, 1980b; Clay et al., 1974). The tolerance of pendimethalin, propachlor and

ethofumesate was subsequently confirmed in field experiments at other research stations in the UK. (Lawson and Wiseman, 1978; UK Horticultural Centre, Loughgall, 1976, 1977). The type of information obtained on damaging herbicides in the tests on container grown plants can prove valuable for selecting herbicide treatments for otherwise intractable weed problems. Growers are often prepared to use drastic treatments but to make the best choice they need to be able to compare efficacy with likely damage.

Table 2

The effect of four herbicides applied to the roots of apples in sand culture

Methazole	Metribuzin	Oxyfluorfen	Simazine
4.8*	1.2	> 100	2.7

* Dose (mg/pot) causing 50% reduction in dry wt shoots

TESTING VARIETAL TOLERANCE

Experimentation and commercial use has shown that differences in varietal tolerance occur with some herbicides in fruit but that care is needed in interpreting results. Cultivars may be more susceptible at high doses but adequately tolerant at recommended doses, e.g. lenacil/strawberry cv. C. Favourite (Table 3). Other cultivars may be damaged by the recommended application rates but still might be treated if there is no effective alternative e.g. 2,4-D and ethofumesate on strawberries (Davison and Bailey, 1978; Clay, 1981, 1982). Thus at an early stage in the introduction of new cultivars there is a need for information on herbicide tolerance.

Table 3

The effect of lenacil on strawberry cultivars Cambridge Favourite (F), Senga Gigana (G) and Montrose (M) in sand culture and in the field

	Sand culture leaf dry wt (% untreated)					Field			
Lenacil dose (mg/pot)	F	FS*	G	M	Lenacil dose (kg/ha)	F	FS*	G	M
2.0	90	73	62	95	2.0	109	102	113	105
5.0	64	39	23	66					
12.5	28	36	8	37	6.0	100	81	60	120
31.2	10	5	4	25					
Untreated	100	100	100	100	Untreated	100	100	100	100
(Actual value g/plant)	(10.1)	(7.0)	(8.4)	(4.7)	Actual value g/plot)	(75.8)	(69.0)	(63.8)	(57.2)
S.E. ± (treated v. untreated)		10.6					6.1		

* FS, small plants, C. Favourite

To give maximum information tests of varietal tolerance need to include standard tolerant and susceptible cultivars and to induce damage in both. Only then can there be reliable ranking of susceptibility. Damage to susceptible cultivars can often be induced in field trials using high doses but damaging more tolerant cultivars may be difficult.

Because higher doses are more practicable in tests of foliar activity on container grown plants the likelihood of success is higher. Similarly, testing varietal tolerance to soil-acting herbicides in dose/response experiments in sand culture is more reliable.

A number of factors may lead to differences in results between tests. With strawberries plant size needs to be typical for the cultivar since this can greatly affect response (Table 3). Growth rate may also be a factor; the greater susceptibility of cv. Redgauntlet to ethofumesate in winter may be due to it being less dormant (Clay, 1982). Growth habit may affect the amount of spray received; in apples certain pollinator varieties have been more damaged by glyphosate than fruiting trees, because their weeping branches received more spray (Stinchcombe and Stott, 1980). Differences in crop vigour and rooting depths of cultivars might lead to differences in response but this has not yet been established. All these factors need to be appreciated in designing and interpreting tests of varietal tolerance to herbicides.

FURTHER PLANT AND HERBICIDE FACTORS AFFECTING TOLERANCE

The use of containerised plants for tolerance experiments at WRO has enabled investigation of some of the plant and herbicide factors involved. These have included the effects of plant size, timing of treatments, and the effect of herbicide formulation and mixtures.

Plant size and treatment timing

Apples pruned in different ways varied in tolerance to simazine applied to roots in sand culture. Simazine applied in April caused more damage to lightly pruned one-year-old trees than to severely pruned trees in which shoot growth was initially slower (Table 4). However, when simazine was applied to similar trees in June the severely pruned trees were more damaged. Differences in growth rate and water uptake during the treatment period were probably responsible. The experiment also showed the different speed at which simazine can affect trees depending on when it is applied to the roots. With trees treated on April 30 there were no visible leaf symptoms for two months, whereas treatments on June 21 resulted in severe damage within one month.

Table 4

The effect of simazine applied on April 30 or June 21 to roots of 1-year-old apples (cv. Cox on M 26) in sand culture following light (L) or severe (S) pruning of the trees

Application date	Plant condition score, September 14+			
	April		June	
Pruning	S	L	S	L
Simazine dose (mg/pot)				
12	107	100	67	100
24	107	63	41	63
48	87	48	14	30
96	71	33	11	30
SE +		5.8		

+ % untreated, 0 = plant dead, 100 = plant healthy

Herbicide formulation

There is little published information on relative tolerance to different herbicide formulations. Ester formulations of herbicides such as 2,4-D and 2,4,5-T

have been known to give damage in tree fruit because of volatility while amine formulations are safer. Oxadiazon as a wettable powder or granule was shown to be less toxic than the emulsifiable concentrate to strawberries (Clay, 1980c). The recent change of several commonly used herbicides from wettable powder to flowable (suspension concentrate) formulations has raised queries about crop safety. In experiments with strawberries s.c. propachlor has given more necrosis of sprayed leaves than the w.p. at a volume rate lower than recommended (Table 5). This damage occurred on the youngest expanded leaves shortly after spraying and was subsequently outgrown.

Table 5

The effect on strawberries of s.c. and w.p. formumulation of propachlor applied at two volume rates on 28 June assessed 21 July

Volume rate (Dose (kg/ha)	Number of necrotic leaves/plant+ 240 l/ha s.c.	240 l/ha w.p.	475 l/ha s.c.	Score of plant condition+ 240 l/ha s.c.	240 l/ha w.p.	475 l/ha s.c.
4.5	11	0	0	90	100	100
9.0	26	6	2	75	92	91
SE +						2.4

+ % untreated (actual leaf number 18; score 0 = dead, 100 = healthy)

Tolerance to herbicide mixtures and sequences

There are some warnings in manufacturers' herbicide recommendations against the use of products in mixture or in sequence with others, but so far no published information about any damage problems has been seen for fruit crops. Experiments with strawberries in sand culture have not shown any adverse interactions with mixtures of commonly used residual herbicides. With sequences on strawberries there were no synergistic effects when phenmedipham was sprayed on to plants pre-treated with ethofumesate (Clay, 1982). Recent work with strawberries has indicated that there may be adverse interactions with mixtures of lenacil and new grass weedkillers. Mixturs of lenacil with alloxydim sodium or fluazifop-butyl applied to strawberries at three times the commercial rate were severely damaging while alone they were safe (Table 6). Mixtures of standard rates were much less damaging.

Table 6

The effect on strawberries of lenacil (L), alloxydim sodium (A) and fluazifop-butyl (F) alone or in mixture sprayed at 240 l/ha

	Leaf fresh wt , % untreated L	A	F	LA	LF	Untreated (actual value g/plant)
Dose 1+	100	95	90	84*	82*	100 (61.8)
Dose 2	97	100	97	60***	32***	
SE +			4.9			

+Actual doses (kg/ha)

	L	A	F
Dose 1	2.0	1.5	0.75
Dose 2	6.0	4.5	2.25

*, **, *** indicates values significantly different from untreated at P = 0.05, 0.01, 0.001.

FUTURE REQUIREMENTS AND OPPORTUNITIES

As with all screening systems there are a number of features of the WRO methods which may mean that potentially safe treatments are not selected for further testing, or damage is underestimated. Some of these are listed below:-

1) Herbicides may be safe in winter but not in the growing season when most tests are done.

2) With foliage acting herbicides the tests may not allow for the toxicity of herbicides washed into buds or shoot apices by rain.

3) Entry through the bark of young trees may not be allowed for.

4) Damage from herbicide vapour.

5) Damage from rain splashing of herbicide from soil to foliage.
There is some evidence of this causing leaf damage with oxadiazon on strawberries and oxyfluorfen on plum suckers.

6) Differential herbicide adsorption, persistence and leaching in soil is not allowed for by the sand culture method.
These major factors must be considered in deciding the likely safety of the herbicide in the field.

7) Toxicity of herbicides inhibiting root development may be underestimated since plants rarely encounter moisture stress in sand culture.

8) Herbicides could be less active in sand if toxic breakdown products present in soil are not formed.

Many of these problems can be anticipated if the herbicide mode of action is known and more detailed study made if required. In spite of these limitations the system is proving a useful method of evaluating tolerance and reducing the number of new herbicide treatments that require field testing.

There should be scope for further improvement in screening efficiency by including soil and "rain" in the containerised-plant system. Work is planned at WRO in which plants in large containers have herbicide applied to the soil and then receive different rainfall treatments under a rain simulator. Preliminary tests using soil columns have indicated the rainfall treatments that are likely to maximise downward movement of herbicides (Pinho, 1981). If the relative importance of the various soil and rainfall factors can be determined more precise prediction of field tolerance to soil-acting herbicides should be possible. Another development that may improve efficiency is the use of micropropagated plantlets for evaluating herbicides and varietal tolerance. Collaborative work between East Malling Research Station and WRO is in progress to investigate its potential.

This review has indicated that while progress in tolerance testing has been made there is scope for even greater efficiency. This must be done to make the best use of the limited resources available for developing and servicing minor uses of herbicides and solving new weed problems of major importance to growers.

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THE TOLERANCE OF NEWLY PLANTED PLUM AND APPLE TREES TO A NUMBER OF GRAMINICIDES

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Summary. Crop tolerance of newly planted plum and apple trees to the grass herbicides fluazifop-butyl (PP009), sethoxydim (NP55) and propionic acid:2-(4-((3-chloro-5-(trifluoromethyl)-2-pyridinyl)oxy)phenoxy):2-ethoxyethyl ester (Dowco 453EE, apples only) applied either to the soil or to a 30 cm length of leafy one-year-old shoot has been assessed. Applications of paraquat, simazine and glyphosate were made to plums and simazine to soil and glyphosate to shoots in apple as standards.

In 1981 neither fluazifop-butyl, sethoxydim or simazine, as foliar applications, produced any symptoms in plums. Paraquat and glyphosate caused obvious damage. No treatment, as a soil application, produced damage.

In 1982 results for plum were similar to those for 1981 except that foliar applications of simazine caused chlorosis.

In apples foliar glyphosate, but none of the other treatments, damaged new growth.

In 1981 foliar applications of both simazine and glyphosate reduced plum shoot growth. Fluazifop-butyl and sethoxydim had no effect, although the shoot growth of most trees receiving foliar application was less than when applications were made to the soil. Fluazifop-butyl, Sethoxydim, herbicide management, grass, damage, Dowco 453EE.

INTRODUCTION

Fruit trees are adversely affected by weed competition. Atkinson and White (1981) showed that competition from grass weeds or annual weeds in a herbicide strip area around 10-yr Cox/M.26 reduced crop weight by up to 49%.

Modern intensive orchard systems on dwarfing rootstocks (spindle bushes, continuous rows, high density plantings) (Oberhofer, 1981) result in substantial proportions of leaves and fruit being placed close to the soil surface. The traditional separation of sensitive parts of the tree and weeds which occurred in bush orchards

no longer exists. Tolerance to accidental contact with the chemical is important to the tree.

Good weed control in plum orchards is difficult with current chemicals because of low tolerance of the trees. When simazine is applied to established plum trees, even at half the dose recommended for apples, leaf chlorosis frequently results. There are only seven approved herbicides for plum (MAFF, 1982), which are for established trees only, so an improved range is needed.

Results are presented from trials using a range of new grass herbicides. Tolerance to both soil applications and deliberate sprays to a single branch so as to simulate accidental contact have been assessed.

The response of crops to herbicides varies between years so alternative grass-controlling materials (paraquat, simazine and glyphosate) which could damage plums, were included as standards against which new materials could be evaluated.

METHODS AND MATERIALS

Trees of Victoria/Pixy planted in Spring 1981 at a spacing of 2.5 x 4 m received the following treatments in both 1981 and 1982:

1)	Fluazifop-butyl	1 kg ai ha^{-1}
2)	Sethoxydim	3 kg ai ha^{-1}
3)	Paraquat	1.1 kg ai ha^{-1}
4)	Simazine	2.0 kg ai ha^{-1}
5)	Glyphosate	2.5 kg ai ha^{-1}

A small quantity of 'Lisapol N' was added as a wetter in treatments 1 and 2 although this is not a recommended addition for 2.

All treatments were applied in May either to a 2 x 2 m square around the tree or as a directed spray to a 30 cm target of leafy one-year-old shoot using an Oxford Precision Sprayer. Treatments were replicated six times in a fully-randomised block design. Supplementary paraquat sprays were used to keep all trees weed free.

Trees of Cox/MM.106 were planted in Spring 1982 and treatments were applied as above except glyphosate was applied only to shoots and simazine to soil. The paraquat treatment was replaced by propionic acid:2-(4-((3-chloro-5-(trifluoromethyl)-2-pyridinyl)oxy)-phenoxy):2-ethoxyethyl ester (Dowco 453EE) at 0.5 kg ai ha^{-1}. For the apples a low dose of simazine (1 kg ha^{-1}) and paraquat as required were applied to give overall weed control.

Damage was assessed on a 0-5 scale as follows: 0 = healthy, 1 = slight chlorosis, 2 = relatively severe chlorosis on one or more shoots/odd patches of dead leaf material, 3 = almost all shoots chlorotic, 4 = severely damaged but not dead, 5 = dead.

Damage to plums was assessed on three occasions, June and August 1981 and following leaf emergence in May 1982.

Damage to the apples was recorded in June 1982.

RESULTS

In 1981 neither the new herbicides fluazifop-butyl and sethoxydim or simazine caused any obvious damage to the plum trees (Table 1). Shoots treated with paraquat were either dead or dying although the rest of the tree was unharmed. The effects glyphosate applied to the shoot became apparent soon after spraying and persisted through 1982; both the sprayed shoot and the remainder of the tree showed severe damage. None of the soil applications caused damage.

Table 1

The effect of a range of herbicides on plum trees. August 1981. Damage scores on a 0 (healthy) to 5 (dead) scale

Treatment	Method of application		
	Soil	Foliar	
	Whole tree	Whole tree	Treated shoot
Fluazifop-butyl	0.0	0.2	0.3
Sethoxydim	0.3	0.0	0.2
Paraquat	0.2	0.5	4.3
Simazine	0.0	0.3	0.3
Glyphosate	0.0	3.3	5.0

In 1982 there were effects similar to those observed in 1981 for plum except simazine also affected new growth (Table 2).

Table 2

The effect of a range of herbicides on plum trees. May 1982. Damage scores on a 0 (healthy) to 5 (dead) scale

Treatment	Method of application			
	Soil	Foliar		
	Whole tree	Whole tree	Treated shoot 1981	1982
Fluazifop-butyl	0.0	0.0	0.0	0.0
Sethoxydim	0.0	0.0	0.2	0.2
Paraquat	0.0	0.0	3.3	4.2
Simazine	0.2	0.0	0.0	1.5
Glyphosate	0.0	3.5	5.0	4.2

Table 3

The effect of a range of herbicides on apple trees. June 1982. Damage scores on a 0 (healthy) to 5 (dead) scale

Treatment	Method of application		
	Soil	Foliar	
	Whole tree	Whole tree	Treated shoot
Fluazifop-butyl	0.0	0.0	0.2
Sethoxydim	0.0	0.2	0.5
Dowco 453EE	0.0	0.0	0.0
Simazine	0.2	NA	NA
Glyphosate	NA	0.2	3.8

NA - no application

Neither fluazifop-butyl, sethoxydim or Dowco 453EE caused harm to the apples regardless of the method of application. The soil application of simazine caused no damage. The foliar glyphosate spray had damaged the treated shoot but not the remainder of the tree in June 1982.

Table 4

The effect of a range of herbicides on total plum shoot growth and the mean length of an individual shoot (cm) 1981

Treatment	Total shoot length		Mean shoot length	
	Method of application			
	Soil	Foliar	Soil	Foliar
1 Fluazifop-butyl	208	291	18.5	18.7
2 Sethoxydim	230	202	20.9	15.2
3 Paraquat	286	291	20.7	21.6
4 Simazine	255	161	20.8	13.3
5 Glyphosate	211	71	17.8	11.6
LSD 5% (applications)	99.7		5.96	

In 1981 there were significant treatment effects on growth (Table 4). Paraquat increased shoot growth relative to the other chemicals, while glyphosate reduced growth. There was a significant effect of spray target, due mostly to the reduction of growth in trees receiving foliar applications of glyphosate and simazine. Generally, foliar applications reduced shoot growth in comparison with soil applications. The difference in mean shoot length between trees having soil and foliar sprays was significant with simazine and glyphosate.

Table 5

The effect of a range of herbicides on number of plum fruit buds 1982.

Treatment	Method of application	
	Soil	Foliar
1 Fluazifop-butyl	63.3	77.5
2 Sethoxydim	81.5	74.2
3 Paraquat	78.2	64.8
4 Simazine	70.7	69.0
5 Glyphosate	81.2	57.3
LSD 5% (applications)	46.9	

The fruit bud counts showed no significant difference between treatments at the 5% level although tree-to-tree variation within treatments was fairly high (CV = 32%). However, for four of the five treatments mean fruit bud numbers are higher for trees with the soil applied treatment.

DISCUSSION

In plum and apple orchards grass weed competition can effectively reduce both crop and growth potential. In intensive orchard systems, where crop and foliage are located near the ground, there is a clear need for chemicals which will eliminate weeds but will not damage the tree if it is accidentally sprayed.

Fluazifop-butyl and sethoxydim applied to a single shoot did not produce any type of damage symptoms or adversely affect plum growth. There were similar results for Dowco 453EE in apple. In contrast, in these trials glyphosate caused extensive damage and paraquat severely damaged the sprayed shoot (Tables 1-4).

No adverse effects occurred as a result of a soil application of any of the chemicals. The higher growth obtained on plums receiving soil rather than foliar applications of simazine in 1981 suggest that damage, ie reduced growth, can occur with herbicide treatments even in the absence of visible symptoms.

These results suggest that all the new graminicides tested would be of value in intensive or other orchard systems where there is substantial risk of accidental contact between herbicide and tree and can be sprayed up to the tree using rates likely to give good weed control.

When interpreting any results on herbicide effects it is important to compare damage occurring as a result of the chemical treatments to that from weed competition. Growth reductions of up to 69% (Atkinson *et al.*, 1982) have been reported due to grass competition with young fruit trees. Here there were no significant reductions in growth with either fluazifop-butyl or sethoxydim and even with simazine, taking the soil value as 100%, the reduction was

only 37%. However, with glyphosate which was extensively translocated, growth reduction due to herbicide damage (66%) approximated to that likely to be due to weed competition.

The new herbicides therefore appear promising for optimal control of grass weeds without crop damage.

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WEED MANAGEMENT SYSTEMS AND HERBICIDE RESIDUES IN NURSERY TREES AND NEWLY PLANTED PEACH ORCHARDS

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Summary. A weed management system for nursery trees and newly planted peach trees was developed which incorporated considerations of availability of labour, safety of fruit trees to herbicides, ease and uniformity of herbicide application, spectrum and duration of weed control, herbicide residue carry over, and versatility of chemicals to permit intercropping during orchard management.

Nursery trees of peach, pear or apricot could be protected from weed competition by granular simazine at 1.25 kg/ha plus 4 kg/ha napropamide, without injury or risk of residue carry over to other crops.

Weed control in the year of orchard establishment was achieved by pre-emergence application of simazine with napropamide or oryzalin. Post-emergence application of paraquat or glyphosate with simazine or linuron provided excellent weed control, but foliar injury by paraquat and glyphosate was observed. Excellent weed control was observed in pre-plant incorporated treatments of metribuzin and trifluralin which permit intercropping during the early years of orchard establishment. Intercropping, seedling, pear, apricot.

INTRODUCTION

Previous studies have demonstrated good season long pre-emergence control of weeds in established peach orchards with combinations of simazine, oryzalin, napropamide, oxadiazon and methazole, supplemented by paraquat or glyphosate. (Arnold and Aldrich, 1979; Young and Welker, 1981). Few studies have been concerned with weed control in a peach nursery the year after budding the rootstocks or in the first year of planting a new orchard. Removal of weeds from nursery trees and newly planted orchards during establishment increased tree vigour, growth survival, and winter-hardiness of bark and woody tissue (Ahrens, 1978; Marriage and Quamme, 1980). For several years we have evaluated various weed control treatments to assess their phytotoxicity and efficacy in the nursery and in young peach orchards. The elements of the management program included availability of labour, safety of herbicides to young peach trees, ease and uniformity of herbicide application, spectrum and duration of weed control, herbicide carry over, choice of herbicide to permit intercropping during orchard establishment, and effects of weed competition on tree growth.

METHODS AND MATERIALS

Nursery site

The tree nursery trial was located on a Fox sandy loam (1.5-2.0% organic matter, pH 6.7 to 7.4, 70% sand, 16% silt). Nursery rows were 1.0 to 1.2 m apart and had been planted with seedlings that were grown for about 2 months in peat pots in the greenhouse prior to planting in early summer. They were budded in late summer and top growth above the bud was removed the following spring. Thus new growth in the year of herbicide application was from the scion bud. Granular simazine at 1.1 to 2.5 kg/ha was applied alone or in combination with a directed spray of napropamide at 4.0 to 4.5 kg/ha. Napropamide was applied in early March and simazine mid April

to early June. Pear, peach and apricot seedlings were included in the three replicate experiment which was repeated for four years.

Granular applications were made to clean-cultivated areas with a tractor-mounted modified Gandy granular applicator system (Gandy Company, Owatonna, Minn.). An inverted metal V, to protect the trees, was added to the horizontal bar where the 25 cm outlets were mounted. Tree clearance was 10 cm and the corner flanges of the inner outlets were adjusted to ensure deposition of granular herbicide into the tree row. A 50 cm band of herbicide was distributed on either side of the tree row.

Major weed species in the nursery area were Portulaca oleracea, Amaranthus retroflexus, A. powellii, Chenopodium album, and Digitaria sanguinalis. In addition to the above weed species Ambrosia artemisiifolia, Vicia spp., and Cenchrus longispinus were prevalent in the orchard site.

Orchard site

Plots 2 m by 6 m were planted with three peach trees in mid April with the treatments arranged in a completely randomized design replicated four times. Granular herbicides were applied with a hand applicator and liquid formulations with a hand sprayer calibrated to deliver 1,000 l/ha at 270 kPa. Plots received 530 kg/ha of a 10-20-20 fertilizer prior to planting and trees were pruned according to normal cultural practices. Incorporated treatments were applied prior to planting, pre-emergence treatments six days after planting, and post-emergence treatments the first week of June. Trunk diameters were measured with a caliper about 50 cm from ground level at planting and throughout the growing season. Weed control ratings were assessed according to Hamill et al. (1977).

Soil residues were determined to a depth of 10 cm on selected treatments throughout the season by compositing $\simeq$ 25 soil cores of 22 mm diameter per plot. Soil samples were air dried, ground to pass a 2 mm sieve and stored at -5°C.

Simazine, napropamide, oxadiazon, oryzalin, metribuzin and trifluralin residues were determined by appropriate gas chromatography methods. See for example Gaynor and MacTavish (1981).

RESULTS AND DISCUSSION

Nursery site

Application of granular simazine with the modified applicator gave an adequate distribution of herbicide over the soil surface. Deposition was less in the tree row than between rows but the lower dosage was sufficient to give excellent weed control within the row. A single application of granular simazine at 2.2-2.5 kg/ha provided season long weed control. Some injury to pear and apricot seedlings was observed and residues the following spring were unacceptable for triazine susceptible crops (Table 1). However, a lower dose of simazine of 1.1-1.25 kg/ha in combination with 4 kg/ha napropamide provided good broadleaf control and improved grass control (Table 2). Simazine residues were reduced and no injury to the trees was observed.

Orchard site

Weed competition has an immediate and long term effect on growth of peach trees in the year of planting (Skroch et al., 1971). In this study, weeds restricted tree growth as shown by the smaller trunk diameter (Table 3). When weed pressure in the unweeded plots was reduced the year after planting by mowing (Y2=1978) or by a single application of glyphosate (2.25 kg/ha; Y2=1981) trunk diameter was still significantly less than those grown for two years without weeds.

Various herbicide treatments and timings were evaluated for season long weed control in the year of planting the peach trees. The pre-emergence application time, a week after planting in April, coincided with the availability of labour when

Table 1

Weed control in nursery trees, tree injury and soil residues the following spring after application of 2.2-2.5 kg/ha granular simazine 1975-1978

Species	Weed control rating[a] Broadleaf	Grass	Tree injury[b]	Simazine residue, ug/g
Peach	9.6	9.8	0	0.34 ± 0.04
Pear	9.4	7.5	2	0.30 ± 0.12
Apricot	9.1	9.8	1	0.26 ± 0.09

a. 0-10 scale: 0 = no weed control; 10 = complete kill.

b. Injury rating of leaf chlorosis and percentage of trees affected: 0 = none; 1 = very slight, < 10%; 2 = slight, 20-40%.

Table 2

Weed control in nursery trees, tree injury and simazine residue the following spring after application of 1.1-1.25 kg/ha simazine + 4.0-4.5 kg/ha napropamide 1977-1978

Species	Weed control rating[a] Broadleaf	Grass	Tree injury	Simazine residue, ug/g
Peach	9.6	10	0	0.05
Pear	9.6	10	0	0.08
Apricot	9.0	10	0	0.03

a. 0-10 scale: 0 = no weed control; 10 = complete kill.

Table 3

Residual effects of weeds on peach tree growth

Treatment	Trunk diameter, mm[a] Y0	Y1	Y2
		1977-1978	
Hand weeded	17.3	25.3*	34.5*
Not weeded (Y0 to Y1)[b]	19.0	20.3	25.0
		1980-1981	
Hand weeded	12.7	21.9*	35.5*
Not weeded (Y0 to Y1)[b]	13.1	14.8	24.2

a. Y0 = year of planting; Y1 = end of year one; Y2 = end of year two.

b. Plots mowed; (Y2 = 1978). Glyphosate at 2.25 kg/ha; (Y2 = 1981).

* Significantly greater (P = 0.05).

Table 4

Weed control in newly planted peach with selected herbicides and combinations (1975-1980)

Treatment	Rate kg/ha	Time of application	No. of years	Weed control rating (range)[a] Broadleaf	Grass	Trunk diameter increase, mm
Hand weeded			6	10	10	6.2 ± 2.3
Not weeded			6	0.3(0-1.4)	7.4(3.2-9.5)	1.6 ± 0.4
Simazine	2-2.5	pre	3	7.1(1.5-10)	5.2(4.7-5.8)	2.9 ± 0.7
Simazine	4-4.5	pre	3	7.9(3.8-10)	5.6(2-8)	2.6 ± 0.7
Simazine + oryzalin	1.25-2+2	pre	4	9.0(6.7-9.7)	8.7(6.5-9.8)	6.4 ± 3.5
Simazine + napropamide	1.25-2+4	pre	5	5.8(1-9.5)	9.2(7.5-9.9)	4.3 ± 1.8
Oxadiazon	0.75-4	pre	3	3.9(2-7.4)	6.3(2-9.8)	3.5 ± 1.7
Glyphosate	3-3.5	post	4	3.0(0-5.2)	4.8(3-6.7)	3.2 ± 1.1
Paraquat	1	post	3	2.3(1.3-3.3)	4.3(2-5.6)	2.6 ± 0.2
Simazine + paraquat	4 + 1	post	2	9.5 b	9.8 b	6.1 ± 2.3
Simazine & glyphosate	2-2.5&3-3.5	pre/post	2	9.7(9.3-10)	8.4(7.3-9.4)	4.4 ± 0.4
Linuron + paraquat	4 + 1	post	3	9.7(9.4-9.9)	9.1(8.4-9.8)	6.2 ± 0.7
Simazine & paraquat	4 & 1	pre/post	2	9.4 b	7.0 b	5.0 ± 2.8

a. 0-10 scale; 0 = no weed control; 10 = complete kill.

b. Weed control not rated 1977.

dormant pruning had been completed. Post-emergence treatments applied about a month later in June occurred after intensive culture of other crops which began in May. A pre-emergence application of simazine plus napropamide or oryzalin provided season long weed control (Table 4).

In some years control, especially of broadleaf weeds, was reduced because of a strong dependence on rainfall to activate the chemicals, particularly with napropamide and oryzalin. No pre-emergence treatments were effective in 1977 probably because of heavy rainfall before planting (133 mm) and low rainfall (7 mm) in the two weeks following herbicide application. Broadleaf weed control was generally good with oxadiazon, but its failure to control ragweed resulted in reduced tree growth.

Simazine alone provided an average of 70 to 80% control of broadleaf weeds but control of grasses was only in the range of 50% and weed competition led to a marked reduction in tree growth (Table 4). Single applications of paraquat or glyphosate did not provide acceptable control of grass or broadleaf weeds because of weed regrowth and tree growth was similar to the nonweeded control.

Pre-emergence simazine in combination with a post-emergence application of paraquat or glyphosate provided very good broad spectrum weed control. Trees were occasionally injured when their foliage was contacted by glyphosate or paraquat during application. In this study, tree injury from glyphosate was not serious enough to permanently affect tree growth, but Putnam (1976) reported mortality of newly planted peach trees receiving a basal or foliage application of glyphosate at 4.4 kg/ha. A single post-emergence application of linuron or simazine with paraquat provided residual weed control with some tree injury from the paraquat evident but tree growth was comparable to hand weeded (Table 4).

Lack of income during orchard establishment has prompted intercropping of newly planted orchards by many growers. Trifluralin and metribuzin preplant incorporated provided season long weed control in newly planted peach trees; up to twice the recommended rate for the Fox sandy loam soil did not cause noticeable tree injury (Table 5). Trees were placed in the holes with minimal regard to placement of treated soil on the tree roots. Both the herbicides were required to provide adequate control and to ensure maximum increase in tree growth, especially in southwestern Ontario where ragweed and grasses predominate.

Table 5

Weed control and increase in trunk diameter for preplant incorporated treatments in newly planted peach trees 1979-1980

Treatment	Rate kg/ha	Weed control rating[a]		Trunk diameter increase, mm	
		Broadleaf	Grass	1979	1980
Hand weeded		10	10	4.0	9.2
Not weeded		0	9.1	1.7	1.7
Metribuzin	0.75	6.5	8.8	9.9	7.6
Trifluralin	1.0	7.5	9.9	8.3	1.8
Trifluralin	2.0	6.8	9.9	7.0	1.5
Trifluralin + metribuzin	1.0 + 0.375	8.3	10	10.1	5.4
Trifluralin + metribuzin	2.0 + 0.75	9.3	10	7.4	11.5
L.S.D. (P=0.05)				3.9	3.6

a. 0-10 scale: 0 = no weed control; 10 = complete kill.

Herbicide residues

The persistence of simazine, oryzalin, napropamide and oxadiazon was monitored in the peach orchard for three years and metribuzin and trifluralin for one year (Table 6). Herbicide persistence, as reflected by the half life, was lowest in 1978 probably because of physical movement of the herbicide by wind erosion from the treated area shortly after application (Gaynor and MacTavish, 1981). A detailed study of one of the simazine treated plots indicated up to 45% of the herbicide was transported about 2.5 m downwind of the plots.

Table 6

Half life from first order rate constant and residues of selected herbicides in newly planted peach trees

Year applied	Application rate, kg/ha	Half life, days	Residue at days ( ) after application, kg/ha
		Napropamide	
1978	4	11	0.20 ± 0.03 (56)
1979	4	32	1.08 ± 0.34 (78)
1980	4	28	1.99 ± 0.34 (79)
1980	4	34	0.82 ± 0.23 (143)
		Simazine	
1978	2	11	0.16 ± 0.15 (56)
1979	2	28	1.09 ± 0.72 (78)
1980	2	29	0.74 ± 0.17 (79)
1980	2	34	0.26 ± 0.15 (143)
		Oryzalin	
1978	2	19	0.50 ± 0.14 (71)
1979	2	44	0.98 ± 0.20 (78)
1980	2	44	0.57 ± 0.08 (79)
1980	2	70	0.29 ± 0.19 (169)
		Oxadiazon	
1978	1	11	0.08 ± 0.06 (56)
1979	0.75	33	0.33 ± 0.05 (78)
1980	4	58	1.46 ± 0.03 (171)
		Trifluralin	
1980	2	83	0.67 ± 0.27 (90)
		Metribuzin	
1980	0.75	23	0.03 ± 0.03 (90)

An assessment of the half lives in 1979 and 1980 showed the dinitroanilines, trifluralin and oryzalin, to be the most persistent, with simazine, napropamide and oxadiazon being of equal persistence. No difference in persistence was measured between granular and wettable powder formulations of simazine, oxadiazon and napropamide (data not shown). Metribuzin was the least persistent of the herbicides studied.

For all treatments except metribuzin, soil residues were sufficient to persist into the next year at concentrations phytotoxic to susceptible crops. Therefore, a total orchard management system should include compatibility of interplanted crops with the chemical weed program.

From these data a weed management system for nurseries and newly planted peach trees has been developed incorporating the criteria initially outlined.

Simazine plus napropamide at 1.25 and 4 kg/ha respectively, provided the required long term weed control for peach, pear and apricot nursery plantings without tree injury or carry over of residue to the next growing season.

Simazine at 2 kg/ha in combination with 2 kg/ha oxyzalin or 4 kg/ha napropamide provided adequate weed control in newly planted peach trees, but herbicide efficacy depended upon precipitation or irrigation within a week of herbicide application.

Trifluralin and metribuzin can be safely used in newly planted peach orchards and extension of their registration to include this crop would permit intercropping with transplanted tomatoes or soybeans during orchard establishment.

Paraquat or glyphosate in combination with simazine or linuron provided adequate post-emergence weed control, but the difficulty to completely prevent herbicide contact with tree shoots precludes their use with currently available spray equipment.

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TOLERANCE OF RASPBERRY TO NEW HERBICIDES FOR CONTROL OF PERENNIAL WEEDS

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Summary. Spring treatment with oxadiazon at 2 or 6 kg a.i./ha desiccated young raspberry canes and delayed and stunted growth of replacement canes. This herbicide is likely to be acceptable only for very limited spot-treatment to control Convolvulus arvensis. 3,6 dichloropicolinic acid at 0.2 and 0.6 kg a.i./ha caused malformation of young canes, which was rapidly outgrown, but reduced the numbers of tall canes available for subsequent fruiting. Spring application to dense infestations of Cirsium arvense may be justified, but spot treatment may be preferable for light infestations. The grass-killers alloxydim sodium and fluazifop butyl at 1.5 and 1.0 kg a.i./ha respectively had no adverse effects on the crop, although treble dosing caused some reduction in numbers of tall canes per stool. These herbicides both appear safe for overall application after cane emergence in spring. Oxadiazon, 3,6 dichloropicolinic acid, alloxydim sodium, fluazifop butyl.

INTRODUCTION

Perennial weeds, particularly those which do not produce aerial shoots until after cane emergence in spring, are an increasing problem in non-cultivated raspberry plantations. Treatment with bromacil, dichlobenil and chlorthiamid in late winter can control a range of grass and broad leaved species. However, the need to apply the herbicides at a time when no active weed foliage is present makes accurate cover of weed patches difficult. Promising results with several new herbicides for application against emerged perennial weeds in strawberry (Clay, 1981; Lawson & Wiseman, 1982a) stimulated interest in their possible use in raspberry. In these experiments the objective was primarily to evaluate the tolerance of the major cultivar Glen Clova to applications made after cane emergence.

METHODS AND MATERIALS

Two experiments were carried out at Invergowrie in established plantations of cv. Glen Clova, on sandy loam soils with organic matter contents of 7.6% (as determined by chromic acid oxidation). Plots consisted of single rows 5 m long and 1.85 m apart, each comprising 8 stools. The plantations were managed with no soil cultivation. Annual weeds were controlled with simazine at 2.2 kg a.i./ha applied in late winter. Suckers growing in the alleys and between the stools were removed mechanically 2-3 times between mid-April and mid-July. There were no perennial weeds at either site.

Treatments were arranged in randomised blocks, with four replications in Expt. I and three in Expt. II; an extra set of untreated plots was included in the latter. Experimental herbicides were applied in medium volume by Oxford Precision Sprayer, using fan jets, to 30 cm bands on either side of the centre of the row, when young canes were 12-15 cm tall. All raspberry foliage below 45 cm, whether on laterals or young canes was thoroughly wetted. Treatments were applied on 18 May, 1979 in Expt. I and on 1 May, 1980 in Expt. II at the rate suggested by

manufacturers for control of perennial weeds and at three times that dosage. Oxadiazon was included in Expt. I, but was replaced by fluazifop butyl in Expt. II. Alloxydim sodium and 3,6 dichloropicolinic acid were applied in both experiments. Records were taken of both fruit and cane production during the year of treatment.

RESULTS

Expt. I

Treatments were applied in warm, sunny conditions. No rain fell in the next 48 h but 35 mm were recorded during the following 6 days. Oxadiazon produced 80% desiccation of all treated foliage within 3 days at both rates of application. Replacement canes emerged erratically and their growth was retarded, particularly at the treble rate (Table 1). There was no evidence of translocation into the fruiting zone of fruit canes. Yield on these plots was significantly higher than on untreated plots, due mainly to the production of more berries per cane. End-of-season records showed that adverse effects on young cane production had persisted, with numbers of canes reaching tipping height (155 cm) being very considerably reduced in comparison with those on untreated plots. Alloxydim sodium had no visible effect on treated foliage or on early growth of young canes. Fruit yield was also unaffected, but in late November there were 30% fewer canes at tipping height on plots treated at the treble rate than on the untreated standard. 3,6 dichloropicolinic acid caused "hormone-type" leaf rolling and stem twisting of young canes and sprayed laterals, particularly at the higher dose, but these effects were outgrown and symptoms could no longer be detected by fruit harvest. There was no evidence of translocation into or up fruiting canes and fruit production was not significantly affected. Numbers of canes reaching tipping height at the end of the growing season were 40% fewer than on untreated plots with both rates of application.

Expt. II

This was an earlier growing season and treatments were applied in warm sunny conditions. No rain fell for 10 days. Fluazifop butyl and alloxydim sodium produced no visible symptoms of treatment and the latter had no effect on cane or fruit production, even at the treble rate. There was some evidence, however, that fluazifop butyl affected cane heights, and significantly fewer canes reaching tipping height were recorded at the end of the growing season on plots treated at the treble rate. The performance of 3,6 dichloropicolinic acid in this experiment was similar to that described for Expt. I and the effect on numbers of tall canes at the end of the growing season was almost as severe, particularly at the treble rate. Growth of young canes was recorded during 1981, with no further experimental treatments being applied. There was no evidence of delayed emergence, malformed foliage or retarded growth on any treated as compared with untreated plots.

DISCUSSION

Oxadiazon desiccated all young canes and delayed and stunted the growth of replacement canes. The former effect makes its overall use in spring as a selective herbicide unacceptable. The latter effect rules out its use as a potential replacement for dinoseb-in-oil for cane vigour control, despite the increased fruit yield resulting from the reduction in competition between fruiting and vegetative phases (Lawson & Wiseman, 1976). Since control of Convolvulus arvensis requires spring treatment (May & Baker Ltd. - personal communication), this herbicide may be suitable only for very limited spot-treatment in raspberry.

3,6 dichloropicolinic acid had no desiccant effect and the visible effects on young canes were rapidly outgrown. Nevertheless it did reduce the amount of

Table 1

Expt. I Fruit and cane records 1979

Herbicide	Dose kg a.i./ha	Yield t/ha	Berries /cane	Mean wt(g)/ 100 berries	Mean ht(cm) young canes (11 July)	Canes/stool (27 Nov.) Total no.	Canes/stool (27 Nov.) No. >155 cm
Untreated		14.9	40.8	393	143	21.8	12.5
Oxadiazon	2.00	21.1***	60.8***	417	86***	13.3**	6.8***
	6.00	21.8***	63.0***	418	32***	9.3***	3.5***
Alloxydim sodium	1.50	14.7	41.8	382	137	22.6	12.5
	4.50	15.1	44.9	370	133	19.5	8.7*
3,6 dichloropicolinic acid	0.20	16.5	47.0	380	141	16.2*	7.5***
	0.60	15.7	43.9	382	127	17.0	7.2***
S.E. mean ±		1.07	3.71	11.9	6.7	1.85	1.05

Table 2

Expt. II Fruit and cane records 1980

Herbicide	Dose kg a.i./ha	Yield t/ha	Berries /cane	Mean wt(g)/ 100 berries	Mean ht(cm) young canes (9 July)	Canes/stool (4 Dec.) Total no.	Canes/stool (4 Dec.) No. >155 cm
Untreated		10.2	78.9	339	119	16.5	8.9
S.E. mean ±		0.46	4.47	11.0	3.5	0.70	0.57
Fluazifop butyl	1.00	10.5	86.0	325	103*	16.8	8.7
	3.00	10.3	77.3	346	126	16.0	6.5*
Alloxydim sodium	1.50	10.0	76.4	343	129	16.4	8.2
	4.50	11.7	87.1	348	127	16.4	8.2
3,6 dichloropicolinic acid	0.20	10.9	80.2	357	127	14.8	7.2
	0.60	9.7	70.9	346	99*	17.9	5.6**
S.E. mean ±		0.65	6.31	15.6	4.9	0.99	0.80

*, **, *** - Significantly different from Untreated at the 5%, 1% or 0.1% level.

good quality fruiting cane available for the following year, particularly at the treble rate. There was, however, no carry-over of injury into the next season's young canes. The risk of incurring severe adverse effects with this herbicide on raspberry is probably no greater than that recorded with strawberry (Bailey & Clay, 1980; Lawson & Wiseman, 1982a). Control of dense infestations of Cirsium arvense in both crops may justify the risk of overall spring treatment commercially, but spot treatment would obviously be preferable. Autumn application of 3,6 dichloropicolinic acid in raspberry is less feasible than in strawberry, because of obstruction by sprawling canes after harvest. Further work is needed to evaluate its safety on less vigorous raspberry cultivars and to monitor the effects on the crop from overall treatment in two successive springs.

Both alloxydim sodium and fluazifop butyl appear to have adequate safety margins for spring use as overall treatments for the control of perennial grasses. There was an indication in 1980 that there may have been slightly less crop tolerance to spring treatment with fluazifop butyl. This confirms findings on several other crops (Lawson & Wiseman, 1982a,b), but is unlikely to be of commercial significance in raspberry. Tolerance by other cultivars should be tested.

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A COMPARISON OF RESIDUAL HERBICIDES ON NEWLY PLANTED AND ESTABLISHED STRAWBERRIES IN IRELAND

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Summary. Several residual herbicides were compared for the control of germinating weeds in newly planted and established strawberries in seven different trials. Metamitron gave consistently good weed control without causing damage to strawberry plants. Ethofumesate is useful for the control of some difficult weeds but caused damage in two trials. Trietazine + simazine mixture caused economic damage in these trials, whilst oxadiazon applied to newly planted strawberries in winter caused severe damage. Pendimethalin and napropamide also gave promising results. Several herbicides were applied as tank mixes without encountering any problem. Strawberries, metamitron, ethofumesate, oxadiazon, pendimethalin, napropamide, trietazine + simazine.

INTRODUCTION

Although simazine gives excellent control of most annual weeds a number of important weeds are resistant and it cannot be used safely on newly planted strawberries without protecting them with a charcoal dip. Lenacil is very safe on newly planted strawberries but does not give prolonged or adequate control of several important weeds. The necessity to apply it to moist soil is sometimes a problem when dry weather follows spring planting. Thus, there is a need for residual herbicides less affected by soil conditions than simazine and lenacil.

Previous trials at Clonroche failed to find more satisfactory herbicides for Irish conditions (Rath and O'Callaghan, 1976). A number of new products have become available and promising results have been obtained in Great Britain with ethofumesate, pendimethalin and propachlor (Clay, 1978; Lawson and Wiseman 1978). Clay (1980) showed that oxadiazon could be safely applied to established strawberries during the dormant season. Metamitron and napropamide have not been widely tested on strawberries in the British Isles. This series of trials was laid down to test the tolerance of strawberries to these herbicides and their weed control under Irish conditions.

METHODS AND MATERIALS

All the trials were carried out at the Soft Fruit Research Station at Clonroche. The soil is classified as a loam to clay loam and contained in the 0-15 cm depth 18% coarse sand, 11% fine sand, 41% silt and 30% clay. Only the standard cultivars Cambridge Vigour and Cambridge Favourite were used in these trials. The strawberries were always planted on ridges which were 0.25 m high and were 0.87 m apart. Cambridge Vigour plants were spaced at 0.45 m, while Cambridge Favourite plants were at 0.4 m. The plots comprised of two adjacent ridges, each carrying 25 plants. Cambridge Vigour were maintained as spaced plants, while matted rows were established on the Cambridge Favourite by the autumn following planting. Sprays were applied at 1000 l/ha with an Azopropane sprayer which had a 1.75 m boom fitted with seven 1.2 Birchmeier Helico Sapphire

nozzles operated at 500 kPa.

Experiment 1. Cambridge Favourite were planted on 13 April, 1977. The treatments listed in Table 1 were applied on 3 May, 1977 and were reapplied on 5 May, 1978, on 5 April, 1979 and on 26 February, 1980. The second application of phenmedipham was respectively 2, 2, 5 and 6 days later. All plots were cleaned up by hand and treated simazine during August 1977, October 1978 and August 1979.

Table 1

Effect of ethofumesate, trietazine + simazine and phenmedipham on crop yield of Cambridge Favourite

Treatment	Dose kg/ha	Crop yield, tonnes/ha 1978	1979	1980
Untreated		7.4	11.5	7.3
Ethofumesate	2.0	7.3	12.0	9.3
Ethofumesate + phenmedipham	2.0 + 1.1	16.0	16.9	11.6
(Trietazine + simazine)*	1.0	10.3	16.9	11.1
(Trietazine + simazine) + phenmedipham	1.0 + 1.1	14.6	16.3	10.6
Phenmedipham + Actipron 5.6 l/ha	1.1	16.2	15.8	10.3
Phenmedipham + repeat application 2-6 days later	0.5	16.6	15.8	10.8
F test		***	***	NS
S.E. (df = 18)		0.38	0.57	1.10

*Applied as the product Remtal.

Experiment 2. Cambridge Vigour were planted on 26 April, 1978. The treatments listed in Table 2 were applied on 8 June and 29 August, 1978, on 3 April and 11 September, 1979 and on 8 April, 1980. In 1980 the "ethofumesate alone" treatment was omitted to observe the carry-over effect of damage caused by the application in the previous September.

Table 2

Effect of residual herbicides with phenmedipham on crop yield of Cambridge Vigour

Treatment	Dose kg/ha	Crop yield, tonnes/ha 1979	1980
Lenacil + phenmedipham	2.0 + 1.1	15.7	20.5
Ethofumesate	2.0	10.2	4.2
Ethofumesate + phenmedipham	2.0 + 1.1	11.0	3.7
Metamitron	5.3	16.0	18.8
Metamitron + phenmedipham	5.3 + 1.1	15.0	18.4
Ethofumesate + metamitron + phenmedipham	1.0 + 2.6 + 1.1	13.5	6.4
F test		**	***
S.E. (df = 15)		0.63	0.69

Experiment 3. Cambridge Favourite were planted on 26 April, 1978. The plantation was maintained weed-free until the following September with routine applications of lenacil. The treatments listed in Table 3 were applied on

7 September, 1978 and reapplied to the same plots on 14 March and 1 October 1979 and on 8 April, 1980.

Table 3

Effect of residual herbicides with phenmedipham on crop yield of Cambridge Favourite

Treatment	Dose kg/ha	Crop yield tonnes/ha 1979	1980
Lenacil	2.0 + 1.1	12.6	14.4
Ethofumesate	2.0	11.0	11.3
Ethofumesate + phenmedipham	2.0 + 1.1	11.3	10.4
Metamitron	5.3	11.8	13.6
Metamitron + phenmedipham	5.3 + 1.1	11.9	12.4
Ethofumesate + metamitron + phenmedipham	1.0 + 2.6 + 1.1	10.2	11.5
F test		NS	**
S.E. (df = 15)		0.69	0.55

Experiment 4. Cambridge Favourite were planted on 4 December, 1978. The treatments listed in Table 4 were applied on 10 April, 1979. The trial area was cleaned up by hand and treated with simazine in August 1979. The treatments were reapplied to the same plots on 13 April and 18 December, 1980.

Table 4

Effect of residual herbicides on Cambridge Favourite

Treatment	Dose kg/ha	Crop yield, tonnes/ha 1980	1981
Lenacil + phenmedipham	1.8 + 1.1	21.5	16.6
Lenacil + phenmedipham + handweeding	1.8 + 1.1	23.8	18.3
Propachlor	4.6	20.1	16.2
Propachlor + phenmedipham	4.6 + 1.1	22.3	17.4
Metamitron	5.3	20.6	19.4
Metamitron + phenmedipham	5.3 + 1.1	20.1	15.7
Ethofumesate	2.0	15.1	17.5
Ethofumesate + phenmedipham	2.0 + 1.1	13.4	17.5
F test		***	NS
S.E. (df = 21)		1.39	1.01

Experiment 5. Cambridge Favourite were planted on 11 April, 1979. The treatments listed in Table 5 were applied on 3 May, 1979. The trial area was cleaned up by hand and treated with simazine in August 1979. The treatments were again applied to the same plots on 3 April and 19 December, 1980.

Table 5

Effect of residual herbicides on Cambridge Vigour

Treatment	Dose kg/ha	Crop yield, tonnes/ha 1980	1981
Lenacil + phenmedipham		23.1	17.1
(Trietazine + simazine)* + phenmedipham		21.4	16.8
Propachlor		22.1	13.8
Propachlor + phenmedipham		25.5	17.2
Metamitron		22.5	17.6
Metamitron + phenmedipham		21.3	16.9
Ethofumesate		22.3	16.2
Ethofumesate + phenmedipham		21.5	17.0
F test		*	NS
S.E. (df = 21)		0.75	1.09

*Applied as the product Remtal.

Experiments 6 and 7. Cambridge Favourite (experiment 6) and Cambridge Vigour (experiment 7) were planted on 12 November, 1981. The treatments listed in Table 6 were applied on 3 December, 1981 and 5 February, 1982. The metamitron treatment was omitted from experiment 7. The trial areas received an overall application of phenmedipham at 1.1 kg/ha plus lenacil at 1.8 kg/ha in early May to control the seedling weeds which were then emerging on all plots.

Table 6

Comparison of herbicides on newly planted strawberries

Treatment	Time of application	Dose kg/ha	No. crowns/plant August 1982 C. Favourite	C. Vigour
Lenacil	December	1.8	5.0	6.0
Pendimethalin	December	2.0	4.5	5.8
Pendimethalin + lenacil	December	2.0 + 0.9	4.8	5.3
Oxadiazon	February	2.0	4.2	5.8
Oxadiazon + lenacil	February	2.0 + 1.8	4.5	5.7
Napropamide	December	4.5	4.8	6.0
Napropamide + lenacil	December	4.5 + 0.9	4.5	5.7
Metamitron	December	5.3	4.7	-
F test			NS	NS
S.E. (df = 21)			0.25	-
S.E. (df = 18)			-	0.32

RESULTS

Experiment 1. When the plots were sprayed in April 1977, seedling weeds were emerging on all plots. All treatments involving the use of phenmedipham gave excellent control of these weeds. During summer 1977 the alleys of the plots treated with phenmedipham alone and phenmedipham plus Actipron became lightly

infested Poa annua and Lolium perenne. The plots treated with ethofumesate plus phenmedipham and with trietazine + simazine plus phenmedipham remained almost completely free of weeds until August. The plots which received no application of phenmedipham became completely covered with broadleaved annual weeds, principally Viola arvensis, and when the trial area was cleaned up in August the strawberry plants on these plots were severely stunted. V. arvensis was again the dominant weed in 1978. Applications of phenmedipham in spring 1978 and 1979 also gave good control of seedling broadleaved weeds. Ethofumesate alone gave very poor weed control in both 1978 and 1979. Trietazine + simazine gave moderate control of V. arvensis 1978 and 1979. During 1980 all herbicides initially controlled broadleaved weeds, although subsequently these weeds developed on plots not treated with residual herbicide. These plots were also badly infested with P. annua during 1980. The untreated control plots were badly infested with P. annua and V. arvensis in 1977, 1978, 1979 and 1980.

The herbicide treatments did not appear to cause any direct herbicide damage to the strawberry plants at any stage. Crop yield was significantly affected by treatment in all three cropping seasons and appeared to be directly related to the degree of weed control attained.

Experiment 2. All treatments gave good control of the weeds present during 1978. During late autumn 1978 some P. annua established on the ethofumesate - treated plots. The treatments had no obvious effect on the growth of the strawberry plants in 1978. Weed control during 1979 and 1980 was again good although some Senecio vulgaris plants established on plots treated with ethofumesate alone in both years.

During late April 1979 severe crinkling of strawberry foliage appeared on all plots treated with ethofumesate. The plants became severely stunted in May, but during summer 1979 the plants recovered. Similar damage appeared on the same plots during March 1980. The stunting became very severe during April and May, with little difference between September and September plus April treatments. During May 1979 the foliage of the metamitron treated plots were slightly paler than the lenacil treated plots. All ethofumesate treatments caused large and significant reductions in crop yield in 1979 and particularly in 1980.

Experiment 3. All herbicide treatments gave good weed control for the duration of the trial. The treatments had no obvious effect on the growth of the strawberry plants in 1978 or 1979. During April and May 1980 the ethofumesate treated plots were all slightly stunted. While crop yield in 1979 was unaffected by herbicide treatment, it was reduced in 1980 by all treatments containing ethofumesate (Table 3).

Experiment 4. All treatments gave good weed control during spring 1979. In the summer of 1979, the propachlor treated plots became badly infested with V. arvensis. This also became established on the lenacil treated plots. Metamitron and ethofumesate treatments gave good weed control throughout the summer of 1979. During 1980 the propachlor treated plots again became infested with broadleaved weeds, particularly V. arvensis. Although the other herbicide treatments gave good weed control, some S. vulgaris became established on the ethofumesate treated plots. No herbicide treatment maintained good weed control throughout the summer of 1981 and at fruit picking all plots were lightly infested with V. arvensis and S. vulgaris. Propachlor was again the least effective of the herbicides in 1981.

The herbicide treatments had no obvious effect on plant growth in 1979. During April and May of 1980 the strawberry plants on the ethofumesate plots were slightly stunted and crop yield in 1980 from those plots was also reduced (Table 4). No treatment had any effect on strawberry growth or cropping in 1981.

Experiment 5. All herbicide treatments gave good control of seedling broadleaved weeds in summer 1979, although in the beginning August 1979 the propachlor and the lenacil treated plots were lightly infested with V. arvensis and P. annua.

Propachlor was again the least effective of the residual herbicides in 1980 and 1981 and when the trial was harvested in 1981 these plots were severely infested with grass weeds.

During early June 1979, slight marginal leaf scorch occurred on the strawberry plants treated with metamitron and with trietazine + simazine. These made a complete recovery from mid-June onwards. In June 1979 stunting of the strawberry plants and crinkling of the foliage also occurred on the ethofumesate treated plots. These plants recovered from July onwards. No symptoms of herbicide damage occurred during 1980 and 1981. The treatments had no effect on crop yield in 1980. The plots treated with propachlor alone yielded less heavily than the other plots in 1981.

Experiment 6 and 7. All treatments gave good weed control until early May. The principal weeds present, volunteer barley and blackcurrant seedlings, were best controlled with the oxadiazon and napropamide treatments. The oxadiazon treatments and pendimethalin at 2 kg per ha also controlled the few Galuim aparine plants which occurred.

During late February severe scorching of the strawberry foliage of both cultivars occurred on all plots treated with oxadiazon. Although this damage became very severe during March no plants were killed. The plants recovered well from April onwards and plant measurements taken in early August did not indicate that any permanent damage had occurred (Table 6). No symptoms of herbicide damage occurred on the plots receiving other herbicide treatments.

DISCUSSION

Metamitron gave excellent results in this series of trials. No damage to the strawberry plants occurred under a wide range of conditions. However, in a more recent trial, metamitron has caused scorching of strawberry foliage at Clonroche when applied during very warm weather in late May 1982. This herbicide is now a very well established for use on sugar beet and its possible usefulness on strawberries needs to be more widely investigated. The low dose, high pressure application technique might make this a safer and more effective herbicide for use on newly planted strawberries during the critical April - May period.

Variable results were obtained with ethofumesate. In experiment 2 an April application in 1979 caused severe damage to Cambridge Vigour while autumn applications to the same plots caused severe damage in 1980. Although less severe than on Cambridge Vigour, spring applications of ethofumesate caused damage to Cambridge Favourite in 1980 in experiments 3 and 4. These results are in agreement with the manufacturer's recommendation of applying ethofumesate to Cambridge Favourite only during the autumn - early winter period.

Propachlor was safe when used on strawberries at Clonroche. The comparatively poor performance was probably due to its relatively short residual life. A likely use on strawberry crops is to complement the range of weeds controlled by other herbicides.

Oxadiazon caused unacceptable damage to newly planted strawberries in 1982. Weather conditions caused the oxadiazon to be applied later than intended and the February application resulted in damage similar to that reported by Clay (1980).

Although pendimethalin was only tested in two trials, results were very promising. This is in agreement with results reported from Great Britain by Clay (1978), Lawson (1978) and Davison and Bailey (1980). Pendimethalin should be a very useful herbicide for strawberries as it controls many of the weeds resistant to simazine and lenacil.

Napropamide also gave very encouraging results but also needs to be tested under a much wider range of conditions.

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THE RESPONSE OF FIELD HORSETAIL (EQUISETUM ARVENSE) TO PROPYZAMIDE AND ASULAM

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Summary. Propyzamide at doses up to 6.8 kg/ha was applied to Equisetum arvense in the winter. In experiments at three sites 1.7 and 3.4 kg/ha suppressed the development of vegetative shoots until early in the following July. But in a subsequent experiment regrowth developed sooner and by early July there was no difference between the untreated area and propyzamide even at 6.8 kg/ha. Chlorthiamid at 6 kg/ha and dichlobenil at 5.4 kg/ha gave season-long control.

Asulam at 1.8 kg/ha applied to E. arvense in June or July failed to kill the treated shoots and to prevent regrowth the following season. Adding an adjuvant oil gave some reduction of regrowth. Aminotriazole at 4.5 kg/ha alone, mixed with 2,4-D or following asulam gave good initial control and prevented regrowth in the year after treatment. Dichlobenil, chlorthiamid, aminotriazole, 2,4-D, adjuvant oil.

INTRODUCTION

Observations by the authors and reports by advisers (Jones, 1982) indicate that field horsetail (Equisetum arvense) is becoming more troublesome in fruit, especially strawberries, and nursery stock in England. It is also reported to be widespread in horticultural establishments in west and central Scotland (Marshall, 1980a).

Control measures are available for certain crops (Fryer and Makepeace, 1978). The sprayed shoots can be killed with foliage-applied herbicides. With some, including MCPA and 2,4-D, there is considerable regrowth in the year after treatment, but with aminotriazole there is much less regrowth (Hoyt and Carder, 1962). The soil-applied benzonitrile herbicides, chlorthiamid and dichlobenil, prevent shoot development of E. arvense for a year or more (Williams, 1973; Ryan, 1976).

Alternative control measures are needed for situations in which the current recommendations cannot be used or are too expensive. This report presents the results from experiments with propyzamide, which is claimed to give some control (PBI, 1982) but is not widely used by growers, and asulam which is reported to be highly active against E. arvense (Holly, 1969).

METHODS AND MATERIALS

Commercial formulations of propyzamide (50% w.p.) and asulam (40% a.c.) were applied to E. arvense in otherwise weed-free perennial crops or fallows that had not been cultivated for at least one year. Table 1 gives application dates, soil type, plot size and replication details for the four sites. Herbicide treatments are given with the results in Tables 2-5. Chlorthiamid and dichlobenil granules which were applied in early April as standard herbicides in two of the 1980/81 experiments contained 7.5 and 6.75% a.i. respectively. The adjuvant oil used with asulam was a 97% emulsifiable paraffinic oil (BP 'Actipron'). Aminotriazole (20% a.c. plus ammonium thiocyanate) and 2,4-D amine (32% a.c.) were also included.

Table 1

Site and application details

Location	Soil type*	Treatment dates	Plot size (m)	Replicates
Propyzamide 1980/81				
Appleton	VFSL	15 Dec. 1980	5 x 2	4
Chearsley	SCL	3 Feb. 1981	5 x 2	3
Long Compton	ZCL	10 Dec. 1980	5 x 1.8	3
Propyzamide 1981/2				
Appleton	VFSL	13 Nov. 1981 25 Jan. 1982	4 x 3	2
Asulam 1981				
Long Compton	ZCL	18 June 1981 10 July 1981	4 x 2	2
Knaphill	LS	8 May 1981) 22 June 1981) 26 Aug. 1981) 12 Oct. 1981)	4 x 4	2-3

*V very, F fine, S sandy, C clay, L loam, Z silty, LS loamy sand

Sprays were applied with a carbon dioxide pressurized knapsack sprayer fitted with Spraying Systems 6502 or 8002 fan jets and operated at 200 kPa. The volume was 330 and 660 l/ha for 1.7 and 3.4 kg/ha of propyzamide respectively in the 1980/81 experiments. All other sprays were applied at 200 l/ha. The chlorthiamid and dichlobenil granules were applied with a hand shaker.

The amount of E. arvense at the time of treatment was expressed as percentage ground cover of dead shoots (propyzamide experiments) or living cover (asulam experiments). Growth in the season after treatment was assessed at intervals and expressed as either shoot numbers per m^2 or percentage ground cover.

RESULTS

Propyzamide. In the three 1980/81 experiments there were similar numbers of spore bearing shoots on the treated and untreated areas, but nearly all those on the treated areas were stunted and dead or dying at the May assessment. At that time there were 20 to 30 vegetative shoots per m^2 on the untreated areas but not more than 1.2 per m^2 with propyzamide at 1.7 kg/ha or 0.3 per m^2 with propyzamide at 3.4 kg/ha or the benzonitriles.

By mid-June there were large differences in the percentage of untreated ground covered by vegetative shoots at the three sites. However, the results in Table 2 show that all the treatments reduced significantly ($P = 0.05$) the amount of E. arvense. The reductions ranged from 73 to 98 percent with propyzamide and 96 to 100 percent with the benzonitriles. These levels of control were maintained until early July. Thereafter there was an appreciable amount of regrowth on the propyzamide treated areas and by late August they were almost indistinguishable from the untreated areas. However, there was still only one percent ground cover with the benzonitriles.

Table 2

Percentage ground covered with Equisetum arvense at three sites in mid-June, 1981
Winter 1980/81 applications

		Herbicide (kg/ha a.i.)				
		Propyzamide		Chlorthiamid	Dichlobenil	SE
Location	Untreated	1.7	3.4	6.0	5.4	±
Appleton	53.3	2.7	1.0	0.0	-	4.46
Chearsley	18.3	5.0	2.3	-	0.7	1.18
Long Compton	91.7	12.3	2.0	-	-	2.64

Table 3 shows that in 1982 all the propyzamide treatments at Appleton reduced significantly (P = 0.05) the ground covered by vegetative shoots in late May. By early June there was a considerable amount of regrowth even though on all but the 0.85 kg/ha doses there was significantly less cover than on the untreated areas. By early July the only treatment that differed significantly from the untreated areas was the propyzamide at 3.4 kg/ha applied in November. There were no differences due to the timing or splitting of the applications.

Table 3

Effect of dose and timing of propyzamide on the percentage ground cover of Equisetum arvense at Appleton

Assessed	applied		total dose (kg/ha a.i.)				SE
(1982)	(winter 81/82)	Untreated	0.85	1.7	3.4	6.8	±
21 May	early		10.0	3.0	1.0	-	
	late	32.5	3.7	4.7	1.7	-	2.40
	early + late		-	3.0	1.0	1.0	
7 June	early		36.7	18.3	10.7	-	
	late	50.0	35.0	27.3	15.0	-	6.68
	early + late		-	25.0	21.7	13.3	
2 July	early		83.3	65.0	36.7	-	
	late	73.3	76.7	80.0	68.3	-	8.44
	early + late		-	70.0	71.7	66.7	

Asulam. At Long Compton the areas that received asulam alone, or asulam with the adjuvant oil were indistinguishable from the untreated areas in the year of treatment. In contrast, there was complete or almost complete kill of shoots with the aminotriazole and 2,4-D treatments. The results in Table 4 show that in early July of the following year all treatments except asulam and 2,4-D alone had reduced significantly (P = 0.05) the percentage of ground covered with E. arvense. Although not significant statistically there was least growth with treatments containing aminotriazole.

Asulam also failed to kill treated shoots at Knaphill or to reduce ground cover in the year after treatment. Aminotriazole gave almost complete kill in the year of treatment and the results in Table 5 show that, with the exception of the May application, there was very little regrowth in the year after treatment regardless of whether it was applied alone, mixed with asulam or applied two months after asulam.

Table 4

Percentage ground cover of Equisetum arvense in July 1982, a year after application of foliage-applied herbicides at Long Compton

Herbicide	Dose (kg/ha a.i.)	application date 10 June	18 July
asulam	1.8	43.2	33.2
asulam + adjuvant oil	1.8 + 5 l/ha	13.2	18.2
aminotriazole	4.5	5.0	2.5
2,4-D amine	2.0	-	27.5
aminotriazole + 2,4-D amine	4.5 + 2.0	10.0	6.0
untreated		45.0	
SE ±		4.95	

Table 5

Percentage ground cover of Equisetum arvense in late June 1982, a year after treatment with asulam and aminotriazole at Knaphill

		aminotriazole (4.5 kg/ha a.i.)				
		nil	8 May	22 June	26 Aug	12 Oct
asulam (1.8 kg/ha a.i.)	nil	90	50	< 1	0	1
	22 June	63	-	< 1	0	-
	26 Aug	60	-	-	1	1
	12 Oct	90	-	-	-	6

DISCUSSION

The results presented confirm the effectiveness of chlorthiamid, dichlobenil and aminotriazole in reducing the shoot growth of E. arvense in the season after treatment.

The period of control achieved with propyzamide in the 1980/81 experiments would ensure the easy harvesting of early maturing fruit such as strawberries and gooseberries in most seasons. The more rapid recovery of the E. arvense in the 1981/2 experiment at Appleton would be unacceptable to most growers. Therefore, until the difference in control between the two years results can be explained and predicted, it is unlikely that propyzamide will be used specifically for the control of E. arvense.

The failure of asulam to give an adequate reduction in shoot growth in the year after treatment reflects the limited reduction of rhizomes reported by Marshall (1980b). Greater activity may be attainable by increasing the dose, adding an adjuvant oil or additional wetters, or applying to wet foliage as reported by Coupland and Peabody (1981). However, they also obtained comparable enhancement with aminotriazole or glyphosate, both of which Marshall (1980b) found to be more effective than asulam in killing the rhizomes of E. arvense.

From the results presented neither propyzamide nor asulam appear to have any practical advantages over chlorthiamid, dichlobenil or aminotriazole in situations where these herbicides can be used. More favourable results may result from a better understanding of those conditions necessary for maximum activity.

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THE EFFECT OF MECOPROP ON SHOOT AND ROOT GROWTH AND MINERAL NUTRITION OF YOUNG APPLE TREES

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Summary. Applications of mecoprop at 6.7 kg ha^{-1} reduced the shoot growth of Cox/MM.106 apple trees grown under field conditions. This effect was, however, smaller than that due to competition from a grass sward. With grass competition, where shoot growth was severely reduced, mecoprop had little additional effect although it reduced root growth and leaf nitrogen. Apple, mecoprop, herbicide management, grass, growth, roots, nitrogen, manganese, potassium.

INTRODUCTION

Studies involving the use of herbicides in fruit plantations have shown that eliminating competition from weeds, even with mature trees, can in some years increase yield by up to 49% and economic returns by up to 55% (Atkinson and White, 1981). Similarly by removing inter-row grass and converting from herbicide strip to overall-herbicide management yield can be increased by up to 32% (Atkinson and White, 1976). The response to soil management with herbicides can therefore be substantial. However, herbicide use may damage fruit trees without visible symptoms. Tottman and Davies (1978) found that applications of mecoprop at commercial rates either to the soil or as a foliar spray, deformed the roots of spring wheat. Root deformation was sometimes associated with reduced root or shoot weight. Gurung (1979), found in a trial where mecoprop had been applied around apple trees annually for 14 years, that root growth, compared with untreated trees, was absent from 0-5 cm depth and reduced by 43% at 5-10 cm depth. As both sets of trees were under overall grass differential weed control effects were not a factor.

These results suggest that in the absence of visible damage or leaf symptoms herbicides can have adverse effects and so a field trial was initiated to investigate the effect of mecoprop, applied annually, on the growth and performance of newly-planted apple trees grown either under grass or bare soil management.

METHODS AND MATERIALS

Trees of Cox/MM.106 were planted in April 1978 at a spacing of 2 x 2 m in a randomized block design with 12 replicates. In each block trees received the following treatments:-

1) Grass + mecoprop. S50 timothy, Phleum nodosum was sown in May 1978 and mowed so as to maintain a short turf. Mecoprop at 6.7 kg a.e. ha^{-1} was applied as a uniform spray in July 1978 and again in June 1979 and 1980.

2) Grass. Grass was established in 1) but no mecoprop was applied.

3) Overall bare + mecoprop. Paraquat was applied in June 1978 to maintain the soil weed-free and then mecoprop in July 1978 and subsequently as in 1). Basic weed control in 1979-1982 was with simazine at 2.0 kg ha^{-1} and paraquat as needed.

4) Overall bare. Trees were treated as for 3) but no mecoprop was applied.

The soil on the site used was a fine sandy loam of the Malling series (Furneaux, 1954).

Shoot growth was measured directly on the trees at annual intervals. Root growth was assessed as the length of white root present at 0-50 cm depth as seen using an endoscope (P.W. Allen and Co., London) which allowed the inside of 50 cm long x 8 cm diameter circular glass observation tubes inserted 30 cm to the south of all trees in four of the experimental blocks (Gurung, 1979) to be viewed.

Leaf mineral nutrient levels were determined on a sample of 20 leaves, without petioles from mid-way along the current year's extension shoots in August. Analyses were carried out by a service laboratory using standard methods.

RESULTS

In the year of planting, the mean length of individual shoots was reduced by mecoprop under bare soil but not significantly under grass. The reduction due to mecoprop, 31%, was smaller than that due to grass competition, 69%, (Table 1). Under grass, but not bare soil, mecoprop reduced root growth.

Table 1

The effect of mecoprop on shoot length (cm) and length of white root on 1st September (cm) for newly-planted trees

	Grass		Bare soil		
	+ mecoprop	- mecoprop	+ mecoprop	- mecoprop	LSD 5%
Shoot length	4.3	5.0	11.2	16.3	3.9
Root growth	1.0	19.0	12.9	4.5	18.0

During 1979-81 shoot growth continued to be reduced under bare soil but only to a small extent under grass. As in the year of planting, the reduction due to mecoprop (3.5 - 17.2% bare soil, 0 - 5.1% grass) was much less than that due to grass competition (48.2 - 71.3%) (Table 2). In 1979 and 1981 effects on total growth under bare soil conditions were mainly or totally due to an effect on shoot numbers rather than mean shoot length. In 1979 mean shoot length was increased in the presence of mecoprop, due to the large effect on numbers. In 1980 when numbers were similar mecoprop significantly ($P = 0.01$) decreased mean shoot length, from 49.7 to 43.1 cm overall.

Despite the effects upon growth mecoprop had no major effects on mineral nutrition. Grass competition reduced leaf nitrogen in 1979 but subsequently only to a smaller extent (Table 3). Under grass mecoprop reduced leaf nitrogen in all years but had little effect under bare soil conditions. Although leaf phosphorus was significantly, $P \geq 0.001$, increased under grass in all years, from 0.26 to 0.44% on average, mecoprop had no additional effect. Neither grass nor mecoprop consistently affected leaf calcium. Under both soil management treatments mecoprop slightly increased leaf potassium levels while leaf manganese was either reduced or unaffected by mecoprop.

Table 2

The effect of mecoprop on a total shoot length (m tree^{-1}) and shoot number

Treatment	Mecoprop	1979		1980		1981	
		Total length	Number	Length	Number	Length	Number
Grass	+	1.9	8.8	8.1	17.9	20.6	42.5
	-	1.6	8.3	8.6	17.5	20.9	43.3
Bare soil	+	5.5	10.8	16.9	40.9	36.6	87.3
	-	5.7	14.8	20.4	41.0	40.3	100.2
LSD 5%		0.9	2.8	3.6	7.5	8.9	20.3

Length: Effect of grass significant at P>0.01 all years. Number: Effect of grass significant at P>0.001 all years and grass/mecoprop interaction in 1979.

Table 3

The effect of mecoprop on leaf nitrogen, potassium (% DW) and manganese (ppm) concentrations

		Grass		Bare soil		LSD
		+ mecoprop	- mecoprop	+ mecoprop	- mecoprop	5%
Nitrogen	1979	1.92	2.14	3.19	3.22	0.15
	1980	2.84	2.92	3.10	3.00	0.09
	1981	2.46	2.55	2.65	2.60	0.25
Potassium	1979	2.79	2.56	1.97	1.89	0.16
	1980	2.34	2.30	2.32	2.2[illegible]	0.15
	1981	2.31	2.25	2.19	2.15	0.16
Manganese	1979	65	67	75	82	11
	1980	74	72	69	76	8
	1981	61	60	65	64	9

Nitrogen: Effect of grass significant at P>0.001 1979, 1980, mecoprop P>0.05 1979. Interactions P>0.01 1980. Potassium: Effect of grass significant at P>0.001 1979; mecoprop P>0.05. Manganese: grass significant P>0.01 1979.

DISCUSSION

The results presented here generally confirm those previously reported for pot experiments with cereals (Tottman, 1978). There, applications of 10 kg a.e. ha^{-1} to plants in sand caused severe root abnormalities and reduced shoot growth. Lower concentrations, 2.5 kg ha^{-1}, did not inhibit shoot growth. The root abnormalities produced in cereals (excessive number of lateral premordia and stunting of crown roots) were different from the root damage found in apple where mecoprop damaged the cortex and epidermis so the remaining tissues were opened to the soil fauna, as evidenced by enchytracid worms found in the stele (Gurung, 1979). Damage this severe presumably lead to the absence of roots from the soil surface

(Gurung, 1979). In the current study, in the initial year under grass, but not bare soil, mecoprop significantly reduced root growth.

The growth of apple trees in bare soil, but not under the stressful conditions induced by grass competition, was adversely affected by mecoprop. With grass, growth was greatly reduced and mecoprop produced no significant additional effect. The adverse effect of mecoprop was always much smaller than that of grass competition. Where there is severe weed competition any adverse effect of a growth regulator herbicide is likely to be inconsequential. The severity of effects of the different 'hormone type' herbicides may, however, vary (Tottman and Davies, 1979).

Effects on root growth might be expected to influence mineral nutrition. Grass competition initially reduced leaf nitrogen although in late years effects were small perhaps due to the compensating effect of greatly reduced shoot growth. Here, but not under bare soil where available nitrogen is much higher (Gurung, 1979), mecoprop produced a further small reduction in leaf nitrogen.

Manganese uptake is often increased by the reduced pH in the soil surface which occurs as a consequence of herbicide produced bare soil. In 1979 and 1980 leaf manganese levels were slightly reduced by mecoprop perhaps due to reduced root activity near the soil surface (Gurung, 1979).

Mecoprop can adversely affect the growth of field-grown apple trees although effects are smaller than those due to competition.

Care is needed in selecting the best chemical for broad-leaved weed problems.

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TOLERANCE OF STRAWBERRY TO NEW HERBICIDES FOR CONTROL OF PERENNIAL WEEDS

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Summary. Oxadiazon applied in May or August caused severe injury and yield reduction in matted rows of strawberry cv. Cambridge Favourite. Although the crop recovered virtually completely in the next growing season, spot treatment may be the only acceptable commercial method of application of this herbicide for control of Convolvulus arvensis. Alloxydim sodium and fluazifop butyl had relatively little adverse effect on the crop at either date of application, even at twice the recommended rates. Both herbicides should be sufficiently safe for overall application at rates necessary for the control of Agropyron repens. 3,6 dichloropicolinic acid was more damaging with May than with August treatments. From the crop safety aspect, the control of Cirsium arvense may be best achieved by overall application post-harvest followed by spot treatment in spring. Oxadiazon, alloxydim sodium, fluazifop butyl, 3,6 dichloropicolinic acid.

INTRODUCTION

Perennial weeds have become an increasing problem in strawberry plantations, with the advent of effective programmes of control for annual weeds and the virtual disappearance of soil cultivation. They are particularly troublesome in pick-your-own plantations, where the public is easily discouraged by unpleasant picking conditions. Several new herbicides have shown promise for the control of perennial weed species in broad-leaved crops, including strawberry. Preliminary trials on the use of 3,6 dichloropicolinic acid to control Cirsium arvense were reported at the last Conference (Bailey & Clay, 1980; Lake & Bennett, 1980). Varying degrees of crop injury were observed, but effects on thistles were sufficiently promising to merit further investigation. Reports on oxadiazon, used primarily to control Convolvulus arvensis (Clay, 1980), have suggested that although treated strawberry crops suffered initial leaf necrosis they recovered rapidly and showed no long-term adverse effects. The systemic grasskillers alloxydim sodium and fluazifop butyl have shown adequate tolerance by strawberry in screening trials (Ingram et al, 1978; Plowman, Stonebridge & Hawtree, 1980). All four herbicides were evaluated at SCRI during 1979-81 for their effects on crop growth in matted row plantations of strawberry, cv. Cambridge Favourite.

MATERIALS AND METHODS

Two experiments were carried out at Invergowrie on sandy loam soils with organic matter contents of between 6% and 7% (as determined by chromic acid oxidation). Plots consisted of single matted rows of cv. Cambridge Favourite, 45 cm wide by 6.75 m long, with 45 cm alleys between rows. Treatments were arranged in randomised blocks, with four replications in Expt. I and three in Expt. II; an extra set of untreated plots was included in the latter. There were no perennial weed species on the sites and annual weeds were controlled with lenacil applied in late winter at 2.24 kg a.i./ha, supplemented by hand weeding. Runners growing outside the matted rows were controlled with paraquat at 1.12 kg a.i./ha applied in early autumn.

Experimental herbicides were applied in medium volume by Oxford Precision Sprayer, using fan jets, to a 90 cm band centred on the middle of the matted row. Treatments were applied in May or August 1979 in Expt. I and records were taken in 1979 and 1980. In Expt. II, treatments were applied in August 1980 or in May 1981, with fruit records taken only in 1981. Oxadiazon, alloxydim sodium and 3,6 dichloropicolinic acid were applied in both experiments; fluazifop butyl was included only in Expt. II. Doses were once and twice those initially suggested by manufacturers and are shown in the tables. Agral at 0.1% by volume was added to spray solutions containing fluazifop butyl. Spring applications were made to the first flush of actively growing foliage, just before the onset of flowering. Autumn treatments were applied 2-3 weeks after the end of fruit picking.

Crop diary	Expt. I	Expt. II
Planted	13 April, 1978	30 April, 1978
Treatments applied	9 May, 1979 29 August, 1979	19 August, 1980 8 May, 1981
Fruit harvested	July 1979 & 1980	July, 1981

RESULTS

Expt. I

Spring treatments were applied in warm, damp conditions, with no rain for 24 h after application. A total of 5.5 mm fell in the next 12 h, followed by several days of fine sunny weather. Oxadiazon at both rates desiccated all treated foliage within two weeks of application. Subsequent recovery was slow although no plants were killed; there was little ground cover by foliage at fruit harvest. Alloxydim sodium had no visible effect on the crop, but 3,6 dichloropicolinic acid caused leaf-rolling and malformation of treated leaves. This became severe on plots treated at the double rate, so that the leaf canopy was very much reduced before and during fruit harvest, although leaves were not actually killed. At both rates, flowers produced reflexed petals. Fruit records (Table 1) showed large effects on all components of yield with oxadiazon;the reduced yield with 3,6 dichloropicolinic acid (double rate) resulted from fewer and smaller berries. Alloxydim sodium did not affect yield.

No rain fell within 48 h of application of the post-harvest treatments. Oxadiazon again caused virtually complete desiccation of strawberry foliage. Only occasional leaves showed typical leaf-rolling following the double rate of 3,6 dichloropicolinic acid. Neither rate of alloxydim sodium produced any visible effect on the crop.

Crop records in 1980 (Table 2) showed no significant adverse effect on fruit yield of any of the treatments applied in May 1979. Plots treated with 3,6 dichloropicolinic acid had no malformed leaves, while those treated with oxadiazon appeared to have more vigorous growth and produced bigger berries than the untreated plots. There was an indication that alloxydim sodium had increased numbers of fruit trusses at the expense of numbers of berries produced per truss. Following the August application, plots treated with oxadiazon produced normal healthy foliage in 1980, but adverse effects on yield were recorded due mainly to small berries (Table 2). Neither of the other herbicides significantly affected yield, but there were fewer berries per truss on plots treated with the double rate of 3,6 dichloropicolinic acid than on untreated plots.

Expt. II

Oxadiazon and 3,6 dichloropicolinic acid applied in autumn 1980 produced foliar effects similar to those described for Expt. I. Alloxydim sodium and fluazifop butyl had no visible effects. Rain fell within 2 h of application,

Table 1

Expt. I 1979 fruit records following treatment on 9 May, 1979

Herbicide	Dose kg a.i./ha	Yield t/ha	Mean berry wt(g)	No. fruit trusses /m^2	No. berries/ truss
Untreated		18.0	8.20	104	3.50
Oxadiazon	2.00	4.8***	6.26***	60***	2.17**
	4.00	1.5***	5.81***	40***	1.09***
Alloxydim sodium	1.50	17.2	8.38	104	3.37
	3.00	19.0	8.29	119	3.31
3,6 dichloropicolinic acid	0.20	16.8	7.90	110	3.25
	0.40	12.9**	7.04**	115	2.66*
S.E. mean ±		1.04	0.223	8.0	0.279

Table 2

Expt. I 1980 fruit records following treatment in spring or autumn 1979

Herbicide	Dose kg a.i./ha	Yield t/ha	Mean berry wt(g)	No. fruit trusses /m^2	No. berries/ truss
Untreated		24.9	9.97	120	3.50
Treated 9 May, 1979					
Oxadiazon	2.00	28.0	11.39***	119	3.43
	4.00	23.2	11.40***	105	3.27
Alloxydim sodium	1.50	22.6	10.38	133	2.79*
	3.00	29.3	10.18	156*	3.14
3,6 dichloropicolinic acid	0.20	24.3	10.28	111	3.60
	0.40	20.8	9.45	106	3.44
Treated 29 August, 1979					
Oxadiazon	2.00	20.7	9.09*	125	3.02
	4.00	18.7*	8.33***	110	3.62
Alloxydim sodium	1.50	26.4	10.23	130	3.28
	3.00	24.7	10.41	121	3.38
3,6 dichloropicolinic acid	0.20	25.3	9.78	136	3.11
	0.40	21.4	9.36	133	2.80*
S.E. mean ±		1.79	0.237	10.5	0.246

*,**,*** - Significantly different from Untreated at the 5%, 1% or 0.1% level.

Table 3

Expt. II 1981 fruit records following treatment in autumn 1980 or spring 1981

Herbicide	Dose kg a.i./ha	Yield t/ha	Mean berry wt(g)	No. fruit trusses /m^2	No. berries/ truss
Untreated		23.4	9.80	109	3.60
S.E. mean ±		1.07	0.176	7.2	0.191
Treated 19 August, 1980					
Oxadiazon	2.00	16.4***	8.96**	95	3.15
	4.00	14.8***	8.47***	70**	4.11
Alloxydim sodium	1.50	19.0*	9.48	99	3.32
	3.00	19.4*	9.84	114	2.83*
3,6 dichloropicolinic acid	0.20	21.4	9.71	94	3.90
	0.40	19.7	9.68	88	3.89
Fluazifop butyl	1.00	23.6	9.53	104	3.87
	2.00	23.0	9.45	123	3.23
Treated 8 May, 1981					
Oxadiazon	2.00	6.4***	7.89***	47***	2.77*
	4.00	1.9***	7.04***	31***	1.41***
Alloxydim sodium	1.50	24.3	10.18	118	3.32
	3.00	23.4	9.66	110	3.61
3,6 dichloropicolinic acid	0.20	22.9	9.63	112	3.44
	0.40	23.3	9.40	134	2.98
Fluazifop butyl	1.00	22.4	9.69	110	3.45
	2.00	19.4*	10.17	93	3.34
S.E. mean ±		1.52	0.249	10.1	0.270

*,**,*** - Significantly different from Untreated at the 5%, 1% or 0.1% level.

reaching a total of 4 mm in a 24 h period. The next 7 days were dry and sunny. Spring applications were followed by dry weather for 12 h, 1.0 mm rain in the next 12 h, and then by fine sunny conditions for several days. Oxadiazon again desiccated the foliage rapidly, while 3,6 dichloropicolinic acid caused rolling and distortion of leaves and reflexing of petals, but not to the same extent as in Expt. I. There were no visible symptoms from fluazifop butyl, but the double rate of alloxydim sodium caused some reddish blotching of leaves.

Fruit records (Table 3) showed no adverse effects of the autumn treatment with 3,6 dichloropicolinic acid or fluazifop butyl. Alloxydim sodium reduced fruit yield significantly at both rates, partly due to the production of fewer berries/truss than on untreated plots. Oxadiazon again severely reduced fruit production following autumn treatment and especially from the spring treatment. Foliar effects of spring application of 3,6 dichloropicolinic acid were not sufficiently severe to reduce yield in this experiment. The double rate of fluazifop butyl applied in May caused a significant yield reduction, whereas alloxydim sodium had no adverse effect at either rate.

DISCUSSION

Spring applications of oxadiazon at either rate had a very severe effect on subsequent growth and yield, but in Expt. I the crop showed complete recovery during the following year. This agrees with the findings of Clay (1980) who reported that timing and formulation were of more importance than dose in determining the degree of injury obtained. In our experiments the 25% e.c. formulation was used. August treatments still resulted in considerable, although less severe, yield loss in the following summer, but Clay (1980) reported that December application when the crop was dormant had no adverse effects on growth or yield. For optimum control of Convolvulus arvensis, however, spring treatments are necessary (May & Baker Ltd., personal communication). The speed of recovery of the crop in the following growing season suggests that local spot-treatment in spring may be an acceptable method of using this herbicide in strawberry. Overall treatment would be much too damaging for commercial use.

Treatment with alloxydim sodium in spring had no adverse effect on subsequent yield at double the recommended rate in either experiment. This also applied to August application in Expt. I, but both rates of application reduced fruit yield following this date of treatment in Expt. II. This is contrary to previous reports by the manufacturers, ADAS and WRO (Clay, 1981). The leaf blotching resulting from the double rate applied at SCRI in May 1981 did not lead to any yield reduction from that treatment. No such symptoms occurred following the equivalent autumn application. By contrast, fluazifop butyl caused injury to spring-treated plots, but not to those sprayed in the previous August. The causes of yield reductions with these two herbicides require further investigation, but the evidence suggests that under most circumstances both herbicides have sufficient safety margin for overall use to control perennial grasses in strawberry in either spring or autumn. Since these trials were initiated, the rate of fluazifop butyl recommended for control of perennial grasses has been reduced to 0.75 kg a.i./ha.

3,6 dichloropicolinic acid produced much less foliar distortion following August than following May application and autumn treatment had no significant effect on subsequent fruit yields in either experiment. Spring application was more severe in Expt. I than in Expt. II, the double rate reducing fruit yield by 28%. Both crops were at a similar stage of growth when treated and the difference between years may have been due to environmental conditions after treatment. However, there was no evidence of significant residual effect in the following season from even the most severe treatment. Bailey & Clay (1980) and Lake & Bennett (1980) reported a similar plant response to treatments made in the pre-flowering period, which coincides with the optimum growth stage of C. arvense for effective control. Earlier application was, however, safer to the crop. Autumn application, while

offering no solution to the current season's problem had less effect on the crop in our experiments. It may be preferable to apply an overall treatment at that time, followed by spot-treatment of surviving plants in the spring in order to minimise crop injury, rather than to risk overall application in May.

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THE TOLERANCE OF STRAWBERRY CULTIVARS TO ETHOFUMESATE ALONE AND IN MIXTURE WITH LENACIL AND PHENMEDIPHAM

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Summary. The effects of ethofumesate alone or in mixtures were tested on strawberries in pot experiments in 1980 and 1981. Ethofumesate applied in December or February generally caused less damage than in March or April. Addition of lenacil to ethofumesate had no effect on the degree of damage. When phenmedipham was applied to plants previously treated with ethofumesate, adverse effects were less than additive.

Cv. Cambridge Favourite was less damaged than Montrose or Redgauntlet by ethofumesate applied in December or February. Gorella and C. Vigour were intermediate. Differences between cultivars were smaller when ethofumesate was applied in March, April or May and when ethofumesate + phenmedipham was applied in April or May. Application of ethofumesate specifically to the foliage or to roots of plants growing in sand showed that damage was caused by both methods of treatment; cv. Montrose was more damaged than C. Favourite with both methods.

INTRODUCTION

Ethofumesate is recommended for pre- and post-emergence weed control in strawberries in the UK (UK, MAFF, 1979) . Its use is restricted to treatment in autumn on established crops of cv. Cambridge Favourite. Some other cultivars may be less tolerant of ethofumesate (UK Horticultural Centre, Loughgall, 1977; Ireland, 1980; Clay, 1981). In the work reported here the tolerance of the most commonly-grown cultivars in the UK was compared with that of cv. C. Favourite.

In sugar beet ethofumesate is also recommended in mixture with lenacil or phenmedipham (Fryer and Makepeace, 1978). These mixtures may be safe in strawberries (Lawson and Wiseman, 1980; Clay, 1981) but there is little information on varietal differences. Tolerance of different cultivars to these mixtures was also tested.

METHODS AND MATERIALS

Pot experiments were carried out at Begbroke Hill in 1980 and 1981. Two main experiments were carried out in which runners of the cultivars listed in Tables 1 and 2 were planted in a sandy loam soil (pH 6-7, organic matter content 2-3%) using 18 cm diameter pots. Fertilizer and benomyl (630 mg/l Benlate) were incorporated into the soil before planting. Experiment 1 was planted on 25 October 1979 and Experiment 2 on 28 October 1980 and the plants grown outdoors. Lenacil at 0.5 kg/ha was sprayed over all the pots shortly after planting to control weeds. Herbicides were applied at the dates shown in Tables 1 and 2 using a laboratory pot-sprayer with an 80015E TeeJet at 210 kPa pressure and a spray volume of 230 l/ha. Ethofumesate (20% e.c.) was sprayed at 1, 2 and 4 kg a.i./ha, lenacil (80% w.p.) at 0.4, 0.8 and 1.6 kg/ha and phenmedipham (11.4% e.c.) at 0.8, 1.6 and 3.2 kg/ha. Plants were returned outside as randomised blocks immediately after spraying. There were six replicates of each treatment (12 for untreated controls) in all experiments.

In all experiments plant condition was assessed at intervals after treatment using a 0-9 scoring scale (0 = plant dead, 3 = very stunted, 5 = 50% growth reduction, 7 = obvious growth reduction, 9 = plant normal). In Experiment 1 fruit trusses were removed on 10 June 1980 and fresh weight recorded. At the end of the experiments leaf fresh weight was recorded. In Experiment 2, on untreated plants, the youngest expanded leaf at the time of planting was tagged and subsequently the number and length of leaves recorded at intervals. The number of leaves showing distortion due to ethofumesate damage was recorded on 16 June 1981.

An additional experiment (3) was carried out at the same time as experiment 1 to compare the tolerance of two cultivars (C. Favourite and Montrose) to ethofumesate applied specifically to the foliage or to the roots. The methods used were those described by Clay (1980). In the test of foliar activity the plants were grown as for Experiment 1 but after spraying with ethofumesate on 20 March 1980 they were protected from rain by a mobile transparent cover and all subsequent watering was on to the soil surface. In the investigation of ethofumesate activity through the roots, similar plants but with washed soil-free roots were transplanted into silica sand in 25 cm diameter pots on 29.2.80 and watered. A range of doses of ethofumesate was applied on 20.3.80 to the sand surface of pots standing in individual foil saucers. The doses were 0, 0.3, 1.3, 5.0, 20.0 and 80.0 mg/pot. Pots were subsequently protected from rain and watered on the sand surface with quarter strength Hewitt's nutrient solution.

RESULTS

Experiment 1. Damage symptoms. Effects of ethofumesate treatment were seen on new leaf growth (Tables 1, 2) which became stunted and thickened with tip burn. With severe damage leaves emerging subsequently were thickened, the laminae being folded and often stuck together. Petioles were shortened. Plants subsequently recovered but distorted leaves were still noticeable when the experiment was finished in early July. There was no leaf damage with lenacil treatments. Phenmedipham caused chlorosis and slight necrosis of sprayed leaves but effects were soon outgrown. With ethofumesate + phenmedipham the main effects were chlorosis and necrosis of youngest sprayed leaves and distortion of subsequent leaves.

Table 1

The effect of ethofumesate applied on 18 February at three doses on the cultivars C. Favourite(F), Gorella(G), Montrose(M), Redgauntlet(R) and C. Vigour(V) (Expt. 1)

Herbicide dose (kg/ha)	Plant condition score* 22/4					1/7					Leaf fresh wt* 16/7				
	F	G	M	R	V	F	G	M	R	V	F	G	M	R	V
1	85	75	65	67	77	100	100	93	95	100	114	103	101	111	122
2	82	71	52	61	62	100	89	78	93	100	108	116	98	118	117
4	76	65	52	55	60	100	76	61	65	81	114	102	106	100	119
SE ±			2.1					3.3					6.0		

* % untreated

Treatment effects. Most of the results presented (Tables 2, 3, 4) are mean values for the three doses, to save space and because variability of individual dose values for measurements was high. There was little difference in the overall effects of ethofumesate applied in February or March (Table 2) except that fruit weight was reduced by March treatment (Table 3). Ethofumesate applied in May, to C. Favourite only, caused longer term damage than the earlier treatments (data not shown). The lenacil treatments had no overall adverse effects or interaction. Where phenmedipham was applied in May to plants treated with ethofumesate in February short-term leaf damage was increased compared with plants sprayed with one herbicide. There were generally significant reductions in fruit weight.

Table 2

The effect of ethofumesate(E), lenacil(L), and phenmedipham(P) on the strawberry cultivars Cambridge Favourite(F), Gorella(G), Montrose(M), Redgauntlet(R) and Cambridge Vigour(V) (Experiment 1)

Herbicide o	Application date+	Score of plant condition* 22/4 F	G	M	R	V	mean	19/5 F	G	M	R	V	mean	1/7 F	G	M	R	V	mean
L	Feb.	100	105	98	88	94	97	101	105	101	95	98	100	99	99	100	100	103	100
E	Feb.	81	70	56	61	67	67	96	91	68	64	84	81	100	88	77	84	94	89
E	Mar.	81	60	56	61	68	66	102	79	83	80	89	87	98	80	83	80	93	87
E P	Feb. May	-	-	-	-	-	-	76	63	59	54	64	63	95	87	79	83	90	87
EL P	Feb. May	-	-	-	-	-	-	79	70	63	62	67	68	99	83	80	75	88	85
E+P	May	-	-	-	-	-	-	74	57	66	67	68	66	94	67	69	78	90	80
P	May	-	-	-	-	-	-	85	79	86	79	85	83	99	92	95	91	101	96
SE ±	herbicides						0.9						1.5						1.0
	herb x cv.		2.1							3.4						2.3			

o Mean of three doses; E, 1, 2 and 4 kg/ha, L, 0.4, 0.8, 1.6 kg/ha; P, 0.8, 1.6, 3.2 kg/ha
+ Actual dates, Feb. = 22.2.80; Mar. = 20.3.80; May = 10.5.80
* as % of the value for untreated plants of that cultivar; 0 = dead, 100 = healthy

The mixture of lenacil with ethofumesate, sprayed in February, did not increase the damage caused by subsequent phenmedipham spraying. The mixture of ethofumesate + phenmedipham caused appreciable damage particularly at the highest dose. Phenmedipham alone caused only slight leaf damage.

Final leaf fresh weight was not significantly lower than untreated with any treatment and was significantly increased with most treatments (P = 0.05) (Table 3).

Table 3

The effect of ethofumesate(E), lenacil(L) and phenmedipham(P) on the fruit yield and leaf fresh wt of cv. C. Favourite(F), Gorella(G), Montrose(M), Redgauntlet(R) and C. Vigour(V) (Experiment 1)

Herbicide	Application date	Fruit wt (10/6/80)*						Leaf fresh wt (16/7/80)*					
		F	G	M	R	V	Mean	F	G	M	R	V	Mean
L	Feb.	103	106	105	79	97	97	106	107	110	110	107	109
E	Feb.	100	120	96	67	100	96	112	107	101	110	116	109
E	Mar.	94	93	87	49	84	82	118	97	107	106	121	110
E) P)	Feb. May	86	77	72	45	69	70	103	101	100	104	108	104
E+P	May	81	62	57	44	52	59	106	95	104	111	115	106
P	May	99	84	79	68	83	83	101	103	108	100	112	105
SE±			9.7				4.3			4.2			1.9

* % untreated

Differences between cultivars. There were significant differences with ethofumesate treatments in February and March (Table 1, 2). C. Favourite was least affected, plants recovering completely by June whereas Montrose and Redgauntlet showed severe damage at all doses. Gorella and C. Vigour were intermediate with the February treatment but Gorella was more damaged by the March treatment. The addition of lenacil to ethofumesate did not generally affect the relative tolerance. Differences in damage were much smaller where phenmedipham was mixed with or followed ethofumesate. C. Favourite showed appreciable short-term leaf damage (necrosis/distortion of youngest sprayed leaves) but recovered more quickly. Redgauntlet was the most severely affected with fruit weight being reduced by over 50% compared with controls (Table 3). There was little differential effect of phenmedipham or lenacil alone on cultivars.

Experiment 2. There was little difference in the damage from December and February ethofumesate treatments and no effect on final leaf weight (Table 4). April spraying caused more leaf distortion and leaf weight reduction at harvest. Phenmedipham applied in April to plants treated with ethofumesate in February caused no increase in leaf damage. The ethofumesate + phenmedipham mixture applied in April caused more severe leaf damage than in Experiment 1 and final leaf weight was reduced appreciably. Phenmedipham alone caused severe leaf chlorosis and necrosis at the highest doses but final leaf weight was unaffected.

Differences between cultivars. Effects of winter treatments on cultivars were similar to Experiment 1, C. Favourite being least affected and Redgauntlet being the most damaged (Table 4). C. Vigour and Gorella were intermediate although Gorella was more damaged by February treatment. With April treatment effects were more severe and differences between cultivars less than the earlier sprays, but by the end of the experiment C. Favourite was significantly less damaged than Gorella

Table 4

The effect of ethofumesate (E) and phenmedipham (P) on the strawberry cultivars Cambridge Favourite (F), Gorella (G), Redgauntlet (R) and Cambridge Vigour (V) (Experiment 2)

Herbicide o	Application date+	Score of plant condition* 24/4/81 F	G	R	V	mean	2/6/81 F	G	R	V	mean	No. of distorted lves[x] 16/6/81 F	G	R	V	mean	Leaf fresh wt* 16/6/81 F	G	R	V	mean
E	Dec.	84	68	62	78	73	94	79	69	87	82	6	31	102	60	50	118	92	108	109	107
E	Feb.	80	58	63	72	68	94	81	69	78	80	7	43	79	40	42	115	71	102	103	98
E	Apr.	60	57	53	62	59	60	51	44	54	52	45	64	65	74	62	87	72	61	86	77
E P	Feb.) Apr.)	68	52	53	69	61	86	71	65	80	75	-	-	-	-	-	103	70	94	95	90
E+P	Apr.	54	49	51	59	53	58	49	39	53	50	57	73	64	73	67	68	63	59	87	69
P	Apr.	63	65	65	81	69	82	97	94	100		-	-	-	-	-	90	91	98	98	94
SE ±	herbicides					1.0					1.2					4.6					2.7
	herb x cv.			2.1					2.5					9.3					5.4		

o Mean of the three doses; E, 1, 2 and 4 kg/ha; P, 0.8, 1.6 and 3.2 kg/ha
+ Actual dates; Dec. = 16/12/80; Feb. = 18/2/81; Apr. = 9/4/81
* As % value for untreated plants for that cultivar
x Based on counts on highest doses only, as % of mean number of leaves on corresponding untreated plants

or Redgauntlet. There were only slight differences in varietal response to ethofumesate + phenmedipham applied in April.

Experiment 3 - Foliage treatment. Ethofumesate at 4 kg/ha caused slight stunting on C. Favourite but more severe stunting on Montrose with one or two leaves emerging after spraying showing distortion. Final leaf weight was not affected (Table 5).

Table 5

The response of strawberries, cv C. Favourite(F) and Regauntlet(R), to ethofumesate applied to the foliage only or to the roots of plants grown in sand (Experiment 3)

	cv.	Plant condition score[x]			Fruit fresh wt[x]	Foliage fresh wt[x]
		22/4	19/5	1/7	10/6	16/7
Foliage treatment[+]						
Ethofumesate	F	89*	100	101	109	88
4 kg/ha	M	70***	87*	101	106	98
LSD (P = 0.05)		7	10	8	32	15
Sand treatment[+]						
Ethofumesate+	F	82*	99	85*	119	81*
5.0 mg/pot	M	76**	64**	54***	51*	76*
LSD (P = 0.05)		13	13	9	38	17

+ Foliage treatment applied 20/3/80; sand treatment 20/3/80 x % untreated
*, **, *** indicates significant differences from control at P = 0.05, 0.01, 0.001 respectively

Sand treatment. Ethofumesate caused similar effects to those of Experiments 1 and 2, with particularly severe leaf distortion developing after treatment. The main differences in the two cultivars were seen at the middle dose (Table 5), Montrose being more damaged than C. Favourite. At the lower doses damage was only slight.

DISCUSSION

The type and degree of damage caused by ethofumesate applied in winter and spring was similar to that reported (Clay, Rutherford and Wiseman, 1974; Clay, 1980). As in previous field experiments application of 2 kg/ha in spring resulted in more damage than winter treatment (Clay, 1981), but even severely-damaged plants subsequently recovered. Although damaged plants showed no final leaf weight reduction, leaf symptoms were often still obvious at harvest, as shown by the scores. This indicates that scores may give valuable information about the effects and acceptability of a treatment which are not shown by measurements. The increase in leaf weight/plant with many of the ethofumesate treatments probably resulted from their leaf thickening effect.

There was no indication that the low rate of lenacil applied after planting adversely affected growth. Lenacil is recommended on newly planted strawberries at four times this dose (Fryer and Makepeace, 1978).

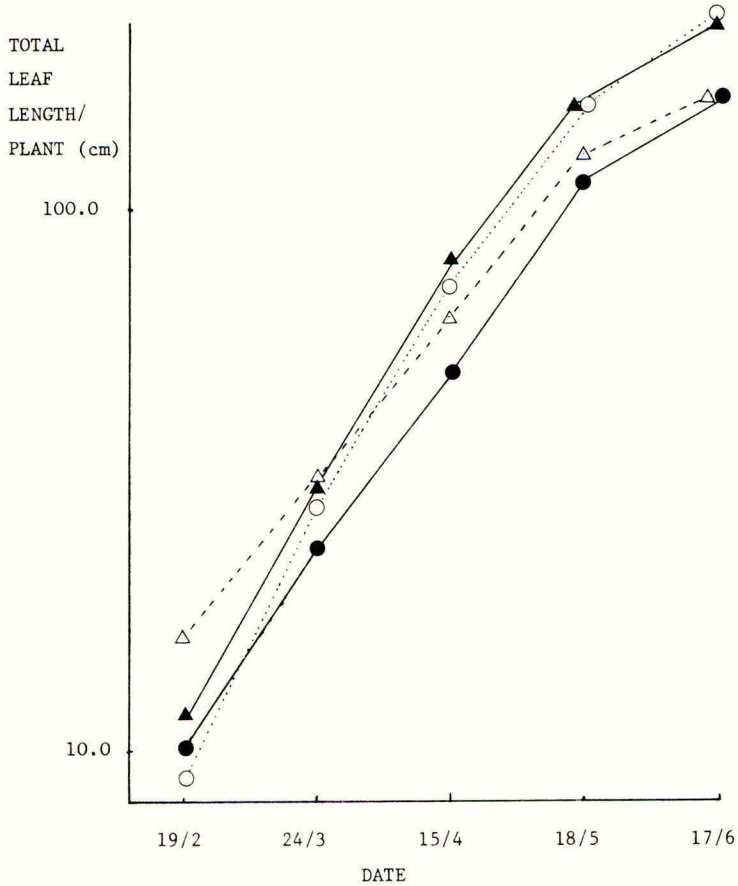
There was no evidence of adverse results from the mixtures of ethofumesate with lenacil, but even the highest dose of lenacil used did not result in leaf damage; the possibility of adverse interactions in situations where lenacil uptake approaches toxic levels cannot be excluded. Lawson and Wiseman (1980) reported no damage from mixtures with a low proportion of lenacil but there has been severe damage on newly-planted runners from mixtures containing more lenacil (UK, MAFF, 1979b).

The type of damage from the ethofumesate/phenmedipham mixture was similar to that found previously but generally more severe than at comparable doses in field experiments (Lawson and Wiseman, 1980; Clay, 1981). There was no evidence of adverse interactions where damaging rates of phenmedipham were applied to plants showing different degrees of damage from previous ethofumesate treatment.

The relative tolerance of the cultivars to ethofumesate was in the same order in both years. C. Favourite was consistently tolerant to winter treatment at high rates whereas Redgauntlet was damaged in both years. The differences in the response of C. Favourite, Gorella, Montrose and C. Vigour confirm earlier work (Clay, 1981; UK Loughgall Horticultural Centre, 1977; Ireland, 1980).

Fig.1

Total leaf length/plant for strawberries cv. C. favourite (●) Gorella (○), Redgauntlet (△) and C. vigour (▲) (Experiment 2)



Various factors may be responsible for differences in varietal tolerance to ethofumesate (Clay, 1981). Where the herbicide was applied separately to roots and leaves of two cultivars of contrasting tolerance (Experiment 3) the susceptible cultivar was more damaged with both methods of application indicating that a

metabolic factor may be involved. Damage from the foliage treatment of Montrose was not as great as in the main experiment where herbicide was sprayed over both plant and soil suggesting that root uptake may be the more important cause of damage.

Initial size and subsequent growth rate of the cultivars may also be involved. At planting there was little difference in the weight of runners except for C. Vigour which was 25% heavier. Small runners can be more susceptible to herbicides (Mason and Dudney, 1978). After planting Redgauntlet produced more leaves in the winter and had a larger leaf area whilst Gorella and C. Vigour grew more rapidly in the spring (Fig. 1). Redgauntlet also produced more flower trusses in winter and early spring, which may have led to the greater fruit weight reduction with this cultivar. Thus rate of growth at time of treatment may be an important factor in the occurrence of damage.

The results of these experiments confirm the tolerance of Cambridge Favourite to ethofumesate; it is the only cultivar for which there is currently a recommendation. Use in autumn on other cultivars may give subsequent leaf damage and fruit yield reduction and so could only be considered for weed situations where no alternative treatment is available. The mixtures of ethofumesate + lenacil or phenmedipham could be useful treatments but more work is needed to establish acceptably safe conditions of use.

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THE RESPONSE OF WILLOWHERBS (EPILOBIUM ADENOCAULON AND E. OBSCURUM) TO PRE- OR POST-EMERGENCE HERBICIDES

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Summary. A range of herbicides was applied pre- or post-emergence to Epilobium adenocaulon in pots and post-emergence to E. obscurum in the field.

E. adenocaulon was controlled pre-emergence by diuron, oxyfluorfen, propachlor, chloroxuron, hexazinone, bifenox and dichlobenil but not by simazine and chlorthal-dimethyl. It was controlled post-emergence by atrazine, bromacil, diuron, terbacil, 2,4-D, MCPB, mecoprop, glyphosate and triclopyr.

E. obscurum at the rosette stage was controlled by atrazine, diuron, terbacil, 2,4-D, MCPB, 3,6-dichloropicolinic acid, glyphosate and triclopyr. At flowering it was more tolerant to the same herbicides except diuron, which, together with paraquat, gave complete control.

The implications for control in perennial crops is discussed.

diuron, paraquat, simazine, atrazine, terbacil, 2,4-D, MCPB, triclopyr, chloroxuron, propachlor.

INTRODUCTION

Epilobium spp. (willowherbs) are an increasing problem in perennial crops in the UK in which herbicides are used. They are perennials which spread mainly by seed, and some species (e.g. E. angustifolium) produce up to 80,000 seeds per plant (Fryer and Makepeace, 1977)

There is very little information on their response to herbicides. Rowell and Wallis (1976) reported them to be resistant to simazine and Karhiniemi (1977) found E. montanum to be tolerant of chlorthiamid and dichlobenil. However, Lasscock (1975) found that E. billardieranum was eliminated by chloroxuron.

Davison (1972) reported excellent control of E. hirsutum with glyphosate and Turner and Richardson (1980) controlled E. angustifolium with triclopyr. Atkinson (1976) killed the tops of Epilobium spp. with glyphosate and aminotriazole in June but they regrew by autumn.

This paper reports on pot and field experiments on the pre- and post-emergence control of E. adenocaulon and E. obscurum, two of the species that occur in fruit.

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METHODS AND MATERIALS

E. adenocaulon: pre-emergence control (Expt. 1)

The treatments in Table 1 were applied on 24 November 1981 in 386 l/ha to pots of sandy loam soil at field capacity. Twenty five seeds were then sown in each pot and lightly covered with silver sand. The three replicates were arranged in randomised blocks in a heated glasshouse and watered overhead as necessary. The seedlings that emerged were counted and scored for vigour at the intervals shown in Table 1.

post-emergence control (Expts. 2 and 3)

The treatments in Table 2 were applied on 19 January 1982 to plants that were 10-16 cm tall (Expt 2) or on 19 February 1982 to plants that were 20 cm tall (Expt 3). On both occasions the plants, which were growing singly in pots of sandy loam soil, were well branched but not flowering. The method of application, replication and growing conditions except for the watering, were as for Expt. 1. For the first two days water was directed onto the soil, avoiding the foliage, thereafter the foliage was also wetted. The plants were scored for damage at the intervals shown in Table 2.

E. obscurum: post-emergence control at the rosette stage (Expt. 4)

The treatments in Table 3 were applied on 24 March 1982 to a natural stand of overwintered plants with rosettes of leaves up to 10 cm diameter and an average density of 5 per m^2 in a sandy loam soil at Begbroke. The sprays were applied at 200 l/ha from a pressurized knapsack sprayer fitted with TeeJet 8002 fan nozzles and operated at 200 kPa. Plots were 3 x 1.5 m and there were three replicates. Plots were scored for damage at the intervals shown in Table 3.

post-emergence control at flowering (Expt. 5)

The treatments in Table 3 were applied on 14 June 1982 to a natural stand of overwintered plants in a sandy loam soil at Begbroke. There were 20-30 plants per m^2 with a mean height of 50 cm and they were flowering profusely. Plot size, replication, method of application and assessments were the same as in Expt. 4.

RESULTS

E. adenocaulon: pre-emergence control (Expt. 1)

The results in Table 1 show that diuron, propachlor, chloroxuron, dichlobenil, hexazinone, oxyfluorfen and bifenox all gave complete control but only oxyfluorfen and bifenox prevented seedling emergence. 43 days after treatment (DAT), propyzamide and asulam had significantly ($P = 0.05$) reduced both numbers and vigour. Pendimethalin, diphenamid, aziprotryne and napropamide had only reduced seedling vigour but simazine and chlorthal-dimethyl reduced neither numbers nor vigour.

post-emergence control (Expt. 2)

The results in Table 2 show that 29 DAT there was complete kill with 2,4-D, MCPB, triclopyr, atrazine and bromacil and almost complete kill (a score of one or less) with mecoprop, diuron and terbacil. Glyphosate caused very severe damage. The plants treated with oxyfluorfen and paraquat were recovering from the initial damage.

Table 1

The response of Epilobium adenocaulon to a range of herbicides applied pre-emergence - Expt 1

Values expressed as % of untreated

Herbicide	Dose kg/ha	Days after treatment (DAT) 16 Numbers	43 Numbers	43 Vigour*
diuron	0.5	87	0	0
"	1.0	66	0	0
"	1.5	61	0	0
oxyfluorfen	0.25	0	0	0
bifenox	1.5	0	0	0
propachlor	4.4	38	0	0
chloroxuron	3.6	103	0	0
dichlobenil	3.3	10	0	0
hexazinone	0.25	71	0	0
propyzamide	1.0	21	35	39
asulam	1.0	61	52	19
pendimethalin	1.3	120	121	29
diphenamid	4.5	136	109	19
aziprotryne	2.0	103	98	47
napropamide	2.0	103	103	24
simazine	1.0	110	133	90
chlorthal-dimethyl	4.5	169	162	100
untreated	-	100 = 6.1	100 = 5.8	100 = 7
SE ±		16.5	16.9	3.4

* 0 - 7 scale; 0 = dead, 7 = as untreated

post-emergence control (Expt. 3)

The results in Table 2 show that 25 DAT all herbicides except 3,6-dichloropicolinic acid, oxyfluorfen and paraquat gave complete or almost complete kill (a score of one or less) at least at the higher dose. The lower doses of paraquat, atrazine and diuron were significantly (P = 0.05) less effective. There was regrowth with both doses of oxyfluorfen and paraquat. Simazine, the least effective treatment, still had a statistically significant (P = 0.05) effect .

E. obscurum: post-emergence control at the rosette stage (Expt. 4)

The results in Table 3 show that 52 DAT all treatments gave complete kill except 3,6-dichloropicolinic acid at 0.2 kg/ha where the surviving plants were moribund. At the 23 DAT assessment the only single herbicide treatments which had given complete shoot kill were the soil-acting herbicides diuron and terbacil.

Table 2

The response of Epilobium adenocaulon in pots to a range of herbicides applied post-emergence

Scores for amount of damage on 0-9 scale*

Treatment	Dose kg/ha	Expt. 2 Days after treatment 29	Expt. 3 (DAT) 25
2,4-D amine	2	-	0.0
	4	0.0	0.7
MCPB	3	-	0.7
	6	0.0	0.0
mecoprop	2	-	1.3
	4	0.7	1.0
3,6-DPA•	0.2	-	2.7
	0.4	-	1.7
glyphosate	2	-	0.0
	4	2.0	0.3
oxyfluorfen	1	-	2.7
	2	3.0	2.3
trichlopyr	1	-	0.0
	2	0.0	0.3
paraquat	1	-	4.0
	2	3.5	0.3
atrazine	2	-	2.3
	4	0.0	0.0
bromacil	2	-	0.0
	4	0.0	0.0
diuron	2	-	3.0
	4	1.0	0.0
terbacil	2	-	0.0
	4	0.3	0.0
simazine	2	-	5.0
	4	-	4.3
untreated	-	9.0	9.0
SE ±		0.23	0.63

• 3,6-DPA = 3,6-dichloropicolinic acid
* 0 = complete shoot kill, 1 = moribund, 3 = very severe damage
7 = obvious damage, 9 = as untreated

Table 3

The response of natural infestations of Epilobium obscurum to a range of herbicides applied post emergence

Scores for amount of damage on 0-9 scale (see Table 2)

Treatment	Dose kg/ha	Expt. 4 Days after treatment (DAT) 23	52	Expt. 5 Days after treatment (DAT) 24	46
aminotriazole	4.5	-	-	4.0	2.7
2,4-D amine	1	-	-	6.7	3.7
	2	2.0	0.0	6.7	3.0
MCPB	1.5	-	-	7.7	7.7
	3	1.6	0.0	6.7	5.7
mecoprop	1	-	-	8.3	9.0
	2	-	-	7.0	8.7
3,6,-DPA*	0.2	7.0	1.0	7.0	8.3
	0.4	5.3	0.0	6.3	7.3
glyphosate	1	-	-	6.0	5.3
	2	1.3	0.0	4.3	3.3
triclopyr	0.5	3.6	0.0	7.3	8.3
	1	0.6	0.0	6.3	4.3
paraquat	1	-	-	1.7	0.0
atrazine	0.5	-	-	8.7	6.7
	1	-	-	4.3	2.0
	2	0.3	0.0	-	-
atrazine + paraquat	0.5 + 1	-	-	0.7	0.3
	1 + 1	-	-	2.3	1.7
	2 + 1	0.0	0.0	-	-
diuron	0.5	-	-	3.0	0.3
	1	-	-	1.3	0.0
	2	0.0	0.0	-	-
diuron + paraquat	0.5 + 1	-	-	2.0	0.3
	1 + 1	-	-	0.3	0.0
	2 + 1	0.0	0.0	-	-
terbacil	1	0.0	0.0	-	-
terbcil + paraquat	1 + 1	0.0	0.0	-	-
simazine	2	-	-	6.0	3.7
simazine + paraquat	2 + 1	-	-	0.7	0.0
untreated		8.0	9.0	9.0	9.0
SE ±		0.56	-	0.28	0.42

* 3,6-DPA = 3,6-dichloropicolinic acid

post-emergence control at flowering (Expt. 5)

The results in Table 3 show that the only single herbicide treatments 46 DAT that had given complete control were paraquat and diuron at 1 kg/ha. Paraquat also gave complete control when mixed with simazine and diuron, but not with atrazine. Simazine alone at 2 kg/ha also had a considerable effect but it was not as damaging as atrazine at 1 kg/ha or diuron at 0.5 kg/ha which gave almost complete control. The foliage-applied herbicides other than paraquat were generally less effective, although aminotriazole, 2,4-D and the high dose of glyphosate caused severe damage.

DISCUSSION

Epilobium spp. tend to be restricted to open fertile sites and they germinate at or near the soil surface whenever there is adequate moisture (Myerscough and Whitehead, 1966 and 1967). Thus they are well suited for establishment in perennial crops in which weeds are controlled chemically. The natural populations of E. obscurum in the two field experiments had established in similar conditions.

The tolerance of E. adenocaulon to pre-emergence simazine (Expt. 1) helps to explain its increased incidence in perennial crops where this is the only soil-applied herbicide used. The results show that, under glasshouse conditions, it can be controlled with other soil-applied herbicides already used in fruit and other perennial crops. It was also controlled by several post-emergence herbicides used in these crops. Thus the prospects for field control are promising but require confirmation.

E. obscurum responded similarly to E. adenocaulon in that it was controlled post-emergence with soil-applied herbicides. Notable amongst these was diuron which gave complete kill even at the flowering stage at doses well below those recommended for fruit and other perennial crops. Rosettes of E. obscurum were also controlled by several foliage-applied herbicides which are also used in these crops but flowering plants were much more tolerant. The kill of the flowering plants with paraquat is inconsistant with the results achieved commercially but growers do not usually spray at such a late growth stage.

The field work with E. obscurum revealed that there can be natural mortality. There were large numbers of seedlings on all plots in Experiment 4 all of which died during the course of the experiment, probably due to low night temperatures. The much larger plants at the rosette stage were unaffected.

From the evidence presented, E. adenocaulon and E. obscurum appear to respond similarly to the herbicides tested, the differences probably being related to the stages of growth. However, it should not be assumed that all species will respond similarly. For instance, in Experiment 5, the only plant of E. tetragonum tolerated diuron at 0.5 kg/ha which killed E. obscurum.

It is difficult to relate the limited amount of published information on Epilobium spp. to the results presented here because the species are either different or not specified. In addition some of the results are from tests on containerised plants grown in peat-based media. Comments from growers and advisers is also confused due to the difficulty in distinguishing between the many species that occur in the U.K.

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SESSION 4B

MODE OF ACTION OF HERBICIDES

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THE MODE OF ACTION OF GLYPHOSATE

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Summary. Glyphosate is a specific inhibitor of the shikimate pathway, preventing the formation of the aromatic amino acids phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Tryp). This is a unique site of interference for a herbicide and important secondary consequences of such action are the arrest of both protein synthesis and the formation of diverse phenolic compounds. Two other phenomena have potential importance: the enhanced oxidative destruction of indolyl-3-acetic acid (IAA) and inhibition of formation of the chlorophyll precursor 5-aminolevulinic acid (ALA), suggesting that the synthesis of all porphyrin-containing molecules may be inhibited. Inhibitor, shikimate pathway, aromatic amino acids, chlorophyll, indolyl-3-acetic acid.

INTRODUCTION

Glyphosate has presented a formidable challenge to biochemists by virtue of its structural uniqueness and its failure to influence to any substantial degree a number of processes which are well known primary sites of action for successful herbicides. However, over the last ten years, a considerable literature has accumulated on mode of action, much of which has been specifically concerned with effects on the metabolism of aromatic amino acids and secondary phenolic compounds. Early findings that supplemental feeding of exogenous aromatic amino acids could in many cases alleviate the toxic symptoms of the herbicide have culminated in the establishment of glyphosate as a specific and powerful enzyme inhibitor. Alleviation of toxicity in this manner is by no means ubiquitous and this provided the impetus to search for other important effects and possible alternative primary modes of action. However, the argument for a single primary site of action at the shikimate pathway remains convincing although several independent secondary effects resulting from this interaction can be envisaged and it is the additive effects of these secondary phenomena which bring about cell death.

A particular problem which is evident when attempting to collate the data on the biochemistry of glyphosate is the wide range of plant materials used, with a corresponding variation in suitability. Since glyphosate is known to accumulate only in growing areas of the plant, data from meristems or cell cultures must be regarded as more meaningful than those from non-growing areas or stationary single cells isolated from such areas. Also, while glyphosate is an effective inhibitor of photosynthesis (in vivo) and appearance of chlorophyll, it is abundantly clear that such effects are not directly related to a mode of action often exerted in non-green areas of the plant. However, recent advances have now made it possible to be able to present a reasonably unified picture of glyphosate's mode of action.

INHIBITION OF FORMATION OF AROMATIC AMINO ACIDS, SECONDARY COMPOUNDS AND PROTEIN

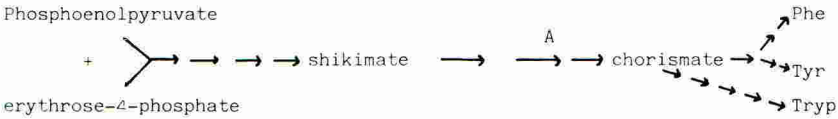
In many instances, the inhibitory effects of glyphosate can be negated partially and sometimes completely by the concomittant supplemental feeding of aromatic amino acids (e.g.Jaworski 1972, Haderlie et al 1977, Gresshoff 1979). Casein hydrolysate can also substitute for these. The hypothesis that this was indicative of impaired aromatic amino acid synthesis was introduced by Jaworski although his conjecture about

the exact site of inhibition, based upon the partial alleviatory effects of certain shikimate pathway intermediates was inaccurate. If a common primary site of action is to be proposed, it is likely that in cases where exogenous aromatic amino acids failed to modify glyphosate toxicity, (e.g. Duke and Hoagland 1978, Cole et al 1980) potential antidotal compounds did not influence the particular pools depleted by glyphosate. It is well known that free amino acids are distributed between several pools within the cell which can be modified independently. Some pools may be metabolically inactive and can account for a substantial proportion of the total cell complement (Berlin and Widholm 1978, Sasse et al 1979). However, in soybean, which did not respond to supplemental feeding, ^{14}C-Phe and Tyr were readily absorbed and metabolised in the presence of glyphosate (Duke and Hoagland 1981). A corollary to the depletion of metabolically active pools of Phe,Tyr and Tryp, thereby arresting protein synthesis, should be a rise in the free levels of other protein precursor amino acids which are unable to be incorporated into protein, since the synthesis of proteins will be arrested at a point which requires an aromatic amino acid. Again, such changes may be partially masked due to the existence of independently modified pools, but from several studies made of the effect of glyphosate on free amino acids, a reasonably consistent pattern has emerged of decreases in Phe and Tyr and increases in the total free amino acid content, often accounted for by considerably elevated levels of glutamate, aspartate, glutamine and asparagine (Haderlie et al 1977, Nilsson 1977, Hoagland et al 1978,1979, Holländer and Amrhein 1980).

The derepression of phenylalanine ammonia-lyase (PAL) in several species (Duke and Hoagland 1978, Hoagland et al 1979, Cole et al 1980)provided further evidence for an interference in aromatic amino acid formation. PAL is a key regulatory enzyme linking primary and secondary metabolism and enzyme level is thought to be controlled by end product pool size. Marked decreases in the levels of secondary phenolic compounds occur in response to glyphosate (Holländer and Amrhein 1980, Berlin and Witte 1981, Hoagland and Duke 1982), suggesting reduced availability of substrate (Phe and Tyr). Elevation of PAL activity does not in itself have important consequences since the use of PAL inhibitors failed to ameliorate the toxicity of glyphosate (Duke et al 1980, Cole et al 1980). The major progress on the interaction of glyphosate with the shikimate pathway (Fig.1) has been made in Amrhein's laboratory. The

Fig.1

The shikimate pathway for the production of aromatic amino acids.
Each arrow represents a separate enzymatic step.



transformation of ^{14}C-shikimate into all three aromatic amino acids was strongly suppressed (Holländer and Amrhein 1980, Berlin and Witte 1981) and indicated inhibition of the triple-branched pathway before the initial bifurcation. Further evidence for inhibition of chorismate formation was provided by inhibition of chorismate-derived anthraquinone accumulation in Galium mollugo cells (Amrhein et al 1980). This was accompanied by an enormous accumulation of shikimate which was also observed in buckwheat (Amrhein et al 1980) and tobacco cells (Berlin and Witte 1981)), thus the site of glyphosate's action was narrowed down to three enzymes transforming shikimate to chorismate. This was resolved by observing the specific inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (Fig.1, enzyme A) in cell-free preparations of Aerobacter aerogenes (Steinrücken and Amrhein 1980) and there is satisfactory evidence that this is the target enzyme in glyphosate's interference with aromatic amino acid synthesis.

An important secondary consequence of reduced aromatic amino acid synthesis is the arrest of protein synthesis. A rapid decline in the protein content of wheat root

tips was paralleled by inhibition of respiration, suggesting that the arrest of protein synthesis is an important factor in toxicity (Cole et al 1980). The strong inhibition of ^{14}C-leucine incorporation into protein compared with the relative insensitivity of ^{14}C-Phe incorporation in single node budes of Agropyron repens rhizome supported inhibition of protein synthesis primarily be a deficit of aromatic amino acids (Cole et al 1980) although no such differential inhibition or decline in protein occurred in carrot cells (Haderlie et al 1977).

SENSITIVITY OF OTHER CELL PROCESSES

An important factor in glyphosate toxicity may be the enhanced destruction of IAA. Glyphosate accelerated the destruction of IAA in tobacco callus (Lee 1982a) which was ascribed to the promotion of IAA oxidase and increased destruction was correlated with herbicide toxicity in this tissue (Lee 1982b). Since many phenolic compounds are inhibitors of IAA oxidase, this phenomenon may be a direct consequence of reduced secondary product synthesis.

A notable property of glyphosate is the prevention of chlorophyll appearance. Accumulation in pre-treated etiolated material is highly sensitive in several species (Holländer and Amrhein 1980, Kitchen et al 1981a, Lee 1981) and appears to be due to an inhibition of the synthesis of ALA, a common precursor of all porphyrins, including chlorophyll. The subsequent transformation of ALA into chlorophyll is unaffected (Kitchen et al 1981b). the exact site of inhibition is unknown and is complicated by the uncertainty over the relative importance of two distinct pathways for the formation of ALA (Meller and Gassman 1982). Although the prevention of chlorophyll formation is unlikely to be an important factor in phytotoxicity in the field, where glyphosate prevents development of non-green meristems, a general inhibition of ALA synthesis thus preventing formation of porphyrin-containing molecules such as cytochromes, catalase and peroxidase may be of considerable importance in mode of action. Whilst convincing evidence for a role of glyphosate in inhibition of chlorophyll formation has been presented, it is highly probable that achlorophyllous regrowth following sub-lethal doses occurs by photodestruction of chlorophyll in the absence of carotenoid pigments. Such foliar regrowth of A.repens lacks secondary pigments and cannot re-green although new chlorophyllous basal growth can occur (Cole, Caseley and Dodge, unpublished observation). Decline in the chlorophyll content of green tissue is synergised by light (Abu-Irmaileh and Jordan 1978, Lee 1981) suggesting the involvement of photodestruction. This phenomenon occurs in response to several herbicides of unrelated structure which inhibit carotenoid synthesis (Fedtke 1982). Achlorophyllous growth caused by glyphosine is almost certainly due to photodestruction (Croft et al 1974). It therefore seems that glyphosate can influence chlorophyll content in two distinct ways.

Photosynthesis is a primary site of action for many herbicides but glyphosate has no effect on photosynthetic reactions of isolated chloroplasts (Richard et al 1979). Inhibition of photosynthesis in vivo has invariably been observed but there is no evidence to suggest that such a phenomenon is of great importance and this must be viewed as an indirect effect (Sprankle et al 1975, Shaner and Lyon 1979, Pihakaski and Pihakaski 1980). Similarly, respiration is not an initial target (Sprankle et al 1975, Abu-Irmaileh et al 1979) and the rapid effect observed in wheat roots (Ali and Fletcher 1978) only reflected the rapid translocation of the herbicide from the site of application. In the absence of comparison with other effects, studies on respiration in vivo are of little value. Despite insensitivity in vivo, Olorunsogo et al (1979) have demonstrated uncoupling of oxidative phosphorylation in vitro. Some glycolytic intermediates and other compounds which feed into the TCA cycle alleviated growth inhibition in carrot cells (Killmer et al 1981) and it is likely that these were substituting for carbon which was continually feeding into the shikimate pathway in the absence of feedback control in the early part of the inhibited pathway.

Glyphosate has negligible effects upon the permeability of the plasma membrane (Fletcher et al 1980, Brecke and Duke 1980) which is unsurprising in view of the high polarity of the compound. Nevertheless a rapid inhibition of inorganic ion uptake

occurred (Brecke and Duke 1980) but this is unlikely to be due to inhibition of monovalent ion-linked membrane ATPases (Cole, Caseley and Dodge unpublished). Significantly reduced uptake of labelled macromolecule precursors has been observed which largely explained the inhibitory effects upon macromolecule synthesis (Haderlie et al 1977, Brecke and Duke 1980).

Ultrastructural investigations have been inconclusive and restricted to mature, green areas which do not accumulate the herbicide. Changes in leaf mesophyll cells of A.repens preceded visual leaf yellowing and included chloroplast degeneration, accumulation of osmiophilic plastoglobuli, swelling of the rough endoplasmic reticulum and wrinkling of the plasmalemma. There was no obvious initial site of action (Campbell et al 1976). Disruption of chloroplasts also occurred in white mustard (Uotila et al 1980). Pihakaski and Pihakaski (1980) have presented an extensive, high resolution study of the liverwort Pellia epiphylla and noted vesicular and tubular structures in the cytoplasm, thought to have derived from the chloroplast envelope; granular bodies associated with the chloroplast envelope and degeneration of the large oil bodies. Notable characteristics of advanced damage were chloroplast and plasmalemma disruption. Although these workers have published the most satisfactory record of glyphosate's ultrastructural effects to date, caution must be exercised in extrapolating these observations on a bryophyte to higher plants.

CHELATION AS A POSSIBLE MODE OF ACTION

The known chelating properties of phosphonic acids (Carter et al 1967, Kabachnik et al 1974) invited speculation that glyphosate may exert its effects by complexing biologically important divalent cations but there is no evidence that such reactions occur within the plant to a deleterious extent (Gresshoff 1979, Holländer and Amrhein 1980). The ability of glyphosate to inhibit several enzymes of the shikimate pathway appears to be due to an ability to remove divalent co-factors but the very high concentrations required for this indicate that this is not the mechanism whereby glyphosate inhibits this pathway (Roisch and Lingens 1980).

CONCLUSIONS

Glyphosate is a powerful inhibitor of 5-enolpyruvylshikimate-3-phosphate synthase, causing the depletion of aromatic amino acids which results in the arrest of protein and secondary compound synthesis. The apparent specificity of action has rendered the compound useful as a tool with which to inhibit the pathway for experimental purposes. The occurrence of this pathway only in plants and microorganisms ensures low toxicity to animals. Enhanced destruction of IAA may be related to reduced phenolic compound synthesis, but it is not clear whether inhibition of ALA formation is related to interference with the shikimate pathway.

A fairly comprehensive picture of this remarkable compound's mode of action now exists but there are certain areas of deficiency, notably ultrastructural effects which are poorly understood. Nothing is known about ultrastructural changes at sites of accumulation and ultimately it is desirable that biochemical and ultrastructural changes can be correlated in order that a unified sequence of events can be determined. It should be stressed that many of the important observations on mode of action have been obtained using cell cultures and microorganisms which are amenable systems biochemically, but in the final instance these observations must be seen to be applicable to the intact higher plant. In particular, weed species and their target organs, to which glyphosate is particularly effective deserve more attention from biochemists (Cole et al 1980) and the mechanisms by which the herbicide prevents germination of dormant rhizome buds may be more complicated than hitherto assumed, on the basis of work which invariably involves the termination of growth as an experimental approach.

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THE MECHANISM OF SELECTIVITY OF CHLORTOLURON BETWEEN CEREALS AND GRASSWEEDS

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Summary Data indicating the likely mechanisms of chlortoluron selectivity between wheat and barley, and Avena fatua L. and Alopecurus myosuroides Huds. is presented. Metabolism studies demonstrated that chlortoluron was susceptible to degradation by both N-demethylation and ring-methyl oxidation pathways, followed by conjugation. Ring-methyl oxidation predominated in the cereals, unlike the grassweeds in which the N-demethylation pathway constituted the major metabolic route. Studies on the inhibition of the Hill reaction by chlortoluron metabolites indicated that the only compound retaining significant phytotoxicity was N-monodemethylated chlortoluron. Uptake of chlortoluron was considerably more rapid in A.fatua than for wheat and barley in which the rates were very similar. The greater uptake by wild oat plants was reflected in the amount of phytotoxic material associated with their chloroplasts, which was significantly larger than for cereals. Ring-methyl oxidation, N-demethylation, absorption, photosynthesis, wild oat, blackgrass, wheat, barley.

INTRODUCTION

The two grassweeds, wild oat (Avena fatua, Avena ludoviciana, Avena sterilis and Avena barbata) and blackgrass (Alopecurus myosuroides) constitute a serious threat to the successful cultivation of winter-sown cereals in particular (Wormell, 1972). Because of the economic importance of cereals a number of herbicides have been developed for the selective control of these troublesome weeds. The basis for the selective action of several of these compounds has been reviewed recently (Pallett, 1980). Among the chemicals specifically recommended for the control of these weeds is chlortoluron (N'-(3-chloro-4-methylphenyl)-N,N-dimethylurea). The efficacy of chlortoluron for selective weed control in cereals has been investigated in considerable detail (Van Hiele et al, 1970; Skorda, 1974; Guillemenet, 1975).

Several excellent accounts of the metabolism of substituted phenylureas are available in the literature (Frear et al, 1972; Geissbühler et al, 1975; Naylor, 1976). Stepwise N-demethylation, often followed by conjugation of the intermediates as O-glycosides, has generally been considered the major degradation pathway for dimethylphenylureas in plants. However, recent studies of the degradation of chlortoluron in cereals (Gross et al, 1979; Ryan et al, 1981) clearly demonstrated the susceptibility of the ring-methyl group to oxidative attack, the products of which predominated amongst the extractable metabolites.

The aim of the present study was to establish a biochemical/physiological basis for the different reactions to chlortoluron shown by cereals and some important grassweeds, and, in particular, to see whether these are reflected in relative rates of detoxification of the herbicide.

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METHODS AND MATERIALS

Growth and Treatment of Plant Material

The plant species used in the present study were wheat (var.Atou), barley (var. Astrix), A.fatua L. (type USA) and A.myosuroides Huds. Plants treated with herbicide via the soil were grown in pots of soil type 'stein', the composition of which has been previously described (Ryan et al, 1981). For studies of the intracellular distribution and metabolism of absorbed radioactivity such plants were treated with [carbonyl-^{14}C]chlortoluron (sp.act. 2.4 x 10^5 Bq/µmol.) at rates of 1.2 mg and 0.6mg/pot for cereals and grassweeds respectively, corresponding to field applications of 1.5 & 0.75 kg/ha. Plants required for uptake studies and for manometric measurements of oxygen evolution were grown in nutrient solution (Hewitt, 1966) supplemented with 2.6 mg/l [^{14}C]chlortoluron (sp. act. 7.8 x 10^3 Bq/µmol.) or 3.1 mg/l unlabelled chlortoluron respectively. Untreated plants used for metabolism studies with leaf discs and for chloroplast isolation were grown in pots of John Innes No 2 compost. All plants were grown in controlled environment cabinets maintaining a 16-h-day regime (light intensity 10 000 lx, r.h. 60%) and day and night temperatures of 20°C and 14°C respectively. Plants used for experimental studies were at growth stage 11 or 12.

Manometric Measurements

Oxygen evolution by leaf discs (5 mm diam.) was measured using a Warburg apparatus fitted with a bank of incandescent lamps providing illumination of 10 000 lx. In addition to the plant material, the main compartment of each flask contained 2.5ml of 0.05 M phosphate buffer pH 7.0, whilst the centre well contained 0.6ml of a CO_2 buffer (Umbreit et al, 1972) absorbed onto convoluted Whatman No 42 filter paper to maintain a CO_2 atmosphere of 1% at the experiment temperature (25°C).

Hill activity of isolated chloroplasts

The methods used for the preparation of chloroplasts and for subsequent measurement of their rates of DCPIP photoreduction were those described previously by Owen et al (1975). Chlortoluron and its metabolites were added to assay mixtures as aliquots of stock solutions in 5% methanol. All control assays incorporated an equal volume of 5% methanol not containing herbicide.

Extraction and Analysis of absorbed radioactivity

The methods employed for the extraction and analysis of [^{14}C] chlortoluron and its metabolites have been described previously (Ryan et al, 1981).

Sub-cellular organelle preparation

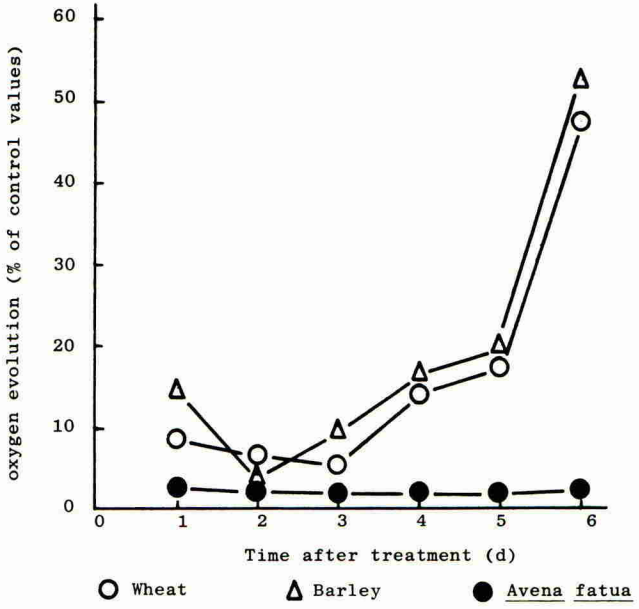
Harvested plants were homogenised with 5 ml/gm fresh weight of a buffer containing sorbitol, 0.3 M; KCl, 0.01 M; tricine, 0.05 M, pH 7.3, in an atomix blender (MSE, U.K.) operated at maximum speed for 10 s. The resulting homogenate was filtered through two layers of cheesecloth and the filtrate centrifuged at 200 xg for 5 min. Chloroplast, mitochondrial and microsomal fractions were sedimented from the 200 xg supernatant by centrifugation at 1500 xg (10 min), 9000 xg (20 min) and 100 000 xg (80 min) respectively. Each fraction was washed with homogenising buffer (10 ml) and re-sedimented prior to lyophilisation and extraction of associated radioactivity.

RESULTS

Since it is well documented that the phenylureas exert their phytotoxicity through potently inhibiting photosynthetic electron transport (Good, 1961; Moreland & Hill, 1963) our initial studies concentrated on photosynthetic phenomena. However, a preliminary comparison of the in vitro effect of chlortoluron on photosynthetic electron transport in chloroplasts from wheat, barley, A.fatua and A.myosuroides showed no significant difference in pI_{50} values (Ryan, 1981). In contrast to other types of herbicide action, inhibitory effects on photosynthesis can be assessed by measuring

Fig. 1

Recovery of oxygen evolution by leaf segments from plants treated with chlortoluron (3.1mg/l)



gas exchange. Fig. 1 presents data for the rates of oxygen evolution by leaf discs of susceptible and resistant species obtained at various time intervals following an initial 24 h treatment of the plants with chlortoluron (3.1 mg/1). The onset of inhibition of oxygen evolution was rapid in all three species examined, indicating effective absorption and translocation of the herbicide. After removal of the plants to fresh nutrient solution not containing herbicide, oxygen evolution continued to decrease over the subsequent 24 h period probably due to herbicide already within the apoplast being transported to the chloroplasts and reinforcing inhibition. Subsequently, over the next four days, there was a rapid and parallel recovery of oxygen evolution capacity in both cereals to some 50% of control values. In contrast A.fatua showed no recovery over this period.

The data in Fig. 1 were indicative of an effective detoxification mechanism for chlortoluron in wheat and barley. The ability of such leaf discs from the cereals and A.fatua to metabolise chlortoluron was assessed over a 24 h period following a pretreatment with [carbonyl-^{14}C]chlortoluron. The results obtained (Table 1) indicated that metabolism occurred in the case of all three species, N'-(3-chloro-4-methylphenyl)-N-methylurea (I) and N'-(3-chloro-4-hydroxymethylphenyl)-N,N-dimethylurea (II) being the only detectable metabolites. A quantitative difference was shown to exist between the species with respect to chlortoluron metabolism, the parent herbicide being reduced to 53 and 57% respectively of the extracted radioactivity 24h after treatment in the case of wheat and barley, and to 72% in A.fatua. Of particular interest, however, was the finding that there was also a qualitative difference in metabolism between wild oat and the cereals. Whereas in wheat and barley degradation occurred predominantly by ring-methyl oxidation to give (II), in A.fatua this pathway is of only minor importance and metabolism was essentially restricted to N-demethylation which gave rise to (I) as the major metabolite.

Table 1

Quantitative metabolism of chlortoluron by leaf discs

Species	Time from cessation of treatment (h)	Chlortoluron	Metabolite I	Metabolite II
Wheat	0	98.1	N.D.	1.0
	8	82.5	5.9 (2.1)	11.6 (4.1)
	24	52.6	12.2 (5.5)	34.9 (12.3)
Barley	0	98.0	N.D.	0.8
	8	82.7	3.6 (1.2)	12.3 (3.4)
	24	57.3	11.2 (5.3)	29.3 (11.4)
A.fatua	0	97.3	0.9	N.D.
	8	86.2	10.9 (0.7)	1.8 (0.5)
	24	72.0	21.3 (2.6)	5.4 (1.2)

The values given are the percentages of the total radioactivity found in each metabolite at the various harvest times. Values in parenthesis are the amounts of each metabolite present as conjugates. N.D. = not detectable.

The results obtained in a more extensive study of chlortoluron metabolism are shown in Table 2 which also includes data for A.myosuroides. Radio-labelled herbicide was applied to the soil in amounts corresponding to field application rates of 1.5 and 0.75 kg a.i./ha for cereals and weeds respectively. At these rates chlortoluron caused no phytotoxicity to the plants so that metabolic activity was expected to be normal. These data confirm the results of the short-term study with leaf discs in that the overall degradation of chlortoluron correlated well with the susceptibility of the plant, the herbicide being more efficiently degraded in the tolerant wheat and barley than in sensitive Alopecurus and Avena. Chlortoluron was shown to be degraded by both N-demethylation and ring-methyl oxidation pathways in all four species resulting in the formation of a variety of ring-methyl derivatives with various degrees of N-demethylation. Metabolites detected in addition to (I) and (II) were N'-(3-chloro-4-methylphenyl)urea (III), N'-(3-chloro-4-hydroxymethylphenyl)-N-methylurea (IV), N'-(3-chloro-4-carboxyphenyl)-N,N-dimethylurea (V), N'-(3-chloro-4-carboxyphenyl)-N,-methylurea (VI) and N'-(3-chloro-4-hydroxymethylphenyl)urea (VII). Metabolites (III) and (VII) were detected only in A.fatua which did not form (VI). The data in Table 2 again reveal qualitative differences between the cereals and grassweeds. Ring-methyl oxidation derivatives predominated in the non-polar metabolite fraction of wheat and barley, (II) being the major metabolite. In contrast, in Alopecurus and Avena

Table 2

Degradation products of chlortoluron in treated plants

Species	Chlor-toluron	Metabolite fraction I	II	III	IV	V	VI	VII	Un-known	Non-extract-able
Wheat	22.2	2.9 (0.8)	54.3 (50.7)	N.D.	12.4 (11.8)	2.2 (0.7)	2.9 (0.8)	N.D.	-	3.1
Barley	29.3	6.1 (1.6)	39.3 (33.3)	N.D.	13.0 (11.5)	7.0 (5.8)	1.5	N.D.	-	3.7
A.fatua	38.7	24.7 (2.1)	7.0 (4.2)	5.3 (3.4)	8.8 (6.7)	1.7	-	1.1 (1.1)	6.1	6.6
A.myo-suroides	39.9	18.9 (13.1)	22.2 (20.3)	N.D.	6.6 (5.3)	0.4	1.0 (0.4)	N.D.	1.2	9.8

Values are given as the percentage of total radioactivity found in whole plants after 10 days of treatment. N.D. = not detectable

N-demethylation activity predominated over ring-methyl oxidation. In addition, varying proportions of most metabolites were also extracted as polar conjugates. Of particular significance is the high percentage of metabolite (II) released by β-glucosidase from the conjugate fraction from wheat and barley.

The significance of the enhanced ability of wheat and barley compared to the grassweeds to oxidise the ring-methyl group of chlortoluron is apparent from the data presented in Table 3 which compares the abilities of the various metabolites to inhibit the Hill activity of isolated chloroplasts. All metabolites involving an oxidation at the ring-methyl position were essentially non-inhibitory. Though N-didemethylated chlortoluron was also without effect on Hill activity, this metabolite has little relevance to the interpretation of the present data in that it was only detected in A.fatua where in the free form it constituted 1.9% of the total radioactivity (Table 2). In contrast, however, a considerable ability to inhibit photosynthesis was retained in the N-monodemethylated derivative (I) which caused an inhibition which was only some 10-18% less than for the parent herbicide.

TABLE 3

Effect of chlortoluron and its metabolites on the Hill reaction of isolated chloroplasts

Source of chloroplasts	Chlortoluron	Metabolites					
		I	II	III	IV	V	VI
Wheat	53	67	92	93	101	97	96
Barley	53	62	92	94	102	97	98
A.fatua	50	66	93	96	98	98	94

Results are expressed as a percentage of control values, the concentration of each metabolite being equal to the chlortoluron I_{50} value.

In the course of the metabolism studies it was noted that the total radioactivity extractable from plants of all four species was similar despite the fact that because of their greater sensitivity, the grassweeds were treated with half the quantity of [^{14}C]herbicide applied to the cereals. Since the implication from these observations was that the grassweeds absorbed chlortoluron more effectively, an experiment was designed to examine uptake more specifically. Comparison of the results obtained for wheat, barley and A.fatua (Table 4) indicated a very substantial difference in uptake, there being a greater amount of radioactivity present in the wild oat at all harvest times. This difference is particularly striking at short times after treatment, when the amount of chlortoluron absorbed by A.fatua can be as much as 4-5 fold greater than for wheat and barley. Radioautographs prepared from plants at each harvest time indicated that for each species the absorbed radioactivity was translocated to all plant parts.

TABLE 4

Uptake of [^{14}C]chlortoluron by plants grown in nutrient culture (μg/g fresh weight)

Time (days)	Wheat	Barley	A.fatua
0.5	5.1	4.2	19.7
2	14.1	11.4	33.3
6	23.0	26.7	51.5

The initial concentration of chlortoluron in the nutrient solution was 3.1 mg/l for all plants

Such differences in uptake are only of significance, however, where they can be shown to result in similar differences in the amounts of phytotoxic material associated with the site of action in the plants. The results of a study on the intracellular distribution of phytotoxic compounds in wheat and wild oat following uptake of

[^{14}C]chlortoluron by soil-grown plants are given in Table 5. It is apparent from these data that the 2.5 fold greater amount of phytotoxic material recovered from homogenates of A.fatua compared to wheat is reflected in similar differences between most sub-cellular fractions. However, the largest difference (some 3 fold) amongst the particulate organelle fractions occurred between chloroplasts.

Table 5

Intracellular distribution of phytotoxic compounds in wheat and A.fatua

Sub-cellular fraction	% Phytotoxic material[a]	
	wheat	A.fatua
Crude homogenate	33.0	84.0
Debris	6.5	12.1
Chloroplasts	6.5	18.7
Mitochondria	4.9	12.4
Microsomes	0.9	2.1
Post-microsomal supernatant	14.2	38.4

a. calculated as the sum of the parent herbicide and its N-monodemethylated metabolite (I). The percentages given are of the total identified radioactivity/g tissue after 10 days treatment

DISCUSSION

The results of studies on the recovery of photosynthesis in leaf discs of wheat and barley subsequent to chlortoluron treatment correlated well with the capacity of these species to metabolise the herbicide. Essentially similar findings have been reported previously for related phenylureas in other plant species (Swanson & Swanson 1968 a & b; Eshel, 1969; van Leeuwen & van Oorschot, 1976). Probably as important as the overall rate of chlortoluron degradation in the cereals, however, is the fact that the major metabolites were products of ring-methyl oxidation. The data showing that such compounds were ineffective as Hill reaction inhibitors clearly indicate that the tolerance of wheat and barley has a sound biochemical basis. However, the observation that the N-monodemethylated metabolite of chlortoluron is also an effective inhibitor of photosynthesis implies that the greater contribution of N-demethylation to overall metabolism in A.fatua and A.myosuroides results in a less efficient detoxification mechanism in these species.

In both metabolism studies most metabolites were isolated in both free and conjugated forms. However, in the case of the short term study using leaf discs conjugated metabolites constituted a much smaller percentage of total extractable radioactivity implying that initially, rates of conjugation were slower than the primary degradative steps. All metabolism data presented for Avena fatua showed that this species possessed an apparently poor ability to conjugate metabolites, a phenomenon which might further contribute to selectivity.

The results of our studies on uptake of chlortoluron showed that compared to wheat and barley, A.fatua absorbed substantially larger amounts of the herbicide, this difference being especially pronounced at early times after treatment. A similar result was recorded previously (Shimabukuro et al, 1976) for barban uptake in wild oat and wheat. Whereas one day after treatment the wild oat contained significantly higher amounts of barban, at two days the uptake was similar in both species. This short time difference was considered important in selectivity because of the rapid inhibitory effects of barban.

The greater uptake of chlortoluron in A.fatua recorded in the present studies was reflected in higher amounts of phytotoxic material associated with subcellular organelle fractions, particularly chloroplasts, prepared from this species compared to wheat. Though care must be taken in the interpretation of such data due to the possibility of relocation of radioactivity during organelle preparation, our overall conclusion from the present studies is that the enhanced sensitivity of the grassweeds to

chlortoluron is based on a rapid and extensive inhibition of photosynthesis resulting from a faster rate of herbicide uptake in combination with a less effective detoxification mechanism.

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STUDIES INTO THE DIFFERENT RESPONSE OF THREE WEED SPECIES TO THE HYDROXYBENZONITRILES

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Summary. The chlorophyll content of three weed species, Matricaria inodora, Stellaria media and Viola arvensis were compared after treatment with 0, 0.28, 0.56, 1.12 and 2.24 kg a.i./ha ioxynil and bromoxynil salts. Bromoxynil caused a greater chlorophyll loss than ioxynil with M. inodora, whereas ioxynil caused greater loss in S. media. The chlorophyll content of V. arvensis was similarly reduced with both herbicides. In chloroplasts isolated from the three species ioxynil was 2-3 times more inhibitory than bromoxynil towards electron transport. Sigmoidal type inhibition curves occurred with DCPIP (dichlorophenolindophenol) as electron acceptor. It is proposed that the two binding sites of the hydroxybenzonitriles and the two electron acceptor sites of SiMo (silicomolybdate) resulted in biphasic inhibition curves which occurred with this electron acceptor. The greater inhibition of electron transport by ioxynil may contribute to the response of S. media. However other factors are involved in the response of M. inodora and V. arvensis. Hydroxybenzonitriles, Matricaria inodora, Stellaria media, Viola arvensis, binding sites, electron transport inhibition.

INTRODUCTION

Ioxynil and bromoxynil are postemergence contact herbicides which control a wide range of annual broad-leaved weeds in cereals (Carpenter et al., 1964). The selective action of ioxynil and bromoxynil may be partly due to a greater herbicide degradation in barley and wheat (Davies et al., 1968; Shafer and Chilcote, 1970). Somerville (1972) reported differences in the retention and penetration of ioxynil and bromoxynil, however, this alone could not explain the differential susceptibility of certain weed species to these herbicides.

This paper reports initial data in which the biochemical and physiological effects of ioxynil and bromoxynil salts were compared on three important weed species, Matricaria inodora, Stellaria media and Viola arvenis.

METHODS AND MATERIALS

Plants were grown in seed compost (J. Arthur Bowers) in 3 inch pots (spray experiment) or seed trays (chloroplast isolation) in a greenhouse. Plants were used for experimentation when 6-8 leaves had developed on the main shoot of S. media and 3-4 leaves had developed on the main shoots of M. inodora and V. arvensis.

Plants were sprayed with ioxynil Na or bromoxynil K salts in an experimental sprayer at rates equivalent to 0.28, 0.56 (field rate), 1.12 and 2.24 kg a.i. ha^{-1} and a volume rate of 200 l ha^{-1}. Spray retention was determined using an aqueous solution of tartrazine (Pallett and Caseley, 1980). Four replicate plants were used for each treatment and harvested 7 and 14 days after spraying. Plants were weighed and chlorophyll determined by the method of Arnon (1949).

Chloroplast fragments (Type E, Hall, 1972) were isolated from the three species. The following isolation conditions were employed to maintain optimum photochemical activity for 45-60 min:

(a) S. media and V. arvensis 5g of leaf tissue was homogenised in 20ml of media containing 50mM Tricine-NaOH buffer (pH 7.5); 300mM NaCl; 3mM $MgCl_2$ and 0.01% bovine serum albumin (BSA). After filtering through 4 layers of muslin, cell debri was removed by centrifugation at 1000g for 1 min. The supernatant was recentrifuged at 3000g for 10 min and the chloroplast pellet resuspended in media containing 5mM Tricine -NaOH buffer (pH 7.5); 100mM sucrose; 3mM $MgCl_2$; 2mM EDTA and 0.1% BSA. All procedures were carried out at 4°C.

(b) M. inodora 5g of leaf tissue was homogenised in 20ml media containing 50mM Tricine-NaOH buffer (pH 7.5); 300mM NaCl; 3mM $MgCl_2$; 2mM EDTA; 0.01% BSA and 0.1% polyvinylpyrrolidone. After filtering through 8 layers of muslin and centrifugation at 3000g for 1 min the pellet was immediately resuspended in the above media.

Photosystem II activity was monitored by measuring O_2 evolution in an O_2 electrode (Hansatec Limited). The reaction chamber was maintained at 20°C and illuminated by a projector lamp giving 3000μE $m^{-2}s^{-1}$ at the electrode. The reaction medium contained 0.3ml 300mM Tricine NaOH buffer (pH 8.0); optimum concentrations of either, 10mM dichlorophenolindophenol (DCPIP) (M. inodora, 75μl; S. media, 40μl; V. arvensis, 100μl) or silicomolybdate (SiMo), (M. inodora, 50μl; S. media, 50μl; V. arvensis, 75μl); chloroplasts equivalent to 100μg chlorophyll; variable concentrations of pure ioxynil Na or bromoxynil K salts; and water to 3ml.

RESULTS

A major symptom of the hydroxybenzonitriles is chlorosis of treated foliage (Carpenter et al., 1964). This is shown in Figure 1, which presents the chlorophyll content of plants treated with ioxynil and bromoxynil. S. media appeared least affected by the two herbicides, whereas M. inodora showed considerable chlorophyll loss at the 0.28 kg a.i./ha doses. Chlorophyll loss was greatest with bromoxynil in M. inodora, compared with ioxynil. Ioxynil was more effective in S. media and both herbicides caused similar effects in V. arvensis. Similar responses to the two herbicides were apparent from fresh and dry weight values (data not presented).

The spray retention values for the three species were: M. inodora, 540.6μl/g Dry Wt. (7.9μl/plant); S. media, 645.6μl/g Dry Wt. (23.9μl/plant); and V. arvensis, 986.9μl/g Dry Wt. (18.0μl/plant). These values cannot explain the results shown in Figure 1.

The primary site of action of the hydroxybenzonitriles is well established as an inhibition of chloroplast electron transport (the so-called Hill reaction). This inhibition can be monitored with isolated chloroplasts using artificial electron acceptors such as DCPIP and SiMo. Figure 2 shows inhibition curves of ioxynil and bromoxynil salts with chloroplast fragments isolated from the three weed species and DCPIP and SiMo as electron acceptors. Ioxynil was more inhibitiory than bromoxynil in all three weed species. With DCPIP as electron acceptor the inhibition curves were sigmoidal, however with SiMo they were biphasic.

The I_{50} values calculated from Figure 2 are shown in Table 1. They are similar in the three species for ioxynil however the values for bromoxynil are lower for M. inodora, particularly with SiMo as electron acceptor.

DISCUSSION

The loss in chlorophyll following treatment with herbicides that inhibit electron transport is due to photoxidative processes (Pallett and Dodge, 1980). When electron transport is prevented, light energy absorbed by chlorophyll in the photosystems cannot drive electron transport and unless channelled elsewhere, chlorophyll destruction will occur. Carotenoids, particularly β-carotene, can dissipate some of this energy harmlessly, however when electron transport is inhibited the carotenoid system becomes overloaded and chlorophyll breakdown and toxic species, such as singlet oxygen are generated, which lead to chloroplast and subsequently cell

Fig. 1

The chlorophyll content of plants 7 (□) and 14 (▨) days after treatment with ioxynil Na or bromoxynil K salts at doses equivalent to 0, 0.28, 0.56, 1.12 and 2.24 kg a.i. /ha.

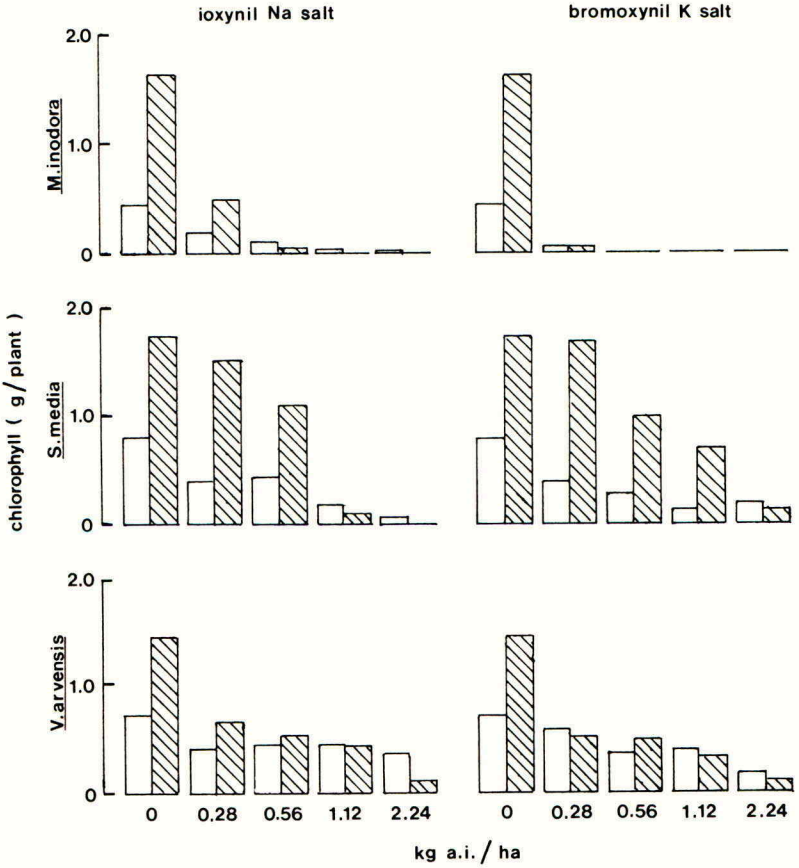


Table 1

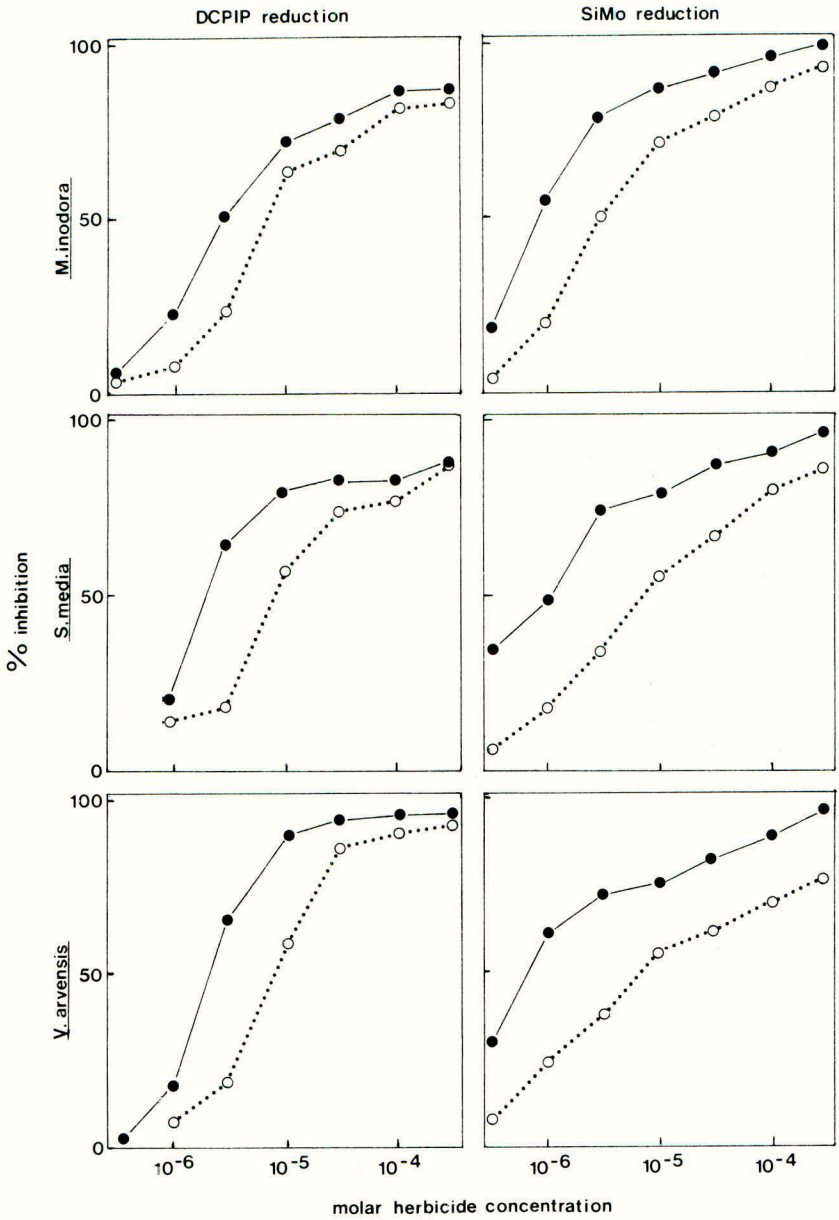
The concentration of herbicide necessary to give 50% inhibition of electron transport (I_{50}) with DCPIP and SiMo as electron acceptors

(determined from Figure 2)

	I_{50} value (μM)					
	Ioxynil			Bromoxynil		
	M. inodora	S. media	V. arvensis	M. inodora	S. media	V. arvensis
DCPIP	2.6	2.2	2.0	6.8	8.3	9.0
SiMo	0.9	1.1	0.7	3.5	8.3	7.3

Fig. 2

The effect of ioxynil Na (●) and bromoxynil K (○) on oxygen evolution from chloroplast fragments with DCPIP and SiMo as electron acceptors.



destruction. In addition the activity of ioxynil may be enhanced by free radicals produced by the breakdown of ioxynil in the leaf (Zaki et al., 1967; Pallett, 1978).

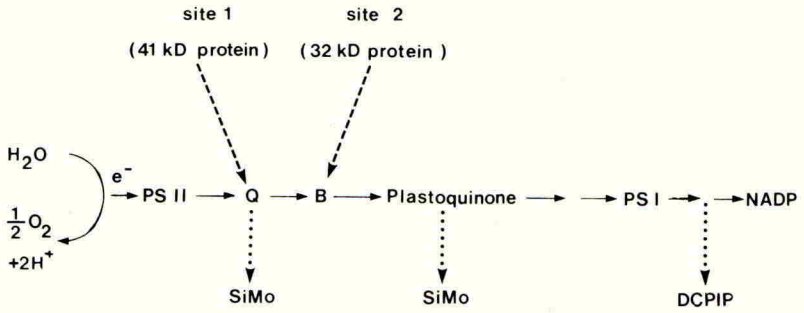
The degree of electron transport inhibition is obviously an important factor in the development of herbicide symptoms. The wide range of herbicides that inhibit electron transport vary considerably in their effectiveness as electron transport inhibitors (Moreland, 1969). Resistance that has developed in recent years, to the triazine class of herbicides is due to a greatly reduced ability to bind to, and inhibit electron transport (Pfister and Arntzen, 1979).

In all three weed species ioxynil was a more effective inhibitor of electron transport (Fig. 2 and Table 1). This supports previous reports that the inhibition of electron transport by the hydroxybenzonitriles depends on substitution and decreases in the order I>Br>Cl (Trebst et al., 1979).

The binding site for photosynthetic inhibitor herbicides is well established as a proteineous component situated between Q and plastoquinone. This site was believed to be common for all classes of inhibitor herbicides, however techniques, including trypsin treatment of isolated chloroplasts, binding studies and photoaffinity labelling have revealed two binding sites (Pallett and Dodge, 1979; Oettmeier et al., 1982) Site 1, a 41kD protein probably associated with Q and the PSII reaction centre which binds only phenolic type herbicides (hydroxybenzonitriles) and site 2, a 32-34 kD protein possibly associated with B which can bind the DCMU-type herbicides (ureas, triazines, uracils) and the phenolic type compounds (Fig. 3). Phenolic inhibitors

Fig. 3

A scheme showing the proposed herbicide binding sites and electron acceptor sites. For explanation see Text.



can displace DCMU-type inhibitors and vice versa and therefore the two sites are likely to be closely associated. It has been proposed that the 32-34 kD protein is located on the surface of the thylakoid membrane, and is more accessible, with the 41 kD protein hidden underneath, and consequently less accessible (Oettmeier et al., 1982).

The biphasic nature of the inhibition with SiMo as acceptor (Figure 2) may be explained by the two binding sites of the herbicides and two electron acceptor sites of SiMo (Figure 3). Site 2 will have a higher affinity for the herbicide than Site 1 because of its location on the membrane surface. At lower herbicide concentrations

electron acceptance of SiMo from plastoquinone will only be inhibited, however at higher concentrations acceptance from both Q and plastoquinone will be inhibited (Fig. 3). Inhibition of DCPIP reduction will occur irrespective of which binding site is occupied.

The I_{50} values would be expected to be the same for the two electron acceptors or possibly higher values would be necessary for SiMo. However, the values for SiMo were 2-3 times lower than DCPIP for ioxynil (Table 1). A possible explanation for this may be that the close proximity of the two binding sites with the electron acceptor sites may lead to an interaction of ioxynil with SiMo rendering its reduction more sensitive to this herbicide.

In conclusion ioxynil is the more effective inhibitor of electron transport. This may contribute to the greater symptoms induced by this compound with S. media, however other factors such as penetration, translocation and metabolism must be responsible for the different responses of M. inodora and V. arvensis. These factors are currently under investigation.

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AC 222,293 - TRANSLOCATION AND METABOLIC SELECTIVITY

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Summary: Radiotracer studies showed that AC 222,293 is both xylem and phloem mobile. Metabolic studies indicate that this herbicide is extensively metabolized by wheat, Avena fatua, Alopecurus myosuroides and Brassica kaber, but the pattern of metabolites produced differ among the species. The herbicidal activity of the two positional isomers that compose AC 222,293 are different. The p-isomer, AC 239,589, is more active on B. kaber and less active on A. fatua and A. myosroides than the m-isomer, AC 252,767. The metabolic selectivity of the isomers of AC 222,293 appears to be determined by the level of the free acids formed from the parent esters.

Postemergent cereal herbicides, Alopecurus, Avena, Brassica, metabolism.

INTRODUCTION

AC 222,293 is a new postemergent herbicide being developed by American Cyanamid for the control of Avena spp., Alopecurus myosuroides and certain dicotyledonous weeds in wheat and barley. The general herbicidal properties of AC 222,293 based on greenhouse and field results are described by Shaner, et. al. (1982) and Kirkland and Shafer (1982).

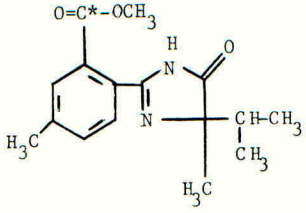
AC 222,293 is a mixture of two different chemicals, methyl 6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-m-toluate (AC 252,767) and methyl 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-p-toluate (AC 239,589). The herbicidal properties of these two positional isomers may not be identical. In order to understand better the herbicidal activity of AC 222,293, investigations were carried out to study the absorption, translocation, and metabolism of AC 222,293 and its component isomers, respectively.

METHODS AND MATERIALS

Chemicals and plant material: All studies were preformed with compounds that were labeled in the carboxyl carbon on the benzene ring (Figure 1). The plants used were in the Zadocks 13 growth stage. The wheat was a hard red spring wheat, variety Era.

Structural formulae:

a.)



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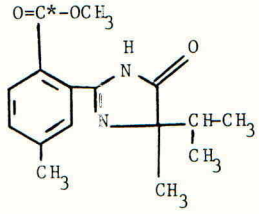

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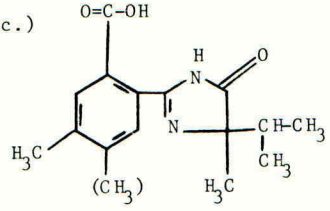

Figure 1: a. AC 252,767 b. AC 239,589
a+b. AC 222,293 c. AC 263,840
*^{14}C-Label

Translocation:

1. Leaf applied: ^{14}C-labeled AC 222,293 (20 uCi/ml, specific activity 20 uCi/mg) was dissolved in H_2O with 0.25% DM710 surfactant. Twenty droplets (0.5 ul/droplet) were applied to the first leaf of either wheat or A. fatua. Plants were harvested 3 days after treatment, divided into the treated leaf and the remainder of the shoot and the roots, pulverized with dry ice, lyophilized, and burned with a biological oxidizer. The treated leaf was washed in 20 ml of H_2O:MeOH (9:1) before being pulverized. To identify the chemical nature of the radioactivity, extraction and identification procedures were used as described in the metabolism section.

2. Root applied: A. fatua and wheat were grown hydroponically in 0.1 strength Hoagland's solution. ^{14}C-labeled AC 222,293 (0.5 uCi/plant) was added to the rooting media and the plants allowed to take up the radiolabeled compound for 24 hours. After this time period the plants were transferred to fresh media with no radiolabeled compound. Plants were harvested 3 days after treatement and the distribution of the ^{14}C-activity was determined as previously described.

Metabolism studies: The metabolic profiles of AC 222,293, AC 239,589, and AC 252,767 were determined either by applying ^{14}C-labeled material to the roots or leaves of intact plants as described for AC 222,293 above or by using excised leaves and supplying the radiolabeled compound to the leaf via the transpiration stream. In the latter case, 0.5 uCi of AC 239,589 (115 uCi/mg) or AC252,767 (141 uCi/mg) was supplied to the leaf over a 17 h period after which the leaf was transfered to fresh solution with radiolabeled material for an additional 48 hours. After that time period, the leaves were extracted in MeOH:1% Acetic Acid (9:1). After centrifugation an aliquot of the supernatant was applied to reverse-phase TLC plates. The plates were developed with MeOH:1% Acetic Acid (60:40), air-dryed, and exposed to X-ray film to determine the location of the radiolabel. On all plates 5 ug of the parent ester (AC 222,293) and its free acid (AC 263,840) were also applied as separate spots to act as reference points. Radioactive spots that co-chromatographed with the ester and the acid were positively identified by mass spectroscopy.

Herbicidal efficacy: The herbicidal efficacy of the positional isomers and the free acid was determined by procedures described in Shaner et al. (1982).

All tests were replicated 3 to 5 times and repeated at least once.

RESULTS

Radioactivity could be detected throughout the plants when ^{14}C-AC 222,293 was applied to the root system of either species, with apparent accumulation in the foliage. There were no obvious differences in the pattern of translocation of radioactivity between the two species (Table 1). When ^{14}C-AC 222,293 was applied to a single leaf, a small amount of radioactivity was translocated out of the treated leaf, nevertheless three times more radioactivty was translocated out of the A. fatua leaf as translocated out of the wheat leaf.

Table 1

Absorption and distribution of ^{14}C-activity from ^{14}C-AC 222,293 three days after application

Species	Site of application	Absorption (%)	Distribution of ^{14}C-activity (% of absorbed)		
			Root	1st Leaf	2nd Leaf
Wheat	Root	100	7.0	66	27.0
A. fatua	Root	100	9.0	51	40.0
Wheat	Leaf	90	0.4	99[a]	0.4
A. fatua	Leaf	75	0.3	97[a]	2.7

[a] Treated Leaf

Metabolism: Several bands of radioactivity were apparent in extracts from both species. Only two bands were positively identified, the parent esters, AC 222,293, and the acids, AC 263,840, formed from the esters. Table 2 shows that both wheat and A. fatua can metabolize AC 222,293. However, the pattern of metabolism was quite different, especially in the tissue to which the radioactivity was translocated. The level of the acids derived from AC 222,293 was much higher in A. fatua than in wheat, particularly when the herbicide was applied to the leaves. The level of these acids may determine the selectivity of AC 222,293. When the acids were sprayed onto wheat and A. fatua, they were herbicidally active but were not selective on the wheat (Table 3).

Table 2

Metabolic profile of ^{14}C-activity from ^{14}C-AC 222,293 in wheat and A. fatua three days after application

Species	Site of application	Plant part	Distribution of radioactivity (%) Esters[a]	Acids[b]	Other metabolites[c]
Wheat	Root	Root[d]	-	-	-
		Shoot	52.5	6.6	40.9
A. fatua	Root	Root[d]	-	-	-
		Shoot	69.6	20.4	10.0
Wheat	Leaf	Treated leaf	52.9	< 0.1	47.1
		Rest of plant	37.0	< 0.1	63.0
A. fatua	Leaf	Treated leaf	71.4	8.7	19.9
		Rest of plant	48.3	51.7	0.0

[a] AC 222,293 - methyl esters
[b] AC 263,840 - acids formed from AC 222,293
[c] Unidentified metabolites
[d] Not enough ^{14}C-activity was extracted to characterize

Table 3

Comparison of herbicidal efficacy of AC 222,293 and its postional isomers

Compound	Control rate (kg/ha)			Safe rate (kg/ha)	
	A. fatua	A. myosuroides	B. kaber	Wheat	Barley
AC 222,293	0.60	0.60	0.4	4.0	4.0
CL 239,589[a]	2.00	2.00	0.3	4.0	4.0
CL 252,767[b]	0.40	0.40	0.4	4.0	4.0
CL 263,840[c]	0.25	0.25	0.1	< 0.1	< 0.1

[a] p-isomer in AC 222,293
[b] m-isomer in AC 222,293
[c] Acids of AC 222,293

AC 222,293 is composed of two positional isomers. Since these are two different chemicals, their herbicidal activity might also be different. AC 239,589, the p-isomer, was not as active as the m-isomer, AC 252,767, on A. fatua or A. myosuroides, but was more active on B. kaber (Table 2). Both isomers were selective on wheat. A metabolic profile was determined for each positional isomer in each species. In 3 of the 4 species the level of the free acid formed from the parent ester correlated with the herbicidal activity of the positional isomer, the higher the level of the free acid, the more active the isomer (Table 4). The only species for which this relationship did not hold was A. myosuroides in which the level of the free acid was higher with the p-isomer but the m-isomer was more active. However, the level of the acid from AC 222,293 from either positional isomer was much higher in A. myosuroides than in the wheat.

Table 4

Metabolic profiles for positional isomers of AC 222,293 in an excised leaf system[a]

Compound	Species	Days after application	Distribution of radioactivity (%) Ester	Acid	Other metabolites
CL 239,589	Wheat	1	76.3	2.5	21.2
		3	67.7	2.7	29.6
	A. fatua	1	70.6	4.8	24.6
		3	44.5	4.4	51.1
	A. myosuroides	1	49.7	28.0	22.3
		3	33.9	25.4	40.7
	B. kaber	1	77.5	11.3	11.2
		3	63.4	15.6	21.0
CL 252,767	Wheat	1	14.0	3.1	82.9
		3	9.0	2.8	88.2
	A. fatua	1	35.4	9.6	55.0
		3	26.0	6.9	67.1
	A. myosuroides	1	58.1	8.0	33.9
		3	37.7	8.9	53.4
	B. kaber	1	47.4	2.6	50.0
		3	28.5	4.8	66.7

[a] 0.5 uCi of either isomer was supplied to excised leaves via the transpiration stream.

DISCUSSION

Radiotracer studies have shown that AC 222,293 is both xylem and phloem mobile based on radiotracer studies. The selectivity of AC 222,293 on wheat cannot be attributed to a difference in the pattern of translocation of the herbicide within wheat and A. fatua. However, there is a difference in the metabolism of AC 222,293 between A. fatua and wheat in that the level of the free acids formed from AC 222,293 is much higher in A. fatua than in wheat. Furthermore, when the two positional isomers that make up AC 222,293 are separated, the herbicidal activity is different, and the difference is correlated with the level of free acid formed in three of the four species tested. The data strongly suggest that the mechanism of activity and selectivity of AC 222,293 is similar to that found for other wild oat herbicides such as dichlofop methyl (Shimabukuro et al., 1979), flamprop isopropyl (Jeffcoat and Harries, 1975) and benzoylprop ethyl (Hill et al., 1978) in that the active herbicide is the acid formed from the applied ester. The tolerance of wheat to each of these herbicides is due to the low level of the acid that is produced in the crop as compared to the higher level produced in the susceptible weed. This relationship appears to hold not only for A. fatua, but also for A. myosuroides and B. kaber for AC 222,293. However, the difference in the herbicidal efficacy of the two positional isomers that make up AC 222,293 cannot be fully explained by the level of the acid formed in A. myosuroides. There is more acid formed with the p-isomer, but the m-isomer is more herbicidal. There are apparently other differences in the behavior of these two isomers in A. myosuroides that relate to their herbicidal activity.

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MODE OF ACTION AND METABOLIC FATE OF THE HERBICIDE FENOXAPROP-ETHYL, HOE 33171

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Summary. The post-emergence herbicide fenoxaprop-ethyl (Hoe 33171) caused rapid destruction of plant meristems. Though only about 1 % of the foliar applied compound was translocated, it had systemic effects on Sorghum halepense. Maize leaf discs fed with ^{14}C-labeled acetate and treated with 5×10^{-5} M fenoxaprop-ethyl incorporated considerably less radioactive material into lipids than untreated leaf discs. In plants the herbicide was rapidly degraded to polar products. In seeds of soybean and rape radioactivity from one fenoxaprop-ethyl-^{14}C application was not detectable (Quantification limit for the radiotracer method 0.005 µg/g). After oral administration to rats, the ^{14}C-labeled compound was completely absorbed, rapidly converted to polar metabolites and excreted via urine and faeces in about equal amounts. In different soils rapid saponification of fenoxaprop-ethyl-^{14}C was followed by complete degradation with evolution of 10 - 20 % $^{14}CO_2$ during a period of 32 days.
Fenoxaprop-ethyl, uptake, translocation, physiological effects, metabolism, soil, plant, rat.

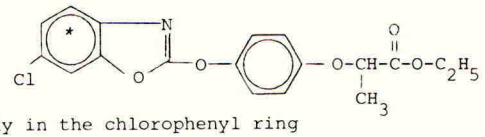
INTRODUCTION

The herbicide ethyl-2-(4-(6-chloro-2-benzoxazolyloxy)-phenoxy)-propanoate (fenoxaprop-ethyl, Hoe 33171) selectively controls a broad spectrum of grass weeds in dicotyledonous crops, and is predominantly applied post-emergence (Bieringer et al., 1982).

It is our objective to review the present state of knowledge on the mode of action of this herbicide and its metabolic fate in plants, soil and warm-blooded animals.

Fig. 1

Chemical structure of fenoxaprop-ethyl



*^{14}C-label uniformly in the chlorophenyl ring

Herbicide symptoms

Leaves of susceptible grass species treated with fenoxaprop-ethyl showed a reduction in growth within one day after treatment, followed by the appearance of chlorotic zones on the blades of the youngest leaves. Leaf growth was completely arrested usually after three days, as was the growth of secondary roots. These symptoms were followed by a discoloration of the intercalary and apical meristems of the shoot. Between five and seven days after treatment leaf necrosis developed. Complete death of the plant usually occured after 2 weeks.

In laboratory experiments with grass seedlings grown in aqueous herbicide solutions, fenoxaprop-ethyl had a strong inhibitory activity on root growth. A concentration of 10^{-7} M gave 50 % inhibition of root growth of Setaria italica seedlings. The free acid fenoxaprop had approximately the same level of activity in this test system.

Uptake, translocation and systemic effects

Plants were grown in the greenhouse at 24 - 26°C (day), 16 - 18°C (night) and an average of 60 % r. h. Sorghum halepense plants were raised from seeds, unless otherwise mentioned and taken for the experiments in the four-to five-leaf stage, and soybean plants were taken when one trifoliate leaf had developed. For tracer studies, ^{14}C-labeled fenoxaprop-ethyl was used (Fig. 1) with a rediochemical purity of > 99 % and a specific activity of 973 MBq/g, synthesized by Dr. Ganz, Radiochemical Laboratory Hoechst AG. Both, labeled and unlabeled active ingredient were formulated as a 12 % e. c. and applied to the plant surface by microsyringe. Each treatment consisted of five to six replicates. Tracer studies were evaluated by liquid scintillation counting and autoradiography.

Foliar penetration was studied by applying 15 µl ^{14}C-labeled herbicide solution (0.2 % a.i.) on the adaxial surface of the fourth leaf (blade) of Sorghum halepense. Radioactive material which could not be removed from the plant by 3 consecutive short washes with dichloromethane at the end of the experimental period, was defined as having penetrated. One day after treatment, about 35 % had penetrated, and 85 % after 7 days. Only 0.75 % of the total radioactive material was translocated from the treated leaf blade to other parts of the plant (Table 1).

Table 1

Foliar uptake of ^{14}C-labeled fenoxaprop-ethyl

DAT	Percentage of total radioactivity		
	In treated leaf	In leaf washes	Translocated
1	34.5 ± 12.5	65.3 ± 12.3	0.19 ± 0.03
7	84.5 ± 3.9	14.7 ± 3.8	0.75 ± 0.30

The distribution of the translocated material in the different plant parts was examined by applying 10 µl ^{14}C-labeled herbicide solution (20 µg a.i. per plant) adaxially to the third leaf of Sorghum halepense, either on the proximal or the distal half of the blade. Plants grown from both seeds and rhizome pieces were treated. Proximal application resulted in more radioactive material in the nontreated plant parts than did distal application.

Total translocation rates were low, 2.5 % after seven days being the highest value found (Table 2). Chemical identification of the translocated radioactive material was not attempted.

Soybean, a plant species highly tolerant to fenoxaprop-ethyl, showed a translocation rate and pattern similar to Sorghum halepense (Table 2).

Table 2

Translocation (%) of foliar applied ^{14}C-labeled fenoxaprop-ethyl

Species	Site of application on leaf blade	DAT	Blade of treated leaf*	Rest of lower	shoot upper	Roots/ rhizomes	Total
S. halepense	proximal	3	0.79	0.18	0.07	0.10	1.14
		7	1.50	0.16	0.11	0.08	1.85
(from seeds)	distal	3	0.22	0.13	0.02	0.05	0.42
		7	0.39	0.11	0.07	0.05	0.62
S. halepense	proximal	7	0.89	0.83	0.25	0.55	2.52
(from rhizomes)	distal	7	0.55	0.32	0.08	0.21	1.16
Soybean	center	3	-	0.48	0.29	0.07	0.84
		7	-	0.77	0.59	0.08	1.44

* tip of blade after proximal, base of blade after distal treatment

The biological effect of selective placement on Sorghum halepense was examined by treating the adaxial surface of the third leaf with 5 µl herbicide solution on the distal or proximal half of the blade or the abaxial suface of the leaf sheath. Contact of the meristematic areas with the treatment solution was prevented.

After 12 days the best systemic effects were found after leaf sheath application; 5 µl of 0.05 % (a.i.) herbicide solution caused 100 % plant damage. The same dosage resulted in 60 % damage after application to the proximal half of the blade, and 12 % damage when applied to the distal half of the blade. With a 0.2 % (a.i.) solution, 100 % plant damage was obtained after application to the proximal and 52 % after application to the distal half of the blade (Table 3).

Table 3

Influence of placement on systemic action of fenoxaprop-ethyl

Site of placement	Concn of a.i. (%)	Damage rating (%)	Fresh wt/plant (g)
Distal half of leaf blade	0.20	52	0.48 ± 0.11
	0.05	12	2.02 ± 1.42
Proximal half of leaf blade	0.20	100	0.27 ± 0.03
	0.05	60	0.45 ± 0.05
Leaf sheath	0.20	100	0.23 ± 0.05
	0.05	100	0.27 ± 0.09
Untreated, intact	-	0	2.09 ± 0.23
Untreated, third leaf removed	-	0	2.00 ± 0.26

This result shows that despite the low rate of basipetal transport sufficient physiologically active material reached the meristematic areas at the shoot base to cause the death of the plant. It also shows that the distance between the site of herbicide deposition on the leaf and the meristems at the shoot base is inversely related to the systemic activity.

To determine the translocation period which is required to obtain a systemic effect, Sorghum halepense was treated on the blade of the third leaf with 5 µl of herbicide solution (0.2 % a.i.).
Removal of the treated leaf at 24 h after application resulted in no systemic effect within a two week period. However, removal at 72 h after application resulted in a systemic effect comparable to plants with the treated leaf remaining attached throughout the two week period of the experiment (Table 4).

Table 4

Systemic action of fenoxaprop-ethyl after removal of treated leaf

Treatment		Damage rating (%)	Fresh wt/plant (g)
Treated leaf removed	0 DAT	0	2.66 ± 0.78
	1 DAT	0	2.43 ± 0.35
	3 DAT	95	0.16 ± 0.03
Treated leaf not removed		100	0.19 ± 0.07
Untreated control		0	2.32 ± 0.93

Physiological and biochemical effects

Sorghum halepense plants which received a spray treatment with fenoxaprop-ethyl showed, after five days treatment, a dose-dependant reduction in chlorophyll content. In contrast, the content of free amino acids and soluble sugars markedly increased in the shoots of treated plants. These effects were also present at the lowest dosage of 25 g a.i./ha. However, the protein content was not influenced by fenoxaprop-ethyl (Table 5).

Table 5

Relative concentrations of plant components in shoot

Treatment (g a.i./ha)	Free amino-acids	Soluble sugars	Protein	Chloro-phyll a	Chloro-phyll b
250	303 ± 20	239 ± 4	99 ± 1	53 ± 1	64 ± 1
100	247 ± 9	245 ± 8	98 ± 1	61 ± 2	67 ± 2
25	195 ± 12	170 ± 15	101 ± 3	78 ± 3	83 ± 4
Untreated	100 ± 6	100 ± 10	100 ± 3	100 ± 3	100 ± 3

Net CO_2-fixation, measured with the infra-red gas analyzer, initially showed a constant rate, followed by a sharp decline 4 days after spraying with fenoxaprop-ethyl. The rate of decline paralleled the increase in visible plant damage. The result suggests that inhibition of photosynthesis is only a secondary effect of this herbicide. The increase in free amino acids and soluble sugars appears to be a result of continued synthesis for several days after treatment and reduced utilisation resulting from rapid inhibition of plant growth.

The influence of fenoxaprop-ethyl on plant lipid synthesis was examined by incubating leaf discs from young maize in buffer containing the herbicide and acetate-^{14}C for a 4 h-period (Zacher, 1982). Lipid extracts of discs kept in buffer containing 10^{-4} M or 5×10^{-5} M fenoxaprop-ethyl had a considerably lower percentage of

^{14}C-labeled material in the lipophilic phase than extracts from herbicide-free controls (Table 6).

These results suggest an interference of fenoxaprop-ethyl with lipid synthesis. Investigations into the primary biochemical effects of this herbicide are continuing.

Table 6

Partitioning of radioactive material after feeding of ^{14}C-labeled acetate to maize leaf discs

Treatment	Percent of radioactive material in	
	Lipophilic phase	Water phase
Control	56.5	43.5
Fenoxaprop-ethyl 5×10^{-5} M	32.9	67.1
Fenoxaprop-ethyl 1×10^{-4}	22.8	77.2

DEGRADATION IN PLANTS

Kinetics

Treatment of the plants (soybean, rape) with fenoxaprop-ethyl [chlorophenyl-U-^{14}C] under field conditions led to extremely low levels of radioactivity in the seeds at the day of harvest. Treatment of soybean plants, 18 days after sowing, with a rate of 0.25 kg a.i./ha resulted in no residues (limit of quantification was 0.006 µg/g, measured as radioactivity) in the beans at the day of harvest (120 days p. appl.). A second application to another plot 40 days after the first treatment (= 80 days before harvest) caused a radioactivity level in beans corresponding to 0.03 µg/g. The situation in rape was similar.

Treatment of rape plants in the four-leaf stage with fenoxaprop-ethyl-^{14}C at field rate resulted in no detectable radioactivity above background in the seeds at the day of harvest which was 77 days after treatment. The limit of quantification was 0.005 µg/g. The extremely low total residue in the seeds is in agreement with the finding of a low degree of translocation from the treated leaves to other parts of the plants.

Metabolites

Because of the extremely low level of radioactive compounds in the mature seeds their chemical nature could not be determined. Nevertheless, for metabolism studies, soybean plants were treated when 2 to 3 trifoliate leaves had developed. The metabolite spectrum in the directly treated leaves was investigated 15 days later. The results showed rapid metabolization to watersoluble transformation products. Of the total radioactivity in/on the leaves 25 % was removed from the leaf surface with a diethylether rinse and was identified as the parent compound. An additional 5 % of the parent compound was extracted from the interior of the leaves and 3 % was the free carbonic acid fenoxaprop.

Non-conjugated chlorobenzoxazolyl-2-ol and polar conjugates of this compound accounted for 1 % and 5 %, respectively, of the total radioactivity while 2 % was identified as the non-conjugated hydroxylated chlorobenzoxazolyl-2-ol. More than 12 additional polar transformation products were separated but none of them exceeded 8 % of the total radioactivity present in the leaves. Assuming that the metabolite spectrum in the leaves on day 15 post applicationem is comparable to that in the seeds at the normal date of harvest, then the following conclusion can be drawn: in the case of two applications (rate 0.25 kg a.i./ha each) the highest concentration of one degradation product in the seeds of soybean or rape does not exceed 0.003 µg/g.

FATE OF FENOXAPROP-ETHYL IN RATS

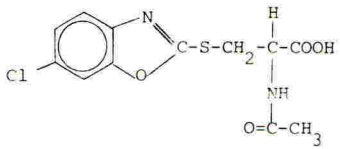
Kinetics

The distribution of fenoxaprop-ethyl in rats was investigated in a pharmacokinetic test over a period of seven days after single oral and intravenous application of two mg/kg body weight of the ^{14}C-labeled test compound. Comparison of the renal/faecal excretion ratio after oral and intravenous application indicated complete absorption of the oral dose. Of the absorbed radioactivity 54 % and 71 % was excreted via urine by male and female animals, respectively. The corresponding values for faecal excretion were 44 % and 25 %. Thus, the compound was almost completely eliminated from the rats.

The half-life values for the biphasic excretion processes via urine were 8 (♂) and 13 h (♀) and 37 (♂) and 70 h (♀), respectively. The levels of radioactive material 7 days after application in the blood, kidney and liver ranged from 0.2 to 0.3 µg/g. The concentration in the adipose tissue corresponded to 0.1 µg/g, showing that fenoxaprop-ethyl does not tend to accumulate in this tissue. This can be explained by the fact that the carbonic ester undergoes rapid hydrolysis in living systems, resulting in salts of fenoxaprop with high water solubility at a physiological pH value of 7.

Metabolites

Fenoxaprop-ethyl-^{14}C was applied orally to female rats in a single dose and the degradation products in urine and faeces were determined. The predominant metabolite was chlorobenzoxazolyl-2-mercapturic acid representing about 50 % of the radioactivity in the urine.



This conclusion was based on the extraction behaviour from acidified water and mass spectral analysis of the methylester showing a molar mass of 328 g/mole and containing one chlorine atom. After cleavage of the thioether bond, producing chlorobenzoxazolyl-2-thiol, and subsequent methylation or acetylation, the g. c. m. s. data were identical with those of the authentic reference compound chlorobenzoxazolyl-2-thiol. In addition to 4 % non-conjugated chlorobenzoxazolyl-2-ol, 6 % hydroxylated chlorobenzoxazolyl-2-ol were extracted from urine and identified by g. c. m. s. The remaining radioactive compounds were found to be nonextractable from the water phase.

Apart from 33 % of the radioactivity not extractable from faeces and 30 % represented by highly watersoluble compounds, 30 % was found to be a conjugate which contained chlorobenzoxazolyl-2-ol as a structural element. The labile conjugate was cleaved on methylation, silylation and acetylation, respectively, resulting in the methylated, silylated and acetylated chlorobenzoxazolyl-2-ol, respectively.

DEGRADATION IN SOIL

In our experiments rapid hydrolysis of fenoxaprop-ethyl was observed in the three soils used resulting in the formation of the main metabolite, the free carbonic acid fenoxaprop. The half-life values of the parent compound were measured to be below one day for each of the three soils. Further degradation resulted in the intermediary appearance of 4-(6-chloro-benzoxazolyl-2-oxy)-phenol and 6-chlorobenzoxazolyl-2-ol which amounted to less than 3 % and 5 %, respectively, and subsequent rapid and complete mineralization. Depending on the soil used, 10 - 20 % of the applied radioactivity was evolved as $^{14}CO_2$ during 32 days.
Other end products of degradation were bound residues in the soil amounting to 40 -

60 % at the end of the 32 days experimental period. These products probably resulted from microbial degradation and subsequent incorporation into cellular material via the intermediary metabolic pathways. The quantitative and qualitative findings indicate that fenoxaprop-ethyl is rapidly dissipated from field soils, thus, excluding the risk of long term effects on the environment. In addition, no radioactive residues were found in rotational crops which were grown in fenoxaprop-ethyl-^{14}C treated soils.

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EXPERIMENTS INTO THE MECHANISM OF ACTION OF THE EXPERIMENTAL HERBICIDE M&B 34552 IN CYPERUS ROTUNDUS AND ZEA MAYS

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Summary. M&B 34552 (benzyltrimethyl ammonium chloride) is an experimental herbicide which has been screened for activity in two species Cyperus rotundus (purple nutsedge) and Zea mays (maize). Applied as a foliar spray the primary response was chlorosis of the shoots in both species but this was more severe in nutsedge than in maize. Shoot and root growth in maize was inhibited together with shoot number and shoot and rhizome growth in nutsedge. Uptake and distribution of the ^{14}C-labelled herbicide has been investigated. This was rapid in both species with greater absorption occurring in maize but there was a higher level of translocation in nutsedge. The ^{14}C-label accumulated at active meristems. Chlorophyll synthesis was inhibited in etiolated maize. Preliminary investigations suggest a higher level of herbicide metabolism in maize. Possible sites of action are discussed. Nutsedge, maize, M&B 34552, uptake, distribution, metabolism, chlorosis, chlorophyll synthesis and meristems.

INTRODUCTION

M&B 34552 (benzyltrimethyl ammonium chloride) is a well known quarternary ammonium compound. Its weak selective suppression of Cyperus spp. in Zea mays (maize) was discovered by May and Baker in the course of random screening. Purple nutsedge is an important weed of many crops and persists in a broad zone from 0° to 30°-35° North and South (William, 1976). It has been listed as the World's "worst weed" (Holm, 1969) and is together with Cyperus esculentus a serious problem in maize in many parts of the world. The primary method of propagation is by a substantial underground system of rhizomes and tubers (Holm et al., 1977). Mature tubers can contain up to 12 dormant buds which are largely inaccessible to foliar applied herbicides (Mercado, 1979).

This report discusses the mode of action of M&B 34552 and the basis for its apparent selectivity between nutsedge and maize. It involves the nature of herbicide activity in relation to ^{14}C-labelled herbicide uptake and translocation together with the effect of the herbicide on chlorophyll synthesis.

METHODS AND MATERIALS

Both species were grown in plastic pots (13cm) containing John Innes No. 3 compost, maize seeds (var. Caldera 535) being first germinated in plastic trays containing vermiculite. Three mature dormant nutsedge tubers were planted per pot. Four weeks after emergence nutsedge and maize (four emerged leaves) were sprayed with M&B 34552 (2,4,8 Kg/ha) using a Mardrive overhead pneumatically propelled constant speed (0.9 m/sec) sprayer which was calibrated to deliver 200 l/ha using a Teejet 8004 spraying system. All treatments including a water control contained the surfactant Ethylan C.P. (0.1%). Plants were kept in a greenhouse at 25°C (± 20%). Each treatment had four replicates and was repeated twice.

Two weeks after treatment the shoots of nutsedge and the shoots and roots of maize were dried and weighed. Four weeks after treatment, the nutsedge regrowth was cut, dried and weighed. The rhizome system was washed and the rhizomes dried and weighed.

Using 80% acetone, chlorophyll was extracted from the shoot samples of plants two weeks after herbicide treatment and measured using the method of Anderson and Boardman (1964). Four replicates were used.

The uptake and movement of ^{14}C-ring labelled M&B 34552 was examined in both species over a time course of 3, 7 & 10 days. Nutsedge and maize plants were treated with ^{14}C-labelled M&B 34552 (0.06 µCi, 0.025 mg) applied as a series of 0.5 µl droplets to the adaxial surface on either side of the midrib. The youngest fully expanded leaf of nutsedge (two weeks after emergence) was treated and the second leaf of maize (four emerged leaves) treated. At harvest the treated leaf was washed in distilled water for 5 min to remove the surface residue, dipped in chloroform for 30s to remove any material associated with leaf surface wax. Aliquots from both samples were removed for liquid scintillation counting in 5 ml Picofluor 30 (toluene based liquid scintillator). The plants were then divided into sections as later described, freeze dried, pelletised and combusted using a sample oxidiser (Packard B306). The activity associated with each region was determined by liquid scintillation counting. Each treatment had four replicates and the experiment was repeated three times.

In a further experiment ^{14}C-labelled M&B 34552 (0.094 µCi, 0.04 mg) was applied to nutsedge 8 weeks after emergence (youngest fully expanded leaf treated) and to maize (8 leaved plants the fourth leaf being treated). After 7 days plants were harvested by the above method. Four replicates were treated. In all experiments plants were kept in a growth cabinet at 25°C (± 0.5%) and 65% rh (± 5%).

The effect of M&B 34552 (50-2,500 ppm) on chlorophyll synthesis in excised etiolated maize leaves was studied using the methods of Fletcher and Drexler (1980). Each treatment had four replicates and the experiment was repeated three times.

Where relevant the results of these experiments were subjected to an analysis of variance and to Duncan's Multiple Range Test (D test).

RESULTS

Foliage application of M&B 34552 inhibited shoot growth of both nutsedge and maize (Table 1). The root growth of maize was also inhibited. The number of shoots produced by nutsedge was also affected by M&B 34552.

Table 2 shows the effect of the herbicide on the regeneration ability of nutsedge shoots after removal of the existing shoots. At the highest rate used (8 Kg/ha) the shoot dry weight was less than 10% of the control. This application rate was also the only treatment where the number of shoots did not increase after shoot removal. The herbicide also reduced the rhizome dry weight. These effects increased with herbicide concentration.

The effect of the herbicide on chlorophyll levels in the shoots following foliar application is shown in Table 3. The level of chlorosis in nutsedge was greater than in maize. Increasing rate of application resulted in a more chlorotic appearance of the shoots in both species.

Uptake and distribution of the ^{14}C-labelled herbicide at 3, 7 and 10 days after application is shown in Table 4. Uptake in maize (> 70%) was greater than in nutsedge (< 60%). However the level of translocation in nutsedge (> 25%) was substantially higher than the level of translocation in maize (< 6%), (region/species interaction, $P = 0.001$). Over the time course used, the rate of uptake in each species was similar, indicating that uptake of the herbicide was rapid. The results in Table 5 show the distribution of the ^{14}C-label after 7 days in plants treated at an older

growth stage. These indicate a similar distribution to plants treated at a younger growth stage. Uptake in maize was greater than in nutsedge but translocation was less (region/species interaction P = 0.001).

Chlorophyll synthesis was inhibited by M&B 34552, (Table 6) the degree of inhibition increasing with herbicide concentration (treatment P = 0.001).

D. Tests. In all tables prescripts indicate absence of significance between means within a column and postscripts indicate no significance between means in a row.

Table 1

The effect of M&B 34552 as a foliar spray on nutsedge and maize 14 days after treatment

Treatment (Kg/ha) Per pot	0		2		4		8	
	Maize	Nutsedge	Maize	Nutsedge	Maize	Nutsedge	Maize	Nutsedge
No shoots	-	15.60	-	14.60	-	10.70	-	8.90
g.d.m. shoots	0.37	3.50	0.35	3.30	0.29	2.61	0.15	2.45
g.d.m. roots	0.13	-	0.10	-	0.09	-	0.08	-

Table 2

The effect of M&B 34552 on nutsedge regrowth 28 days after treatment

Treatment (Kg/ha) Per pot	0	2	4	8
No shoots	19.60a	17.30a	11.30	6.30
g.d.m. shoots	1.06	0.65	0.21	0.07
g.d.m. rhizomes	0.29	0.18	0.13	0.11

Table 3

The effect of M&B 34552 on chlorophyll content in nutsedge and maize 14 days after treatment

Treatment (Kg/ha)	Chlorophyll content (% of control) 2	4	8
Maize	94.24	a58.16a	52.31a
Nutsedge	87.09	a35.46a	18.60a

Table 4

Uptake and translocation of ^{14}C-M&B 34552 in nutsedge treated 2 weeks after emergence and maize treated with 4 emerged leaves

Time days	^{14}C-distribution (% of applied does)					
	3		7		10	
Region	Maize	Nutsedge	Maize	Nutsedge	Maize	Nutsedge
Water wash	21.09	48.91	19.16	45.02	19.01	49.69
Chloroform wash	1.31	1.40	1.23	1.11	1.45	1.37
Treated leaf	73.65	20.01	72.92	19.52	73.01	18.48
Other leaves	0.88	7.33	1.12	11.31	1.24	5.69
Stem	1.67	5.79	2.46	6.41	3.88	4.99
Roots	1.14	1.03	1.74	1.36	1.56	0.98
Rhizomes	-	7.34	-	8.02	-	4.53
Tuber rhizomes	-	6.27	-	7.11	-	8.61
Tuber	-	1.12	-	1.32	-	1.40
% Absorbed	77.34a	48.89	78.24	55.05	78.69a	44.68
% Translocated	3.69	28.88	5.32	35.53	5.68	26.20
% Recovery	99.74	99.20	98.63	101.18	99.15	95.73

Table 5

Uptake and translocation of ^{14}C-M&B 34552 in nutsedge treated 8 weeks after emergence and maize treated with 8 emerged leaves, 7 days after treatment

Region	^{14}C-distribution (% of applied dose)	
	Maize	Nutsedge
Water wash	17.51	43.13
Chloroform wash	1.22	1.31
Treated leaf	77.34	20.40
Leaves above treated leaf	1.19	2.69
Leaves below treated leaf	0.05	0.10
Stem	1.16	3.91
Roots	0.77	0.92
Rhizomes	-	2.24
1st daughter plants	-	7.71
2nd daughter plants	-	9.30
Tuber rhizomes	-	5.65
Tuber	-	0.74
% Absorbed	80.51	a53.66
% Translocated	3.17	a33.26
% Recovery	99.24	98.10

First daughter plants are shoots attached to the treated shoot.
Second daughter plants are shoots attached to first daughter plants.

Table 6

Effect of M&B 34552 on chlorophyll synthesis in excised etiolated maize leaves

Herbicide concentration (ppm)	Chlorophyll (% of control)
50	75.40
100	64.30
1000	53.80
2500	26.60

DISCUSSION

Chlorosis of the shoots in both species was the main visible symptom following foliar applications of M&B 34552, however, the development of chlorosis differed in the two species. In nutsedge, chlorosis occurred along the entire length of the leaves at all application rates, but in the case of maize was restricted at the lower application rates and only at the highest rate occurred along the entire leaf length. These differences are indicated in Table 3 which shows the extent of chlorosis to be greater in nutsedge at all application rates.

The visible response between the two species also differed where in nutsedge any shoots appearing after treatment were chlorotic. With maize any foliage appearing after treatment did not become chlorotic. These observations suggest that the mobility of the herbicide within maize is restricted. Results obtained with the ^{14}C-labelled herbicide confirmed that translocation of the herbicide in maize was restricted with under 6% of the applied dose leaving the treated leaf. The possibility of herbicide binding to plant constituents has been considered as a factor to explain the low level of translocation in maize. Results obtained (unpublished) with binding studies using the ^{14}C-labelled herbicide, indicate that most of the material within the treated leaf is not bound. In contrast the herbicide appears to be highly mobile within nutsedge. Translocation of the ^{14}C-labelled herbicide followed the "source to sink" pattern, accumulation occurring at the active rhizome meristems and also in the younger leaves above the treated leaf. Results in Table 5 show that secondary daughter shoots accumulated more of the ^{14}C-label than primary daughter shoots. These shoots are younger than primary daughter shoots and may be more dependent on photosynthates from the parent shoot. The ^{14}C-label is translocated via the phloem with photosynthates. Over 30% of the applied dose can be translocated out of the treated leaf in nutsedge. The vascular system within nutsedge is entire continuing through the tuber (Wills and Briscoe, 1970). The ^{14}C-label is therefore able to accumulate in rhizomes and shoots sprouting from tuber buds other then treated shoots. Such movement is indicated by the ^{14}C-label accumulating in tuber rhizomes (Tables 4 and 5). Accumulation within the tuber was low although translocation to dormant buds may have occurred after sprouting. Autoradiographic studies (unpublished) confirmed the pattern of translocation shown by these quantitative results. These differences in translocation of the herbicide in the two species may be involved in herbicide injury to the underground region of the plants. The inhibition in root growth recorded in maize could be a possible response to reduced shoot vigour rather than by a direct inhibition caused by translocated herbicide. With nutsedge direct growth suppression of the underground portions may be possible due to the higher levels translocated particularly to the active rhizomes.

Since penetration of the herbicide was greater in maize the extent of herbicide injury might be expected to be more severe than in nutsedge, however in nutsedge chlorosis was more extensive than in maize. The possibility of herbicide degradation within the plant has been studied using the ^{14}C-labelled herbicide. Results (unpublished) indicated that over an 18 day period both species metabolised the herbicide but the level of metabolism was greater in maize than in nutsedge. If the metabolites formed are inactive, metabolism may contribute as a means of selectivity between the two species.

The chlorotic appearance associated with foliar application of M&B 34552 could be the result of herbicide interference with one or more systems. Chlorophyll synthesis in excised etiolated maize leaves was inhibited by M&B 34552 (Table 6); other possible sites of interference include carotenoid synthesis and photosynthetic electron transport. Carotenoids are considered to have a protective function which involves deactivation of excess photosynthetic excitation energy (Moreland, 1980). Several herbicides are known to inhibit carotenoid synthesis resulting in chlorosis due to destruction of chlorophylls which are no longer protected by carotenoids (Moreland, 1980). M&B 34552 at concentrations above 1000 ppm has been shown to reduce carotenoid levels in etiolated maize seedlings (unpublished data). However, concentrations below this level inhibited chlorophyll synthesis in excised leaves (Table 6). In addition, although carotenoid levels were reduced, carotenoid precursors were not detected. Carotenoid precursors were present in maize seedlings treated with the carotenoid inhibitor herbicide amitrole (unpublished data). Reduction in carotenoid levels may represent an indirect inhibition.

Experiments with isolated chloroplasts (unpublished data) indicated that M&B 34552 did not inhibit photosynthetic electron transport. The development of chlorosis would not then appear to be involved with events following inhibition of this process (Ridley, 1977).

These and other aspects of the mechanism of action of M&B 34552 are currently being investigated.

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