RESULTS

Experiment 1. The growth of the simazine-resistant biotype (R) of C.suaveolens from Luddington was unaffected by doses of simazine up to 24 kg/ha (Table 1). The susceptible biotype (S) was killed by a dose of 0.07 kg/ha and the 0.01 kg/ha dose reduced shoot fresh weight by 30%. Experiment 2. Numbers surviving and shoot fresh weight of R biotypes of C.suaveolens and S.vulgaris were not reduced by a dose of 100 kg/ha simazine (Table 2) whereas doses of 0.1 kg/ha or more killed the S biotypes and 0.01 kg/ha reduced shoot fresh weight and numbers surviving. Simazine doses of 0.1 kg/ha or more killed S E.ciliatum and 0.01 kg/ha reduced shoot growth. With the R biotype doses of 10 kg/ha or more reduced shoot weight but plants appeared healthy. With all species there was no difference between the biotypes in the number of untreated plants surviving or shoot weight/plant except that plant number was 60% less with the S C.suaveolens (Germoe).

TABLE 1

The effect of simazine* applied pre-emergence to a resistant (R) and susceptible (S) biotype of C.suaveolens (Experiment 1)

Shoot Fresh wt (% untreated) 5 weeks after treatment								
Dose (kg/ha)	0.01	0.07	0.49	3.45	12.0	24.0	0	Actual value**
Biotype R	80	102	103	106	91	84	100	3.82
S	69	0	0	0	0	0	100	4.29

* 50% a.i., s.c. in all experiments **g/pot

TABLE 2

The effect of simazine applied pre-emergence on resistant (R) and susceptible (S) biotypes of C.suaveolens (Cs), E.ciliatum (Ec) and S.vulgaris (Sv)(Experiment 2)

		% untreated (3 weeks after treatment)						
Dose (kg/ha)		0.01	0.10	1.0	10.0	100	0	Actual value
Spp.	Biotype							(untreated)
				Seedling Number				Number/pot
Cs	R	101	95	94	85	102	100	82
	S	80	0	0	0	0	100	35
Ec	R	119	78	78	107	105	100	58
	S	48	0	0	0	0	100	54
Sv	R	99	99	100	102	101	100	102
	S	58	1	0	0	0	100	96
				Shoot fresh wt				wt/plant
Cs	R	108	83	95	59	85	100	156
	S	53	0	0	0	0	100	150
Ec	R	118	73	70	36	27	100	44
	S	15	0	0	0	0	100	46
Sv	R	113	79	92	88	99	100	119
	S	47	0	0	0	0	100	111

TABLE 3

The effect of simazine at different doses on C.suaveolens from different sites at Luddington E.H.S, (L), Begbroke (B) and Germoe(G) assessed 5 weeks after treatment (Experiment 3)

Dose (kg/ha)	Site	L1*	L2*	L3	L4	L5	L6*	L7*	L8*	L9*	L10	L11*	B	G
		Seedling Number (% untreated)												
0.02		169	100	148	116	93	130	152	103	113	97	150	101	130
0.14		174	110	1	7	0	110	102	106	98	0	136	0	0
0.98		333	148	0	1	0	99	119	104	163	0	94	0	0
6.86		272	118	0	0	0	76	146	83	138	0	119	0	0
48.0		139	103	0	0	0	89	104	99	98	0	84	0	0
Untreated		100	100	100	100	100	100	100	100	100	100	100	100	100
Actual no./pot		6.6	22.1	27.0	28.5	36.9	15.1	15.9	39.0	17.8	36.7	10.1	32.1	6.9
		Shoot fresh wt (% untreated)												
0.02		93	110	87	98	77	92	85	101	92	80	134	74	28
0.14		155	97	1	1	0	95	115	113	117	0	91	0	0
0.98		109	74	0	2	0	69	102	88	79	0	56	0	0
6.86		88	81	0	0	0	95	88	117	70	0	43	0	0
48.0		69	67	0	0	0	90	77	88	78	0	59	0	0
Untreated		100	100	100	100	100	100	100	100	100	100	100	100	100
Actual wt/plant(mg)		59	441	176	217	328	516	453	318	417	231	273	340	636

* Sites receiving annual application of simazine for >10 years

Experiment 3. Growth of C.suaveolens plants from seven out of 11 locations at Luddington was not inhibited by simazine doses up to 48 kg/ha (Table 3). Plants from the remaining four sites were killed or severely inhibited by doses of 0.14 kg/ha or more but largely unaffected by 0.02 kg/ha. Numbers of plants emerging were variable between replicates and particularly between locations; mean shoot weight/plant in untreated pots also varied considerably but this was not related to the response of that biotype to simazine.

Experiment 4. The simazine resistant biotypes of C.suaveolens and S.vulgaris were also more resistant to metribuzin and lenacil although killed or severely inhibited at the higher doses used (Table 4). The S biotype tended to be slightly more susceptible to diuron but there was no difference in the response of the two biotypes to diphenamid, napropamide and pendimethalin.

Experiment 5. Vigour of the R biotype of E.ciliatum was little affected by simazine at up to 64 kg/ha (Table 5), although final shoot weight was reduced. A dose of 0.06 kg/ha killed the S biotype. Diuron was somewhat less toxic to the R biotype but there was little difference in biotype response to diphenamid.

Experiment 6. Oxyfluorfen, paraquat and pyridate caused leaf necrosis and growth reduction to both biotypes of E.ciliatum (Table 6) but effects were more severe on the simazine resistant biotype. Pyridate at 6 kg/ha killed the R biotype.

Experiment 7. Atrazine at 1 and 4 kg/ha severely damaged the S but not the R biotype of E.ciliatum (Table 7). Oxyfluorfen caused more damage to the R than the S biotype. Paraquat at 1 kg/ha caused only transient damage to the S biotype but killed the R biotype. At 4 kg/ha it resulted in a 40% growth reduction of the S biotype.

TABLE 4

The effect of herbicides applied pre-emergence to simazine resistant (R) and susceptible (S) biotypes of C.suaveolens and S.vulgaris (Experiment 4)

		Fresh wt shoots (% untreated) 3 weeks after treatment					
		C.suaveolens			S.vulgaris		
		Dose (kg/ha)					
Herbicide formulation	Biotype	0.25	1.0	4.0	0.25	1.0	4.0
Simazine	R	94	67	74	80	63	62
(50% SC)	S	0	0	0	0	0	0
Metribuzin	R	62	1	0	50	11	0
(70% WP)	S	0	0	0	0	0	0
Lenacil	R	10	15	8	39	28	12
(80% WP)	S	0	0	0	0	0	0
Diuron	R	12	0	0	78	24	0
(50% WP)	S	1	0	0	42	47	0
Diphenamid	R	20	3	6	11	5	6
(70% WP)	S	16	3	4	7	2	2
Napropamide	R	12	8	2	15	7	6
(45% SC)	S	15	2	1	26	5	3
Pendimethalin	R	12	2	1	97	60	17
(33% EC)	S	18	0	1	64	59	15

TABLE 5

The effect of herbicides applied pre-emergence to simazine-resistant (R) and susceptible (S) biotypes of E.ciliatum (Experiment 5)

Herbicide	Dose (kg/ha)	Results as % untreated Vigour score (6 weeks)** R	S	Fresh weight (8 weeks)** R	S
Simazine	0.06	108	0	114	0
"	0.25	94	0	78	0
"	1.0	100	0	94	0
"	4.0	96	0	74	0
"	16.0	94	0	77	0
"	64.0	84	–	53	–
Diuron	0.016	104	97	112	88
"	0.012	46	0	36	0
"	0.25	0	0	0	0
Diphenamid	0.04	98	101	103	106
"	0.20	22	19	24	20
"	1.00	12	11	7	4
Untreated		100	100	100	100
Actual value		8.33*	8.75*	9.6	15.3 g/pot
SE±		4.3	2.5	7.0	6.0

* Vigour score, 0–9 scale ** Weeks after treatment

TABLE 6

The effect of oxyfluorfen, pyridate and paraquat on a simazine-resistant (R) and simazine-susceptible (S) biotype of E.ciliatum* (Experiment 6)

Herbicide	Dose (kg/ha)	Results as % untreated 4 weeks after treatment Vigour score R	S	Max. plant height R	S	Shoot fresh wt R	S
Oxyfluorfen (24% EC)	0.25	42	54	40	58	33	52
	1.0	35	41	40	54	34	47
	4.0	26	51	28	60	18	41
Paraquat (20% a.c.)	0.25	55	89	68	102	70	107
	1.0	45	83	52	95	51	83
	4.0	26	54	16	55	8	40
Pyridate (50% WP)	0.375	68	73	80	78	68	54
	1.50	26	73	24	80	21	65
	6.0	0	57	0	62	0	37
Untreated		100	100	100	100	100	100
Actual value		7.7**	7.8**	222	309mm	6.8	5.0g/pot
S.E.±		6.8		10.0		10.4	

* Plant height 80–120 mm at spraying ** Vigour score, 0–9 scale

TABLE 7

The effect of atrazine, oxyfluorfen and paraquat on a simazine-resistant (R) and a susceptible (S) biotype of E.ciliatum* (Experiment 7)

Herbicide	Dose (kg/ha)	Results as % untreated (4 weeks after treatment) Max.plant height R	Max.plant height S	Shoot fresh wt R	Shoot fresh wt S
Atrazine**	1	96	49	100	25
"	4	89	5	89	1
Oxyfluorfen	1	31	44	14	43
"	4	28	44	12	43
Paraquat	1	0	93	0	83
"	4	0	59	0	65
Untreated		100	100	100	100
Actual value		735	819 mm	28.4	27.7g/pot
SE±		15.3	14.2	17.0	15.3

* plant height 160 to 250 mm at spraying ** 50% S.C.

DISCUSSION

Triazine-resistant weeds have become widespread in perennial crops in England since the first confirmation of resistance in S.vulgaris and P.annua (Putwain 1982) and E.ciliatum (Bailey et al. 1982). Triazine-resistance in C.suaveolens has not previously been described in the UK or elsewhere; a few populations resistant to bromacil and simazine are also reported from E. Anglia (A.J.Greenfield, personal communication). The pattern of resistance appears similar with all these species. Very high doses of simazine were tolerated in these experiments compared with other tests (Putwain 1982; Bulcke et al. 1987); this may have resulted from the smaller amounts of simazine available to the plants from applying the herbicide as a spray after sowing compared with pre-planting incorporation. It does however demonstrate that very high doses of simazine will not overcome the resistance. The partial resistance of the R biotypes to metribuzin and lenacil and their susceptibility to herbicides of other chemical groups agrees with earlier work on these and other weed species (Parochetti et al 1982; Clay & Bailey 1985; Clay 1987) and indicates that a number of different herbicides are available to control the weeds albeit at greater cost than simazine.

The correlation between previous simazine use and resistance seen in the plants from different sites at Luddington confirm earlier suggestions that resistant biotypes are unlikely to spread into 'wild' populations possibly because they are less fit (Radosevich & Holt 1982). Not being wind-dispersed C.suaveolens is less likely to be transferred far on seeding but could be spread by machinery etc. The locations sampled at Luddington were all within an area of 0.5 km square. There was no evidence in these experiments of poorer growth of simazine-resistant biotypes of C.suaveolens or S.vulgaris but growth of E.ciliatum was significantly reduced at higher simazine doses (Tables 2,5). Bulcke et al. (1987) found considerable variation in leaf form and vigour of E.ciliatum biotypes but this was not correlated with their triazine resistance.

The occurrence of paraquat resistance in the simazine-susceptible biotype of E.ciliatum from East Malling, Kent agreed with the observations at the site. Paraquat resistance has been reported in other species notably P.annua and Conyza (Erigeron) species (LeBaron & Gressel 1982). The apparent greater tolerance to oxyfluorfen of the paraquat resistant biotype may be explained by its increased capacity to detoxify active oxygen species (Shaaltiel & Gressel 1986). The result with pyridate is unexpected since it is active in photosynthesis on photosystem 11 and therefore could be expected to be less toxic to the simazine-resistant biotype. Pyridate has given unexpected results in other work in that mixtures with simazine have given better post-emergence control of simazine-resistant E.ciliatum (Bailey & Clay 1985) and E.canadensis (Clay 1987) than pyridate alone.

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SESSION 9A

W WEED COMPETITION AND THRESHOLDS – FUNDAMENTAL ASPECTS OF COMPETITION AND POPULATION DYNAMICS

CHAIRMAN PROFESSOR G. R. SAGAR

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THE POPULATION ECOLOGY OF WEEDS - IMPLICATIONS FOR INTEGRATED WEED MANAGEMENT, FORECASTING AND CONSERVATION

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ABSTRACT

The role of population ecology in understanding the dynamics of weed populations is assessed in relation to strategic forecasting and conservation. Approaches to modelling population trajectories under differing weed management practices are briefly discussed and a methodology for predicting long term dynamics illustrated. Future strategic research needs are commented upon in relation to the application of such studies to agriculture.

INTRODUCTION

Population ecology is that branch of ecology (Greek : *oikos*, home / house; *logos* , study) that seeks to expose the factors that regulate species abundance in 'households' through time and space (Begon and Mortimer 1985). In the context of weeds this 'household' is the agroecosystem and it is one which has come under increasingly close scrutiny during this decade. Calls for the rational management of weeds (both economic and ecological) have arisen from the alternate pressures for the need to optimise inputs in agricultural systems (Robinson 1978) and concern for the protection of the environment. This paper briefly reviews the the potential benefits that studies of the population ecology of weeds may bring to agriculture focussing on homogeneous cropping systems (Snaydon 1980).

THE PRAGMATIC CONSIDERATIONS OF WEED MANAGEMENT.

Based on a range of sources (eg Attwood 1980; Elliott 1982) the criteria of decision making in weed control may be summarised under four general questions :

1. In a given cropping and weed management regime, how likely is it that invasion by weed species will occur and conversely can species of conservation value be retained within the agro-ecosystem ?

2. Given the presence of an infestation, how fast will the size of the weed infestation change (increase or decline) under different management strategies and how much damage (in all senses) will the crop suffer ?

3. How much of a proposed control measure is required to
   a) contain the weed infestation at its current size, or
   b) force the population into a decline so as to cause ultimate eradication ?

4. What are the comparative costs of different weed management strategies and what are the risks associated with alterations in control practices?

These questions are couched in general terms and belie the explicit issues that may be raised by consultants and growers. Yet questions such as ' Will minimum tillage favour weed species "A" over "B" ', ' Will chemical "Y" eradicate species "C" quicker than chemical "Z" ' or ' Is it economic to spray every year against species "D" ' are easily seen as specifics within the general scheme.

THE ECOLOGICAL BACKGROUND

Agroecosystems as 'households' for weeds comprise habitat mosaics on a large spatial scale with components often displaying fidelity both in land use and species composition. Such mosaics are a direct consequence of agricultural management practices, each component offering radically different probabilities of mortality and fecundity to a species. Whilst hedgerow and crop flora attest in a historical fashion to this, critical observations and experimental evidence suggest that management practices are very powerful forces of interspecific (eg Haas and Streibig 1982; Froud-Williams and Chancellor 1984) and intergenotypic (eg Putwain et al. 1982) selection. Past management practice may well have reduced the weed community within the crop to a depauperate collection of 'difficult' species and designated surrounding land to the role of 'reservoir' for natural vegetation. Moreover the spatial distribution of weeds within the crop may be patchy. Two particular requirements of population ecology then, are to explain 'how' such forces operate and 'why' vegetation change results, being specifically concerned with changes in species abundance over time and in spatial location. The forthcoming answers are potentially of equal value when applied both to the conservation of natural flora as to the optimisation of crop yield.

A baseline for predicting whether a plant population will increase or decline in the long term requires measurement of its finite rate of increase or per capita multiplication rate, λ. (For $\lambda>1$ the weed population will increase in size, whilst in a static population $\lambda=1$. Conversely $\lambda<1$ indicates a population in decline. In annual plants with discrete generations and no seed bank, measurement of λ is straightforward. It involves census of the population N at intervals (t and t+1) which encompass a complete generation; λ is then the proportionate change, N_{t+1} / N_t. For species with overlapping generations and/or seed banks the procedures are more involved). Any measurement of λ is however a reflection of the the habitat conditions experienced by the population during a generation and of the distribution of resources for growth to individual members of the population. It is now well recognised in theory at least that in a population 'left to its own devices', λ diminishes with increasing population size with the result that a stable equilibrium population size may be reached. The expectation rests on the assumption that all other features of the habitat remain constant and intrinsic biotic regulation within the weed population restricts λ.

Where a species maintains stability in abundance within a habitat (λ=1), Watt (1947) recognised that there was a persistent spatial shuffle of individual plants in the community on a local scale. This view has led to the suggestion, for a species to persist within a habitat there may be a critical minimum habitat size in which the effects of emigration and death are balanced by immigration and birth.The divisive nature of agricultural management practices reinforces the view that habitat islands exist within agro-ecosystems and suggest that species gain and loss may obey the principles of island biogeography (MacArthur1972).

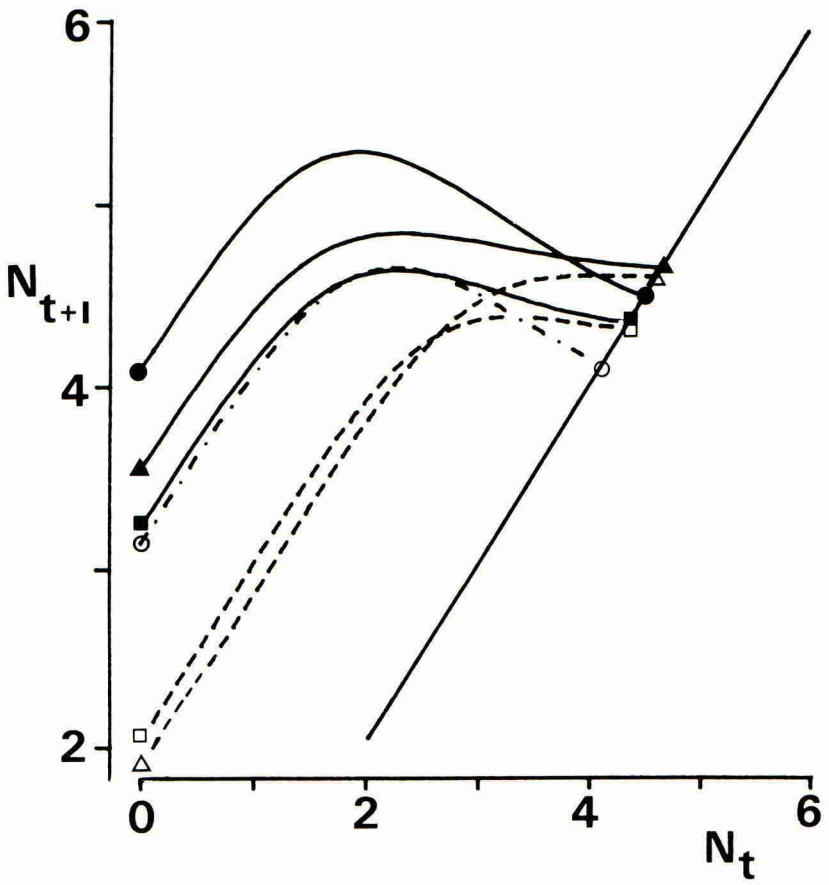

Fig. 1. Changes in population size over generations (N_t to N_{t+1}) in three weed species.Triangles,*Agrostemma githago* in a pot experiment, closed - in monoculture, open - in wheat (data from Watkinson 1981); squares, *Bromus sterilis* in a field trial ,- closed in monoculture, open - in winter wheat (from Firbank et al.1984); circles *Senecio vulgaris* in a pot experiment, closed - biotype sensitive to simazine, open - biotype resistant to simazine (from Watson 1987). Units are 'seed' m^{-2} expressed on $\log_{10}$ scales, curves being derived from fitted models. Solid diagonal line is of unit slope. In all cases no loss of seed from dispersal through to germination is assumed.

APPLICATION OF POPULATION DYNAMICS

What then is the practical relevance of these concepts? Four major points of pragmatic worth immediately arise out of studies of population dynamics.

Calculating rates of increase of weed infestations

Knowing the growth rate of a weed population enables comparative assessment amongst weed species and qualitative prediction of the size of future populations. Fig. 1 gives some examples of the changes in population size that have been measured for three species in the absence of deliberate weed control. They are calculated on the assumption that the habitat conditions other than changes in weed density remain the same. The curves illustrate three important points. First that density dependent regulation can act strongly to regulate the size of populations. Cobwebbing N_t to N_{t+1} successively up the curve leads to the equilibrium point in a very few generations. Second that equilibrium population sizes may be lower than intermediate ones and third that the presence of an associated species (here a crop) may alter the trajectory of population increase.

Similar maps of generation to generation change are given in Figs 2 and 3 for *Avena fatua* and *Alopecurus myosuroides* in field plots of winter wheat experiencing post emergence herbicides. For *A.fatua* (Fig. 2) finite rates of increase greater than one (ie population increase) were recorded across all infestation densities and the herbicide appeared to act in a density independent way. In *A. myosuroides* (Fig .3) different trajectories were observed according to rate of herbicide application. With application of half the recommended rate of chlortoluron, at low infestation densities finite rates of increase were observed to be less than unity (population decrease) at low infestation densities and greater than unity in higher infestations. At recommended rate, however the relationship between successive population sizes was far less clear. Field observations taken during the experiment showed this result to be due to a very few plants surviving herbicide application to set seed (P.F.Ulf-Hansen, pers comm.).

Comparing management practices - sensitivity analysis

The trajectory of the curve governing changes in per capita rates of increase will be governed by the interaction of density dependent and density independent regulatory agencies that are the net result of all crop husbandry and weed management practices used. Families of such curves under a range of management practices provide the opportunity for comparative evaluations of alternative management practices in toto . Their actual construction requires 1) measurement under field conditions of the rates of increase of weed population growth over a wide range of infestation densities; 2) the statistical fitting of an appropriate model to the relationship and; 3) verification of the model. Once achieved, stability analysis can be conducted to examine the sensitivity of the population to changes in parameters that may arise through altering efficiency of control. The difference equation,

$$N_{t+1} = \lambda . N_t \ (1+a.N_t)^{-b} \qquad (1)$$

provides a satisfactory fit to the data in Fig.2 . λ is the finite rate of increase of an isolated plant and a and b are parameters which describe the species response to increasing density. Equation 1 may be extended to include the influence of control practices. One such way is

$$N_{t+1} = \lambda . N_t (1+a.N_t)^{-b} - \Lambda N_t \qquad (2)$$

where $\Lambda = \rho \lambda$. The model assumes that control is exerted as a proportion , ρ , of λ and is independent of weed density. Fig. 4 illustrates the stability domains of the

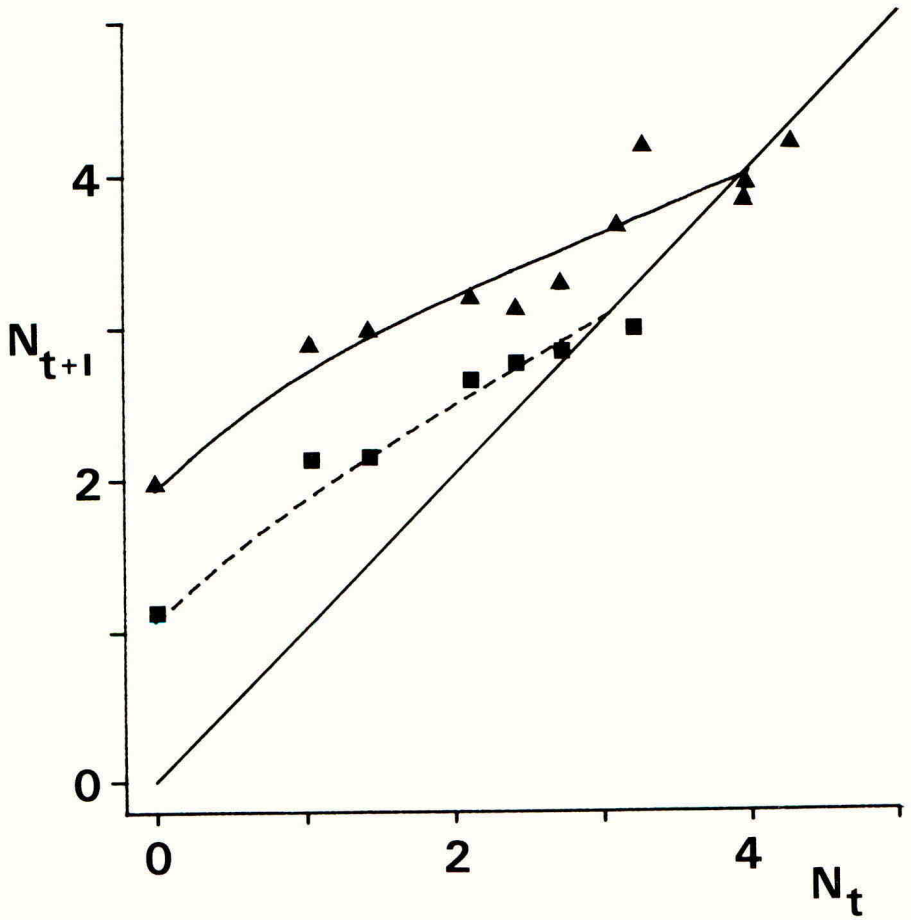

Fig. 2. Changes in population size in *Avena fatua* growing in winter wheat (data from Manlove, 1985). Squares, populations sprayed with I - flamprop - isopropyl at 3 l / ha in 450 l / ha water applied post emergence in late season; triangles, unsprayed populations. Fitted lines are $N_{t+1} = \lambda.N_t.(1+a.N_t)^{-b}$ after Hassell (1975) and census units are seeds m^{-2} after crop harvest, expressed on $\log_{10}$ scales. R^2 is the coefficient of determination.

	λ	a	b	R^2
Control	96.7	0.194	0.593	0.98
Sprayed	13.2	0.228	0.429	0.97

model and allows prediction of the long term outcome under various efficiencies of control. One simple application is as follows. Given description of the population dynamics of a weed experiencing intrinsic regulation in the absence of a proposed control we may predict for a particular intensity of control whether a population will be unable to achieve a steady state equilibrium (and in theory decline to extinction) but if so, what it will be in the long term. A test of prediction may be conducted with the data for *A. fatua* (Fig .2). Given estimates of a and b from unsprayed plots and

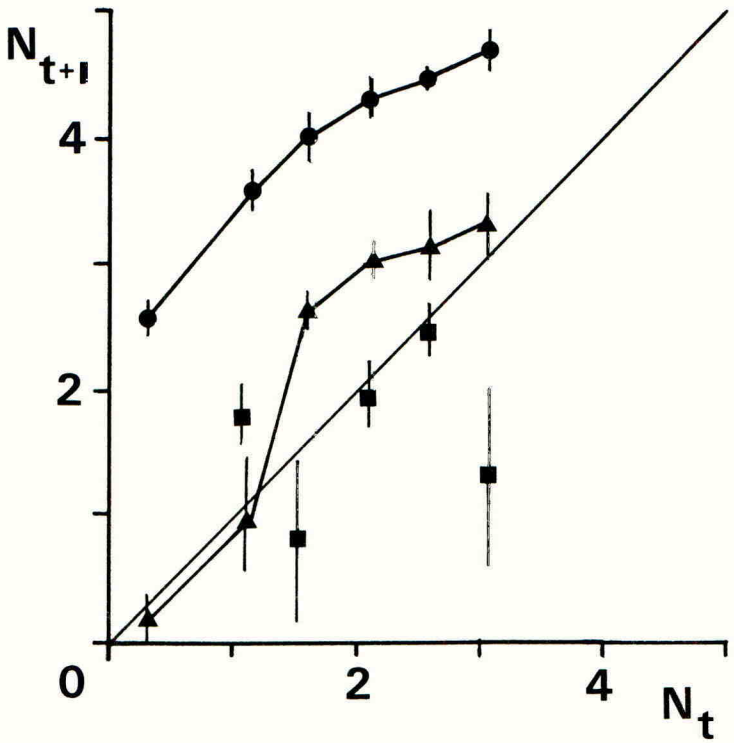

Fig.3. Changes in population size (seeds m^{-2} on $\log_{10}$ scales) in *Alopecurus myosuroides* growing in winter wheat (data of P.F.Ulf-Hansen). Circles unsprayed control; triangles half rate and squares full rate (2.75 l a.i. / ha) chlortoluron. applied post emergence. Means are ± 1 s.e. The population follows the trajectory
$N_{t+1} = 272\ N_t\ (1+ 0.008N_t)^{-0.81}$ when unsprayed ; and
$N_{t+1} = 2029.9\ N_t^2(15190.2+.N_t^2)$ under half rate chlortoluron.

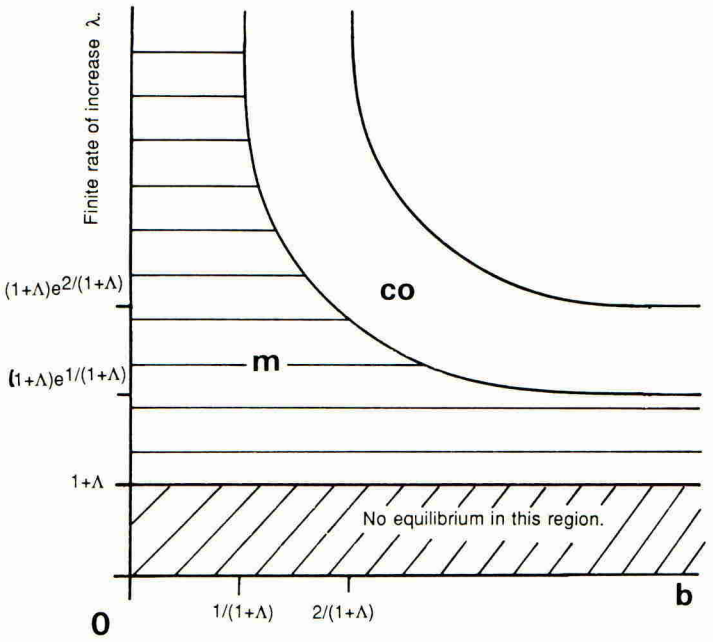

Fig. 4. The stability regions for a population governed by

$$N_{t+1} = \lambda . N_t . (1+a.N_t)^{-b} - \Lambda N_t$$

where $\Lambda = \rho \lambda$ and ρ measures the efficiency of control $0 \leq \rho \leq 1$. In the area bounded by $\lambda > 1 + \Lambda$ and the upper right hand parabola populations achieve a stable equilibrium. The approach to this equilibrium may be monotonic (M) or by convergent oscillations (CO).

Equilibrium population size $N_t = N_{t+1} = a^{-1} [(\lambda / (1+\Lambda))^{1/b} - 1]$.

measuring the reduction in λ achieved by spraying isolated plants in the crop, the equilibrium population size in the long term is calculated to be 125.8 seeds m^{-2}. Clearly this prediction under-estimates the equilibrium observed when spraying (Fig 2). (Note however the comparison is not statistically rigorous as data were collected in the same experiment !).

Controlling to economic threshold levels

Doyle et al. (1986) and Cousens et al.(1986) have pointed the way in using population models to calculate the long term economic benefits of alternative strategies of weed control. If an avowed management aim is to apply weed control to achieve an economic threshold weed density (determined from the relationship between yield loss and weed density at harvest, Cousens 1985), then it becomes necessary to calculate the level of control required. Stability analysis (Fig. 4) of the

appropriate model of the dynamics of the weed population provides one means, given that the model is couched in units of adult plants at harvest.

Evaluating the detailed effects of control measures.

Examining the fates and fluxes of individual plants at particular stages in the life cycle (eg seed ⇒ seedling ⇒ young plant ⇒ adult) and constructing life tables (for example Sagar and Mortimer 1976; Lapham et al.1985) has been shown to expose the importance of agricultural practices on the dynamics of weeds. Wilson and Cussans (1975) have pointed to the importance that straw burning has in the life cycle of *A.fatua* and Mortimer (1985) has illustrated the density dependent survivorship of *Bromus sterilis* seedlings under a herbicide. It is only by detailed comparative analysis in the appropriate way (k-factor analysis) that the relative importance of regulatory agents can be fully examined.

CONCLUDING REMARKS

Forecasting and integrated weed management

Weed management programmes may be considered strategic in the sense that chemical and cultural measures are utilised with the foreknowledge that damage due to weeds will occur within a defined range. Moreover weed control should be practised to maximise the economic return from its application (Elliott 1982). Achieving this goal however requires precise knowledge of the damage done by weeds and the long term behaviour of the weed population. Increasingly the relationships between weed infestation density and crop damage are becoming established (Cousens 1985 ; Cousens et al 1985) and the population ecology of weeds (particularly grass weeds) understood. How far then can such knowledge be used in a predictive way especially where integrated weed management is practised ? Two points need appreciation in answering this question. Firstly that the net effects of integrated weed management programmes may be seen in terms of population trajectories (eg Fig 3) and that if appropriately described mathematically, it becomes possible to examine long term outcomes. This gives the basis for strategic forecasts (see also Mortimer 1983 ; Firbank and Watkinson 1985). Such forecasting methodology is of obvious practical benefit but the precision of the analysis depends on successfull modelling of weed populations with sufficient resolution. Constructing models on the basis of linking phases in the life cycle in discrete stages runs the risk of loss of precision especially when control measures interact in complex density dependent ways and may effect not only mortality of weeds but the competitive ability and fecundity of survivors. The inescapable fact that soil and climate varies, many weed species show episodic germination and show very strong yield compensation means that strategic models need to be applied in worst and best case scenarios and coupled to economic risk analysis to be of worth. This is the next challenge in the application of population ecology in weed science.

A limitation of the above approach is that it is confined to single species dynamics when in reality weeds persist in communities. Stability analysis of the form illustrated in Fig. 4 may be extended to two species interactions and the relative

competitive abilities of species in mixture assessed. (Gould and Mortimer in prep). Further extensions to multi-species assemblages are likely to prove impractable. Developing mechanistic models (eg Spitters and Aerts 1983) may be one alternative although this constitutes a very long term endeavour.

Conservation in agroecosystems.

Evaluating vegetation management programmes to conserve plant species in headlands, field margins or hedgerow bottoms (the habitat islands of agroecosystems) can be approached in a similar way to that outlined above for weed control but with the converse interest in ensuring population growth rates do not fall below unity. Yet designing management programmes is complicated by the fact that the aim is to increase community diversity rather than to diminish it. Whilst alternating strip margins around fields have illustrated that diversity can be markedly increased in relatively short time (Carter 1983), knowledge of how communities will develop is considerably lacking. Detailed studies of the population ecology of individual species, in particular their disperal characteristics, may well prove an invaluable first step in exposing the relevant factors. However, research in this area is likely to be most productive if experimental programmes investigate not only the wide range of habitats that arise from the extremely various uses to which field margins and headlands are put (Fielder 1987) but also to the direct and indirect influences of machinery passage. It is of particular importance to assess whether desirable similar habitats need to be in some way contiguous and what their relevant size should be.

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THE USE OF WEED DENSITY - CROP YIELD RELATIONSHIPS FOR PREDICTING YIELD LOSSES IN THE FIELD

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ABSTRACT

The merits and shortcomings of additive and replacement series experiments for investigating competition between crops and weeds are discussed. Competition between wheat and brome grass (Bromus diandrus) in Western Australia is used as an example to demonstrate that the results of additive experiments can be used to develop weed density - crop yield relationships which can reliably predict yield losses under different cropping conditions encountered over large regions and between seasons.

Various ways in which farmers and their advisers can use the relationships in the field as an aid when making weed control decisions are described.

The level of precision required when developing crop loss predictions for field use is questioned. A need for future work on the effect of mixtures of two or more weed species on crop yields is identified.

INTRODUCTION

Nearly three decades have elapsed since de Wit (1960) introduced a model (replacement series) describing competition between two plant species, based on their growth in monocultures and in various ratios of mixtures at constant total density. Subsequently Baeumer and de Wit (1968) developed a simulation model to predict interference between the components of a binary mixture of plant species through time, on the basis of measurements derived from spacing experiments with the species grown in monocultures and harvested at intervals. Spitters and van den Bergh (1982) placed the Baeumer-de Wit model in a crop-weed context and described how it could be further extended to simulate the effects on crop growth and yield of weed density, time of weed emergence relative to the crop and time of weed removal from the crop.

Although the models based on the replacement series have provided a useful framework in which to consider competition between crops and weeds and have made a valuable contribution towards clarifying thinking in this field, to our knowledge the approach has not been used for predicting the effects of weeds on crops in practical farming situations. One of the reasons for this could be the lack of a biological or physiological foundation for these models. For example, Newman (1982) stated that, 'de Wit's method of analysis was derived from equations applicable to vapour and liquid phase relations of mixtures of two substances. Its application to plant interference has never, to my knowledge, been critically related to the physiological response of plants to shortage of resources'. Furthermore, de Wit (1960) lumped all the factors, for which plants might compete, under the generic term 'space' and suggested that 'to do otherwise is not necessary, always inaccurate and therefore unadvisable'. Such logic does not sit well with physiologists investigating mechanisms involved in crop-weed competition. Donald

(1963) criticised this aspect of de Wit's approach and stated that 'whilst the use of the term space may be a convenient shorthand for mass competition, it evades the need to pursue and recognise real factors for which competition is occurring'. Hall (1974) recognised this problem and suggested a way of combining a de Wit analysis with data on nutrient uptake, to define the causal factors in competition.

Spitters and van den Bergh (1982) commented on the artificiality of this and recommended dynamic simulation as an alternative but they acknowledge that their model serves solely to illustrate the use of systems analysis in weed research and for accurate predictions the model should be extended to include physiological aspects. More recently, van Heemst (1985) pointed out that 'although the behaviour of crops and weeds in mixtures can be described fairly well on the basis of their performance in monocultures, the necessary information for such a quantitiative description is mostly not available, and it is more practical to use empirical relationships based on field studies'. Furthermore, simulation modelling is complex and field agronomists and advisers may distrust this 'black box' packaging of information.

There are other possible reasons for the lack of adoption of the de Wit approach. Because it is also empirical, it is not likely to decrease field experimentation required to develop and verify weed density - yield loss relationships. The data obtained from replacement experiments are not readily translated to weed problems in the field, as much of the data generated are for densities of crop and weed far outside common field occurrence. Furthermore, the practical difficulties of planting replacement experiments may outweigh the disadvantages of additive experiments.

ADDITIVE EXPERIMENTS

The vast majority of weed/crop experiments conducted in the past have used an additive approach. In additive experiments two species are grown together, and the density of one (usually the crop) is held constant while the other (the weed) is varied. This approximates the farm situation where crop density is set by the farmer and weeds are usually unwanted, variable additions. A further common example of an additive (or perhaps 'subtractive') experiment is where a herbicide is used to subtract weeds from a mixed crop-weed population. Information derived from additive and subtractive experiments can be brought together, and described in crop-weed competition relationships which usually take an exponential or hyperbolic shape (Cousens 1985), similar to the one presented in Fig. 1. Notable features of such a general relationship are the near linear loss of yield at low weed densities, which is often the area of commercial interest, and the plateau at high densities.

Additive experiments have been criticised because often they describe the effect of a weed on a crop at a single site/season, or at best a few sites and seasons, and because of this may have very limited predictive value, although in many cases this criticism can be levelled at replacement experiments also. The literature abounds with additive experiments which have little more than historical value and the studies of Dew (1972) and Reeves (1976) who attempted to draw general conclusions from additive experiments and use these to predict the effect of weed infestations, are exceptions. Spitters and van den Bergh (1982) have criticised additive experiments, stating their disadvantage to that there are no adequate mathematical models to quantify the competition effects

and to make predictions on various competitive situations. The analysis of Cousens (1985) suggests that this is not so. We contend that for some crop-weed combinations, additive experiments and simple mathematical procedures can be used to predict losses due to weeds over a wide range of cropping situations. The remainder of this paper describes how additive experiments involving the annual weed, Bromus diandrus and wheat, can be used to predict wheat yield losses in different cropping situations in the wheatbelt of Western Australia, an area of about 10 million hectares.

ADDITIVE EXPERIMENTS WITH WHEAT AND BROME GRASS

Brome grass (B. diandrus) is native to the Mediterranean region. After its introduction to Australia it spread through the temperate agricultural areas, and is now an important weed of cereal crops. Brome grass competes successfully with wheat resulting in large losses of grain yield (Gill & Blacklow, 1984). Recently Gill et al. (1987) and Poole & Gill (1987) have analysed the results of field experiments with wheat and brome grass in Western Australia. Six experiments were carried out over three years at five locations to investigate competition between wheat and different densities of brome grass. Brome grass density was measured within 4-6 weeks of planting. The trials were sown over a range of seasons, soil types and localities. Over different years and locations, the relationship between weed density and grain yield of wheat followed a similar trend. Therefore, data on grain yields were normalized and expressed as a percentage of weed free yield and an exponential curve was fitted to the data on relative grain yield (Y) of wheat and weed densities (X), using the maximum liklihood program (Ross, 1980). Different parameters of the exponential model fitted by the maximum likelihood programme are described below:

$$Y = A + B.\ e^{-kx}$$

where A + B = weed free yield (100% in the present case), B is an estimate of maximum yield reduction, K = a decay constant, BK is an estimate of initial rate of decrease in relative yield, X is brome grass density (plants/m^2) and Y is relative grain yield (predicted).

In a recent review of models used to describe competition between crops and weeds, Cousens (1985) found that rectangular hyperbolae accounted for the greatest proportion of the variation in the data. However, Gill et al., (1987) found the exponential model to be as good as Cousens hyperbolae although the hyperbolae may offer greater flexibility. In Fig. 1(a), the data from six experiments are shown with the fitted curve. The density of brome grass accounted for 87% of the variability in the data on grain yield of wheat (Rectangular hyperbola 86%).

In Fig. 1(b) independent data of Gill and Blacklow (1984) and Gill (unpublished 1985 and 1986) are shown against the curve previously fitted. The experiment conducted in 1986 concentrated on low densities of brome grass as this, from the recommendation viewpoint, is the area of most commercial interest. There is good agreement between the fitted curve and results obtained in later years. These latter data support the contention of Cousens (1987), that rectangular hyperbolae, or in the present case, exponential relationships better describe the relationship between yield loss and weed density than the sigmoidal relationship described by Zimdahl (1980). That is, yield begins falling with the

addition of the 'first' weed, rather than Zimdahl's proposition that low densities of weeds may have no effect on yield. Although it is possible to hypothesize situations of luxury resource supply where a sigmoidal relationship could hold, this will rarely be encountered in the field. In Australia, nutrients and water commonly limit cereal growth and at the close spacing found in wheat or barley crops, the 'first' weed could be expected to capture some of these resources.

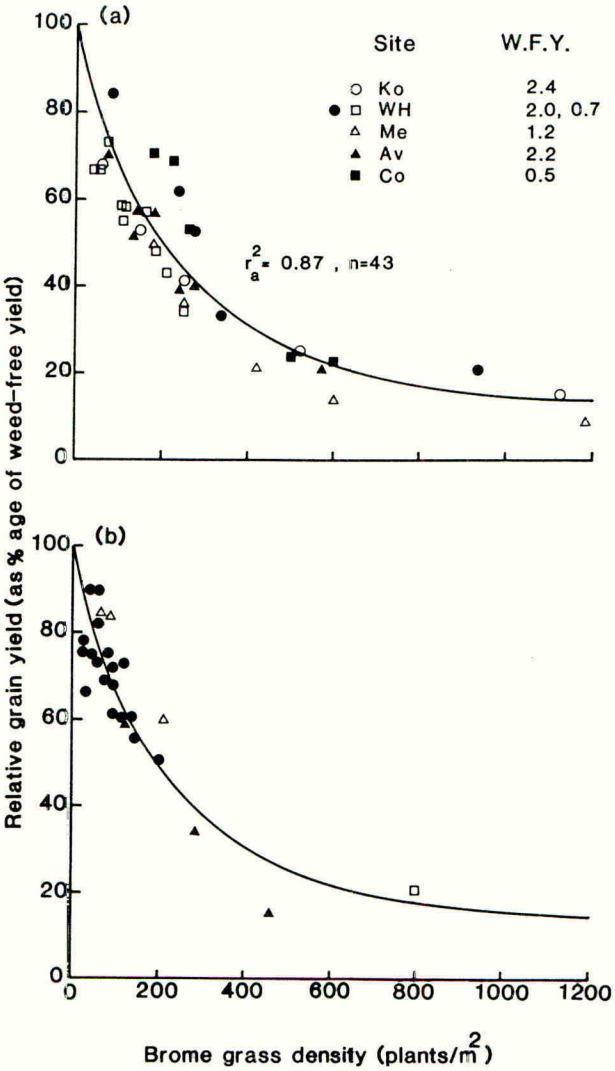

Fig. 1. Relationship between the density of brome grass and grain yield of wheat. (a) Fitted curve to our data from 6 experiments, and (b) comparison of the fitted curve with the independent results of Gill and Blacklow (1984) (□) and our trials during 1985 (▲△) and 1986 (●). W.F.Y. is the weed-free yield in t/ha and r^2_a is the variability accounted for by the relationship.

USE OF RELATIONSHIP FOR PREDICTION OF YIELD LOSS

If the relationship derived is to be used to predict losses of wheat yield at different brome grass densities, it must account for most of the cropping situations likely to be encountered. At this stage, for this combination of brome grass and wheat in Western Australia, the relationship appears remarkably robust. Experiments have been carried out over five seasons and nine sites representing a wide geographical and soil type spread. Weed free yield varied from 0.5 to 2.44 t/ha indicating the range of seasonal and edaphic conditions encountered. In view of the results obtained we are confident that, within the confines of normal cropping practices used in Western Australia, the relationship is a valuable tool for predicting yield loss for crops of different achievable yield infested with different brome grass densities. There are error terms associated with the estimates of weed density and grain yield. Therefore, it is not surprising that there is a spread of data points around the fitted curve (Fig. 1a). Some users may prefer to include a band enclosing the data spread and express a range of yield loss for a particular weed density, but this may be an unnecessary complication when using the curves with the farmers.

Many studies have reported interactions in crop-weed competitiveness between weed density and crop seeding rate, time of seeding and time of weed emergence. These were reviewed by Zimdahl (1980), Harper (1977) and Newman (1982). While it is simple to demonstrate these interactions, frequently the treatments depart so far from common farm practice for a region that they have little practical significance. Brome grass commonly germinates as a single flush with cereal crops at the start of the season (Gill and Blacklow 1984) and for this weed, time of emergence relative to the crop is not normally an important factor. With respect to changes in crop seeding rates, farmers choose seeding rate in keeping with experience and they rarely vary outside a narrow range over large regions. While it is possible to show significant interactions by doubling or tripling normal seeding rates, on the basis of cost effectiveness and deleterious side effects on lodging, water use and disease this is unlikely to be used for reducing weed competition. In summary, provided farmers continue to use normal agronomic practices in a region, the relationship derived appears very valuable for predicting losses of wheat yield due to different brome grass densities.

Will other crop-weed relationships derived in this way will show similar robustness? Elsewhere (Poole & Gill 1987), we have described a similar approach for wheat in competition with other grass weeds. Relationships were derived for three annual grasses, wild oats (A. fatua) barley grass (H. leporinum) and annual ryegrass (L. rigidum). For wild oats the relationship derived was satisfactory ($r^2 = 0.95$) but it has only been tested in fertile, good rainfall conditions. However, when wild oat competition data from elsewhere were plotted against this relationship, in all but one case they fell on or near the curve, providing encouragement that the relationship has some generality. A complicating factor with wild oats is late emergence and it may be necessary to derive a separate relationship for this situation. Also Martin et al., (1986) describe separate curves for widely separated times of planting. Given the construction of several curves, and experience with interpolation, it may be possible to use the wild oat-wheat relationship for prediction in many localities. With annual ryegrass, which has smaller seeds than wheat, it has been necessary to draw two curves, one describing ryegrass emerging with the wheat under normal

seasonal conditions, the other describing late or slow germination in seasons with a dry start. With barley grass, although we reported a good relationship (Poole & Gill, 1987), further work has suggested that soil fertility status may be important, with barley grass competing more strongly with wheat under fertile conditions.

USE OF COMPETITION CURVES IN THE FIELD

We have concluded elsewhere (Poole and Gill 1987) that despite the diversity of the information available it is possible to derive relationships for some important weeds which are useful when making weed management decisions under wide ranging conditions, extending at least to the regional level. It should be stressed however that estimation of crop yield loss in a particular weed situation is only part of the total assessment of the financial loss resulting from weed infestation, although it is usually the one uppermost in farmers minds when making a decision on crop spraying in the cropping year.

When evaluating yield losses derived from crop-weed competition studies, it is tempting to take the yield difference between the weed-free and the weedy situation as the value which will accrue if a control measure is invoked. This will invariably be an overestimate of the likely gains, particularly in the case of herbicides applied after crop emergence. Apart from crop damage and the reduced competitiveness which the herbicide may cause, herbicides are seldom applied early enough to prevent completely the weed reducing crop yield; the herbicide may miss some weeds; herbicides are often not fully effective and may either allow survivors or only suppress weed growth; and tolerance to herbicides may exist in the weed population. Competitive relationships will require adjustment for this in the light of experience and experiment.

Many workers have stressed the importance of assessing the value of weed control practices over time, usually emphasising the importance of weed seeds which are produced in a weedy crop carrying forward to infest crops or pastures in later years. Cousens (1987) has discussed the importance of this recently, although Marra and Carlson (1983) suggest that future benefits from carryover effects of controlling weeds in one year to later years may be so uncertain that it is best to ignore them. Auld and Tisdell (1986), however, suggest that increased uncertainty about the future may be taken into account by applying larger discounts to future costs and benefits, thereby putting a reduced weight on these in decision making. Pannell and Panetta (1986) have placed weed control in a whole farm context and using linear programming to arrive at overall cost of a weed in a farm system. The usefulness of these models depends greatly on the data entered into them. Reasonably accurate estimates of the effects of weeds on crops may be the most important data entered and this appears to be the case in the Pannell-Panetta model.

Firstly, we agree with Cousens (1987) who describes the various 'weed thresholds' used in the literature, that a single critical or threshold density for action has little validity outside a very narrow range of conditions. It is clear from the weed density - yield relationship presented earlier in this paper that for a particular brome grass density, the yield loss will vary greatly depending on the crop size. For example for a density of 100 brome grass plants/m^2, the yield loss for crops with potential yields of 1, 2 and 3 tonne/ha is 300, 600 and 900 kg/ha respectively. A farmer confronted with 100 brome grass plants

in a one tonne crop might make a very different weed control decision to one with a similar density in a three tonne crop.

Experience with farmers and advisers suggests that rather than presenting a fixed critical density, it is more useful, and enlightening, to present data in a way which allows the farmer to gain an idea of the sensitivity of crops of different size to different brome grass densities. Then, even if the farmer is not sure about his likely final yield, or his brome grass density, he can 'cast around' and rapidly gain an appreciation of the sensitivity of the grain losses he will sustain in different size crops infested with different brome grass densities. To allow farmers to do this readily, the information contained in the exponential function described has been presented to them in three ways. This, in itself, allows users to choose the package with which they are most comfortable.

Two-way tables

In Table 1 the information has been placed in a two way table, which shows the likely grain loss for different crops, in this case covering the normal range of yields and brome densities found in Western Australia. The table has been filled out in the lower density range as it is here that most difficulty is found when making weed control decisions. The information can also be presented as monetary loss for a given wheat price, or actual yield achieved. Even if the farmer has only a vague idea of his yield potential and brome grass population, he will usually have a firm cost for a control measure, which he can place against the table, and arrive at combinations of crop size and weed density where it will pay to intervene.

Table 1. Wheat grain yield loss caused by brome grass at different densities in crops of different weed-free yield potentials

Potential weed-free yield (kg ha^{-1})	Brome grass weed density (plants m^{-2})					
	25	50	100	200	300	400
750	67	127	225	367	457	517
1000	90	170	300	490	610	690
1500	135	255	450	735	915	1035
2000	180	340	600	980	1220	1380
3000	270	510	900	1470	1830	2070

These tables allow the farmer to appreciate the impact of brome grass at different densities, and although he may not be able to define his problem further than 'a few' or 'a lot' of brome, or 'one brome for each wheat plant' he can gain an appreciation of likely losses. A single 'critical density' figure does not allow this. In terms of long term weed management, the table may show him that applying a control practice may result in a profit, break-even or loss in that crop, but he can place a judgement on whether to proceed with a practice which will minimise future problems.

Weed cost ready reckoners

The relationships derived have also been placed in ready reckoners (Burgess and Gill 1986) which allow the same 'casting around' as the two way tables, but include different wheat prices also. This method of presentation is shown in Fig. 2. These charts and the tables can be used in the field.

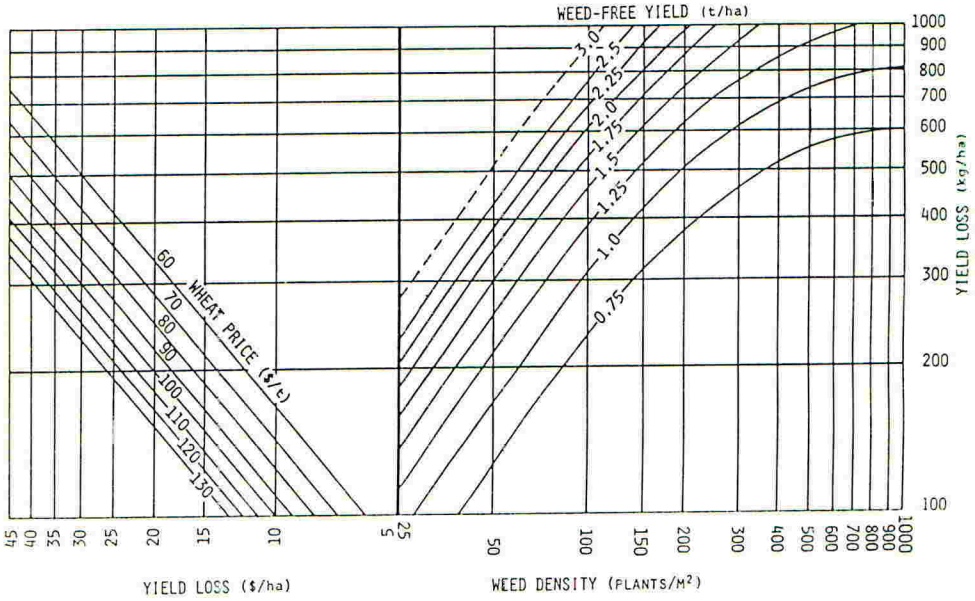

Fig. 2. The 'Weedcost' ready reckoner for brome grass in wheat.

Computer printout

The information has also been programmed for use in microcomputers. The computer prompts the operator with what weed? what weed density? what crop potential? what crop price? and immediately shows losses in kilograms/ha and $/ha. Again, the farmer can rapidly 'cast around' to help his decision making.

Expert systems

The next step, which is in train at present, is to incorporate the relationships within the framework of expert systems technology which will allow farmers to quiz the system about weed control options.

COMMENT

The use of weed density to predict losses due to weeds in crops has been criticized on two grounds. The first is that it is difficult and time consuming to measure (Cousens 1987) and the second that it has limited biological significance, for biomass and leaf area rather than weed density are the key factors which determine uptake of nutrients and water and interception of radiation by a plant species. In this paper we have attempted to show that weed density, measured within 4-6 weeks of

crop emergence, correlates well with yield loss, and can be used to predict crop loss over large agricultural regions and across seasons, provided agronomic practices stay within the range normally used in the region. The difficulty of measurement is acknowledged, as is the variation in density across fields. However, weed density is more easily estimated than weed biomass. Also, when decisions on control practices are made, soon after crop emergence, measurement of biomass of weed seedlings may have little relevance. Gill (1986 unpublished) has shown good correlation between early weed seedling counts and final biomass.

Many workers have shown that weeds which emerge later than a month after the crop emergence have little effect on crop yield, and contribute little to seed production by weeds in the crop. This supports the use of early weed counts for estimates of crop loss. It is acknowledged that for some weed situations, for example where a weed causes tainting, harvesting difficulties or grain contamination, low weed densities and late emerging plants assume importance, and the effect of weeds on yield may then be a secondary consideration.

This paper has not addressed the common problem of mixed weed populations in crops. The impact of controlling one weed species on the competitive relationship of the remaining weeds and crop has been raised by Haizel and Harper (1973), Spitters and van den Bergh (1982) and Poole and Gill (1987). This problem needs to be addressed urgently and is the subject of a present study.

A question which arises when constructing weed competition models is how far to refine them. The spectrum of 'models' available ranges from a single critical weed density figure to complete specification of a physiologically based model (Rijsdijk 1986). The construction of physiological models is difficult and at present may involve so many assumptions that the output is questionable. The models we have described, while being far from satisfying because of their empiricism, are simple mathematically, can be constructed from a combination of historical and easily acquired data, are readily verified under field conditions, are quite robust, and may be all that a farmer wants to help him with his weed control decisions.

While not wishing to diminish the value of simulation or mechanistic modelling, the resources are simply not available to construct such models for even the major crop-weed combinations in an agricultural region. The specification of a model which is sufficiently general to account for most crop-weed situations, yet at the same time has an output which is precise and simple to use in the field appears to be a long way off. It is possible that the two goals are incompatible.

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THE EFFECT OF WEED INTERFERENCE ON THE GROWTH AND YIELD OF WHEAT

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ABSTRACT

The relative competitiveness of Avena fatua, Alopecurus myosuroides and Stellaria media with winter wheat cv. Norman (Triticum aestivum) was compared under uniform glasshouse conditions. Each weed was grown at a range of densities equivalent to 0, 20, 40, 80 and 160 plants/m^2. Weed interference reduced wheat grain yield mainly through a decline in the number of fertile tillers per pot. The order of competitiveness seemed to be A. fatua > A. myosuroides > S. media. The losses in terms of wheat grain yield loss varied in the ranges 27% to 72%, 0.3% to 56% and 0.3% to 32% respectively for the lowest to highest densities of A. fatua, A. myosuroides and S. media.
Linear and non-linear models of yield loss-weed density relationship are compared for each weed species.

INTRODUCTION

Wild-oat (Avena fatua) and black-grass (Alopecurus myosuroides) are two major grass weeds in cereals (Phillipson 1974, Elliott et al. 1979). A. fatua can cause large yield losses in winter cereals (Wilson & Cussans 1978). Interference from A. myosuroides can also cause serious yield losses in cereals (Naylor 1972, Moss 1980). Common chickweed (Stellaria media) is one of the most widespread cereal weeds, although considered to be less troublesome than grass weeds (Mann & Barnes 1950). There is however usually a significant yield response to the control of S. media in winter barley (eg. Orson 1980).

In the study we compare directly the effects of varied densities of A. fatua, A. myosuroides and S. media on the growth and yield of winter wheat cv. Norman. The data are used to evaluate the relative losses due to different weeds, and to test differing models of the yield loss - weed density relationship.

MATERIALS AND METHODS

Additive experiments were carried out under glasshouse conditions (20 ± 6°C; 16 h supplementary lighting). Plants were grown in 25 cm diameter plastic pots with bottom drainage in a sandy loam:peat moss mixture (80:20). Three uniformly pre-germinated seeds of the crop or weed were sown in each position, in a pre-set regular pattern, in the pots. Seedlings were thinned to the required numbers one week after emergence. Standard fertilizers were applied at rates equivalent to 80:60:45 kg/ha N:P:K one week before sowing. Four additional N dressings were applied at rates equivalent to 30, 20, 20 and 10 kg N/ha at tillering (GS 21:Zadoks et al. 1974), stem elongation (GS 30), booting (GS 40) and end of anthesis (GS 70) of wheat plants.

A randomised complete block design with 3 replications was used. A constant density of eight wheat plants per pot was established. Weed

densities were 0, 1, 2, 4 and 8 A. fatua, A. myosuroides or S. media plants per pot, equivalent to 0, 20, 40, 80 and 160 plants/m^2.

The grain yield of both crop and weed plants were harvested at 26-29 weeks after sowing, as they reached maturity. Plant height, number of tillers, number and length of ears, and dry weights of shoots were determined. The ears were threshed: number of grains per ear, and dry weights of chaff and grain were recorded. Data were subjected to analysis of variance using the program Genstat. The data were used to test linear (Dew 1972, Carlson et al. 1981) and non-linear (Wilson & Cussans 1983, Cousens et al. 1984, Cousens 1985) models of the yield loss - weed density relationship. The models were fitted using the program BMDP.

RESULTS

1. Biomass production

The total dry matter yields (as % of plant biomass present in controls) of winter wheat cv. Norman and crop + weed are shown in Fig. 1.

No significant difference (comparisons based on actual yield data) was observed in total biomass production (crop + weed) as the density of A. myosuroides or S. media increased, although it appeared to be slightly lower than that of weed-free control. Avena fatua reduced the dry weight biomass of wheat. The percentage dry weight reduction was significantly more than was caused by the other two weed species. Although there was no significant difference between A. myosuroides and S. media, there is an indication in Fig. 1 of a more rapid rate of yield loss, with increasing weed density, for the grass weed. Total dry matter production by A. fatua was always higher than that of either A. myosuroides or S. media: there was no difference between the latter two species.

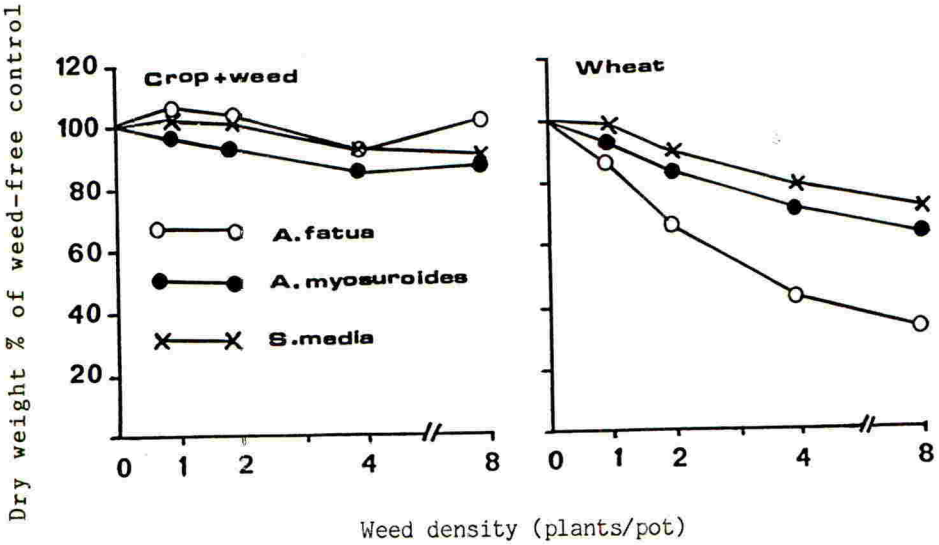

Fig. 1 The effect of different weed species on total dry matter production (crop + weed) and wheat dry weight.

II. Yield and its components

The effects of increasing density of different weeds on wheat grain yield and its components are shown in Table 1.

Grain yield was reduced by increasing weed density. The loss of crop yield was mainly due to a reduction in fertile tiller number. Number of grains per ear, and kernel weight, were also reduced by the presence of weeds in most cases, but to a lesser extent. The greatest yield reduction occurred in *A. fatua* pots. The results of this experiment showed the order of competitiveness to wheat to be *A. fatua* > *A. myosuroides* > *S. media*. The losses in terms of wheat grain yield varied from 27% to 72%, 0.3% to 56% and 0.3% to 32% respectively, for the lowest to highest densities of *A. fatua*, *A. myosuroides* and *S. media*. Wheat dry weight was not reduced to the same extent as grain yield. The losses from these three weeds, in terms of crop total dry weight, respectively varied from 12% to 67%, 5.8% to 37% and 3% to 29% for the lowest to highest weed densities.

TABLE 1

Wheat grain yield and yield components as influenced by different weed species (mean of n = 3 replications; each control replicate taken as the mean of two weed-free control pots)

	Weed density plants/ pot	Grain yield g/pot	No. of ears/ plant	No. of grains/ ear	Kernel weight mg
Weed-free control	0	10.70	1.97	26.60	25.1
Avena fatua	1	7.77	1.57	25.7	23.8
	2	6.43	1.37	26.50	23.0
	4	5.20	1.13	25.10	23.1
	8	2.97	1.00	17.70	21.4
Alopecurus myosuroides	1	10.67	1.93	26.33	25.9
	2	8.37	1.47	26.30	24.5
	4	6.03	1.33	23.80	23.8
	8	4.67	1.17	23.20	20.8
Stellaria media	1	10.67	1.97	26.63	25.5
	2	9.73	1.80	26.57	24.2
	4	9.40	1.80	25.9	24.7
	8	7.20	1.60	23.60	24.1
SE	-	0.57	0.14	1.69	0.67

The goodness of fit of each of the six models to the observed data in terms of residual sum of squared (R.S.S.) varied between the three species (Table 2). For *A. fatua* the best fit was given by the linear model 2 (square root of weed density), with model 6 (non-linear hyperbolic) also giving a very good fit. Only the simplest model (1:linear) gave a poor fit

TABLE 2

Residual sum of squares (R.S.S.) for linear and non-linear models fitted to wheat grain yield loss data.

Weed species	*A. fatua*	*A. myosuroides*	*S. media*
Model	R.S.S.	R.S.S.	R.S.S.
1. $Y_L = Id$ Thurlow & Buchanan (1972)	1233	1296	317
2. $Y_L = b_1 \sqrt{d}$ Dew (1972)	112	1408	608
3. $Y_L = b_1 \sqrt{p}$ Carlson *et al.* (1981)	241	2035	930
4. $Y_L = Ip + cp^2$ Carlson *et al.* (1981)	213	903	313
5. $Y_L = A(1-\exp(-Id/A)$ Wilson & Cussans (1983)	235	965	374
6. $Y_L = Id/(1 + Id/A)$ Cousens (1985)	169	1075	422

d = density
p = weed density/(weed density + crop density)
b_1 = regression coefficient = index of competition as defined by Dew (1972)
I = initial % yield loss
A = asymptotic % yield loss
Y_L = yield loss

TABLE 3

Competitiveness of different weed species with wheat as expressed by initial % yield loss (I)

Weed species	I % per plant/pot	index of competitiveness compared with *A. fatua*
A. fatua	33.8	1.00
A. myosuroides	15.2	0.45
S. media	5.0	0.15

to these data. The observed data for A. myosuroides best described the quadratic model (4), with the two non-linear models (5,6) also giving a relatively good fit. In the case of S. media, again the best fit was provided by model 4, with the simplest linear model (1), and the two non-linear models also giving a good fit to the observed data. The non-linear models were closely similar in terms of goodness of fit for all three data sets. The results of curve-fitting for the hyperbolic model are shown in Fig. 2.

The comparative low-density competitiveness of the three weed species is shown in Table 3. Avena fatua was at least twice as competitive as the other weeds at low densities.

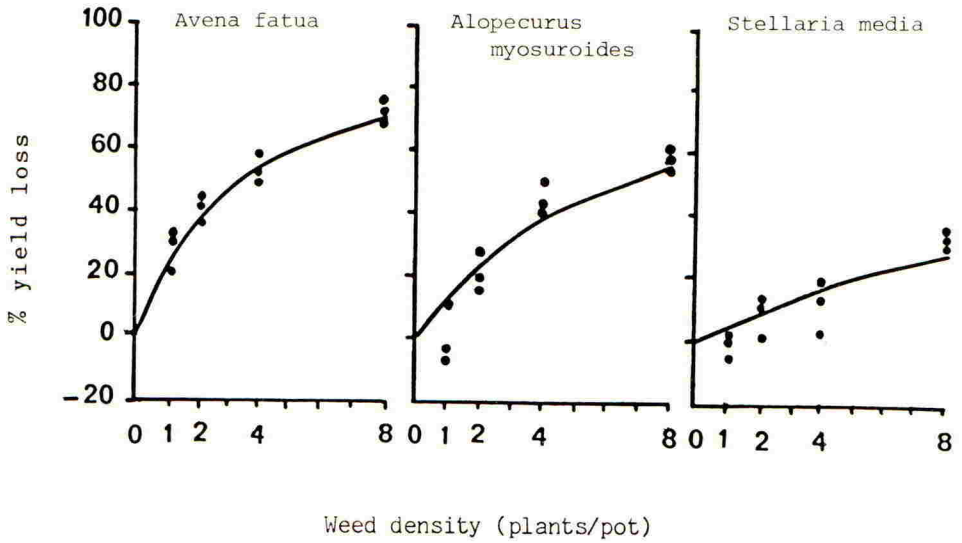

Fig. 2. The results of hyperbolic model (Cousens 1985) applied to different weed species in wheat.

DISCUSSION

The main interference effects of A. fatua, A. myosuroides and S. media on wheat yield were seen in a reduced number of fertile tillers. This accords with long standing observations (e.g. Blackman & Templeman 1938, Aspinall & Milthrope 1959, Wilson & Peters 1982). A. fatua was the most and S. media the least competitive under the conditions of this trial. Shoot interference could occur due to the shading effects of tall-growing A. fatua plants. Willey & Halliday (1971) showed that grain yield was reduced by severe shading as the seed is being filled. The low growth habit of the other two species may reduce the importance of shoot interference with the crop. Other mechanisms, involving root-interference, probably contribute to the severe yield reduction of wheat caused by A. myosuroides at the highest densities and the smaller yield losses caused by S. media. Wellbank (1963) and Naylor (1972) for example have suggested

that A. myosuroides is a good competitor with wheat for nitrogen. However, there are insufficient data to allow conclusions to be drawn here on the differential importance of shoot v. root interference.

Dew's index of competition (Dew 1972) was found to be a good index for comparing the competitiveness of the three weed species. The index of competition for our data was found to be higher than values reported elsewhere, at 5.15, 4.12, and 2.15 for A. fatua, A. myosuroides and S. media, respectively. The index of competition for wild oats in wheat under field conditions is reported to be 3.39 (Dew, 1972) and 4.73 (Carlson & Hill 1985).

In fitting the different models to the observed data, there was no benefit from using relative weed density (p) rather than density (d) itself. This does not agree with the results of Carlson et al. (1981) and Carlson & Hill (1985) who found a better fit resulted from the use of relative proportion of wild oats in the total crop + weed stand. However we used a constant density of crop plants rather than the varying densities of wheat used by these authors.

In general the non-linear models (models 5, 6: Table 2) and Carlson quadratic model (model 4: Table 2) provided a good fit to our observed data. However the simpler linear models could also provide a good fit in certain cases (eg. model 2 and 3 for A. fatua, and model 1 for S. media. The parameter I (initial % yield loss) derived from the non-linear hyperbolic model is perhaps a rather better means of assessing weed competitiveness at low densities (i.e. before intraspecific competition effects come into play). This index has previously shown A. myosuroides to be only 1/4 to 1/5 as competitive as A. fatua according to Cousens (pers. comm.) whilst we found A. myosuroides to be nearly half as competitive as A. fatua, in terms of I, under glasshouse conditions.

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INTERSPECIFIC COMPETITION BETWEEN THREE GRAMINACEOUS WEED SPECIES AND WHEAT

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ABSTRACT

Binary mixtures of wild oats (Avena fatua), bromegrass (Bromus diandrus) and ryegrass (Lolium rigidum) were studied with respect to their effect on the growth and yield of wheat. For each weed species combination, wheat was grown with weed populations of three different total densities. At each density the weed pairs were present in five proportions. There was no evidence in the results that the weeds were interacting in a manner which modified the effect of the individual species on wheat. Comparing the individual species on a per plant basis, annual ryegrass had less effect on wheat than did bromegrass or wild oats. The resulting differences in the behaviour of the weed pairs is discussed.

INTRODUCTION

Wild oats (Avena fatua) and annual ryegrass (Lolium rigidum) are serious and widespread weeds of cereal crops in Western Australia (Paterson 1969, Pearce and Holmes 1976) while bromegrass (Bromus diandrus) is a weed of increasing significance (Holmes 1982, Poole et al. 1986).

A substantial body of information has been compiled regarding the effect on crops of many individual weed species. Such information is important for an economically sound approach to weed control. A number of authors (Moore 1971, Quinlivan 1972, Ennis 1977, Zimdahl 1980 and Gill and Blacklow 1984) have emphasised that in order to improve both the decision making processes involved in, and the efficiency of, weed control a greater knowledge is required of weed biology and the systems of which weeds are a part.

A plant responds to the totality of its environment. The resources available to a given plant are modified by the presence of neighbouring plants which thus constitute a component of the plant's environment. It should not be assumed, therefore, that the growth of a weed and its impact on a crop is independant of the presence of other weed species. Little is known of the influence of weeds on each other or the effect on a crop of a population consisting of a mixture of weeds.

The experiments presented in this paper form part of a study to investigate the interactions among the component species of plant systems which involve wheat grown in the presence of binary mixtures of wild oats, bromegrass and ryegrass. The effect of the weed species on each other and the effect of the mixed weed population on wheat is studied.

The specific aim of the experiments was to determine how the total weed density modifies the interaction between the weed species and between the crop and the weed population. The results presented are for the effect of weed density and proportion on the growth of wheat.

MATERIALS AND METHODS

A set of three experiments was conducted at the School of Agriculture Field Station at Floreat Park, Perth, Western Australia (31°57'S , 115°47' E). The soil was a yellow sand which had been ameliorated by the addition of loam.

To enable the control of moisture, the plots were covered with polythene tunnel houses. Conditions were monitored using thermohygrographs in Stevenson screens within each house and at two adjacent points outside. Rain gauges were installed in each house and irrigation was applied using a sprinkler system. A total of 320mm of water was applied.

Each experiment comprised wheat grown with one of the three binary combinations of wild oats, bromegrass and ryegrass. At each of three total weed densities, replacement series (de Wit 1960) of five proportions were used to investigate the effects of changing the proportion of the mixtures of two weed species on the growth of wheat. The treatment structure was a three x five factorial of total weed density and proportion.

The densities and proportions for all weed pairs were:

Density : 200, 400, 600 plants/m^2
Proportion (as percentage) : 100, 75/25, 50/50, 25/75, 100

Wheat (cv. Gamenya) was present in all plots at a density of 150 plants/m^2. Plots were 1.5m x 1m and contained six rows of wheat. The experimental design was a randomised complete block with two blocks.

The experiment was sown on 24 May 1985. All plots were sampled when the wheat reached anthesis and grain maturity (95 and 197 days after sowing). At each sampling four plants of each species were taken using the two central rows of wheat and the surrounding inter-rows for the weeds. The plants were cut just below ground level and morphological traits measured. The plants were then dried to constant weight at 80°C and weighed.

Transformations were used on the data to stabilize variance: natural logarithms for heights and weights and square roots for counts. The results presented in the tables are back transformed means with transformed data in parentheses. All standard errors are for the difference between transformed means. As this study was of the effect of binary mixtures of weeds on a constant density of wheat, the de Wit forms of analysis were considered inappropriate. All results reported are of wheat. Trends noted in the results are significant at least at the P = 0.05 level. All linear measurements were in mm, dry weight in mg and grain weights per plant in g.

RESULTS

Anthesis

At anthesis the effect of the weeds on wheat growth had become evident.

Both leaf area and plant dry weight were linearly reduced by total density of ryegrass/wild oat combinations. There were no significant main effects of proportion (Tables 1 and 2). The wild oat/bromegrass mixture gave no significant results (Tables 3 and 4).

TABLE 1

The effect of total weed density on wheat characteristics at anthesis for mixtures of wild oats and ryegrass.

Total Density	200		400		600		S.E.
Plant Height (mm)	745	(6.613)	726	(6.587)	668	(6.504)	0.0558
Inflorescence No	1.97	(1.2141)	0.95	(1.2034)	0.95	(1.2034)	0.0200
Dry Weight (mg)	7879	(8.972)	7624	(8.939)	6015	(8.702)	0.1239
Leaf Area (cm^2)	91.9	(4.521)	84.9	(4.441)	68.0	(4.220)	0.0912

TABLE 2

The effect of the proportion of wild oats and ryegrass in the weed population on characters of wheat at anthesis.

Proportion	W.O. 100%	75/25	50/50	25/75	R.G. 100%	S.E.
Plant Height (mm)	722	718	686	700	735	
	(6.582)	(6.577)	(6.531)	(6.551)	(6.600)	0.0720
Inflorescence No	0.96	0.96	0.96	1.00	0.91	
	(1.2070)	(1.2070)	(1.2070)	(1.2247)	(1.1892)	0.0258
Dry Weight (mg)	7052	6761	6393	7809	7708	
	(8.861)	(8.819)	(8.763)	(8.963)	(8.950)	0.1599
Leaf Area (cm^2)	72.9	87.0	84.6	82.8	78.4	
	(4.289)	(4.466)	(4.438)	(4.416)	(4.362)	0.1178

TABLE 3

The effect of total weed density on wheat characteristics at anthesis for mixtures of bromegrass and wild oats.

Total Density	200		400		600		S.E.
Plant Height (mm)	701	(6.553)	736	(6.601)	706	(6.559)	0.0277
Inflorescence No	1.12	(1.272)	1.03	(1.238)	1.05	(1.244)	0.0296
Dry Weight (mg)	8383	(9.034)	6708	(8.811)	8119	(9.002)	0.0774
Leaf Area (cm^2)	66.7	(4.200)	64.6	(4.168)	63.6	(4.153)	0.1038

TABLE 4

The effect of the proportion of bromegrass and wild oats in the weed population on characters of wheat at anthesis.

Proportion	Brome 100%	75/25	50/50	25/75	W.O. 100%	S.E.
Plant Height (mm)	699	702	705	745	721	
	(6.549)	(6.554)	(6.558)	(6.613)	(6.580)	0.0357
Inflorescence No	1.00	1.08	1.09	1.00	1.16	
	(1.225)	(1.257)	(1.262)	(1.225)	(1.287)	0.0382
Dry Weight (mg)	8267	7639	6967	7428	8283	
	(9.020)	(8.941)	(8.849)	(8.913)	(9.022)	0.1000
Leaf Area (cm^2)	61.81	63.05	60.58	74.37	65.89	
	(4.124)	(4.144)	(4.104)	(4.309)	(4.188)	0.1340

Plant height, plant dry weight and leaf area exhibited significant responses in the ryegrass/bromegrass set. The overall weed density reduced plant height. Proportion also affected this variate; there was a linear reduction in height as the proportion of bromegrass increased. There was a significant linear reduction in plant dry weight and leaf area as the proportion of bromegrass increased, but for these variates there was no density effect (Tables 5 and 6).

TABLE 5

The effect of total weed density on wheat characteristics at anthesis for mixtures of ryegrass and bromegrass.

Total Density	200		400		600		S.E.
Plant Height (mm)	718	(6.576)	666	(6.501)	638	(6.458)	0.0262
Inflorescence No	0.97	(1.214)	1.00	(1.225)	0.99	(1.221)	0.0256
Dry Weight (mg)	6355	(8.757)	5914	(8.685)	4434	(8.397)	0.1425
Leaf Area (cm^2)	66.09	(4.191)	61.13	(4.113)	48.52	(3.882)	0.1272

TABLE 6

The effect of the proportion of ryegrass and bromegrass in the weed population on the characters of wheat at anthesis.

Proportion	R.G. 100%	75/25	50/50	25/75	Brome 100%	S.E.
Plant Height (mm)	696 (6.545)	723 (6.584)	654 (6.483)	672 (6.510)	624 (6.436)	0.0338
Inflorescence No	0.91 (1.187)	0.96 (1.207)	1.00 (1.225)	1.00 (1.225)	1.08 (1.256)	0.0330
Dry Weight (mg)	6336 (8.754)	6267 (8.743)	5872 (8.678)	4675 (8.450)	4619 (8.438)	0.1839
Leaf Area (cm^2)	72.24 (4.280)	70.11 (4.250)	53.30 (3.976)	47.66 (3.864)	51.37 (3.939)	0.1643

Grain Maturity

At this stage the wild oat/ryegrass mixture produced a linear density effect in which wheat growth as measured by dry weight, grain number and grain yield per plant all decreased as density increased (Table 7). The dry weight, number of ears and number of spikelets per plant all increased with a decrease in the proportion of wild oats (Table 8). As expected from the anthesis results there were no significant effects due to the wild oat/bromegrass mixtures (Tables 9 and 10).

TABLE 7

The effect of total weed density on wheat characteristics at grain maturity for mixtures of wild oats and ryegrass.

Total Density	200	400	600	S.E.
Inflorescence No	1.12 (1.2731)	1.02 (1.2346)	1.05 (1.2444)	0.0214
Spikelet No	21.54 (4.641)	18.99 (4.358)	18.77 (4.333)	0.1700
Dry Weight (mg)	15788 (9.668)	13521 (9.512)	11814 (9.377)	0.1189
Grain Number	42.00 (6.481)	38.42 (6.198)	35.21 (5.934)	0.2264
Grain Weight (g)	1.757 (1.014)	1.659 (0.978)	1.385 (0.869)	0.0493

TABLE 8

The effect of the proportion of wild oats and ryegrass in the weed population on characters of wheat at grain maturity.

Proportion	R.G. 100%	75/25	50/50	25/75	Brome 100%	S.E.
Inflorescence No	1.00 (1.2247)	1.04 (1.2411)	1.04 (1.2411)	1.08 (1.2575)	1.16 (1.2890)	0.0277
Spikelet Number	18.27 (4.274)	19.24 (4.386)	18.58 (4.311)	20.07 (4.480)	22.74 (4.769)	0.2194
Dry Weight (mg)	11316 (9.334)	13575 (9.516)	12016 (9.394)	14958 (9.613)	16916 (9.736)	0.1534
Grain Number	35.74 (5.978)	39.78 (6.307)	37.52 (6.126)	38.19 (6.180)	41.34 (6.430)	0.2922
Grain Weight (g)	1.421 (0.884)	1.675 (0.984)	1.535 (0.930)	1.581 (0.948)	1.779 (1.022)	0.0636

TABLE 9

The effect of total weed density on wheat characteristics at grain maturity for mixtures of bromegrass and wild oats.

Total Density	200	400	600	S.E.
Inflorescence No	1.05 (1.244)	1.07 (1.252)	1.10 (1.264)	0.0279
Spikelet Number	19.96 (4.468)	19.94 (4.465)	21.09 (4.592)	0.1584
Dry Weight (mg)	12283 (9.416)	12052 (9.397)	11873 (9.382)	0.0867
Grain Number	34.02 (5.833)	33.20 (5.762)	33.17 (5.759)	0.2289
Grain Weight (g)	1.428 (0.887)	1.312 (0.838)	1.323 (0.843)	0.0490

TABLE 10

The effect of the proportion of bromegrass and wild oats in the weed populations on characters of wheat at grain maturity.

Proportion	Brome 100%	75/25	50/50	25/75	W.O. 100%	S.E.
Inflorescence No	1.16 (1.287)	1.00 (1.225)	1.04 (1.241)	1.04 (1.241)	1.12 (1.274)	0.0361
Spikelet Number	21.95 (4.685)	18.91 (4.349)	19.64 (4.432)	19.83 (4.453)	21.37 (4.623)	0.2045
Dry Weight (mg)	11920 (9.386)	11362 (9.338)	11992 (9.392)	12991 (9.472)	12137 (9.404)	0.1119
Grain Number	31.40 (5.604)	32.99 (5.744)	32.58 (5.708)	37.70 (6.140)	32.81 (5.728)	0.2955
Grain Weight (g)	1.237 (0.805)	1.363 (0.860)	1.303 (0.834)	1.545 (0.934)	1.333 (0.847)	0.0633

There was a linear response to density in the ryegrass/bromegrass series for dry weight and spikelet number. In both cases increased density decreased the yield. There was no significant effect of proportion (Tables 11 and 12).

TABLE 11

The effect of total weed density on wheat characteristics at grain maturity for mixtures of ryegrass and bromegrass.

Total Density	200	400	600	S.E.
Inflorescence No	1.02 (1.2346)	1.05 (1.2451)	1.00 (1.2247)	0.0083
Spikelet Number	18.41 (4.291)	18.75 (4.330)	16.54 (4.067)	0.0789
Dry Weight (mg)	12657 (9.446)	11920 (9.386)	9396 (9.148)	0.1279
Grain Number	35.05 (5.92)	32.26 (5.68)	28.52 (5.34)	0.3720
Grain Weight (g)	1.370 (0.863)	1.330 (0.846)	1.119 (0.751)	0.0774

TABLE 12

The effect of the proportion of ryegrass and bromegrass in the weed population on characters of wheat at grain maturity.

Proportion	RG 100%	75/25	50/50	25/75	Brome 100%	S.E.
Inflorescence No	1.00 (1.2247)	1.08 (1.2575)	1.04 (1.2411)	1.00 (1.2259)	1.00 (1.2247)	0.0107
Spikelet Number	17.56 (4.190)	19.56 (4.423)	18.02 (4.245)	16.44 (4.055)	17.91 (4.232)	0.1018
Dry Weight (mg)	11441 (9.345)	14750 (9.599)	11004 (9.306)	8875 (9.091)	10862 (9.293)	0.1651
Grain Number	34.11 (5.84)	34.69 (5.89)	31.02 (5.57)	27.67 (5.26)	32.15 (5.67)	0.4810
Grain Weight (g)	1.396 (0.874)	1.455 (0.898)	1.223 (0.799)	0.994 (0.690)	1.312 (0.838)	0.0999

DISCUSSION

The results of studies on the interaction of annual ryegrass and wheat (Reeves 1976 and Rerkasem et al.1980) led to the expectation that, in the present experiments, at least ryegrass would have had a deleterious effect on wheat growth and yield. There is evidence that similar expectations would apply to wild oats and bromegrass (McNamara 1972, Paterson 1969 and Poole et al. 1986).

As expected, the total density of the weeds reduced wheat growth and yield. Over the range considered, the effect was proportional to the number of weeds present. These results applied to wild oat/ryegrass and ryegrass/bromegrass mixtures only. The wild oat/bromegrass mixture had no significant effect on wheat.

Information on the respective competitive status of these three species of weed against wheat derives from the effect of the proportions. Wild oats had a greater impact on wheat than did the ryegrass in the wild oat/ryegrass experiment while bromegrass did not differ significantly from either ryegrass or wild oats. It is concluded that in these binary mixtures ryegrass is the least competitive species against wheat followed by bromegrass and wild oats; the relative status of these two is unclear.

In the bromegrass/wild oat experiment no effect of total density was found. Results for both the effect of wild oats (Paterson 1969) and bromegrass (Poole et al. 1986) on wheat in Western Australia indicated that the greatest effect of these two weeds occurred at low densities. McNamara (1972) states that in New South Wales the maximum effect of wild oats is attained at a density of 300 plants/m^2. The results of the current study support these findings in that the absence of a significant effect of densities above 200 plants/m^2 (the minimum density used) suggests that these densities are in the flatter part of the response curve to these weed species. By contrast, Reeves (1976), working in Victoria, found a response of wheat yield to ryegrass density which had a more constant gradient over a wide range of densities; this would account for our results when ryegrass is present.

The results discussed with respect to wheat growth appear general, with plant height, dry weight, leaf area and at grain maturity, grain variates being influenced by the weeds in the manner described.

There was little indication that the weeds interacted in a manner which modified the effect of the individual species on wheat. If this had been the case one would have expected marked deviations from the linear trends of proportions within the replacement series.

Further work is in progress to confirm these results and to study the implications for crop growth.

ACKNOWLEDGEMENTS

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RELATIVE TIME OF EMERGENCE, LEAF AREA DEVELOPMENT AND PLANT HEIGHT AS MAJOR FACTORS IN CROP-WEED COMPETITION

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ABSTRACT

Growth analysis data (dry weight, LAI and height) are presented of competition experiments with sugar beet (Beta vulgaris cv. monohill and cv. salohill), Fat hen (Chenopodium album) and Chickweed (Stellaria media), as well as with beet and early and later sown Chenopodium. Yield losses are not related to leaf area, the worst weed Chenopodium having the lower LAI. The paradox is explained by different height growth of the weed species in view of the competition for light.
A time lag of 21 days between the emergence of crop and Chenopodium still leads to yield losses; weeds emerging 30 days later than the crop no longer develop a canopy on top of the crop's and no longer lower yields.

INTRODUCTION

In open and early sown crops such as sugar beet, a group of late summer annuals tends to escape the current mechanical operations and even soil herbicides; mechanical control measures during early crop growth may only be effective between the rows. Remaining weeds in the row, even at low density, often cause substantial damage (Solanum nigrum, Chenopodium album, Echinochloa crus-galli) (Zimdahl 1980). In these circumstances the need is felt for reliable prediction of yield losses and advice for herbicide application (Cousens in prep.)

In the search for practical warning systems and thus in the development of empirical models (Spitters et al. 1983), the following characters (parameters) are put forward as useful descriptors of weed infestation:
- the time of emergence of the weed with respect to the crop emergence date (Cousens 1985, Lapointe 1985, O'donovan et al. 1985, Spitters et al. 1983),
- the weed species (provided that we have data on specific biological and physiological characters such as growth form, height and other morphological responses to competition, a.o.),
- the weed density. This parameter on its own has only a limited value, as demonstrated in many experiments on damage thresholds: large differences in yield loss - weed density relations between experiments are often found (Koch 1974, Kropff et al 1984, Poole et al. 1987, Schweizer 1981, Wahmhoff et al. 1985, Zimdahl 1980).

Plant responses to environmental factors can be quantified. For many crop species these relations are used in growth models, but data on physiological characteristics of weeds are still lacking. Present versions of the crop-weed competition models, equally based on physiological growth parameters, suggest a predominant influence of differences in the times of emergence of crop and weed, and of two biological characters, the leaf area development and plant height (Kropff et al. in prep.). However, there appear to be few complete sets of field data on growth and performance of both weed and crop in monocultures and mixtures, in well monitored environmental conditions, in different years and with different time lag between crop and weed emergence. This labourious type of field experiment is rewarding, since, apart from datasets for validation of simulation models and the testing of

hypotheses generated with the models, it offers a discriminating view on competition-related processes in the course of a growing season.

In this contribution we present the analysis of two field experiments in subsequent years, in order to evaluate the influence of the relative time of emergence, and of plant height and leaf area development, given the crop and weed species and their responses to different weather conditions.

EXPERIMENTAL DESIGN

The field experiments, in split plot design with four replicates, were carried out in Wageningen on a loamy sand (4% soil organic matter) with adequate supply of water and nutrients.

In 1985 sugar beet was grown at 30 cm equidistant spacing (11 plants per m^2); the weeds were grown equally distributed between the crop plants, Fat hen at 5.5 plants per m^2 in mixture and 11 plants per m^2 in monoculture, Chickweed at 11 (clumps of) plants per m^2, both in mixture and monoculture. The plot size was 6 x 1.5 m, allowing harvest of 15 plants. The dates of 50% emergence for sugar beet, Fat hen and Chickweed were 9 May, 21 May and 20 May, respectively.

In 1986 sugar beet was grown in rows 50 cm apart, at distances of 18 cm in the row (11 plants per m^2); Fat hen was equally grown in rows at plant distances of 18 cm in monoculture or in the rows of the crop, alternating with the sugar beet plants. Plot size was 6 x 1.25 m. Fat hen was sown at crop emergence and 15 days later and had final densities of 11 plants per m^2 in monoculture, but in the mixtures of 9.1 and 9.7 plants per m^2, respectively. The dates of 50% emergence for sugar beet, Fat hen (early) and Fat hen (late) were 4 May, 25 May and 3 June, respectively.

RESULTS

The 1985 experiment produced growth curves of the type expected for the monocultures of Beta, Chenopodium and Stellaria (Fig. 1A) and illustrates the shorter life cycles of the weeds, especially Stellaria. The time lag between sugar beet and weed emergence of about 10 days leads to substantial crop losses at final harvest, but even at the beginning of July there is an influence of Beta (by then over 2 t ha-1) on the weed, especially Stellaria, and vice versa. Table 1 shows that total crop biomass was reduced with 21% by Stellaria and with 37% by Chenopodium. The time course of the height development and the leaf area index of Beta-weedfree (B), Beta with Chenopodium (Bc) and B. with Stellaria (Bs) is summarized in Table 2, as well as height and LAI for Chenopodium and Stellaria monocultures (Chm and Stm) and their mixtures with Beta (Chb and Stb). Marked differences were the height development of Chenopodium (more than twice as high as the crop) and the much higher LAI of Stellaria (LAI 2.68, against 0.96 in Chenopodium).

The 1986 experiment offers comparable results, with respect to monocultures of Beta and Chenopodium 1 (early). Apparently weather conditions (August and September being drier, colder and more clouded than 1985) were less favourable for Beta and its final production stayed well behind 1985. The weather did not affect final biomass of Chenopodium 1, emerging on May 25 (four days later than previous year), due to its shorter life cycle. Even the Chenopodium 2 (late) emerging on June 3, produced almost the same final biomass, well over 13 t ha-1.

In the mixtures only the early Chenopodium with a time lag of 21 days,

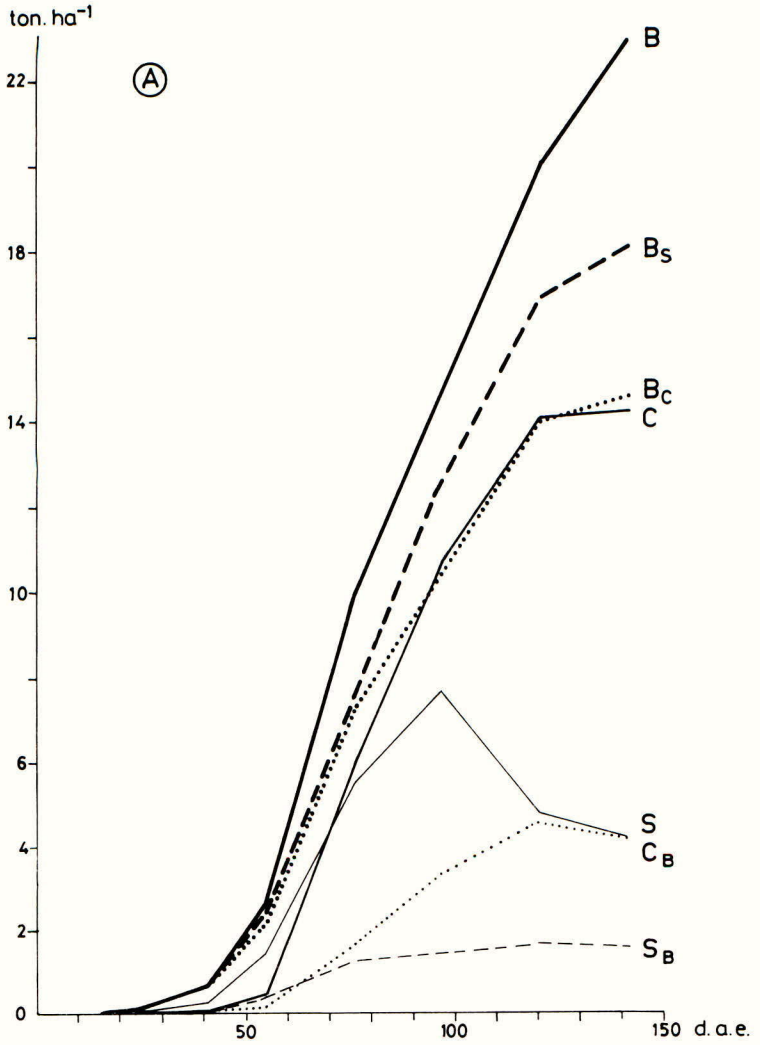

Fig. 1.A. Development of dry weight (t. ha^{-1}) in 1985 of Beta in monoculture (B), in mixture with Stellaria (B_S) and with Chenopodium (B_C). Dry weight of the weeds comprise Stellaria in monoculture (S), in Sugar beet (S_B) and Chenopodium in monoculture (C) and in Sugar beet (C_B); dae: days after emergence of the sugar beet crop.

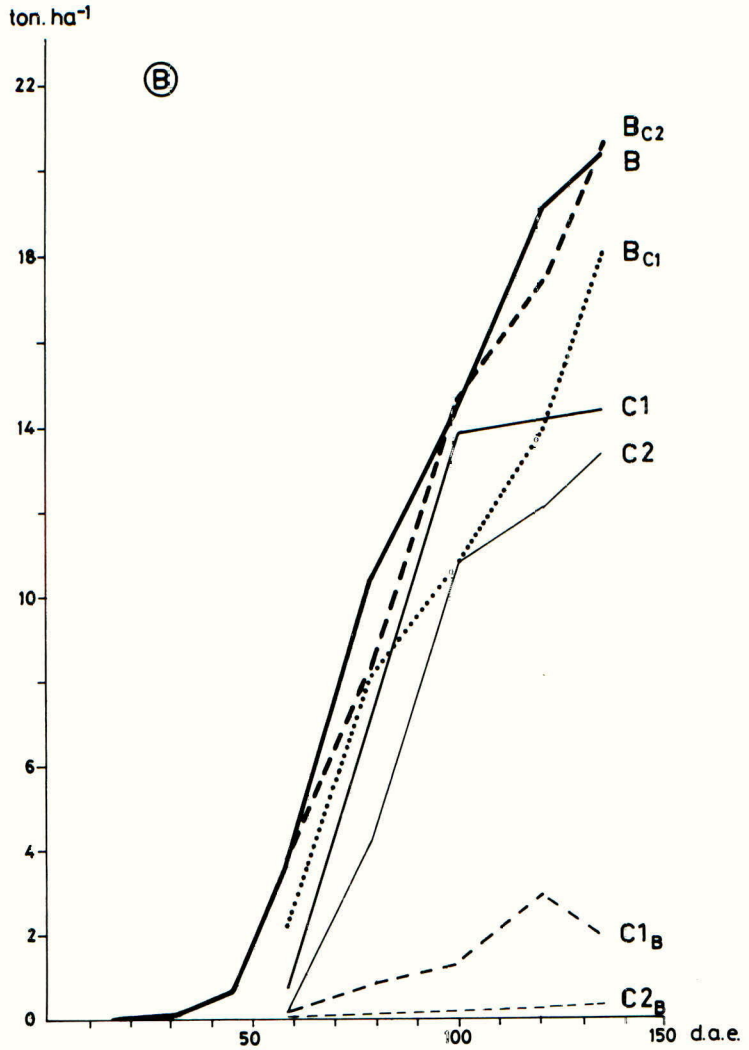

Fig. 1.B. Development of dry weight in 1986 of Beta and Chenopodium (early: C1, and late: C2) in monocultures and mixtures. (Explanation of symbols 1A).

TABLE 2

Height development in Beta (B: mono, B_C: with Chenopodium, B_S: with Stellaria), Chenopodium (C: mono, C_B: with Beta) and Stellaria (S: mono, S_B: with Beta), and development of leaf area index LAI in 1985. For 1986 height and LAI data relate to Beta and Chenopodium only, the latter sown early (C1) and late (C2). Dae: days after emergence of the crop.

	Height cm (1985)							LAI (1985)						
dae	B	B_C	B_S	C	C_B	S	S_B	B	B_C	B_S	C	C_B	S	S_B
39	18	17	17	13	13	10	9	.77	.79	.85	.09	.04	.54	.21
53	34	33	32	53	53	21	22	2.35	1.98	1.94	.81	.24	3.30	1.15
74	50	51	51	142	127	35	50	5.65	3.97	4.03	4.63	.96	10.61	2.68
95	62	57	60	159	145	29	56	5.10	4.17	3.87	4.73	.80	9.33	2.68
118	59	58	60	166	142	-	-	5.10	4.19	4.13	4.24	.59	1.26	.67
140	61	59	56	159	142	11	53	4.66	3.27	3.08	1.15	.12	.58	.17

		Height cm (1986)*				LAI (1986)						
dae C1	dae C2	C1	$C1_B$	C2	$C2_B$	B	B_{C1}	B_{C2}	C1	$C1_B$	C2	$C2_B$
28	38	28	28	12	13	2.29	2.22	2.56	.81a	.18b	.23b	.02c
48	58	136	72	101	31	3.36	2.73	2.40	3.43a	.27a	2.98b	.07c
69	79	158	74	158	43	3.77	2.89	3.34	3.75a	.29a	3.78b	.07c
90	100	159	92	156	43	3.18	2.50	3.15	3.33a	.26a	3.78b	.05c
104	114	156	81	149	45	2.85	2.79	3.11	2.64a	.05a	2.67b	.03c

* Height of Beta compare data 1985

reduced Beta production with 11% at final harvest (Table 1B); already on July 1st a lowering of Beta biomass and of Chenopodium biomass is shown (Fig. 2).

TABLE 1

The 1985 and 1986 sugar beet production at final harvest in mono- and mixed cultures, in t.ha^{-1} and in (%) of weed-free.
Different letters indicate significant difference between treatments (P <0.05, capitals P <0.01).

1985		weed-free	with Stellaria	with Chenopodium
Total dry weight	(t ha^{-1})	23.1 (100) a(A)	18.2 (79) b(AB)	14.6 (63) b(B)
Shoot dry weight	(t ha^{-1})	8.6 (100) a(A)	6.8 (80) b(AB)	6.4 (74) b(B)
Root dry weight	(t ha^{-1})	14.5 (100) a	11.3 (78) ab	8.2 (57) b
Total fresh weight	(t ha^{-1})	61.9 (100) A	48.9 (79) B	33.1 (53) C
Sugar content (%)		15.04 a	15.43 b	15.43 b
Sugar production	(t ha^{-1})	9.3 (100) A	7.5 (81) B	5.1 (55) C

1986		weed-free	with early Chenopodium	with late Chenopodium
Total dry weight	(t ha^{-1})	20.3 a	18.1 b	20.5 a
Shoot dry weight	(t ha^{-1})	7.4 -	6.9 -	7.0 -
Root dry weight	(t ha^{-1})	12.9 a	11.2 b	13.5 a
Total fresh weight	(t ha^{-1})	53.5 a	45.4 b	56.3 a
Sugar content (%)		17.7 -	17.9 -	18.0 -
Sugar production	(t ha^{-1})	8.5 a	7.3 b	9.0 a

CONCLUSIONS

LAI and height

The results of the 1985 experiment show marked differences between the two weedspecies, Chenopodium causing by far the highest yield loss. Supply of water and nutrients taken as sufficient, the competition will have been for light exclusively. This result stands in marked contrast to the lower yield loss by Stellaria, which had a strong leaf area development (LAI 2.7 and in its monoculture even 10) compared to Chenopodium (LAI 0.96 and in monoculture 4.6). This is explained by the data on height development (Table 2). In monoculture Chenopodium plants grew up to a height of 160 cm and up to 150 cm between the sugar beets, which topped at 60 cm, and was able to use its lower LAI more effectively in the light interception. Stellaria in monoculture remained of low statue (35 cm), but part of it used the beet plants to climb up to the same height as the crop.

Relative time of emergence

The results of the 1986 experiment clearly show the strong effect of a difference of 10 days in the period between sugar beet and weed emergence. The latest sown weed did not gain a high statue, reached only modest LAI and finished its growth together with the early sown weed, at the end of a shorter life span and without seriously hampering crop production.

DISCUSSION

Height- and leaf area development, together with emergence date and two differing years, they once more revealed their serious influence on the outcome of crop-weed competition (viz. also Elberse et al. 1979, Lapointe 1985).

On the one hand the relative date of emergence proves to be an indispensible datum in any discussion of competition and it is amazing that in many publications this aspect is neglected.

The results draw attention to the germination and to developmental characteristics of the weeds and above all to the rate of leaf area development and height growth of the weeds relative to the crop canopy. Although Chenopodium is known for its capacity to increase height in a shadowy environment, the plants of the later generation in the 1986 experiment did not develop a canopy on top of the crop's. Shortening daylength urged the onset of flowering and although the weed had a substantial production including a seed crop, it did not interfere with the sugar beet.

The results of the present experiments, although permitting clear conclusions, cannot lead to causal understanding or generalization. This is only to be expected from simulation studies based upon knowledge of the underlying physiological processes, governing photosynthesis and morphological development (height growth, lateral spread, leaf development). The hypotheses generated may lead to relatively simple and less casuistic field experiments.

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SESSION 9B

MODE OF ACTION AND METABOLISM OF HERBICIDES: III

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SESSION ORGANISER DR K. E. PALLETT

INDUCTION OF TETRAPYRROLE ACCUMULATION BY DIPHENYLETHER-TYPE HERBICIDES

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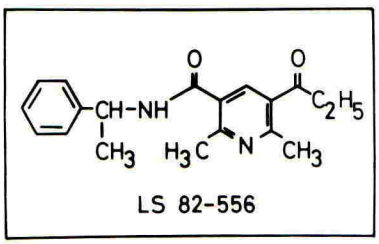
ABSTRACT

The diphenylether herbicide acifluorfen-methyl and the chemically unrelated LS 82-556 (Rhône Poulenc) are toxic to non-chlorophyllous soybean cells. Both herbicides induce the same types of morphological and biochemical symptoms, and their effects are light-dependent. Toxicity of acifluorfen-methyl was observed in soybean cells deprived of carotenoids. Action spectrum showed a peak of activity around 400nm, and notable effects up to 650 nm. Accumulation of tetrapyrroles was observed following a dark treatment with acifluorfen-methyl, and cells could be protected from the herbicidal effect by an inhibitor of tetrapyrrole biosynthesis. Similar conclusions were reached with etiolated cucumber seedlings treated with LS 82-556. These results lead us to propose that the phytotoxicity of diphenylether herbicides is due to their ability to induce abnormal accumulations of tetrapyrroles. These photosensitizing pigments then react with molecular oxygen to produce compounds toxic to living cells.

INTRODUCTION

The diphenylether (DPE) acifluorfen-methyl (AFM) causes bleaching, wilting and dessication of treated plants in a strictly light dependent manner (Orr and Hess 1982). Although belonging to a different chemical family, the pyridine derivative LS 82-556 (Fig. 1) induces exactly the same symptoms (Matringe et al. 1986). Both herbicides are active on green and etiolated seedlings and on non-chlorophyllous cell cultures (Matringe et al. 1986, Matringe and Scalla 1987). In the presence of oxygen, they induce photooxidation of fatty acids, then membrane disruptions and cell death.



LS 82-556

Fig. 1.

In spite of numerous studies, the exact mechanism of the light-activated toxicity is still unclear. Although some experiments support the participation of nitro radicals to the toxic reactions (Draper and Casida 1985), this mechanism is obviously excluded in the case of a chloro-DPE (Orr et al. 1983) and LS 82-556, which nevertheless act in the same way as nitro-DPEs. The nature of the photoreceptor involved is also a matter of controversy. Neither LS 82-556 nor a DPE such as AFM absorb visible light, so participation of cellular photoreceptor(s) has to be postulated. It is commonly accepted that these photoreceptors are carotenoids (Matsunaka 1969). This theory however, does not fit with the sensitivity of treated tissues to red light, i.e. outside the zone of absorption of these pigments (Ensminger and Hess 1985, Matringe et al. 1986).

In order to reexamine the nature of the photoreceptor, we have established the action spectrum for the toxicity of AFM and LS 82-556 on non-chlorophyllous soybean cells and etiolated cucumber seedlings, respectively. As will be shown below, the results of these studies have led us to postulate a participation of tetrapyrrole pigments in the toxicity of DPE-type herbicides.

MATERIALS and METHODS

Cell culture and plant material

Non-chlorophyllous soybean cells were grown in liquid medium as described earlier (Matringe and Scalla 1987). Before experiments, 3-day-old cultures were diluted to 30 mg fresh weight / ml. All chemical treatments were done in the dark during a 14h incubation period. Etiolated cucumber seedlings were grown as described by Matringe et al. (1986).

Norflurazon treatments

Soybean cells were subcultured twice in the presence of 50 µM norflurazon, an inhibitor of carotenoid biosynthesis (Delvin et al. 1976).

Action spectra

Action spectra were obtained using Balzers broad-band interference filters, type Filtraflex-K. The light source was a slide projector delivering a light intensity of 650 $\mu E/m^2/s$ through K 45 to K 70 filters, and 300 $\mu E/m^2/s$ throught the K 40 filter. For white light irradiation, an incandescent lamp (MAZDA PAR, cool beam, 120 W) giving 650 $\mu E/m^2/s$ PAR was used.

Cellular damage

Cellular damage was estimated by three different methods, depending on the plant material. 1: from the amount of ^{86}Rb released by non-chlorophyllous soybean cells into the culture medium, according to Orr and Hess (1982). 2: from the amount of thiobarbituric acid-reacting material (TBARM) in etiolated cucumber hypocot yl, according to Placer et al. (1966). 3: from the amount of electrolytes released from etiolated cucumber hypocothyl sections floating on water (electrolyte leakage was followed using a Hoelzle & Chelius L 17 conductivimeter).

Protective effect of 4,6-dioxoheptanoic acid (DA)

Cell cultures

DA was added twice to cell suspension, at a final concentration of lmM: at the begining of the 14 h dark pretreatment, and one hour before light exposure (650 $\mu E/m^2/s$ PAR). ^{86}Rb efflux was estimated after 8 h in the light.

Etiolated cucumber hypocotyls

They were floated on aqueous solutions of DA (0,5 mM). After 8 h in the dark, they were exposed for 14 h to a light intensity of 400 $\mu E/m^2/s$ PAR. The conductivity of the medium was then determined.

Extraction of protoporphyrin IX

Soybean cells

Extraction was carried out according to Rebeiz et al. (1975). The protoporphyrin content of hexane-washed acetone extracts was determined using a Jobin et Yvon 3D fluorimeter calibrated with a 1 to 8 x 10^{-7} M solution of protoporphyrin IX.

Plant material

Extraction was carried out according to Watson et al. (1960). The protoporphyrin content of 10% HCl extracts was determined by spectrophotometry at 409 nm, with the use of protoporphyrin IX in 10% HCl as a standard.

RESULTS

Effects of AFM and LS 82-556 on the growth of non-chlorophyllous soybean cell cultures.

Treatment by 10 µM AFM in the light resulted in arrested growth and cell death. These effects were not observed when treatment were done in the dark (Fig. 2). LS 82-556 (100µM) induced exactly the same effects (not shown).

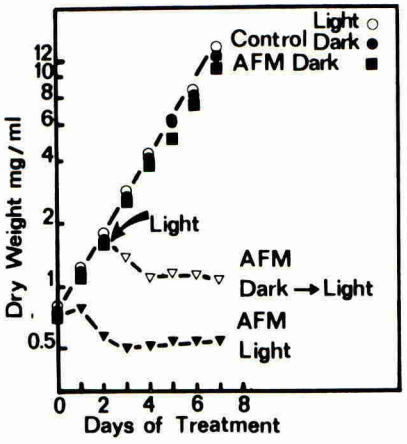


Fig. 2. Response of non-chlorophyllous soybean cells to AFM.

Induction of cellular damage in the absence of carotenoids.

Involvement of carotenoids was examined by monitoring the increase of dry weight / fresh weight ratio in norflurazon-treated cultures, in the presence of AFM or LS 82-556. Although deprived of carotenoids, norflurazon-treated cells remained sensitive to both 10 µM AFM or 100 µM LS 82-556 under white light (Fig. 3).

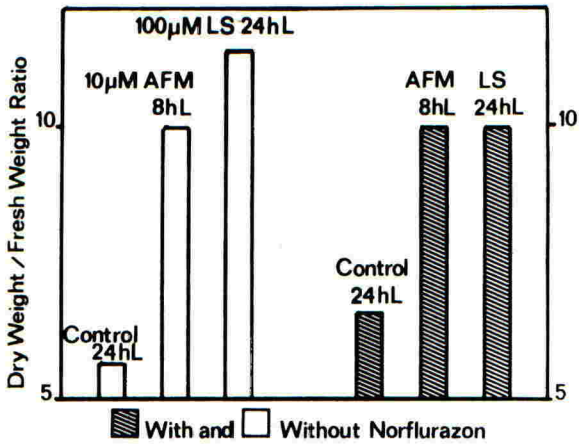


Fig. 3. Effects of LS 82-556 and AFM on soybean cells, either normal or without carotenoids (norflurazon treated). Dry weight/fresh weight ratios were estimated after 8 h (AFM) or 24 h (LS) treatment in the light.

Action spectra of AFM on non-chlorophyllous soybean cells and LS 82-556 on etiolated cucumber hypocotyls.

The action spectrum of AFM on non-chlorophyllous soybean cells was very similar to that of LS 82-556 on etiolated cucumber hypocotyls (Fig. 4). Maximum responses occured in the 350-450 nm region transmitted by a K 40 filter, even though the light intensity delivered by that particular filter was half that of the other ones. Wavelengths between 450 nm and 700 nm also induced toxicity but were less efficient. The light transmitted by the K 70 filter (650-750 nm) did not induce damages, suggesting a drop of activity above 650 nm.

These results indicate that the chromophore(s) implicated in the toxic process strongly absorbs light in the blue region (400 nm) and has secondary zones of absorption at the others wavelengths, ranging from 450 to 650 nm. As the participation of carotenoids was ruled out by the results of Fig. 3, and since soybean cells and etiolated cucumber hypocotyls were devoid of chlorophylls (not shown), a role of these pigments seemed also excluded. Among cellular chromophores, tetrapyrroles have absorption spectra matching the action spectra of AFM and LS 82-556. Therefore, a possible participation of these pigments as photoreceptors was examined.

Protective effect of 4,6-dioxoheptanoic acid (DA)

If tetrapyrroles are the photoreceptors for the light-activated herbicidal toxicity, cells deprived of these chromophores should be tolerant to AFM and LS 82-556. That point was examined using 4,6-dioxoheptanoic acid, which inhibits delta-aminolevulinic acid dehydratase (Meller and Gassman 1981), and consequently stops tetrapyrrole synthesis. As shown in Fig. 5, a 14h pretreatment in the dark with 1 mM DA for non-chlorophyllous soybean cells, or 8 h with 0.5 mM DA for etiolated cucumber hypocotyls, reduced their sensitivity to AFM or LS 82-556, respectively. These results thus

strenghtened the possibility that tetrapyrroles play a role in the toxic process. Tetrapyrrole content of soybean cells treated with AFM and etiolated cucumber seedlings treated with LS 82-556 were thus examined.

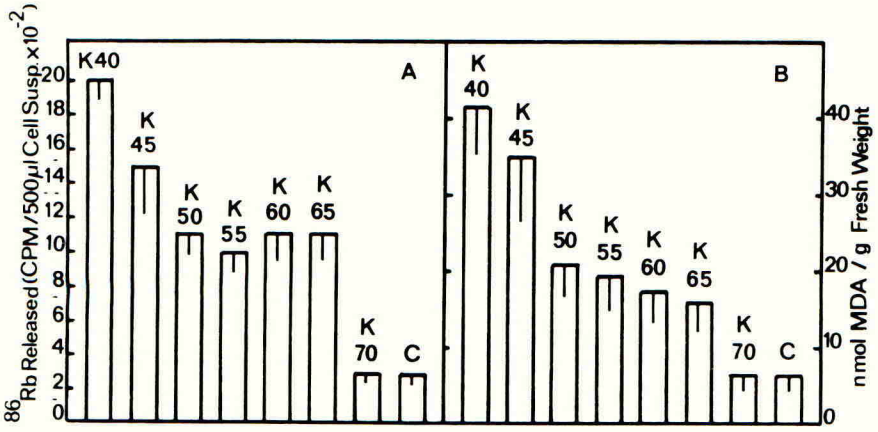


Fig. 4. Action spectra of 10µM AFM on soybean cells (A) and 10µM LS 82-556 on etiolated cucumber hypocotyls (B). Interference filters are designated according to the wavelengths of maximum transmission, e.g. K40 for 400 nm. Half bandwiths are 50 nm. C: control cells (results are the same for all wavelengths). Vertical bars represent standard errors.

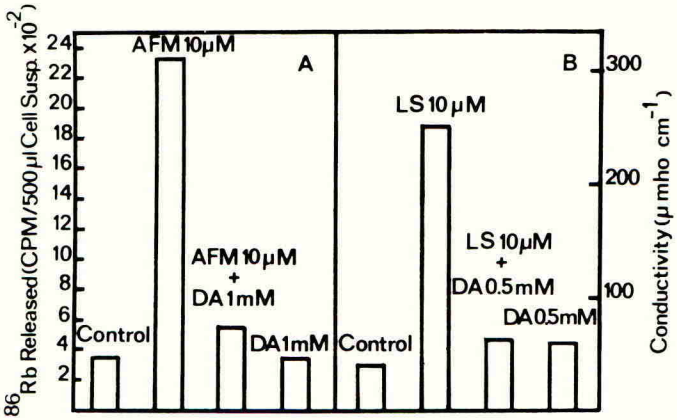


Fig. 5. Effect of 4,6-dioxoheptanoic acid (DA) on the toxicity of AFM on soybean cells (A) and LS 82-556 on etiolated cucumber hypocotyls (B).

Accumulation of tetrapyrroles in AFM-treated cells and LS 82-556-treated cucumber seedlings.

In control soybean cells as well as in etiolated cucumber hypocotyls or cotyledons, the amount of tetrapyrroles was below detection level. By contrast, the extracts of cells or etiolated cucumber seedlings treated with AFM or LS 82-556 in the dark presented fluorescence spectra closely matching the signals of protoporphyrin IX (Fig. 6). Similar accumulation have been found in etiolated cucumber cotyledons (not shown). This accumulation was markedly reduced if AFM treatment was done in presence of DA (Fig. 6).

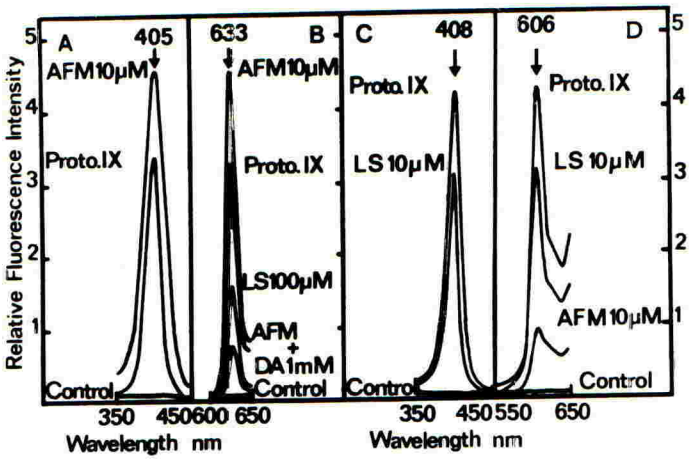


Fig. 6. Fluorescence excitation (A,C) and emission (B,D) spectra of extracts of soybean cells (A,B) and etiolated cucumber hypocotyls (C,D). Spectral differences between the two types of extracts are due to the use of two different extraction solvents (see MATERIALS AND METHODS).

DISCUSSION

Precise identification of the cellular chromophores implicated in the phytotoxicity of DPE could be a clue to the enigmatic mode of action of these herbicides. As mentioned before, the commonly proposed hypothesis is that these chromophores are carotenoids. Since this hypothesis meets some difficulties for chlorophyllous tissues, we have reexamined the question using soybean cell cultures.

AS in green plants, the herbicidal effect of AFM and LS 82-556 on these cells is strictly light-dependent. Our cultures contain carotenoids (0.9 to 1.6 µg / g fresh weight), but are unable to synthesize detectable amounts of chlorophylls, even in the light. For that reason, this material provides a simple and attractive model to reexamine the role of carotenoids. Our results showed that soybean cells deprived of carotenoids remain sensitive to AFM and LS 82-556. Consequently, in non-chlorophyllous cells as well as in green tissues, the role of carotenoid is highly questionable.

Since the action spectrum of DPE in green tissues could be confused by pigments not involved in the toxic process, we have undertaken a reapprai-

sal of this question using our non-chlorophyllous cells. Here again, evidence was not in favour of the participation of carotenoids, but rather of tetrapyrrole-like pigments.

Indeed, spectrofluorometric assays showed that pigment(s) with the fluorescence characteristics of protoporphyrin IX do accumulate in AFM-treated cells. Conversely, cells treated by an inhibitor of tetrapyrrole synthesis were tolerant to AFM.

Several lines of evidence showed that tetrapyrrole accumulation is a characteristic of DPE-type phytotoxicity. First, although AFM and LS 82-556 are chemically unrelated, they exert the same type of phytotoxicity, and similarly induce tetrapyrrole accumulation. Secondly, this last phenomenon is not restricted to cell cultures, but also occurs in plants. Accordingly, these plants are protected if tetrapyrrole synthesis is suppressed, and again the herbicidal action spectrum fits with a participation of tetrapyrroles.

Tetrapyrroles are known as photosensitizers able to generate singlet oxygen in the light (Cox and Whitten 1983). For example, treatment of human erythrocytes by protoporphyrin IX induces photooxidations leading to cell injury (Girotti and Deziel 1983). It has also long been known that in man, metabolic disorders known as porphyrias lead to porphyrin accumulation, and that photosensitivity is one of the main clinical manifestations of these diseases (Schmid et al. 1954). Finally, artificially induced tetrapyrrole accumulation in plants has been shown to result in herbicidal damage, which clearly demonstrate the potential harmful effects of these pigments (Rebeiz et al. 1984).

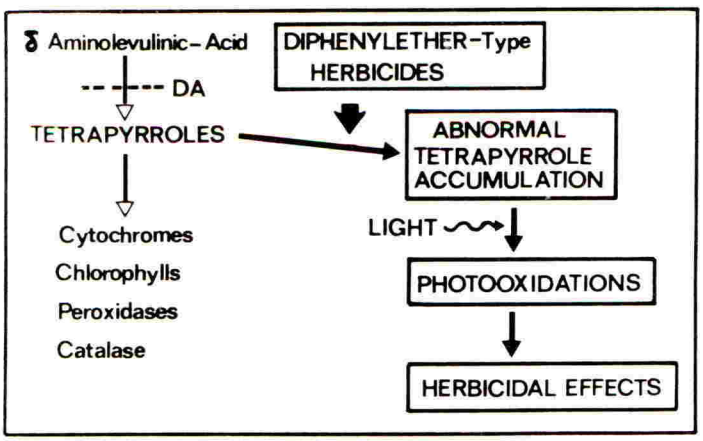


Fig. 7. Postulated mechanism of action of diphenylether-type herbicides.

Our results, together with the photodynamic properties of tetrapyrroles, lead us to propose a new mechanism for the mode of action of DPE-type herbicides (Fig. 7). According to our hypothesis, DPE are not directly involved in any photodynamic reaction. In fact, they would rather induce an accumulation of tetrapyrroles, which would be the actual photosensitizers. The phytotoxic properties of DPE would thus result, not from their elusive photodynamic properties, but from their ability to induce accumulations of these pigments, either in the dark or in the light.

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THE ROLE OF PHOTOSYNTHETIC ELECTRON TRANSPORT IN THE MODE OF ACTION OF NITRODIPHENYL ETHER HERBICIDES

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ABSTRACT

The nitrodiphenyl ether DPEI[1] induces light- and O_2-dependent lipid peroxidation and Chl bleaching in the alga Scenedesmus obliquus. Under conditions of O_2-limitation, inhibition of photosynthetic electron transport by addition of prometryne or diuron, or by mutation, diminishes these effects. Similar symptoms are seen with DPEII, a DPE whose redox properties preclude reduction by Photosystem I. Under conditions of high aeration, diuron does not protect Scenedesmus cells from Chl bleaching induced by DPEI, but does protect against paraquat. These results indicate that the role of photosynthesis in DPE-toxicity in Scenedesmus is not to reduce the herbicide to a radical species which initiates lipid peroxidation but may be to maintain a sufficiently high O_2 concentration. DPEI and DPEII have similar potencies in causing lipid peroxidation in leaves, but while DPEI induces carotenoid bleaching in a non-chlorophyllous "chromoplast" preparation from chrysanthemum petals, DPEII is ineffective, indicating the possibility of multiple bleaching mechanisms in leaves.

INTRODUCTION

Nitrodiphenyl ether herbicides cause a light- and oxygen-dependent membrane lipid peroxidation and pigment bleaching (Orr and Hess, 1982a,b). In the alga Scenedesmus obliquus, the photosynthetic electron transport inhibitor diuron blocks these toxic effects (Kunert and Böger, 1981) and in higher plants partial protection is sometimes observed (Matsunaka, 1969; Orr and Hess, 1982a). These characteristics resemble those of paraquat toxicity, and led Kunert and Boger (1981) to propose that the initial event in nitro-DPE-toxicity is reduction to a radical species. Several lines of evidence argue against this hypothesis. Certain chloro-DPEs which have chemical properties precluding reduction by PSI show similar effects on plants to nitro-DPEs (Ridley, 1983), and plant tissues which are photosynthetically incompetent (non-chlorophyllous soybean cell suspension culture (Matringe and Scalla, 1987); etiolated cucumber seedlings (Duke and Kenyon, 1987); and barley mutants lacking Photosystem I or II (Bowyer et al, 1987)) are highly susceptible to nitro-DPEs. On the other hand oxyfluorfen induces radical formation in a reaction requiring light but inhibited by diuron in isolated spinach thylakoids (Lambert et al, 1984) and the action spectrum of the nitro-DPE aciflurofen-methyl in Chlamydomonas eugametos indicates that light

[1]Abbreviations: DPE, diphenyl ether; DPEI, 5-[2-chloro -4(trifluoromethyl) phenoxy]-2-nitroacetophenone oxime - o - (acetic acid, methyl ester); Chl chlorophyll; PSI, Photosystem I; DPEII, 5-[2-chloro-4-(trifluoromethyl) phenoxy]-methoxyphthalide.

absorbed by chlorophyll can activate the herbicidal effect. A mode of action which does not require photosynthesis, but involves carotenoid in the photo-activation process has been proposed (Orr and Hess, 1982b) on the basis of the absence of nitro-DPE effects on tissue depleted of carotenoid as a result of mutation or treatment with the carotenoid biosynthesis inhibitor nor-flurazon (Matsunaka, 1969). However, reduction of the carotenoid content to undetectable levels by treatment of a soybean cell suspension with nor-flurazon had no effect on the light-dependent toxic action of acifluorfen-methyl (Matringe and Scalla, 1987). It is clear that there are many apparently contradictory results in this field!

In this report we have attempted to clarify some aspects of nitro-DPE action. We show that in Scenedesmus obliquus, the protective effect of diuron does not arise from an inhibition of the photosynthetic reduction of the DPE, but is probably linked to inhibition of photosynthetic oxygen evolution. We also describe a plant system (chrysanthemum petals and "chromoplasts") which is susceptible to a number of nitro-DPEs but which is unaffected by certain DPEs which are potent inducers of light-dependent lipid peroxidation in leaf tissue.

MATERIALS AND METHODS

Algal culture

Scenedesmus obliquus wild type and mutants lacking parts of the photo-synthetic apparatus were obtained from Prof. N.I. Bishop, Oregon State University. Cultures were maintained on an enriched agar medium (modified Kessler's medium (Bishop and Senger, 1971), plus 0.5% w/v glucose and 0.25% w/v yeast extract). Cells in suspension were grown heterotrophically on the same medium at 22°C at a light intensity of 0.27 W/m^2 (100 lux) provided by white fluorescent lights. All cells were grown heterotrophically because the non-photosynthetic mutants are unable to grow autotrophically. Pigment-protein complexes and photosynthetic electron transport chains are produced under these conditions. Cells were grown in 75 ml of medium in 250 ml Erlenmeyer flasks on an orbital shaker rotating at 150 revs/min. They were subcultured every 4 days and each experiment was initiated 4 days after the previous transfer. Cells were harvested by centrifugation and resuspended in fresh sterile growth medium to a concentration of 23 mg wet weight per ml.

Hydrocarbon Formation in Herbicide-Treated Algae

For measurements of ethane formation arising from lipid peroxidation, 5 ml of concentrated cell suspension was placed in a 10 ml Erlenmeyer flask and the required herbicide added. The flask was then sealed with a rubber seal and flushed for 10 min using a hydrocarbon-free synthetic air mixture. The flasks were placed on an orbital shaker rotating at 150 rev/min at 20± 2°C under a light intensity of 330 W/m^2 at the surface of the algal suspension. Illumination was provided by an array of heat filtered 150W floodlights. At intervals 0.5 ml samples of head space were removed from the flasks using a gas tight syringe and analysed for ethane by gas chromatography (Bowyer et al, 1987).

Chlorophyll Bleaching in Herbicide-Treated Algae

5ml samples of algal suspension at 12 mg wet weight/ml in 10 ml conical flasks were set up on an orbital shaker rotating at 200 revs/min at 25°C. The flasks were lightly stoppered with cotton wool, permitting vigorous aeration during the incubation with the herbicide. Illumination for these experiments was provided by an array of 60W bulbs providing an intensity at the surface of the algal suspension of 190 ± 10W/m^2. At intervals, 0.5 ml samples of suspension were removed under sterile conditions, centrifuged,

and pigments were extracted from the algal pellet by heating for 6 min in 96% ethanol at 80°C. The Chl *a* content of the extract was estimated spectrophotometrically (Lichtenthaler and Wellburn (1983)).

Herbicidal effects on Chrysanthemum petals

Horticultural chrysanthemum plants (variety, Bright Golden Princess Ann) in pots were used. For studies on intact petals, detached open blooms with their cut stems in water were coated with a solution of herbicide in 0.01% (v/v) aqueous Triton X-100. Control plants were treated with 0.01% (v/v) Triton X-100. The flowers were exposed to a light intensity of >200 W/m^2 for 51 hours. Damaged was assessed visually. "Chromoplasts" were isolated from mature petals (outer 3 rings of florets) using the method of Falk *et al* (1974) for daffodil chromoplasts. They were visible as yellow bands at the interfaces of the 30%/40% and 15%/30% sucrose layers after centrifugation on discontinous sucrose gradients. The bands were removed, diluted with 5 mM $MgCl_2$, 67 mM phosphate pH7.5 to give a final concentration of 15% sucrose, and pooled. The carotenoid content was determined by centrifuging the chromoplast suspension at 100,000x g for 1 h and resuspending the pellets in ethanol. A white precipitate was removed by centrifugation and an optical absorption spectrum of the ethanol extract taken. An extinction coefficient (E_{1cm} 1%) of 2500 for the predominant peak between 437-440 nm was used to calculate carotenoid content. Comparison of electron micrographs (not shown) of the "chromoplast" preparation and intact petals indicated that the "chromoplast" preparation in fact consisted of large lipid globules probably formed by fusion of the smaller globules within the chromoplasts during homogenisation of the petals. Samples were prepared for electron microscopy as described in Bowyer *et al*, (1987).

General

With the exception of paraquat, herbicide stock solutions were made up in DMSO giving 0.1% v/v DMSO after dilution into the algal suspension. Controls contained 0.1% v/v DMSO. Paraquat was obtained from Sigma Chemical Co. and the other herbicides were kind gifts from agrochemical companies, as listed in the acknowledgements. All results are the means from at least two separate experiments, except where indicated.

RESULTS

The nitro-DPE herbicide DPEI (Bowyer *et al*, 1987) and paraquat caused marked ethane formation resulting from lipid peroxidation in illuminated *Scenedesmus* cells (Fig, 1A, B). DPEI did not induce ethane formation in the absence of illumination (not shown) and the photosynthetic electron transport inhibitors prometryne and diuron both blocked the DPEI and paraquat effects. Neither DPEI nor paraquat induced ethane formation in a mutant which lacked PSI (Fig. 1A). The same result was seen with mutants which lacked either Photosystem II or the cytochrome *b6f* complex (data not presented).

The effects of a novel phthalide diphenyl ether (DPEII) synthesised by the Organic Chemistry Division at Sittingbourne Research Centre are shown in Fig. 2. DPEII induces light-dependent ethane formation and pigment bleaching in leaves of higher plants (P. Camilleri, K. Weaver, J.R. Bowyer and B.J. Hallahan, unpublished observations) and gives a similar primary screen score to DPEI (see Table 1). However, pulse radiolysis studies on DPEII in propan-2-ol/water (1:3 v/v) indicate that it has a one-electron reduction potential of less than -700mV (P. O'Neill, unpublished observa-

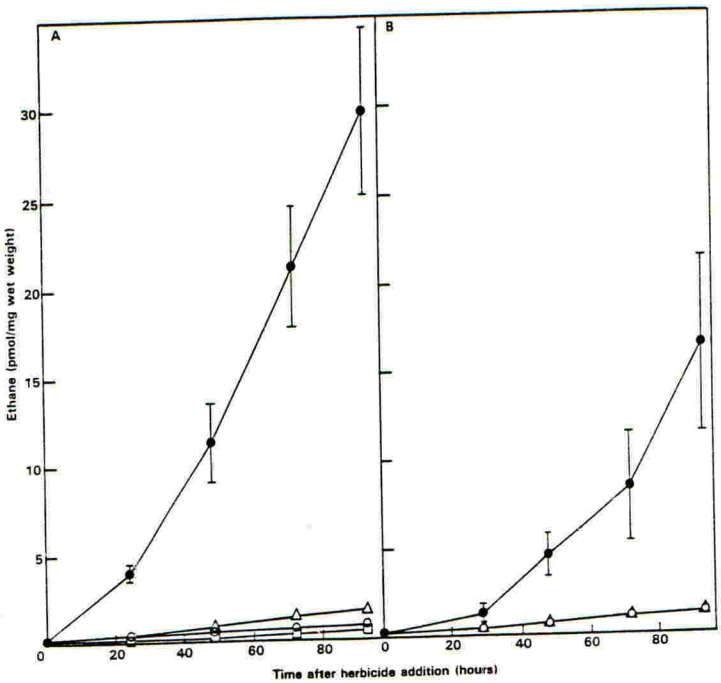


Fig. 1. Effect of inhibition of photosynthetic electron transport on lipid peroxidation induced by herbicides in illuminated Scenedesmus, monitored using ethane formation. A. Effect of 2mM paraquat on wild type (●); effect of 2mM paraquat on mutant lacking PSI (Δ); effect of 2mM paraquat and 10 μM diuron on wild type (O); and control wild type (□). B. Effect of 10 μM DPEI on wild type (●); effect of 10 μM DPEI on mutant lacking PSI (Δ); and superimposed, effect of 10 μM DPEI and 10 μM diuron on wild type (O). The error bars where shown indicate the standard error based on seven measurements.

tions) This property would preclude its reduction by all but the most primary photoreactants of PSI (Hoff, 1982) and reduction by these components can be discounted on kinetic grounds. Fig. 2 shows that DPEII also induced marked ethane formation in Scenedesmus in a light-dependent process, and pre-treatment with diuron provided complete protection. The results in Fig. 2 also indicate that lipid peroxidation induced by both DPEI and DPEII is suppressed when the oxygen concentration is lowered. We have shown that cells suspended in fresh growth medium show a net oxygen evolution rate of around 10μmol O_2/mg Chl/h which increases to 40μmol O_2/mg Chl/h over a period of 2 days. The ethane formation observed in the nitrogen-flushed flasks may therefore be linked to oxygen generated photosynthetically. The results in Fig. 3 show the effects of diuron on chlorophyll bleaching induced by paraquat and DPEI in vigorously aerated cell suspensions. Vigorous aeration was needed to elicit net chlorophyll bleaching by both paraquat and DPEI. Although diuron markedly inhibited the net chlorophyll bleaching induced by paraquat, it did not affect the DPEI-induced bleaching.

Experiments with chrysanthemum petals

In order to further probe the mode of action of nitro-DPEs in a non-chlorophyllous and photosynthetically incompetent plant tissue, we tested the effect of DPEI on chrysanthemum florets. HPLC analysis of extracts of

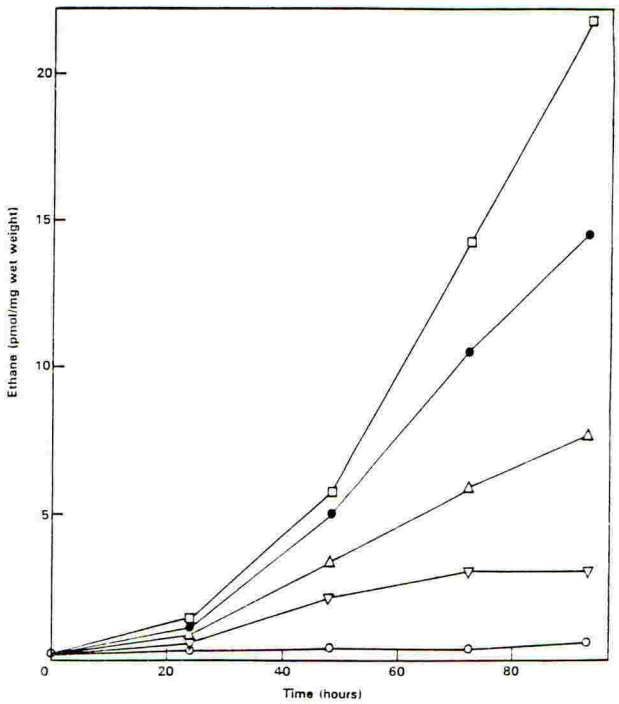


Fig. 2. Effect of O_2 on the ability of DPE's to induce lipid peroxidation monitored by ethane formation in illuminated Scenedesmus cells. At the beginning of the experiment, flasks were flushed with either air or N_2 for 10 minutes. The additions were 10 µM DPEI with air (□); 10 µM DEPII with air (●); 10 µM DPEI with N_2 (▲); 10 µM DEPII with N_2 (▽); 10 µM DPEII with 10 µM diuron and air (○); 10 µM DPEII with air, in darkness, superimposed on (○); and 10 µM diuron with air, superimposed on (○).

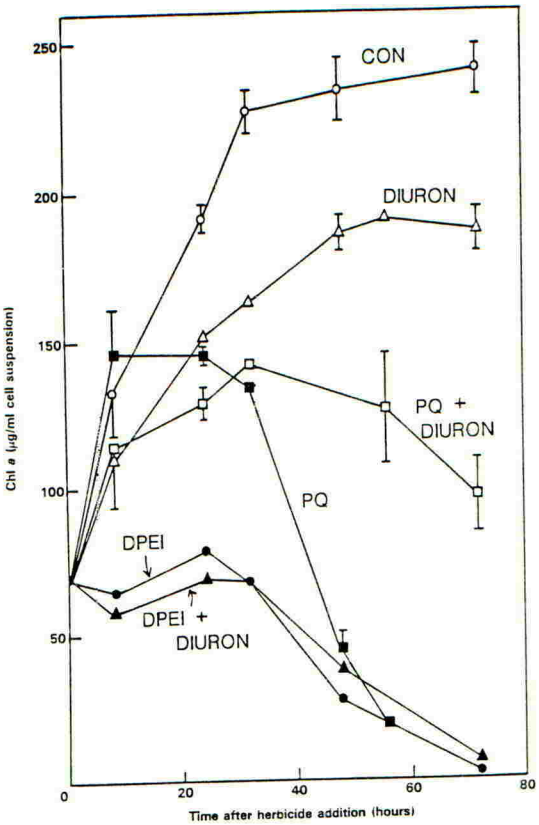
the outer rings of florets confirmed the absence of chlorophylls and revealed a complex mixture of over 40 carotenoid derivatives (not shown). Chrysanthemum flowers treated with DPEI showed necrosis which developed over a period of several days and was strictly light-dependent. Paraquat had no effect on the florets. Electron micrographs of necrotic tissue show that there is extensive membrane disruption which is similar to that seen in DPE-treated leaf tissue (not shown). DPEI also enhanced the light-dependent bleaching of carotenoids in a pigmented fraction ("chromoplasts") isolated from the florets (Table 1). Neither paraquat nor the carotenoid biosynthesis inhibitors norflurazon and diflufenican had any effect on this light-induced bleaching (data not presented). In order to ascertain whether this bleaching and membrane damage were related to the mode of action of nitro-DPEs and related molecules on leaves, we tested a number of different compounds (Table 1). The results show that the structure/activity relationship of the compounds in the "chromoplast" assay was totally different from that on leaves based on a visual assessment of damage in the latter. Of particular interest is that nitrofen was the most potent compound in the "chromoplast" assay, but is a relatively poor nitro-DPE herbicide, whereas the phthalide DPEII which is a very active herbicide with nitro-DPE symptomology, was inactive both in the "chromoplast" assay and also on the intact florets. Both bifenox and nitrofen, although active on the "chromoplast", had no effect on the intact florets (Table 1). α-tocopherol reduced the extent of nitro-DPE induced bleaching in the "chromoplast" suspension, but compounds which would either enhance or reduce the effects of singlet oxygen (deuterium oxide and 1,4-diazabicyclo [2.2.2] octane respectively) or of hydroxyl-radical generating systems (desferrioamine) had no effect (notshown).

DISCUSSION

DPEI and paraquat both induce a light- and O_2-dependent membrane lipid peroxidation in Scenedesmus (cf Kunert and Boger, 1981). This process is

Fig. 3.

Effect of diuron on Chl a bleaching induced by paraquat and DPEI in vigorously aerated illuminated Scenedesmus cells. Effects of 10 μM DPEI (●); 10 μM diuron (▲); 10 μM DPEI with 10 μM diuron (▲); 2mM paraquat (■); 2mM paraquat with 10 μM diuron (□); control (○). The bars where shown indicate the standard error based on at least four measurements.



strongly inhibited when photosynthetic electron transport is blocked either chemically (diuron and prometryne) or by mutation leading to loss of an electron transport component. These effects support the idea that the toxicity of nitro-DPEs may be linked to their reduction by PSI. However, this interpretation can be precluded by the results obtained with DPEII. The redox properties of DPEII prevent its reduction by PSI, but DPEII-induced lipid peroxidation is also inhibited by diuron.

Since lipid peroxidation is suppressed by the removal of O_2, a possible role for photosynthetic electron transport would be to maintain a sufficiently high O_2 content in the medium by water-splitting. Under the conditions of the ethane accumulation measurements, if photosynthetic O_2 evolution is blocked, the respiratory rate of the cells is such that all the oxygen in the flask could be consumed in around 3 hours, and this could therefore be responsible for suppressing lipid peroxidation. In well aerated cultures however, while diuron inhibits the bleaching induced by paraquat, it has no effect on the DPEI-induced bleaching. The inhibitory effect of diuron on the paraquat bleaching can be attributed to an inhibition of the photoreduction of paraquat, an essential step in its toxic action (cf Bowyer et al, 1987). The lack of effect of diuron on DPEI bleaching is consistent with the idea that the role of photosynthetic electron transport is to generate O_2 under conditions when the O_2 concentration may be limiting.

The problem of oxygen supply was not encountered in our own experiments with higher plant tissue (Bowyer et al, 1987) because in the experiments in

TABLE 1

Comparison of the effects of DPE analogues on intact chrysanthemum flowers, and on a "chromoplast" preparation from the chrysanthemum florets, with their herbicide primary screen scores.

Compound	% bleaching induced in chromoplasts[a]	Effect on intact flowers[b]	Primary screen score[c]
Nitrofen	76	-	58
DPEI	43	++	68
Bifenox	30	-	57
DPEII	9	-	70

[a]Bleaching induced by 5 μM compound when added to "chromoplast" suspension at an initial carotenoid concentration of 2.3μg/ml after 18 hours illumination by white light of intensity >200W/m^2. Bleaching is expressed as the carotenoid content as a percentage of that in the control after 18 hours. Typically a 30% bleaching occured in the control during this period.
[b]See Materials and Methods.
[c]Sum of scores on 8 plant species for 1kg/ha foliar spray, max score 72.

which chlorophyll bleaching was monitored in leaf discs, the discs were suspended in water vigorously bubbled with O_2, and in the experiments with barley mutants, the leaves were in contact with the atmosphere during the major part of the herbicide treatment, and only sealed into tubes for 4 hours to measure ethane accumulation. The experimental conditions employed by Kunert and Böger (1981) (autotrophically growing cells bubbled with air) would be less likely to lead to O_2 limitation under conditions of inhibited photosynthesis, but they still observed an inhibition by diuron of oxyfluorfen-induced lipid peroxidation and Chl bleaching. However, the destruction of cytochromes induced by oxyfluorfen was only slightly diminished by diuron, which lead Kunert et al, (1985) to propose O_2-limitation as the cause of the diuron effect. Differences in the degree of protection by diuron would then reflect a balance between the O_2 content of the medium, the toxicity of the DPE, and the O_2 requirement of the particular biochemical effect. Photosynthetic electron transport may play a secondary role in DPE-toxicity by generating lipid radicals, since high concentrations of oxyfluorfen do enhance radical formation in illuminated spinach thlakoids in a diuron-sensitive reaction (Lambert et al, 1984).

The results with the chrysanthemum chromoplasts are of some interest because they suggest that nitro-DPEs can induce light-dependent pigment bleaching by a mechanism not available to other DPEs which are, however, active on leaves. We do not yet know what this mechanism is, but preliminary studies suggest that neither singlet oxygen nor the Fenton reaction are involved. The chemical structures of the non-nitro DPE analogues appear to preclude their conversion by the plant to an active species which would not be generated in the "chromoplasts", The lack of effect of nitrofen and bifenox on intact flowers is presumably due to metabolism or uptake problems.

We do not yet know whether the nitro-DPE bleaching mechanism operating in the "chromoplasts" does occur in leaves, but clearly our results suggest that more than one process may be involved in the bleaching action of DPE herbicides.

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THE EFFECTS OF ACIFLUORFEN ON MEMBRANE INTEGRITY IN GALIUM APARINE LEAVES AND PROTOPLASTS

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ABSTRACT

Acifluorfen phytotoxicity was investigated in excised leaves and isolated mesophyll protoplasts of Galium aparine. Excised leaf photosynthesis was inhibited by 78% after 15h incubation with 100μM acifluorfen, and chlorophyll breakdown, electrolyte leakage and lipid peroxidation were evident after this time. Furthermore, phytotoxicity was apparent at 8 μmoles/m²/s blue light in the absence of photosynthesis. Protoplast viability and photosynthesis over 2h was sensitive to acifluorfen concentration whilst intactness remained unaffected. However, neither electrolyte leakage nor lipid peroxidation were observed. These results are discussed in relation to the current views on DFE action and favour a primary action that is mediated by blue light and the chloroplast envelope which leads to a disruption of membrane integrity.

INTRODUCTION

Acifluorfen has a mode of action in common with other nitrodiphenyl ethers, chlorodiphenyl ethers (Ensminger and Hess, 1985) and a number of compounds which lack the diphenyl ether (DPE) structure (Matringe et al, 1985; Derrick, 1987). These compounds induce rapid bleaching and necrosis in susceptible plants, probably via a peroxidative destruction of membranes in a light-dependent manner (see Orr and Hess, 1982; Duke and Kenyon, 1987 for review). At first glance this would suggest a parallel between the modes of action of bipyridyls (eg. paraquat) and DPE - type herbicides, in that both participate in electron transfer processes between chloroplast thylakoids and membrane lipids via toxic radical species. Indeed, there is some evidence to suggest that oxyfluorfen may be able to participate in such a system (Gillham et al, 1985). It is unlikely however, that this type of mechanism is generally applicable to DPE - type compounds for a number of reasons. Firstly many DPE's are incapable of being directly reduced (Ensminger and Hess 1985) and some DPE compounds lack a nitro- or other comparable reducable group (Matringe et al 1986; Derrick, 1987) often deemed necessary for such reactions (eg Gillham et al, 1985). Secondly, ultrastructural evidence does not favour a paraquat-type mode of action for DPE's, thylakoid disruption occurring only at a relatively late stage in the

development of DPE - induced toxicity (Kenyon et al, 1985; Derrick et al, 1987), whilst the reverse is true with paraquat (Harvey and Fraser, 1980). Thirdly, photosynthetically incompetent plant tissues eg etiolated tissue, and plants grown in far-red light are susceptible to DPE action (Duke and Kenyon, 1986). Nevertheless, evidence for some participation of photosynthetic electron transport in the action of DPE's has been provided by several research groups (see Duke and Kenyon, 1987) in that electron transport inhibitors suppress DPE activity in a number of plant systems. The mode of action of DPE's therefore remains obscure, but distinct from that of the bipyridyls.

In this laboratory we have studied various aspects of the complex action of DPE - type herbicides in the relevant weed species Galium aparine L. (cleavers), using isolated mesophyll protoplasts and intact leaves. This paper reports the novel use of weed protoplasts to examine the effect of acifluorfen on membrane integrity and discusses the involvement of light quality and lipid peroxidation in the action of this herbicide.

MATERIALS AND METHODS

Plant Material

Galium aparine seedlings were grown in peat-based potting compost at 20-25°C under a 14 h photoperiod of 200-400 μmol/m²/s photosynthetic photon flux density (PPFD) provided by high-pressure sodium lamps. Leaves used for experiments were cut from the second whorl of true leaves when the diameter of this whorl was 45-48mm and that of the third whorl 5-10mm.

Studies on excised leaves

To study the effects of acifluorfen on excised leaves, several processes were monitored over a 30h period at 25°C and 50μmol/m²/s PPFD, including leaf photosynthetic competence, chlorophyll content, electrolyte leakage and malondialdehyde (MDA) release. Leaf photosynthesis was measured polarographically (LD2 oxygen electrode, Hansatech Ltd., Kings Lynn, UK), chlorophyll content by acetone extraction (Arnon, 1949) and electrolyte leakage by increase in bathing medium conductivity (PTI-18 conductivity meter, FSA Ltd). This medium was also assessed for MDA accumulation by colour reaction with thiobarbituric acid (Heath and Packer, 1968).

To examine the effects of light quality, ten leaves were floated abaxial surface down on 100 μM acifluorfen solutions in 5cm Petri dishes in an incubation system maintained at 25°C which only emitted light through a window consisting of a colour light filter. Fluorescent light source/filter combinations used were: (1) for blue light, 'natural white' tubes (GEC) plus Cinemoid no. 19A (Rank Strand) filter giving maximum transmission at 440nm and half bandwidth of 50nm. (2) For red light, 'Deluxe Natural' tubes (Thorn-EMI) plus Cinemoid no. 14 filter transmitting wavelengths greater than 610nm. (3) For green light, 'Cool White' (Thorn-EMI) tubes plus Rosco Supergel no. 90 filter (Rosco, Upper Ground, London, SE1 9PQ, U.K.), giving maximum transmission at 515nm and

half bandwidth of 50nm. Light incident on leaves was at 8 μmol/m²/s PPFD. Physiological damage was detected as a conductivity change in the bathing medium and a reduction in leaf chlorophyll content (determined as above). Four Petri dishes in each of six experiments were assessed per treatment, i.e. 240 leaves for each treatment.

Preparation of protoplasts

The abaxial epidermis was peeled from leaves and floated abaxial surface down on a plasmolysing medium (0.5M mannitol, 0.1% wt/vol polyvinylpyrrolidone, 1mM $CaCl_2$ and 10mM MES-KOH buffer pH 5.5) until sufficient leaf material had been peeled. The plasmolysing medium was then replaced with an enzyme medium containing 1% wt/vol Cellulysin (Calbiochem), 0.05% wt/vol Pectolyase Y-23 (Seishin Pharmaceutical) and 0.25% wt/vol bovine serum albumin, dissolved in plasmolysing medium and the pH readjusted to 5.5 with KOH. Following incubation at 25°C under 50 μmol/m²/s PPFD provided by 'natural white' fluorescent tubes (GEC) for 1.5h, the resulting protoplast suspension was passed through two filters (1mm and 200μm mesh), centrifuged at 100 x g for 2 min and the pellet resuspended in a protoplast storage medium (0.4M mannitol, 1mM $CaCl_2$, 20mM MES-KOH, pH 6.0). This crude protoplast preparation was purified by overlaying the suspension onto a stepped gradient of Percoll (Pharmacia) in storage medium (this consisted of 2ml layers of 35%, 30%, and 25% Percoll) and centrifugation at 150 x g for 10 min. Protoplasts were collected from the 0%/25% interface, diluted four fold with storage medium, centrifuged for 2 min at 100 x g and resuspended in storage medium. The protoplast concentration was adjusted to 5 x 10^5/ml of suspension and stored at 25°C prior to use.

Incubation of Protoplasts with Acifluorfen

Aliquots of protoplast suspension (2ml) were incubated in oxygen electrodes (model DW1, Hansatech Ltd). The stirrer was operated at its slowest speed and a 3mm thick spacer placed between the electrode and stirrer base to reduce the stirrer speed sufficient to avoid excessive protoplast breakage, whilst maintaining a suspension and allowing adequate electrode response to changes in oxygen concentration. Under these conditions, protoplast intactness remained stable for over 3h at 25°C (Derrick, 1987). Acifluorfen (97.5% pure), was added from stock solutions in acetone. Final solvent concentration in the storage medium was 1.25% vol/vol.

Estimation of Protoplast Intactness

Intactness, recorded as protoplast number/ml as a percentage of the original protoplast density, was determined by counting on a haemacytometer grid. Counts were made on two grids per sample for each of six experiments, each experiment being performed on a separate protoplast preparation.

Vital Staining of Protoplasts with Fluorescein Diacetate (FDA).

Intracellular hydrolysis of FDA to fluorescein (which emits a yellow-green fluorescence under u.v. light) was exploited as a measure of protoplast viability since only viable cells hydrolyse FDA (Widholm, 1972). Equal volumes (15μl) of protoplast suspension and freshly made 0.01% wt/vol FDA (A.R. grade Koch-Light Laboratories) in storage medium

containing 1% wt/vol acetone, were mixed on a haemacytometer.

Protoplasts were counted under white light and exactly 2 min later, the same protoplasts counted under u.v. light, scoring yellow-green protoplasts as viable. Four microscope fields of view (each equivalent to 0.628 µl of suspension) were assessed per sample in each of six experiments.

Photosynthetic Competence of Protoplasts

Samples (1.5ml) of protoplast suspension were centrifuged at 100 x g for 2 min, the protoplasts resuspended in 1.5ml of 0.4M mannitol, 5mM $CaCl_2$, 25mM $NaHCO_3$ and 50mM tricine-KOH, pH 7.6 and the suspensions returned to the oxygen electrode well. Following a 3 min dark incubation, the protoplasts were illuminated with 500 µmol/m²/s PPFD and oxygen evolution recorded. The chlorophyll content of suspensions was determined by the method of Arnon (1949), to permit expression of data on a chlorophyll basis.

RESULTS

The effect of 100µM acifluorfen on G. aparine excised leaves over a 30h incubation period is illustrated in Fig. 1, from which a possible sequence of events may be deduced. The first deviation from control values was the steady decline in photosynthetic O_2 evolution, so that by 15h this process was inhibited by 78%. However, chlorophyll breakdown

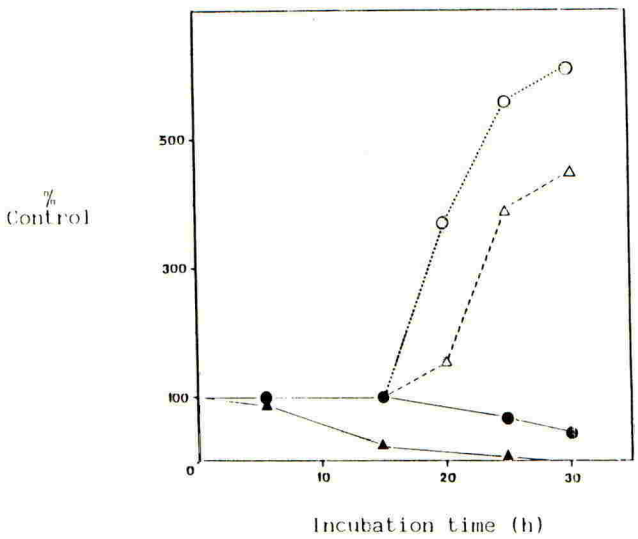

Fig. 1. The effect of 100µM acifluorfen on leaf photosynthesis (▲——▲), chlorophyll content (●——●), electrolyte leakage (O····O) and MDA formation (△- - -△) in excised G. aparine leaves at 25°C and 50 µmoles/m²/s PPFD. Data are expressed as a percentage of control values, which were constant throughout the incubation period i.e. photosynthesis (140 - 150 µmoles O_2 evolved/mg chl/h, determined at 500 µmoles/m²/s, PPFD), chlorophyll (20 - 24 µg/leaf), electrolyte leakage (6µS/cm) and MDA (0.44 nM TBARM after 30h incubation).

only became apparent after this time. Electrolyte leakage markedly increased after 15h, whilst MDA accumulation in the bathing medium followed a similar but lesser pattern. These observations performed with unfiltered white light suggest that photosynthesis in this species is most sensitive to acifluorfen and its inhibition precedes membrane disruption and peroxidation by several hours at 50 μmoles/m^2/s PPFD.

Fig. 2 clearly shows that blue was the most effective light quality in acifluorfen-mediated toxicity after 48h at 8 μmoles/m²/s (PPFD). Photosynthesis was not measurable at such a low flux density and suggests a blue light-sensitive effect that is independent of photosynthesis. These results and others at higher flux densities (Derrick, 1987) are in agreement with those of Ensminger and Hess (1985) who determined an action spectrum for acifluorfen methyl in the green alga Chlamydomonas and found a large peak of activity in the blue region of the spectrum, a minor peak in the red and an inability of green light to generate toxicity.

These observations were further extended by the use of isolated G. aparine mesophyll protoplasts. Fig. 3A illustrates the structural integrity of protoplasts over a 2h incubation period in the presence of 0-750 μM acifluorfen. Intactness was greater than 90% throughout, indicating no significant damage to the plasmalemma in all treatments. However, whilst no acifluorfen - induced lysis was evident, the protoplasts were less able to hydrolyse FDA with increasing acifluorfen dosage, suggesting a decline in metabolic integrity (Fig. 3B). Protoplast photosynthesis was similarly sensitive to inhibition by acifluorfen (Fig. 3C).

Further experiments were also performed incubating protoplasts with acifluorfen to determine lipid peroxidation (by ethane evolution), electrolyte leakage and MDA production. However, none of these products were detected in this experimental system, even after 5h incubation

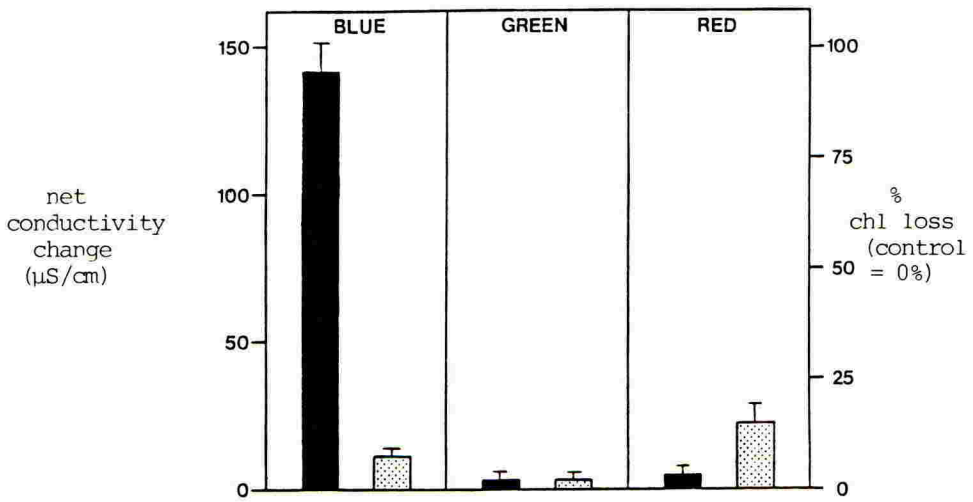

Fig. 2 The leakage of electrolytes (■) and loss of chlorophyll (⊡) from excised G. aparine leaves incubated for 48h with 100 μM acifluorfen under 8 μmoles/m²/s PPFD blue, red and green light. Bars represent S.E.'s.

(Derrick, 1987). Thus, no peroxidative symptoms were detected 3h after a complete loss of photosynthetic activity.

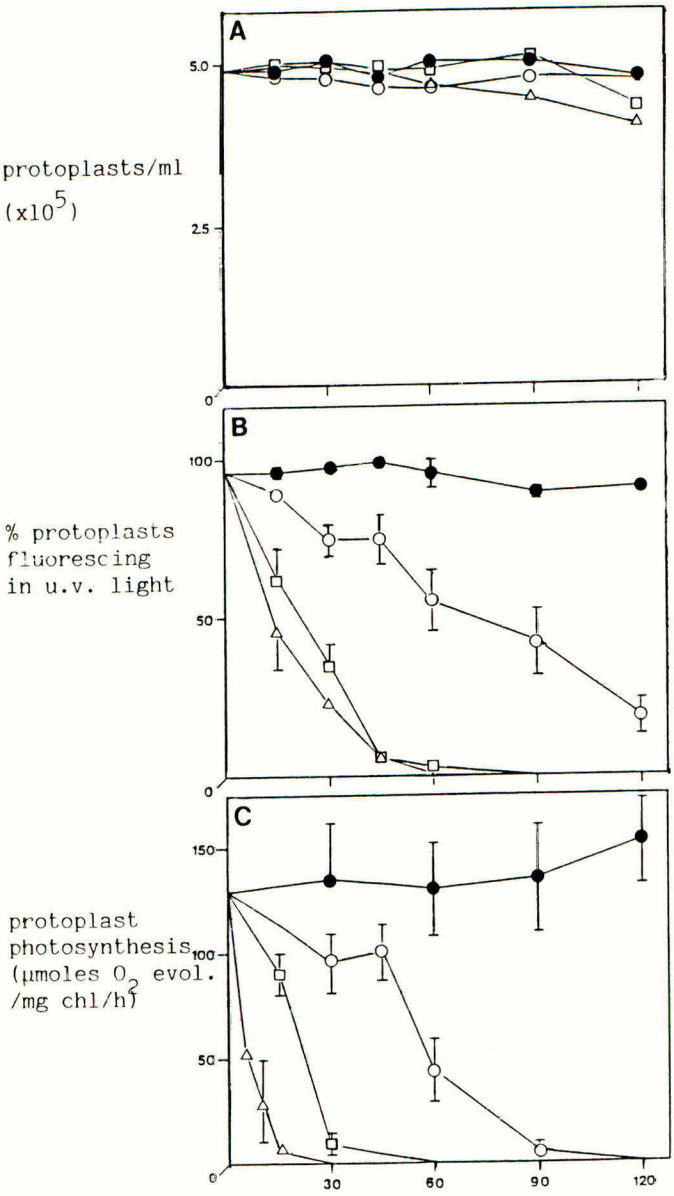

Fig. 3. The effect of 0 (●—●), 375 (○—○), 500 (□—□) and 750 (△—△) μM acifluorfen on protoplast intactness (A), protoplast viability (B) and protoplast photosynthesis (C). Bars represent S.E.'s.

DISCUSSION

Acifluorfen is a post-emergence selective herbicide with a contact action in photosynthetically active tissues of broadleaved weeds, leading to bleaching and necrotic symptoms in these tissues. However, little published information exists on the mode of action of this herbicide in these tissues. This paper reports observations of acifluorfen mode of action in G. aparine leaves and isolated protoplasts and infers a primary effect on membrane integrity prior to lipid peroxidation as shown in Fig. 1. These observations support the findings of an ultrastructural study carried out in this laboratory (Derrick et al, 1988) in which the first observable symptoms of herbicide damage were distorted chloroplast envelopes and later endomembrane disruption. Any such injury to the chloroplast envelope is likely to cause a rapid cessation of photosynthesis as these membranes play a pivotal role in regulating intraplastid homeostasis (see Douce and Joyard, 1979).

Whilst G. aparine mesophyll protoplasts remained intact during acifluorfen incubations over 2h (Fig. 3A), the herbicide clearly had the ability to drastically reduce FDA hydrolysis (Fig. 3B) and photosynthesis (Fig. 3C). As enzymes capable of hydrolysing FDA are presumed present in most cell compartments, such a reduction in FDA hyrolysis suggests a loss of cytoplasmic compartmentation/integrity or a loss of cytoplasmic contents through damaged or a leaky plasmalemma. However, the latter possibility is discounted by the failure to observe electrolyte leakage from protoplasts in the presence of acifluorfen. Furthermore, decreased intactness would have been expected but was not observed.

The finding that acifluorfen induced toxicity is highly sensitive to low flux density blue light (Fig. 2.), indicates the involvement of a chromatophore unrelated to photosynthesis. Indeed, our data strongly suggest that thylakoids are not directly involved in DPE action, but that the initial events occur at the chloroplast envelope. Duke and Kenyon (1987) have recently proposed a model for DPE action in which the herbicide forms a blue-light absorbing photodynamic complex with a 'carotenoprotein' in the chloroplast envelope, which may be able to initiate lipid peroxidation. This study has not detected peroxidative damage in protoplasts and only after a relatively long period after the cessation of photosynthesis in excised leaves. Thus, our data is supportive of the Duke and Kenyon theory but implies a more primary role on membrane integrity rather than peroxidative damage.

ACKNOWLEGEMENTS

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THE MODE OF ACTION OF THE HERBICIDE WL110547

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ABSTRACT

The herbicidal activity of the experimental compound, WL110547, is associated with bleaching of sensitive plants.

More detailed studies of its mode of action were undertaken to establish:

(i) the precise site of action in the chlorophyll or carotenoid biosynthetic pathway;

(ii) any other sites of inhibition which might contribute to the herbicidal effect;

(iii) the reason for variation in activity among closely related analogues.

In vivo and in vitro studies have established that Z-carotene de-saturase and to a lesser extent phytoene desaturase are inhibited, an unusual property among bleaching herbicides.

Linoleic acid desaturase is also inhibited but at relatively high concentrations. This is consequently unlikely to be the more important site for herbicidal activity. Other inhibitors of desaturase enzymes in the carotenoid biosynthetic pathway share this property.

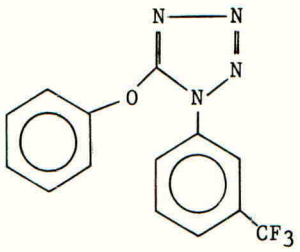
A close analogue of WL110547, WL115531, appears equally active in cell free carotenogenic systems, but is virtually inactive on whole plants. Reasons for this variation have been investigated but a full explanation of the difference has not been obtained.

INTRODUCTION

The experimental herbicide, WL110547 (fig. 1), has shown promise for pre and early post-emergence use in cereal crops. Its herbicidal action is characterised by bleaching of new tissue followed by necrosis. To assist in the optimisation of this compound, we attempted to answer a number of questions:-

a) What type of bleaching is this?
b) What is the precise site of the bleaching action?
c) Are there any additional sites of action?
d) Why are close analogues so much less active?

Fig. 1



WL110547

MATERIALS AND METHODS

Plant material

Barnyard grass seedlings were grown on moist filter paper in covered dishes at 25°C under artificial lights. Capsicum annuum fruits were purchased whilst green and allowed to ripen at 25°C under fluorescent lights (300W/m^2).

Chlorophyll

This was determined from the absorbance of ethanolic extracts at 652nm (Arnon, 1949).

Carotenoids

After saponification of the ethanolic extracts, the carotenoids were extracted into petrol (100-120°C BP) and estimated from their absorbance at 450nm (Tomes, 1963).

Carotenoid Biosynthesis

Incorporation of ^{14}C labelled isopentenyl pyrophosphate into coloured carotenoids was carried out by chromoplasts isolated from ripening fruits of Capsicum annuum (Camara, et al, 1983).

Fatty acid desaturation

Lipids were extracted from plant material (Bligh and Dyer, 1959) and the linoleic and linolenic acid content was estimated by capillary GC.

Catalase and glycolate oxidase

These enzymes were isolated and assayed by the methods of Feierabend and Kemmerich (1983).

Nitrite

This was extracted and assayed by the method of Genichi, et al (1983).

^{14}C labelled compounds

WL110547 (13.6Ci/mole) and WL115531 (16.5Ci/mole) labelled in the phenolic moiety were synthesised at SRC by Dr AN Wright.

TLC analysis

^{14}C labelled compounds extracted from treated plants were separated on silica gel TLC plates developed in chloroform. Radioactive spots were located using a spark chamber, scraped off, and quantified by liquid scintillation counting.

Total ^{14}C content

^{14}C content of treated plant material was estimated by tissue oxidation followed by liquid scintillation counting of the $^{14}CO_2$ evolved.

RESULTS AND DISCUSSION

a) Bleaching of plant tissue may be effected in three ways:-

1) Chlorophyll biosynthesis may be inhibited.

2) Carotenoid biosynthesis may be inhibited, leaving the chlorophyll susceptible to photobleaching.

3) Some form of photoactivation of the herbicide may lead to production of reduced oxygen species or other free radicals which destroy existing pigments.

Treatment of barnyard grass seedlings with WL110547 under low light (.05W/m^2) had little effect on chlorophyll levels but reduced total coloured carotenoids (Table I). Under these conditions, there would be insufficient light to cause photobleaching or photoactivation and so inhibition of carotenoid biosynthesis 2 was indicated.

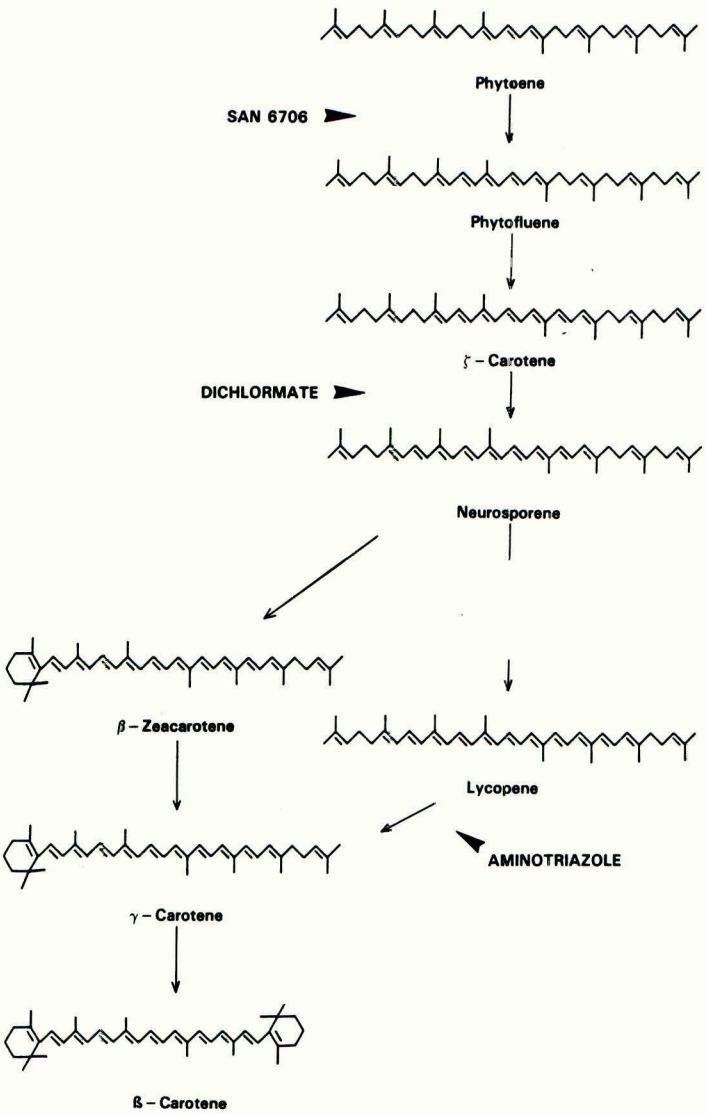
Table I

Effect of WL110547 (10^{-4}M) on pigment content of barnyard grass seedlings after 5 days in low light (0.05W/m^2).

	(average of 2 replicates) chlorophyll (μg/g)	total carotenoids (μg/g)
control	63	11.3
WL110547	69	7.1

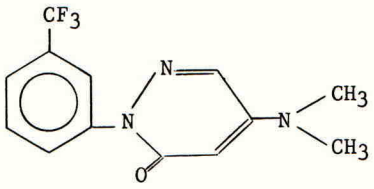
b) The later part of the carotenoid biosynthesis pathway is shown in Fig. 2, together with the sites of action of some well known bleaching herbicides. By far the most common site of action is phytoene desaturase as exemplified by SAN6706 (metflurazon) (Fig. 3).

Fig. 2



Biosynthetic routes for carotenes

Fig. 3



SAN6706

A comparison of the effects of WL110547 and SAN6706 on barnyard grass seedlings showed some differences (Table II). Under low light both compounds caused accumulation of phytoene but WL110547 also caused Z-carotene to accumulate. At higher light intensities, this effect was less clear because the coloured carotenoids were bleached but the loss of chlorophyll by photobleaching was clearly seen.

Table II

Effect of light intensity on pigments in treated barnyard grass seedlings

	% of control values (average of 2 replicates)				
	chlorophyll	phytoene	Z-carotene	β-carotene	lycopene
low (.05W/m^2)					
WL110547.10^{-5}M	97	763	457	38	71
SAN 6705.10^{-5}M	41	1282	12	1	3
moderate (1W/m^2)					
WL110547.10^{-5}M	27	264	135	25	60
SAN6706.10^{-5}M	1	165	5	0	1
high (300W/m^2)					
WL110547.10^{-5}M	29	88	49	23	62
SAN6706.10^{-5}M	1	87	7	0.5	3

SAN6706 is a more active inhibitor of phytoene desaturase and so the absence of Z-carotene accumulation with this compound may have been the result of a total block at the earlier step. At lower SAN6706 and higher WL110547 concentrations, however, the difference was still apparent (Table 3). Thus, WL110547 seems to have a double site of action which is unusual, but not unique.

Table III

Effects of WL110547 and SAN6706 on pigment content of barnyard grass seedlings grown in low light

	% of control values (average of 2 replicates)				
	chlorophyll	phytoene	Z-carotene	β-carotene	lycopene
WL110547					
10^{-5}M	63	230	561	48	82
3 x 10^{-5}M	82	366	918	48	80
SAN6706					
10^{-5}M	67	348	99	39	41
3 x 10^{-6}M	73	447	63	20	14

Using a cell-free carotenogenic system from red peppers, both compounds caused a buildup of phytoene with no evidence of increased Z-carotene. This may be a function of the much shorter incubation time (30 mins. vs. 4-5 days) whereby there was no opportunity for intermediates to leak past the first block.

c) Although WL110547 was some ten fold less active than SAN6706 in inhibiting carotenoid biosynthesis, it was more active herbicidally. The possibility of an additional site of action where it was more active than SAN6706 was therefore explored.

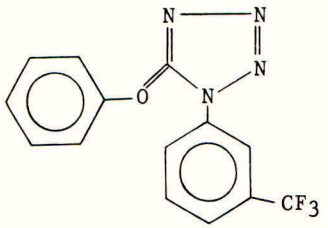
Peroxisomal enzymes such as catalase and glycolate oxidase (Feierabend and Kemmerich, 1983) and also nitrate reductase (Genichi, et al, 1983) are reported to be affected by various bleaching herbicides but WL110547 had no effect on these. Neither did it inhibit photosynthetic electron transport or CO_2 fixation (data not presented).

WL110547 did inhibit fatty acid desaturase activity as exemplified by the conversion of linoleic to linolenic acid (I_{50} = 18uM) but was less active in this system than against carotenoid biosynthesis (I_{50} = 3.5uM).

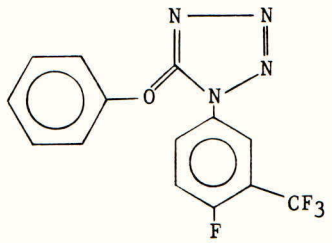
SAN6706 also inhibits fatty acid desaturase (St John, 1976) and is more active (I_{50} = 3μM) than WL110547. Therefore, although this site of action may contribute to the phytotoxic effects of WL110547, it does not explain its greater herbicidal activity than SAN6706. This may be the result of an unknown site of action or superior persistence or distribution in the target plant.

d) A number of close analogues of WL110547 show extremely low phytotoxicity. For example, theoretical calculations show that WL115531 (Fig. 4) is very similar to WL110547 in physical and chemical properties. It is, however, much less active in the herbicide primary screening tests (Table IV). This makes it very difficult to carry out any predictive structure:activity relationship studies. The reason for the low activity of WL115531 was therefore investigated.

Fig. 4



WL110547



WL115531

Table IV

Activity of WL110547 and WL115531 in the herbicide primary screen test at 1kg/ha

(0 = no effect, 9 = total kill)

	maize	rice	barnyard grass	oat	linseed	mustard	sugar-beet	soya
Foliar spray								
WL110547	3	2	7	6	8	8	8	4
WL115531	2	1	2	0	1	2	3	4
Pre-emergence								
WL110547	3	2	9	6	5	7	9	1
WL115531	0	0	0	0	0	0	0	0

Using the cell-free carotenogenic system, WL115531 was shown to be just as active as WL110547. It was argued, therefore, that the difference may reside in:-

a) rates of uptake;
b) rates and pattern of translocation;
c) differential metabolism.

1) Barnyard grass seeds were germinated in the presence of ^{14}C labelled herbicide at 100um in closed vessels in moderate light. Tissue samples were removed at various times up to 6 days and the total ^{14}C content estimated. The seedlings treated with WL110547 were completely bleached whilst those treated with WL115331 were completely green. There was a slightly greater uptake (1.5-2.0 fold) of WL110547 at all times. If the herbicide concentrations were adjusted so that more WL115531 than WL110547 was taken up (Table V), the difference in effect was still apparent.

Table V

Uptake of ^{14}C-labelled WL110547 and WL115531 by barnyard grass seedlings after 6 days in moderate light

	Uptake of ^{14}C (n moles/g. single rep.)	Appearance of seedlings
WL110547		
$5 \times 10^{-5}M$	28.1	very pale green
$10^{-4}M$	42.3	white
WL115531		
$10^{-4}M$	26.2	green
$2 \times 10^{-4}M$	82.7	green

2) Estimates of the distribution of ^{14}C between roots, shoots and seeds of treated barnyard grass seedlings showed little difference between the two compounds (Table VI), although there was again, a slightly greater uptake of WL110547.

Table VI

Distribution of ^{14}C-labelled WL110547 and WL115531 in barnyard grass seedlings after 6 days in moderate light

	% of total ^{14}C taken up (average of 3 reps.)		
	leaf	seed	root
WL110547			
$10^{-4}M$	10	80	10
WL115531			
$10^{-4}M$	12	79	9

3) Extraction and analysis of the ^{14}C labelled components in the treated barnyard grass seedlings showed no detectable breakdown after 3 days. After 6 days, approximately 10% conversion to more polar metabolites was seen, but with little difference between the two compounds (Table VII).

Table VII

Metabolism of ^{14}C-labelled WL110547 and WL115531 in barnyard grass seedlings after 6 days in moderate light

	% of total ^{14}C taken up (single reps.)	
	parent compound	polar metabolites
WL110547		
$5 \times 10^{-4}M$	92	8
$10^{-4}M$	92	8
WL115531		
$10^{-4}M$	89	11
$2 \times 10^{-4}M$	92	8

The reason for the low activity of WL115531 remains elusive unless the carotenogenic system of barnyard grass is very different from that of red peppers.

CONCLUSIONS

Some progress has been made towards understanding the mode of action of WL110547 and this has been helpful to the analogue synthesis programme. Some questions remain unanswered but perhaps it would be naive to hope that all the properties of a herbicide could be explained on the basis of its activity at a single site.

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THE MODE OF ACTION OF DIFLUFENICAN: ITS EVALUATION BY HPLC

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ABSTRACT

The mode of action of diflufenican has been investigated by hplc analysis of pigment extracts of treated plants. The main effect is confirmed as an inhibition of the desaturation reaction of carotenoid biosynthesis, causing the accumulation of phytoene in place of the normal coloured carotenoids. Hydroxy-derivatives of phytoene (and phytofluene) were also present. These may be important in relation to the mechanism of the desaturation reactions. The appearance of β-carotene-5,6-epoxide and a large amount of zeaxanthin suggests that diflufenican also has a minor, photooxidative action. Phytoene was present in all chloroplast pigment-protein complexes, but hydroxyphytoene and zeaxanthin were found only in the free pigment fraction. This information was obtained in only a few days, showing the power of this procedure as a screening method for evaluating the mode of action of potential bleaching herbicides.

INTRODUCTION

Chloroplast carotenoids, especially β-carotene, are essential for protecting plants against the chlorophyll-sensitized photoproduction of singlet oxygen, $^1O_2$, a highly reactive species that can rapidly cause tissue damage (Siefermann-Harms, 1987). Any compound which intereferes with this protective mechanism is therefore potentially useful as a bleaching herbicide. It is already known that many structurally unrelated herbicides cause either white or yellow chlorosis of leaves as a consequence of the total or partial absence of the normal chloroplast pigments, i.e. chlorophylls and carotenoids. These bleaching herbicides can act in two ways, namely by inhibiting the biosynthesis of the carotenoids that are required for photoprotection in the chloroplast pigment-protein complexes (PPC) or by causing the destruction of existing pigments in the PPC following a primary inhibitory action on photosynthetic electron transport.

It is therefore of major importance to have a rapid, reliable and informative screening procedure for elucidating the mode of action of newly synthesized chemicals which cause chlorosis in plants. Much effort is being directed towards the development of model systems, notably algae, plant cell cultures and carotenogenic enzyme preparations from fungi, etc. for use in the evaluation of potential herbicides (Sandmann et al., 1984). We have used an alternative strategy to identify the effects of these compounds directly on the plants. This can be achieved simply by the proper application and interpretation of hplc analysis of chloroplast pigments. This method will immediately distinguish between compounds which act by inhibiting carotenoid biosynthesis and those which interfere with photosynthetic electron transport in a way that leads to destruction of chloroplast pigments. But the method does much more than this. Although we have not yet been able to explore and realize its full potential, it is already clear that this procedure can give information about many aspects of herbicide action, including the site and specificity of inhibition of the biosynthetic pathway, detection of multiple actions, comparison of effects on different plants or of different treatments or doses, specific effects on individual PPC. It also provides a means to study recovery and resistance. A sound first indication of the mechanism by which a new compound acts can be obtained by comparison with other herbicides whose action is well known.

Some of the capabilities of this method are illustrated by the results of a brief evaluation of the effects of diflufenican in comparison with those of other herbicides, e.g. norflurazon, which are believed to have the same primary mechanism of action, i.e. inhibition of the desaturation reactions in carotenoid biosynthesis.

MATERIALS AND METHODS

The plant used was radish (Raphanus sativus L). As a pre-emergence treatment, seeds were soaked overnight in aqueous acetone solutions of diflufenican at the appropriate concentration (O, 1mM, 100µM, 10µM). The seeds were then sown in soil and grown under continuous light (10000 lux light source) for 6 days. Alternatively, for post-emergence studies, seeds were soaked overnight in water and sown in soil and diflufenican was then applied as a suspension in acetone-water (0.5mM) three days after emergence of the radish cotyledons, which were then grown for a further three days in continuous light, as above.

Pigment extraction

Known amounts of leaf material were homogenized in ethanol, the homogenate was filtered through a cotton wool plug and the solvent evaporated under a stream of N_2. The pigment-containing lipid material in the residue was redissolved in diethyl ether, transferred to a clean vial and again evaporated under N_2 ready for hplc analysis.

Hplc analysis

The sample was dissolved in the hplc eluting solvent (acetonitrile-ethyl acetate) and 20µl of this solution injected onto Zorbax-ODS reversed phase hplc column (5µ, 25x0.46 cm). Elution was achieved by a gradient of ethyl acetate in acetonitrile-water (9:1 containing 0.1% triethylamine) as indicated in the legends to the figures. The hplc system used consisted of Kontron pumps and gradient programmer, with a Hewlett-Packard 1040A diode array detector. Chromatograms were monitored simultaneously at 455, 447, 441, 437, 431, 400, 350 and 287nm, and components were estimated quantitatively by integration at each wavelength and by use of established $A^{1\%}_{1\,cm}$ values (Davies, 1976). Compounds were identified by their retention times and absorption spectra. For novel compounds mass spectra and, if possible, ^1H nmr spectra were also determined.

Thylakoid isolation

Thylakoid membranes were isolated as described by Remy et al., (1977), except that Tris-HCl buffer was used throughout the isolation procedure and the isolated thylakoids were resuspended in 0.1M Tris-glycine (pH 9.0).

Separation of pigment-protein complexes

Pigment-protein complexes were separated by our standard polyacrylamide gel electrophoresis procedure. Thylakoid membranes were solubilized for 30 min. at room temperature in 0.1M Tris-glycine (pH 9.2) containing 0.5% (w/v) SDS, to give a final ratio SDS:chlorophyll of 10:1 (w/w). The chlorophyll-carotenoid-protein complexes were then separated by PAGE in 50mM Tris-glycine, pH 9.0, containing 0.06% SDS (w/v) at 3mA/gel for 30 min. Six pigment-protein complexes, plus a free-pigment zone, were separated by the electrophoresis. The pigments were extracted from each individual complex with ethanol and analysed by hplc.

RESULTS AND DISCUSSION

The uptake, transport and metabolism of diflufenican, and its effects on a number of plant species, have been investigated previously (Wightman and Haynes, 1985). The main mode of action has been shown to be on carotenoid biosynthesis. In the present work, the effects of diflufenican on seedlings of radish (Raphanus sativus L) have been investigated. The untreated plants had a normal distribution of coloured carotenoids i.e. β-carotene (approx 25-30% of total carotenoid), lutein (40-45%), violaxanthin and neoxanthin (each approx. 15%), together with chorophylls a and b (chlorophyll a / chlorophyll b approx. 2.0, carotenoid/chlorophyll approx. 0.45). A typical hplc chromatogram of an extract from control (untreated) leaves is illustrated in Fig.1.

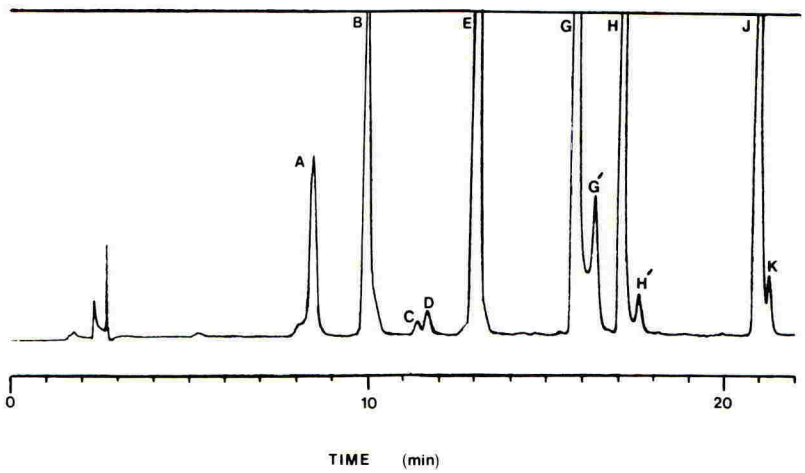

Fig. 1 Reversed-phase hplc chromatogram of an extract from untreated leaves. Monitoring wavelength 445nm. Peak identification: A. Neoxanthin; B. Violaxanthin; C. Lutein-5,6 epoxide; D. Antheraxanthin; E. Lutein; G,G. Chlorophyll b; H,H'. Chlorophyll a; J. β-Carotene; K. cis-β-Carotene.

The effect of diflufenican, applied pre-emergence, was immediately obvious from the reversed-phase hplc chromatograms of the extracts (Fig. 2). The amounts of coloured carotenoids and chlorophylls were greatly reduced, and a substantial amount of the biosynthetic intermediate phytoene was present, thereby confirming that the diflufenican was causing inhibition of carotenoid biosynthesis, in particular blocking the desaturation of phytoene. The chromatographic pattern was easily distinguished from those obtained from plants that had been treated with substances which affect photosynthetic electron transport, such as paraquat and diuron (Fig. 3); in these cases carotenoid levels, especially of β-carotene, were much lower than normal, but no phytoene or other biosynthesis intermediates were present.

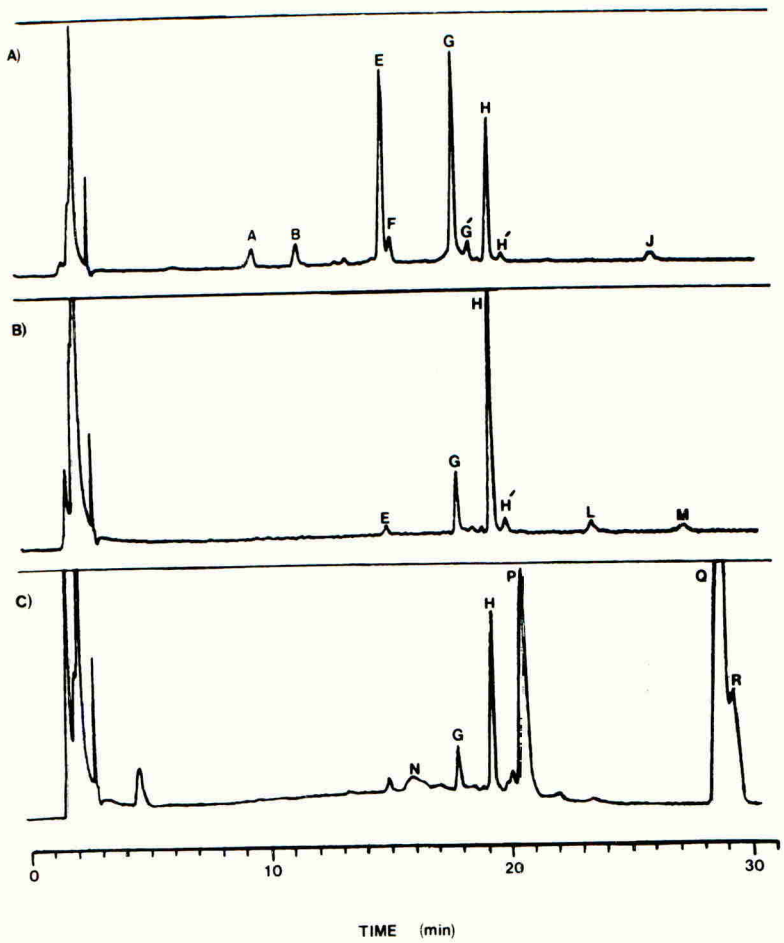

Fig. 2. Reversed-phase hplc chromatogram of diflufenican-treated (pre-emergent) radish. Monitoring at three separate wavelengths a) 445nm, b) 350nm and c) 287nm. Peak identifications: A. Neoxanthin; B. Violaxanthin; C. Lutein; F. Zeaxanthin; G,G'. Chlorophyll b.; H,H'. Chlorophyll a; J. β-Carotene; L. Hydroxy derivative of phytofluene; M. Phytofluene; N. Dihydroxyphytoene; P. Monohydroxyphytoene; Q. 15Z-(cis). Phytoene; R. All-trans phytoene.

The diflufenican-treated plants also contained several other compounds that were not present in the controls, notably ones with absorption spectra identical to those of phytoene and phytofluene. The major one of these compounds has been identified by mass spectrometry and ^{1}H nmr spectroscopy as a hydroxylated derivative of phytoene (probably 12-hydroxyphytoene), and mass spectrometry has also allowed the tentative identification of a dihydroxyphytoene, and a trihydroxy-or dihydroxydidehydro-phytoene, which await full characterization. The phytoene and monohydroxyphytoene were largely the 15Z- (15-cis) isomers. The significance of these hydroxy-phytoenes as possible biosynthetic intermediates remains to be elucidated. It seems likely, however, that they will greatly aid our understanding of the phytoene desaturation reactions.

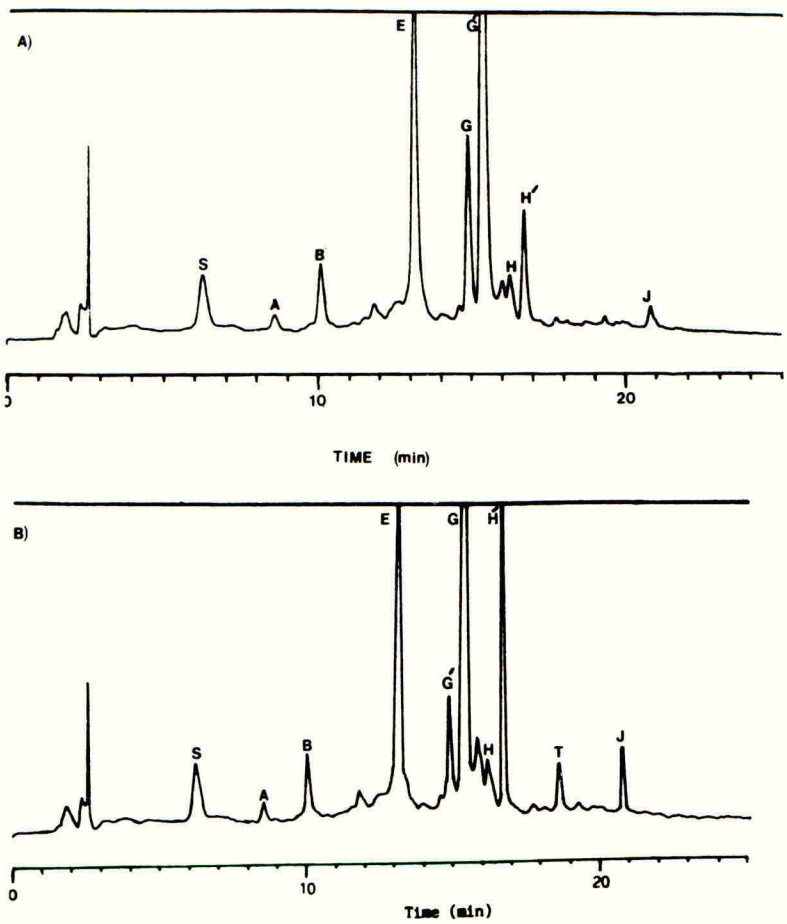

Fig. 3. Reversed-phase hplc chromatogram of barley treated with (a) paraquat and (b) diuron. Monitoring wavelength 445nm. Peak identifications: S. Chlorophyll breakdown product; A. Neoxanthin; B. Violaxanthin; E. Lutein; G,G'. Chlorophyll b; H,H'. Chlorophyll a; T. β-Carotene-5,6-epoxide; J. β-Carotene. Monitoring at 350 nm and 287nm showed that phytoene, phytofluene and their derivatives (compounds L,M,N,P,Q,R) were absent.

Closer examination of the chromatograms showed that, although the pattern of biosynthetic intermediates detected was broadly similar to the pattern obtained for plants that had been treated with other biosynthesis-inhibitor herbicides, e.g. norflurazon, metflurazon, some subtle and possibly diagnostic differences were observed, e.g. in the ratios of cis- to trans-phytoene, phytoene to monohydroxyphytoene or phytoene to phytofluene.

Analysis of plants treated with lower concentrations of diflufenican provided evidence of additional effects on existing pigments. These effects were seen only with diflufenican, not with other carotenogenesis inhibitor herbicides. In

particular, after both pre-emergence and post-emergence treatments and illumination for a few days, small amounts of β-carotene-5,6-epoxide were present (approx. 0.5% of total carotenoid). (Table 1). Our extensive work has shown that this compound is a very sensitive and reliable indicator of the oxidative breakdown of existing β-carotene, the most sensitive carotenoid. Also the 'violaxanthin cycle' (Yamamoto, 1979) appears to be switched on, causing the appearance of abnormally high levels of zeaxanthin and antheraxanthin and a reduction in the violaxanthin content. Both these features are reminiscent of effects of herbicides such as paraquat and diuron which affect photosynthesis and they have not been seen with other inhibitors of carotenogenesis such as norflurazon and metflurazon (data not presented).

TABLE 1.
Comparison of the distribution of carotenoids (expressed as percentage of the total coloured carotenoids) in radish leaves treated, post-em, with diflufenican (DFF) and in untreated leaves (CON).

	β-Car[a]	β-5,6	Lut	Viol	Neo	L-5,6	Anth	Zea	C/X	Non-col	Chla/b
CON	27.8	-[b]	42.5	14.0	12.9	1.9	0.9	-	0.38	-	1.70
DFF	12.4	2.1	51.0	3.2	15.2	1.5	2.5	12.0	0.17	64.0	2.06

[a]Abbreviations: β-Car - β-carotene; β-5,6 - β-carotene-5,6-epoxide; Lut -lutein; Viol - violaxanthin; Neo - neoxanthin; L-5,6 - lutein-5,6-epoxide; Anth - antheraxanthin; Zea - zeaxanthin; C/X - ratio of carotene : total xanthophyll; Non-col - relative amount of colourless carotenoids (phytoene, hydroxyphytoene, etc.) as a percentage of the total; Chl a/b - ratio of chlorophyll a : chlorophyll b.
[b] - indicates below the limit of detection.

The power and scope of this screening procedure become clear when it is realised that the above information about the action of diflufenican was obtained in only a few hours. One analysis is sufficient to give a good indication of the mode of action of any herbicide. This takes only about one hour, including pigment extraction, hplc, and spectral evaluation; several plant species, herbicide concentrations etc. can therefore be examined in a day.

Obviously, for any herbicide a survey of this kind can easily be broadened to investigate, for example, effects on a range of plant species and varieties, plants of different ages and under widely differing growth conditions and also used to look in much more detail at specific features of the inhibition. Thus, over a period of about 5 weeks, much additional information has been obtained about the action of diflufenican. Pre-emergence and post-emergence treatments have been compared and time courses of effects following the application of different herbicide concentrations have been determined. Chloroplasts, thylakoids and individual PPC have been isolated and analysed in an attempt to localize the action (Table 2). The inhibition appears not to be restricted to any particular PPC or carotenoid, although β-carotene was more strongly affected than were the xanthophylls. Appreciable amounts of phytoene were present in all the sub-chloroplast PPC, but hydroxyphytoene was found only in the free pigment fraction. The zeaxanthin that accumulated was also found only in the free pigment. β-Carotene-5,6-epoxide, however, like β-carotene, was localized mainly in the CP1 and CP1a complexes.

TABLE 2.
Percentage distribution of each carotenoid in the chloroplast pigment-protein complexes of untreated radish leaves (CONTROL) and leaves treated, post-em, with diflufenican (DFF).

	CONTROL					DFF			
	CPI/la	LHCPI	CPa/LH2	LHCP3	FP[c]	CPI/la	LHCPI	LHCP3	FP
β-Cara	61.4	8.9	9.3	7.7	12.8	55.9	9.3	12.0	22.8
β-5,6	59.5	6.5	10.2	-	23.8	41.5	12.8	19.0	26.7
Lut	7.5	18.8	5.2	49.1	19.3	12.4	25.7	40.8	21.1
Viol	6.9	7.2	2.1	18.9	64.9	6.6	10.5	26.8	41.4
Neo	3.8	21.6	5.1	62.2	7.3	11.3	30.3	48.6	9.8
L-5,6	11.1	11.4	3.1	25.3	49.1	9.7	13.9	33.9	42.4
Anth	-	-	-	-	-	-	-	41.8	58.2
Zea	-	-	-	-	-	-	-	-	100
Phyt	-	-	-	-	-	18.0	11.2	22.6	45.1
HO-phyt	-	-	-	-	-	-	-	-	100
C/X	2.92	0.17	0.68	0.05	0.16	0.96	0.08	0.06	0.19
Non-col	-	-	-	-	-	19.2	11.2	13.3	35.7
Chla/b	15.08	1.71	3.95	1.46	5.66	5.46	1.57	1.77	3.64

[a]Abbreviations : as in Table l; Phyt - phytoene; HO-phyt - hydroxyphytoene
[b]CPa/LH2 (=CPa/LHCP2) was not detected for the diflufenican - treated plants
[c]FP - Free pigment zone.

CONCLUSIONS

Quite clearly this procedure provides a very powerful means of evaluating bleaching herbicides. In particular it has the potential (i) to distinguish immediately between biosynthesis inhibitors and those which cause reduction in carotenoid levels as a consequence of inhibition of photosynthetic electron transport, (ii) to reveal multiple modes of action and (iii) to detect reliably some features which may be quantitatively minor but which can identify differences or confirm similarities in the action of different compounds which produce the same gross effects. The method is applicable to any potential bleaching herbicide and has the advantages of speed and the amount of information that can be generated, both in terms of breadth and also details of very specific features. It also gives information directly about effects on the plants themselves rather than simply on model systems, and is applicable to any plant species.

When the method has been applied to a wider range of herbicidal compounds, the data base provided will permit deductions to be made about the mechanism of action of any bleaching compound under investigation.

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SESSION 10A

WEED COMPETITION AND THRESHOLDS – PRACTICAL APPLICATIONS OF WEED THRESHOLDS

CHAIRMAN	PROFESSOR G. R. SAGAR
SESSION ORGANISER	DR P. J. W. LUTMAN
INVITED PAPER	10A-1
RESEARCH REPORTS	10A-2 to 10A-6

DEVELOPMENT AND IMPLEMENTATION OF WEED ECONOMIC THRESHOLDS IN THE F. R. GERMANY

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ABSTRACT

Weed economic thresholds have been derived from numerous field experiments measuring the effect of different weed densities and weed coverage on cereal yield. A safety margin was included accounting for possible effects on grain moisture, interference with combine harvesting and influence on weed infestation in subsequent crops. Surveys of weed infestation in typical cereal growing areas indicated a considerable potential for application of such thresholds. The 'fixed' thresholds were evaluated in small and large scale field trials over several years. Based on economic returns, only a low percentage of cases below the thresholds proved to be wrong decisions. Above the 'fixed' thresholds a higher number was wrong, which means that the values may be increased without immediate economic risks. A computer-aided decision model using variable, field and weed specific thresholds is described. The conclusion can be drawn, that without unduly high risks a considerable percentage of chemical weed control measures in cereals can be omitted. Farmers however are using this component of integrated plant protection only reluctantly. More advisory work by the extention services is necessary to acquaint the farmer with this principle.

Research on the development of weed economic thresholds in cereals was started about 15 years ago in the F.R. Germany (Garburg 1974). Subsequently the groups at Hohenheim, Braunschweig and Göttingen intensified their efforts to improve the scientific background of threshold values and to test their reliability in numerous field experiments and under practical conditions (Beer & Heitefuss 1981a, b, Koch & Kemmer 1981, Niemann 1981, Bartels et al. 1983, Wahmhoff & Heitefuss 1985a, b, and others). Soon it became evident, that in typical cereal growing areas of our country with low weed pressure a considerable number of herbicide applications could be omitted. This conclusion was also reached from studies, in which the effect

TABLE 1

Average yield difference, cost of treatment and percentage of uneconomic weed control in field tests of the plant protection service 1977 - 81 (Gerowitt et al. 1984)

Crop	Number of trials	Ø Yield diff. dt/ha	Cost of treatment*) DM/ha	Percentage uneconomic control
winter barley	800	+ 7.4	134,-	25.4
winter wheat	629	+ 5.6	121,-	30.0
winter rye	391	+ 2.9	121,-	47.8
spring barley	431	+ 2.0	82,-	50.4

*) including DM 12,-/ha for spraying; price for grain DM 56,-/dt

of chemical weed control on yield of cereals and net return was calculated (Reschke 1975, Gerowitt et al. 1984). An example of these evaluations is shown in Table 1, in which the results of numerous field trials of the Plant Protection Service over 5 years are summarized.

In winter barley and wheat a considerable average positive yield difference was obtained by weed control. However taking into account the expense of herbicides and spraying, 25 and 30 % respectively of the control measures were not economical; for winter rye and spring barley these figures reached 48 and 50 %! Similar results were reported by other authors (Koch & Kemmer 1981, Niemann 1981).

Development of "fixed" thresholds

Simultanuously work was in progress to quantify the relations between weed infestation and crop yield, in order to derive weed threshold values both for practical implementation and for further evaluation and improvement. Although the general equation,

$$\text{weed economic threshold} = \frac{\text{cost of control, DM/ha}}{\text{b} \cdot \text{yield, dt/ha} \cdot \text{price, DM/dt} \cdot 0.01}$$

contains a number of variables, besides the experimentally derived different regression coefficients b (weed coverage or density / yield or yield loss), practical considerations demanded more or less 'fixed' threshold values. Based on experimental results and experience, and taking into consideration that other possible negative consequences of weeds have to be avoided, such as increase of grain moisture, interference with combine harvesting, danger of higher weed infestations in the following crops, values with a considerable safety margin were recommended for cereals (Table 2):

TABLE 2

Weed economic thresholds recommended for practical use in cereals

Apera spica venti	(loose silky bent)	20 plants/m²
Alopecurus myosuroides	(black-grass)	30 " "
both grasses together		20-30 " "
Galium aparine	(cleavers)	0.5 *) " "
Fallopia convolvolus	(black bindweed)	2 " "
Dicotyledous weeds, density		40 " "
Dicotyledous weeds, coverage		5 % **)

*) or 0.1; **) or 10 % at latest control date

These values may also require adaptation to local needs. In some areas, for example in wet heavy land areas of northern Germany, the threshold numbers for A. myosuroides require considerable reduction, because of the high tillering potential under such conditions. G. aparine is often not tolerated at all because of the risk to the subsequent crop.

Estimation and counting of weed coverage or density can be carried out by the farmer himself with adequate accuracy using a simple metal frame, enclosing 0.1 m² with an attached scale of 1 % and 5 % coverage. One hour is necessary to determine weed density on a 3 - 5 ha field at 20 - 30 sample points in a representative manner, however uneven or patchy weed distribution

should be taken into account as far as possible and headlands if necessary evaluated seperately (Bartels et al. 1983)

This method was also used to obtain information in representative growing areas about the extent of weed infestation and the percentage of fields above or below the thresholds. Table 3 shows the results of a survey covering 4 years in the area of southern Hannover, where the crop sequence sugar beet, winter-wheat, winter-barley is predominant.

TABLE 3

Mean values of weed infestation in winter-wheat, early spring.
Area South Hannover (Blumenberg 1987)

	1982	1983	1984	1985
number of fields surveyed	92	96	91	63
grasses /m²	2.6	10.5	10.2	14.0
G. aparine /m²	0.9	0.6	0.6	0,5
dicotyledous weeds /m²	22.9	17.9	17.6	26.2
weed coverage %	0.2	0.7	0.9	0.9
crop coverage %	5.4	32.5	40.7	23.4

The average weed infestation in wheat following sugar beet was rather low. Except for G. aparine, where the average value was above the threshold, all other criteria were below the recommended values. Due to severe winter conditions the year 1982 shows an extremely low crop coverage, which of course must be taken into account in the control decision. In late sown winter wheat the criterion weed coverage should not be used because the low values, especially from early assessments, give inacurate indications of the thresholds. In these cases one has to rely on density counts. The percentage of cases below the economic thresholds in this survey is given in Table 4.

TABLE 4

Percentage of wheat fields below the weed economic threshold.
Area South Hannover (Blumenberg 1987)

Threshold values		1982	1983	1984	1985
				percentage	
Grasses	25 plants/m²	99	94	91	81
G. aparine	0,5 "	76	72	74	73
Dicot. weeds	40 "	92	93	91	91
Weed coverage	5 %	99	98	97	97
all thresholds considered		70	64	66	57

The data confirm the conclusion from Table 3, that in this typical cereal growing area a high percentage of fields has a weed infestation below the thresholds. In winter barley the conditions are less favourable with 15 - 20 % fields below (Blumenberg et al. 1984). On the other hand, in winter rye fields surveyed in the area around Oldenburg on light soils, a consider-

able percentage was below the threshold (Koenig-Holrah 1985). The prospects of utilizing weed economic thresholds in these and other similar growing areas therefore appear very promising.

Numerous small and large scale field trials have been done evaluating the feasibility of weed control according to threshold criteria, but only a few examples can be mentioned here: In studies over three years yield of winter-wheat was measured in 814 (n) adjacent plot-pairs with and without herbicide applications in 25 field trials. Table 5 shows the results of these studies. Below the threshold only negligable yield differences were recorded except in 1983 when there was a significant decrease of around 1 dt/ha.

TABLE 5

Influence of herbicide application **below** and **above** the weed economic threshold on yield of winter-wheat. 25 field experiments (Springer 1985)

	year	n	yield dt/ha		
			control	herbicide*)	difference
below threshold	82	63	75.3	76.2	+ 0.9
	83	328	66.6	65.5	- 1.1**
	84	269	57.2	57.1	- 0.1
above threshold	82	38	57.3	72.0	+ 14.7**
	83	56	61.4	62.4	+ 1.0
	84	60	47.3	52.6	+ 5.3**

*) Isoproturon 1.5 l a.i. /ha EC 22; CMPP 2.24 l a.i. /ha EC 29-30 (Zadoks-scale)
**) difference significant at p ≦ 1 %, Tukey

In contrast, considerable positive yield differences were reached in 1982 and 84 in plots above the threshold. But even in this category in 1983 the average yield difference was not significant! From these and other field experiments it soon became evident, that application of herbicides in fields below the weed threshold will lead to monetary losses, but even above the threshold weed control leads not in every case to an increase in net return, if the expense for the herbicide and its application is taken into account. The percentage of right or wrong decisions in these categories is given in Table 6, for the experiments described above.

TABLE 6

Percentage of economically right or wrong decisions of weed control in winter wheat according to economic thresholds. 25 field experiments, 814 plot-pairs (Springer 1985)

Weed control decisions			
below threshold		above threshold	
right	wrong	right	wrong
80 %	14 %	52 %	48 %

Development of a computer-aided decision model

The results presented so far, clearly demonstrated that a further improvement of weed economic thresholds is required in order to avoid control measures which are not necessary and could be omitted without an unacceptable higher risk for the farmer. We therefore proceeded to develop a system, which does not use the more less 'fixed' threshold values, but is based on field and weed specific, variable thresholds, which are utilized as components of a computer-aided decision model. A similar computerized management information system for weed control in winter wheat was also proposed by Aarts and De Visser (1985). Field experiments to explore such a system in winter-wheat, - barley and rye, were carried out at different locations of the Federal Republic Germany over 2 years, in cooperation with the German Plant Protection Service. Data from 148 experiments were used and in addition experiences from the preceeding years were also incorporated.

Post-emergence weed control in spring is most suitable for the application of economic thresholds. However in winter barley and in some areas in winter wheat, control measures applied pre-emergence or post-emergence in autumn may have preference. Therefore these applications as based on different criteria not explained here in detail were also included as subroutines into the program.

A simplified scheme of decision steps for the subroutine post-emergence-spring is given in Fig. 1. The output of this system includes:

1. Estimated absolute yield loss
2. Recommendation for a decision based on the calculated profit of weed control
3. Recommendation for a decision as modified by additional criteria.

The latter one includes benefits of weed control not directly related to yield, such as effects on grain moisture and expense for drying, and interference with combine harvesting. For this compartment the fixed threshold values for G. aparine and F. convolvolus were included.

The program stage 'Estimation of crop loss by weeds' is most important for the model and therefore explained here in more detail. The following inputs are included in the estimation:

crop species	(wheat, barley, rye)
crop cover	(%)
development stage crop	(EC-crop)
monocotyledous weeds	(density/m^2)
development stage monocot. weeds	(EC-mono)
dicotyledous weeds	(weed coverage dicots, w.c.d. %)
dicotyledous weeds	(species, density/m^2)

These variables are used according to the following equations and factors:

1. yield loss mono = mono/m^2 · 0,08 · $\frac{\text{EC-crop}}{\text{EC-mono}}$
2. yield loss w.c.d. = w.c.d. % · factor EC-crop

EC-crop	factor
13	0.80
21	0.57
25	0.40
29	0.20

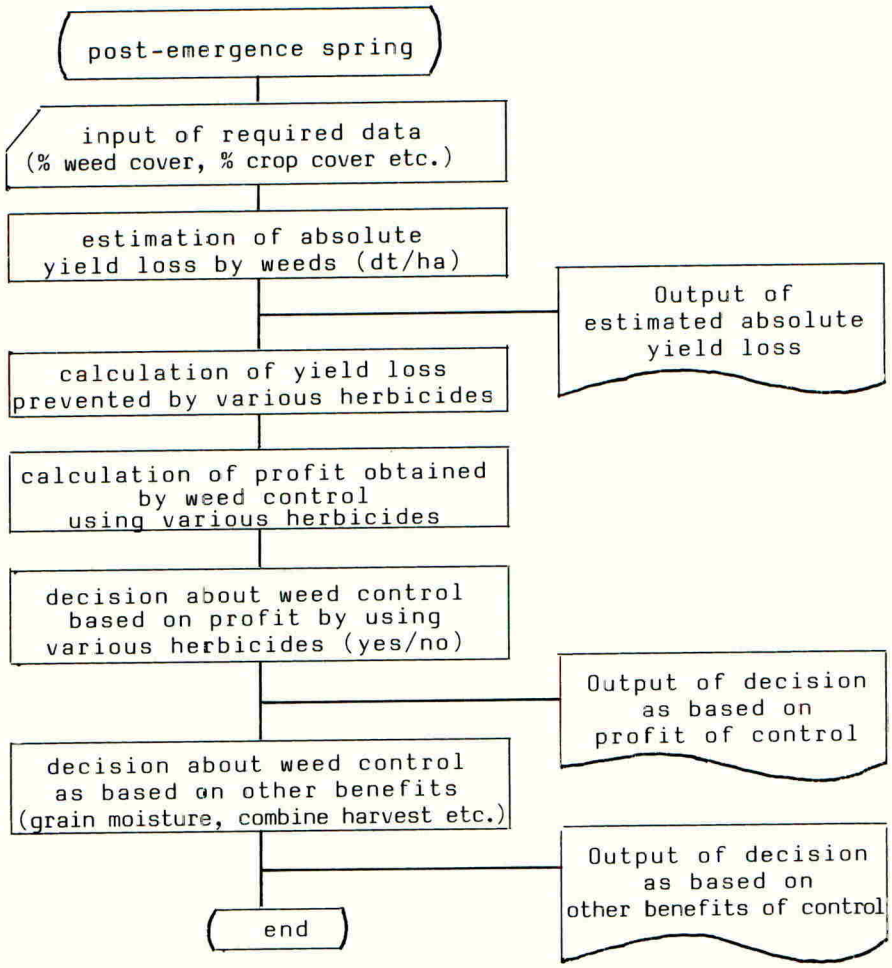

Fig. 1. Flow chart depicting computer aided decision steps in weed control subroutine 'post-emergence spring' (Gerowitt 1987, simplified).

3. yield loss dico = $\sum$dico [species (1,n) · factor species (1,n)]

species		factor
Galium aparine	(cleavers)	0.16
Stellaria media	(common chickweed)	0.08
Lamium sp.	(dead-nettle)	0.06
Veronica hederifolia	(ivy-leaved speed well)	0.05
Viola arvensis	(field pansy)	0.03
Matricaria sp.	(may weeds)	0.03
group 1		0.04
group 2		0.02

group 1 including for example: M. arvensis (field forget-me-not), S. arvensis (perennial sow thistle), F. convolvolus (black bindweed), C. cyanus (corn-flower)
group 2 including for example: C. bursa pastoris (shepherds purse), A. arvensis (parsley piert), F. officinalis (common fumitory), Ch. album (fat hen).

All estimates of yield loss are multiplied by a factor for

$$\text{crop competition} = \frac{\text{actual crop coverage}}{\text{expected crop coverage}}$$

Two independent estimates of yield loss are made:

I based on monocot weeds/m² plus weed coverage %

II based on monocot weeds/m² plus dicot weeds (species)/m²

Estimate I and II are compared, the higher one with less risk is selected for the following calculation.
The agreement between measured and estimated yield loss is sufficient to improve the accuracy of the control decisions.

By means of this system, wrong decisions within the series of 148 field experiments could be further reduced according to our ex post evaluations (Table 7).

The data show a definite improvement with respect to the accuracy of the decisions. The number of false decisions above the weed economic threshold however still remains too high with 28.4 % of the cases in which weed control above the variable thresholds was not profitable. Whether it will be possible by further investigations and complementation of the model to improve this ratio, remains to be shown. The low percentage of 5.4 % wrong decisions below the economic thresholds clearly points out that the risks connected with the omission of weed control by herbicides are very low. A prerequisit, of course, of the use of this threshold model is the accurate estimation of the required parameters both of the weeds and the crop.

TABLE 7

Percentage of wrong decisions as based on 'fixed values' or 'variable values' for weed economic thresholds in the computer model (fixed threshold for G. aparine not considered) (Gerowitt 1987)

	wrong decisions					
	total		below threshold		above threshold	
	n	%	n	%	n	%
fixed thresholds	62	41.9	12	8.1	50	33.8
variable thresholds (decision model)	50	33.8	8	5.4	42	28.4

For scientific reasons, it is important to make the system of weed control based on economic thresholds as accurate as possible. For practical applications the system must be easy to use and the acceptable risk in connection with the decision not to spray as low as possible. On the other hand, public pressure to avoid unnecessary spraying of pesticides due to ecological concerns will probably increase. The farmer is well advised therefore to utilize all available means of minimizing chemical weed control within a system of integrated plant protection, especially those which will give him a higher economic profit compared to routine spraying. Although farmers recently show increased interest in weed economic thresholds, their

overall utilization is still not satisfactory. The reasons are doubts and worry about the accuracy and risk of the decision not to spray in comparison to routine, prophylactic weed control, furthermore the intention to have a weed free field and also the concern about the risk of increased weed infestation in subsequent crops.

Our large scale studies on different farms at representative areas in Niedersachsen from 1983 - 86 (Wahmhoff, in preparation) showed that the utilization of weed economic thresholds is very promising in this regard. Table 8 summarizes the overall data from cereals, comparing conventional routine spraying with plant protection as far as possible according to threshold values for weeds, diseases and aphids. In winter wheat the expenses for routine control reach the highest level with 484,- DM/ha. Savings according to threshold criteria range between 48,- DM for oats (only 1 experiment) and 147,- DM in spring barley. The differences in net return take into account the yields obtained in the two systems. In spite of slight yield reductions in wheat and barley (apparently by delayed fungicide and insecticide sprayings in some cases) there was a small increase in net return from the use of thresholds. On the other hand in spring barley and rye where negative effects of herbicides on yield could be avoided, there was a large increase in net return. These savings in crop protection are mainly due to application of weed economic thresholds (Table 9). About 54 % of the average expense for routine spraying of herbicides was not necessary. In the other groups of pesticides savings were considerably less and especially with fungicides more difficult to obtain.

TABLE 8

Comparison between routine and threshold criteria plant protection in large scale field trials at 8 farms at representative areas in Niedersachsen over three years (Wahmhoff, in preparation)

crop	number of trials	routine plant protection DM/ha	saving according to threshold criteria DM/ha	difference in net return DM/ha
winter wheat	30	484,-	101,-	+ 27,-
winter barley	25	376,-	130,-	+ 82,-
spring barley	8	199,-	147,-	+ 192,-
rye	2	244,-	106,-	+ 181,-
oats	1	114,-	48,-	+ 48,-

TABLE 9

Monetary saving with different groups of pesticides due to threshold criteria spraying in cereals (cf. Table 8) (Wahmhoff, in preparation)

	average expense routine plant protection DM/ha	saving according to threshold criteria DM/ha	%
herbicides	98,-	53,-	54
fungicides	164,-	27,-	16
insecticides	13,-	2,70	21
growth regulators	16,-	2,90	18
total expense	291,-	85,-	29