In conclusion it must be said that integrated control using threshold values certainly requires knowledge and commitment both by the farmer and the advisory service. The efforts however are worthwhile!

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THE EFFECT OF VOLUNTEER BARLEY ON THE YIELD AND PROFITABILITY OF RAPESEED IN WESTERN CANADA

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ABSTRACT

Field experiments designed to determine the influence of different volunteer barley (Hordeum vulgare) densities on yield of spring sown rapeseed (Brassica campestris cv. Tobin) and the yield potential of different volunteer barley densities growing in a rapeseed crop were conducted at Vegreville and Lacombe, Alberta and Scott, Saskatchewan from 1982 to 1986. The data were fitted to a non-linear model (rectangular hyperbola) which provided a good fit to the data in most instances. Rapeseed yield loss and barley yield estimations from the models were used to determine the economics of herbicidal control of volunteer barley. A preliminary cost analysis examined the economics of controlling the volunteer barley with a herbicide. The results suggest that in some situations it may not be economical to control the volunteer barley, especially where densities are relatively low and where the market value of the barley is taken into consideration.

INTRODUCTION

Spring sown rapeseed (Brassica campestris) has become an important cash crop in western Canada. Major weed problems include volunteer cereals which can result from barley (Hordeum vulgare) and wheat (Triticum aestivum) crops sown in the previous year. Two herbicides, sethoxydim and fluazifop-butyl are registered in Canada for the control of volunteer cereals and other grassy weeds such as wild oats (Avena fatua) in rapeseed. The relatively high cost of these herbicides, however, may prohibit their use in some situations, especially when crop prices are low. A mathematical model has been developed to help western Canadian farmers determine the economics of wild oat control in rapeseed with herbicides (Dew and Keyes 1972), but little information is available in Canada on the effects of volunteer cereals on rapeseed yield. This information, as well as an estimation of the yield potential of volunteer cereals growing in a rapeseed crop, could be used to indicate when volunteer cereal control in rapeseed is cost-effective.

The objectives of this paper are to examine a) the relationships between volunteer barley density and both rapeseed yield loss and barley yield and b) the cost-effectiveness of controlling volunteer barley in rapeseed.

MATERIALS AND METHODS

Field experiments were conducted at Scott, Saskatchewan in 1982 and 1984-1986, Lacombe, Alberta in 1984 and 1985 and Vegreville, Alberta in 1985 and 1986. Rapeseed (cv. Tobin) was seeded in spring in rows with a double disc press drill at all locations. Eight population densities of volunteer barley ranged from 0 to approximately 200 plants/m^2 at each location. The method of seeding volunteer barley varied among locations. At Scott and Lacombe the barley was seeded with double disc press drills between the rapeseed rows and at right-angles to the rows, respectively. At Vegreville, it was hand-spread and manually raked into the soil.

Plot sizes were 1 x 5m (Scott), 2 x 3m (Lacombe) and 2 x 2m (Vegreville). Areas 4.0m^2, 1.0m^2 and 0.5m^2, respectively, were established in each plot and plant counts (at emergence) and yield data were taken from these areas.

All experiments were randomized complete block designs with 4 replicates. Data from each experiment were analyzed by analysis of variance to test the effects of location, year and location x year interaction on rapeseed yield loss (%) and barley yield (t/ha). The relationship between rapeseed yield and barley density was investigated for each year at each location using a rectangular hyperbolic model suggested by Cousens (1985):

$$y = Ywf\ [1-((ID)/100(1+ID/A)]$$

where Y is the estimated rapeseed yield (t/ha) per plot, Ywf is the estimated volunteer barley-free rapeseed yield (t/ha), D is the volunteer barley density (plants/m^2), I is the percentage rapeseed yield loss per unit barley density as D approaches zero, and A is the asymptotic rapeseed yield loss as D approaches infinity. The data are presented graphically in terms of percentage yield loss (yL):

$$yL = ID/(1+ID/A)$$

A rectangular hyperbola was also used to describe the relationship between barley yield (t/ha) and barley density.

All data were fitted to the models using non-linear, maximum likelihood iterative procedures from the SAS statistical package.

The cost-effectiveness of controlling the volunteer barley with a herbicide compared to harvesting it as a second crop was determined using the models:

$$D_1 = (RP_1) - (H + A),$$

$$D_2 = RP_1 \text{ and}$$

$$D_3 = [(RP_1) + (BP_2)] - S$$

where D_1, D_2 and D_3 are the cash returns ($/ha) when the volunteer barley is controlled with a herbicide, not controlled and considered to have no economic value, and not controlled but considered to have an economic value, respectively; R is the estimated rapeseed yield (t/ha) as a function of volunteer barley density, P_1 and P_2 are the market prices ($/t) of rapeseed and barley, respectively, B is the

estimated barley yield (t/ha) as a function of barley density, H is the cost of the herbicide ($/ha), A is the application cost ($/ha) and S is the cost ($/t) of separating the barley from the rapeseed.

RESULTS AND DISCUSSION

Rapeseed yield

Analysis of variance of the data (not shown) indicated that rapeseed yield loss due to volunteer barley, averaged over years, differed significantly ($P<0.001$) among locations. Losses were generally greater at Vegreville than at Scott or Lacombe. When averaged over locations, rapeseed yield losses did not differ significantly among years. However, there was a significant ($P<0.01$) location x year interaction indicating that rapeseed yield loss varied from year to year at each of the three locations.

Data were fitted to a non-linear model (rectangular hyperbola), rather than a linear regression model to avoid some of the biologically unrealistic properties associated with linear regression (Cousens 1985). Estimated parameters of the hyperbolic model for rapeseed yield as a function of barley density are presented in Table 1. The model provided a good fit to the data in most instances, except at Scott in 1985 and 1986 where the fit was relatively poor. The maximum percentage yield loss (parameter A) is poorly estimated in some cases as indicated by values over 100. This probably resulted because experiments were not designed specifically to fit the hyperbolic model. The parameter (A) can be highly sensitive to the distribution of weed densities (Cousens 1985). The value of the slope (I) which is a measure of the competitive ability of individual volunteer barley plants, varied from 0.38 at Scott (1986) to 1.29 at Vegreville (1986). The variation can probably be accounted for, partly, by differences in rapeseed densities among experiments. For example, the average rapeseed density varied from only 20 plants/m^2 at Scott in 1985 to 184 plants/m^2 at Lacombe in 1984 (Table 1). It is also probable that variable soil and/or climatic conditions contributed to the variation in yield loss and accounted for the variable volunteer barley-free rapeseed yields among some experiments (Table 1).

Graphical representations of the yield loss models are presented in Figs. 1 and 2. These indicate increasing rapeseed yield losses with increasing volunteer barley densities. Yield loss variation between experiments was generally more pronounced in absolute terms at high, rather than low volunteer barley densities. This may be partly due to the poor estimation of the maximum yield loss (parameter A) in some of the data sets. Decisions on spraying, however, are more likely to be made at the lower volunteer barley populations (<50/m^2) which would normally occur under field situations.

TABLE 1

Estimated parameters of the hyperbolic model for rapeseed* and barley** yield

Location	Year	Rapeseed density*** (plants/m_2)	Rapeseed parameters				Barley parameters		
			Ywf	I	A	R^2	I	A	R^2
Scott	1982	-	2.10	1.21	66.31	0.68	0.099	6.16	0.91
Scott	1984	104	1.11	1.26	84.54	0.77	0.019	2.12	0.82
Scott	1985	20	0.67	0.83	154.71	0.47	0.043	4.19	0.91
Scott	1986	85	1.57	0.38	49.53	0.42	0.034	1.91	0.84
Lacombe	1984	184	2.11	0.57	107.71	0.79	0.021	8.32	0.93
Lacombe	1985	121	2.07	0.74	126.99	0.73	0.034	4.79	0.91
Vegreville	1985	47	1.19	0.91	104.40	0.66	0.044	2.08	0.69
Vegreville	1986	45	4.15	1.29	117.70	0.91	0.101	8.68	0.93

*Y = Ywf [1-((ID)/100(1+ID/A))] and Y = ID/(1+ID/A). Parameters are described in text.

*** Numbers are the average density for each experiment. Rapeseed density was not determined at Scott in 1982.

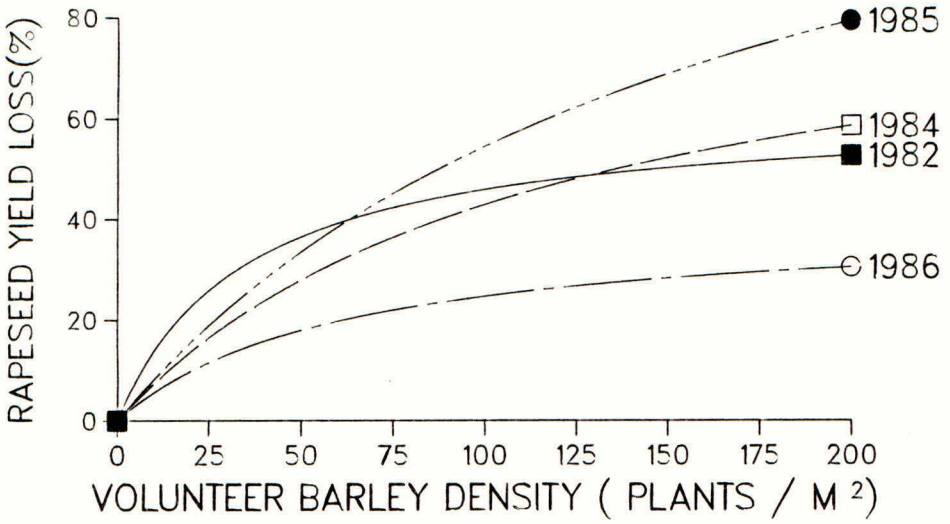

Fig. 1 Influence of volunteer barley density (plant/m²) on percentage rapeseed yield loss at Scott.

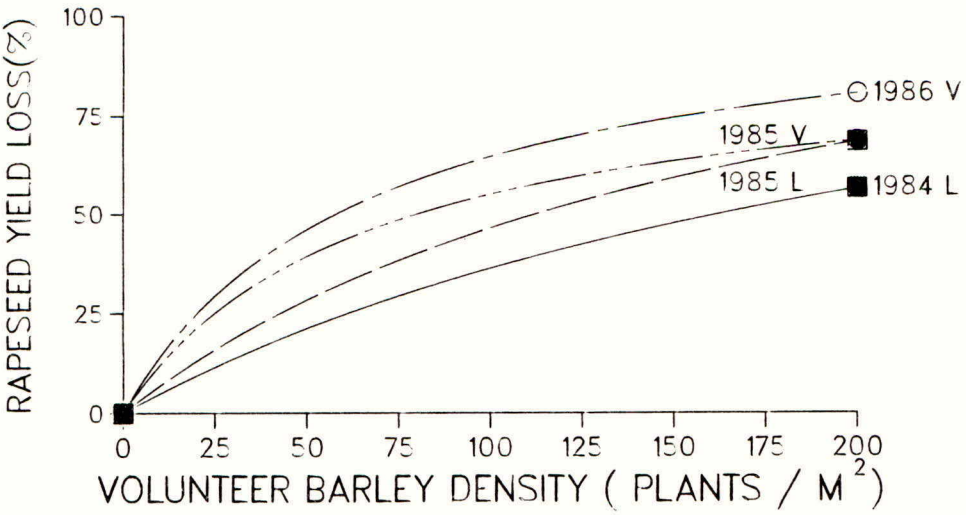

Fig. 2 Influence of volunter barley density (plant/m²) on percentage rapeseed yield loss at Lacombe (L) and Vegreville (V).

Barley yield

Analysis of variance of barley yields (not shown) indicated highly significant ($P<0.001$) effects due to locations and years and a significant interaction between them. Estimated parameters of the hyperbolic model for barley yield as a function of barley density are presented in Table 1. The model provided an excellent fit to the data in most instances. The parameters can be used to estimate barley yields at specific barley densities in each experiment.

Cost analysis of volunteer barley control

A preliminary cost analysis (Fig. 3) examined the economics of controlling the volunteer barley with a post-emergence herbicide. The hyperbolic models for Vegreville (1985) (Table 1) were used to estimate rapeseed yield (R) and barley yield (B) at different volunteer barley densities. This experiment was selected because the estimated volunteer barley-free rapeseed yield (1.19 t/ha) is close to the average yield for most regions of Alberta (Anonymous 1985). It is assumed that market prices of rapeseed (P_1) and barley (P_2) were \$260 and \$75/t, respectively, the herbicide cost (H) was \$33/ha and application cost (A) and seed separation cost (S) were \$7/ha and \$10/t, respectively. These figures represent approximate average prices and costs for western Canada in 1986 and 1987. In the analysis, it is assumed that after herbicide application (D_1), rapeseed yields (and cash returns) were similar regardless of volunteer barley density. This assumption is based on field experiments conducted at Vegreville where rapeseed yields were comparable to weed-free controls when more than 50 plants/m² were sprayed with sethoxydim as late as the 6-leaf stage of growth (O'Donovan 1985).

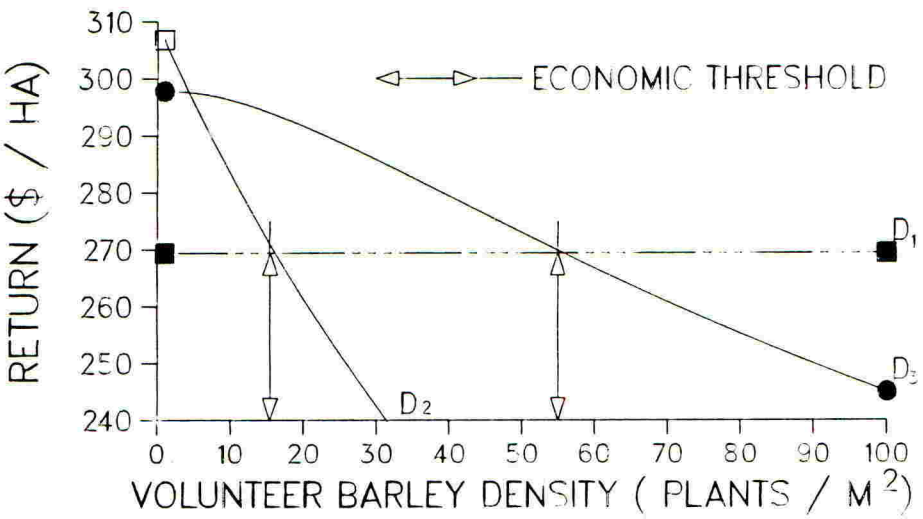

Fig. 3 Economics of volunteer barley control in rapeseed. The models were $D_1 = (RP_1) - (H+A)$, $D_2 = RP_1$ and $D_3 = [(RP_1) + (BP_2)] - S$. See text for details.

The cost-effectiveness of controlling the volunteer barley with a herbicide, as indicated by the economic thresholds, differed considerably depending on whether or not the volunteer barley seed was considered to have a market value (Fig. 3). Where the volunteer barley was considered to have no market value (D_2), spraying was justified economically at approximately 15 plants/m². However, if the potential yield value of the barley was taken into account (D_3), more than 50 plants/m² was required to break even on herbicide application. These results suggest that at current crop prices and herbicide costs, control of volunteer barley with post-emergence herbicides may be difficult to justify economically in some situations in western Canada. This will be so where volunteer barley densities are relatively low and where the barley seed has a market value. The value of the barley will depend on the time the seed matures relative to the rapeseed. In our studies, the maturity times of the barley and the rapeseed were close. Later maturing barley cultivars, however, may have little market value due to poor seed quality.

The economics of controlling volunteer barley in rapeseed, will also be highly dependant on the market price of rapeseed and on the expected weed-free yield. Higher rapeseed prices and yields (compared to these quoted above) will lower the economic threshold and favour control of the volunteer barley. Other factors including the presence of additional grassy weeds such as wild oats should also be considered before a final decision on spraying is reached.

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POPULATION DYNAMICS AND COMPETITIVE EFFECTS OF CYPERUS ESCULENTUS (YELLOW NUTSEDGE) - PREDICTION OF COST-EFFECTIVE CONTROL STRATEGIES

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ABSTRACT

A matrix model of the life-cycle of Cyperus esculentus simulated population fluctuations in a tobacco - grass-ley rotation. Yield - weed density relationships were established in the tobacco crop for each weed contol strategy. The weed dynamics and the effects on tobacco yield were simulated over ten tobacco cropping seasons (40 years). Chemical (metolachlor, 2.0 kg.a.i./ha) control combined with hand-weeding was most beneficial in dense infestations (1225 tubers/m^2). Even in very light infestations (1.5 tubers/m^2) not controlling the weed for one season reduced subsequent economic benefits, indicating an economic optimum threshold of less than 1.5 tubers/m^2. It was only necessary to spray light infestations (2.5 to 7.5 tubers/m^2), that were hand-weeded, if tuber densities increased to greater than 7.1 to 15.4 /m^2. The value of specific threshold values is discussed and emphasis is placed on the ability of the model to compare the consequences of weed control options and cultural practices.

INTRODUCTION

The study of the population dynamics of Cyperus esculentus (yellow nutsedge), has been directed at identifying factors affecting the abundance of the weed, as it has been with other weed species (Sagar & Mortimer 1976). Previous investigations of growth in a monoculture provided the basis for the construction of a predictive model free from the effects of interspecific competition or control measures (Lapham 1985a). This paper describes its use in a common tobacco cropping system in Zimbabwe, tobacco followed by three years grass-ley, which was subjected to various weed control strategies. The model, in association with economic considerations, is viewed as a tool in a more rational approach to weed management.

Model of the population dynamics

The construction of the model followed earlier approaches (Mortimer et al. 1978) based upon the use of matrices described in some population mathematics (Leslie 1945). Cyperus esculentus is a herbaceous perennial that multiplies by means of seeds and tubers and grows as a weed in many parts of the world. Populations can be divided into six categories or states (Fig.1). With time, there is a flux of individuals in each state, by transition to another state, death, or the production of new individuals. The probabilities and fecundities during a time period constitute the elements of a transition matrix while the number of

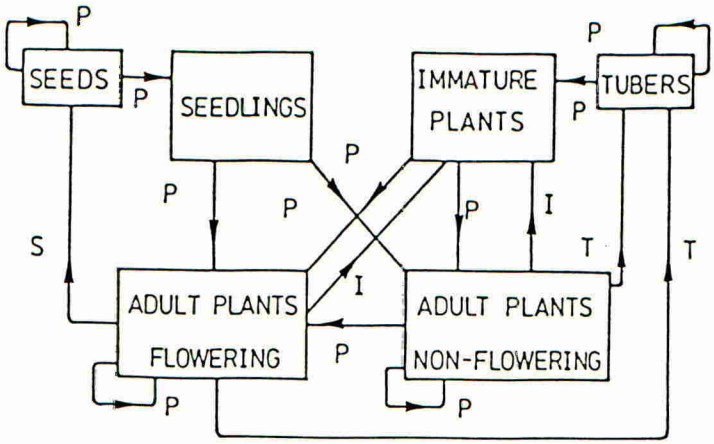

Fig.1. Flow diagram of C.esculentus life-cycle.
(P = transition or survival probabilities,
and the fecundities are:
T = tuber production,
I = immature plant production,
S = seed production).

individuals, present an array or column vector. Post-multiplying the transition matrix by the column vector gives the number of individuals in each state at the start of the next period. Initial development of the model showed that seedling survival was extremely low ($< 3 * 10^{-4}$; Lapham 1985a) and in this study only probabilities of plant and tuber survival were considered together with fecundities of adult plants. The dynamics of the tuber population were considered to reflect that of the weed as a whole. Probability and fecundity parameters were related to the initial density of tubers. Estimation of parameters for each transition period and simulation of population growth was done by computer.

Economic model

Weed growth may have an effect on tobacco quality (Klingman 1979), but it was not considered in economic estimations. Grass-leys can be used for a variety of purposes but their economic contribution is minimal in the rotation and was not analysed. Yield loss of tobacco was related most closely to tuber density (before planting) by a rectangular hyperbolic function as described by Cousens (1985; Fig.2):

$$Yl = ax/(1+ax/b),$$

where Yl is percentage loss, x is tuber density and a and b are parameters of the function. Yield (Yt) will depend upon the yield in weed-free conditions (Ywf),

$$Yt = Ywf(1-Yl/100),$$

and the return (R) will be determined by the price (P),

$$R = P*Yt.$$

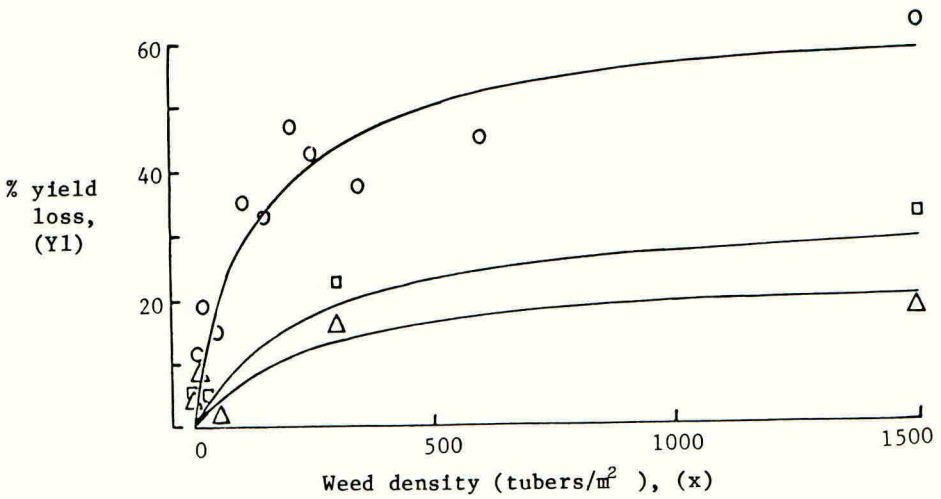

Fig.2 The rectangular hyperbolic function, Y1 = ax/(1+ax/b), relating percentage yield loss to weed density in three weed management systems. (a and b are parameters of the function.
O = no weed control, Y1 = 0.52x/(1+0.52x/62.94);
□ = hand-weeded, Y1 = 0.16x/(1+0.16x/32.28);
△ = herbicide sprayed and hand-weeded Y1 = 0.11x/(1+0.11x/22.56)).

Costs of weed control programmes may include hand-weeding (Cw) and that of the herbicide (metolachlor) and application (Cha). Net return (NR) from a programme including herbicide use and weeding would be,

NR = R-Cw-Cha.

When comparing two weed control strategies the benefit was estimated by comparing the net returns and was expressed as,

% increase in return = (NR1-NR2)/NR2*100,

Calculation of the cost of hand-weeding was based upon the linear relationship between weed density (d) and time spent weeding (t, man hours/ha, where a man hour costs Z$ 0.5),

t = 10.61+1.32d and, Cw = t*0.5.

The number of weeds removed during cultivation were estimated by the population model. Assumptions made and prices used for various items were:

Ywf = 3000 kg/ha,
P = Z$ 2.50 /kg,
Cw = Z$ 0.50 /man hour,
Cha = Z$ 82 /ha (Z$ 72 /ha for metolachlor at 2.0 kg.a.i./ha and Z$ 10 /ha for application).

MATERIALS AND METHODS

Experiments were done at Kutsaga Research Station, Harare, Zimbabwe. The population dynamics of C.esculentus were studied in four systems in 1985-86; a grass-ley (Chloris guyana), tobacco with uncontrolled growth of C.esculentus, tobacco hand-weeded, and tobacco sprayed with metolachlor pre-emergent and also hand-weeded. Appropriate plots were hand-hoed twice, within a month of weed emergence (mid-November 1985) and a month later, irrespective of weed density. Within these main-plots (2.4m * 11.76m) were five sub-plots (1.2m * 0.56m) of 0, 10, 50, 300 and 1500 tubers/m^2. Treatments were replicated eight times. Births and deaths of plants were monitored in quadrats (0.3m * 0.3m) at monthly intervals by marking cohorts with coloured wires. In January and April 1986 further quadrats were excavated to a depth of 0.2m and tubers recovered were counted. Plants were considered to be immature until a month old. Production of new plants was estimated by dividing the number emerging during any one month by the number of living adults. Tuber production was estimated similarly. Dried tobacco leaf from each sub-plot was weighed.

Data from two further experiments were used to establish the relationship between yield and uncontrolled C.esculentus growth from various tuber densities. In 1977-78 tuber densities were 0, 50, 150, 300 and 600 /m^2 and in 1979-80 0, 1, 5, 30, 100, 200 and 400/m^2. These density treatments were replicated 26 times in plots 3.6m * 1.12m. Dried tobacco leaf was weighed.

In another experiment in 1979-80, seven herbicide and three unsprayed treatments were hand-weeded twice in mid-November and December 1979. The time spent cultivating each plot (4.8m * 9.52m) and the C.esculentus plant density were measured. Parameters of the linear relationship between the two variables are described later. The experiment was selected to measure the time spent hand-weeding because the plots (four replicates per treatment) were much larger than in the other experiments. C.esculentus was the dominant weed.

RESULTS AND DISCUSSION

The dynamics of C.esculentus populations were simulated in a rotation of tobacco followed by three years grass-ley because it is the most suited to light soils, reducing nematodes, disease, and soil erosion. Fluctuations in the tuber population were dominated by the dynamics of the weed in the grass-ley. Even if the weed was controlled by metolachlor and hand-hoeing, tuber populations increased in the grass-ley and within 10 tobacco seasons (40 years) were periodically over 1000/m^2 from an initial density of 1.5/m^2 (Fig.3). Simulation demonstrated that in continuous grass-ley the theoretical equilibrium was 1225 tubers/m^2. Of the control strategies studied, spraying of metolachlor with hand-weeding was most cost-effective at this level of infestation (Table 1). Additional and more efficient control measures would necessary to reduce such high weed populations.

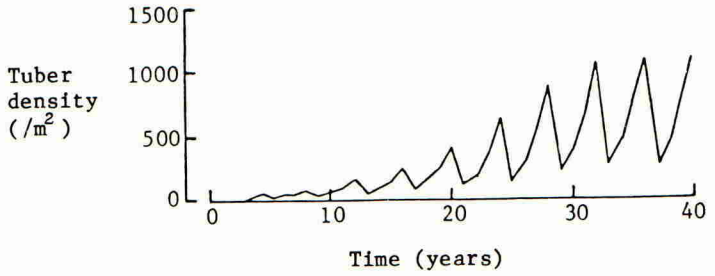

Fig.3 Fluctuations in tuber density, in a rotation of tobacco (sprayed with metolachlor and hand-weeded) followed by three years grass-ley, from a starting density of 1.5/m².

TABLE 1

Comparison between tobacco yield and the percentage increase in returns, compared with no control, of two strategies to control a severe infestation (1225 tubers/m²) of C.sculentus.

Control	Tobacco yield (kg/ha)	Increase in returns, %
Herbicide use & hand-weeding.	2423	85.7
Hand-weeding only.	2169	62.6

The possibility of reducing levels of weed control in light infestations was investigated by simulating various control strategies. The strategy of herbicide use together with hand-weeding every year, was most beneficial in every tobacco crop except the first when compared with a strategy that omitted control in the first year (that is, a strategy that omitted control when tuber densities were $\leq 1.5/m^2$; Fig.4). The economic optimum threshold (Cousens 1987), for implementing such a strategy is obviously below 1.5 tubers/m² but the model was not sufficiently sensitive at these densities to identify the value. The prolific production of tubers in uncontrolled and uncrowded conditions is well documented (Tumbleson & Kommedahl 1961, Lapham 1985b), and some form of control of light infestations is obviously necessary.

As an alternative to not controlling light infestations, farmers can choose between spraying together with weeding or only hand-weeding, an option which is of relevance in developing countries such as Zimbabwe. Strategies employing a range of spray thresholds,

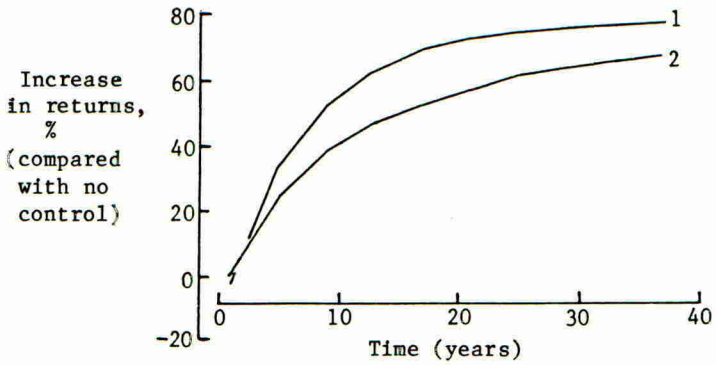

Fig.4 Percentage increase in returns, relative to no control, of two strategies to control a light infestation (1.5 tubers/m^2) over ten cropping seasons.
(1 = herbicide sprayed and hand-weeded every year,
2 = herbicide sprayed and hand-weeded if tuber density $\leq 1.5/m^2$).

TABLE 2

Threshold densities for herbicide (metolachlor) spraying, together with hand-weeding, relative to no control or hand-weeding only.

Threshold	Threshold tuber density ($/m^2$) relative to:	
	no control	hand-weeding
Economic threshold	3	23
Economic optimum threshold	< 1.5	7.1 - 15.4

which were related to tuber density, were examined over ten cropping seasons (40 years), from four starting tuber densities (Fig.5). Tuber populations increased in the grass-ley and spray-thresholds were often widely spaced. The economic optimum threshold (EOT) for herbicide spraying was estimated to be between 7.1 and 15.4 tubers/m^2. From a starting density of 2.5 tubers/m^2 it was apparent that, at the EOT, the most beneficial strategy was to spray in six out of ten cropping seasons (Fig.6). The initial four seasons would be hand-weeded because low tuber densities increase between tobacco crops, in the grass-ley, irrespective of the control strategy (Fig.3). On the other hand at 7.5 tubers/m^2 it was apparent that, at the EOT, it was necessary to spray and weed from the second cropping season.

The economic threshold (Cousens 1987), within one year, is the tuber density at which the differences in returns between two strategies are zero (Table 2). Economic thresholds were larger than EOT's which is

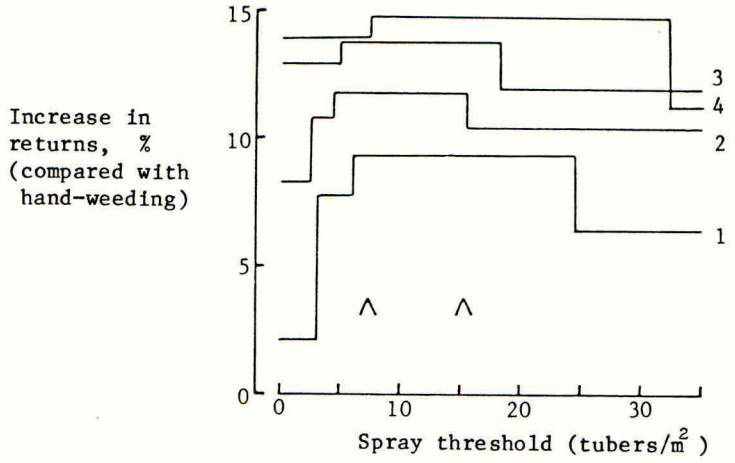

Fig.5 Percentage increase in returns, over only hand-weeding, when a range of spray threshods are applied to four starting densities of tubers, that are hand-weeded, over ten cropping seasons.
(1 = 2.5 tubers/m^2 , 2 = 2.75/m^2 , 3 = 5/m^2 , 4 = 7.5/m^2 .
$\wedge$ = upper and lower range of the economic optimum threshold).

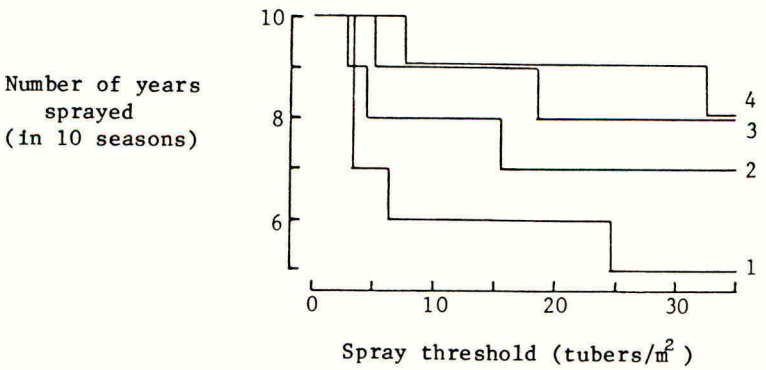

Fig.6 The frequency of herbicide application to four starting tuber densities, that are hand-weeded, over ten cropping seasons.
(1 = 2.5 tubers/m^2 , 2 = 2.75/m^2 , 3 = 5/m^2 , 4 = 7.5/m^2 .
$\wedge$ = upper and lower range of the economic optimum threshold).

normally the case (Cousens et al. 1986). Comparison of thresholds relative to hand-weeding, show that, within one year, it would be more beneficial to hand-weed tuber densities up to 23/m^2 , but spraying and hand-weeding from 7.1-15.4 to 23/m^2 would be most beneficial in the long-term.

In Zimbabwe the rapid increase in populations of uncontrolled C.esculentus growth makes some form of control necessary even at very low weed densities. In tobacco, chemical control of C.esculentus can be up to eight times the cost of annual grass control and the prophylatic use of herbicides is being questioned. However thresholds only provide a guideline for herbicide spraying and the final decision often depends on optimising use of resources in relation to other farm operations on an individual basis. Besides identifying threshold levels this model has indicated that, in the specific rotation, the dynamics of the weed are dominated by its' growth in the grass-ley. Weed control in the rotation could be improved, for example, by removing weeds in relation to their density (that is, if a threshold for weeding was established). On the other hand other practical rotations may reduce levels of infestation more effectively.

More important than identifying specific thresholds, is the models' ability to provide a rational approach to weed control strategies; control programmes and cultural practices can be adopted in relation to their long-term consequences. Further studies will provide data for other management systems and test the validity of the present parameters of the model.

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VARIABILITY IN THE GROWTH OF CLEAVERS (GALIUM APARINE) AND THEIR EFFECT ON WHEAT YIELD.

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ABSTRACT

The presence of cleavers (Galium aparine) in fields of wheat reduced wheat yields significantly in all of 7 experiments, losses ranging from 0.8 to 4.9 t/ha. Losses, when related to weed population, varied from 0.7 to 2.9% per cleavers plant/m^2. Losses were greater than predicted by 'Crop Equivalents' derived from dry weights of crop and weed plants. Cleavers showed a consistent pattern of growth, increasing considerably in dry weight late in the growing season. Variability in plant size, and the rate of population increase, are discussed in relation to thresholds. Due to the extreme competitiveness and high potential rate of increase of low populations, it is concluded that thresholds are not practicable for cleavers.

INTRODUCTION

In recent years cleavers (Galium aparine) has become an increasingly significant weed of winter cereals. This is largely due to poor control by herbicides, attributed to cool and wet weather in spring and early summer (Orson 1985). Surviving plants can impair harvest, contaminate grain and reduce yields. Cleavers has been identified as the most competitive of the common broad-leaved weeds of winter cereals (Wilson 1986). Even low populations can have an appreciable effect, reducing yields and producing seeds to infest future crops (Peters 1984).

Experiments are described which measured the response of wheat yield to the autumn control of cleavers. Additionally, we describe a survey of cleavers showing the extent of variability in the size of plants in wheat crops. The possibility of predicting yield responses from low populations, and the relevance of spray decision thresholds for cleavers, are discussed.

MATERIALS AND METHODS

Experiments

Seven experiments were carried out over 4 seasons from 1981-85, within a 50 mile radius of Oxford. In each experiment the dominant weed was naturally occurring cleavers in wheat, selected as far as possible for low and uniform infestation. Each experiment was a randomised block with six replicates of one sprayed and two unsprayed treatments. Plots of 15-20 m by 3.5 m were set up at right angles to the crop rows, each plot with the same number of tramline wheelmarks. A mixture of ioxynil/bromoxynil/mecoprop esters (Brittox 49.5% e.c.) at 1.73 kg a.i./ha was used in late autumn or

early winter to control cleavers, and re-applied where necessary at a later date to control surviving or late germinating weeds. Other operations such as fertiliser and fungicide applications were carried out by the farmer.

Assessments Crop and weed seedlings were counted at the seedling stage in each experiment in late autumn. In the early experiments (Chesterton 81/82, 82/83, Broadleaze and Wakefield) wheat seedlings were counted in twenty random quadrats each of $0.1m^2$ on each replicate, and cleavers seedlings in ten random quadrats on each plot. From 1984/85 onwards (Farthinghoe, Marlow and Idstone) a pair of $1m^2$ plots was set up within each large plot; within these m^2 plots all crop and weed seedlings were counted. In all experiments cleavers was recounted in the spring, to include late emerging seedlings and to allow for seedling mortality during the winter.

In the experiments from 1983/84 onwards dry weights of crop and weeds were determined on six dates between April and July. Five crop plants (from the weed free plots) and 5 cleavers plants were removed from each replicate from areas adjacent to the m^2 plots (reserved for harvest). These plants were dried and weighed at each date. Average dry weight/weed plant divided by dry weight/crop plant gave a 'Crop Equivalent' value for the cleavers for each sampling date (Wilson 1986). The 'Crop Equivalent' value for the latest date, when cleavers had reached the greatest weight, was used for calculating 'predicted' grain yield losses in each experiment.

In the early experiments grain yields were obtained by combining a 2.1m wide swath from the centre of each plot with a small Claas plot combine. The grain from each plot was weighed, and a sample of 1-2 kg dried at $100^{o}C$ for 16 h and re-weighed. The grain was sieved to remove weed seeds and small grain ($< 2mm$), and yields of clean grain at 85% dry matter were determined. From 1984/85 onwards, yields were obtained from the $1m^2$ plots in which weeds and crop were previously counted. Crop and weeds were cut off at ground level with serrated knives and bagged. Weeds were subsequently separated from the crop, the crop threshed with a stationary thresher and yields of clean grain ($> 2mm$) at 85% dry matter determined.

Survey

During late June and July 1986, unsprayed cleavers growing in wheat were sampled from 19 fields from Somerset through the South Midlands to Essex. In each field 10 crop and 10 cleavers plants were taken, avoiding plants growing in the hedgerow, and dry weights per crop and weed plant determined. The crop was also assessed for growth stage, height and density. Samples of the topsoil were taken for moisture content and analysis. Further information about the sites was obtained from the farmer; this included crop cultivar, drilling date, fertilizer, pesticides and previous cropping.

RESULTS

Experiments

Yield responses

The presence of cleavers reduced yields significantly at all sites. Losses ranged from 0.79 t/ha (Marlow) to 4.87 t/ha (Chesterton 81/82) (Table 1). Regression analyses showed that yield losses per cleavers plant/m^2 ranged from 0.7% (Broadleaze) to 2.9% (Chesterton 82/83).

Growth of cleavers

The pattern of dry weight increase was similar at the four sites where dry weight per plant was measured (Fig.1). Dry weights increased rapidly during June with a further increase during July. Cleavers weights increased more rapidly than the crop weights, so increasing the 'Crop Equivalent' ratio (weed dry weight per plant/ crop dry weight per plant). Crop Equivalents for July ranged from 2.46 (Wakefield) to 4.20 (Farthinghoe). The ultimate size of each cleavers plant differed between sites. Average dry weight per plant in July varied from 12.8g at Wakefield to 27.4g at Farthinghoe (Table 2).

TABLE 1

Wheat Experiments - Details of sites, crop and weed populations and crop yields

Site	Seedlings/m^2		Yield t/ha			% loss per cleavers plant /m^2
	Wheat	Cleavers (spring)	Sprayed	Unsprayed	SED	
1981/82						
Chesterton	198	26	8.57	3.70	0.32	2.2
1982/83						
Chesterton	304	10	7.44	5.29	0.28	2.9
Broadleaze	324	21	8.26	6.98	0.18	0.7
1983/84						
Wakefield	290	16	11.12	7.92	0.31	1.8
1984/85						
Farthinghoe	274	47	6.39	3.07	0.43	1.1
Marlow	252	7	6.56	5.77	0.14	1.7
1985/86						
Idstone	278	4	10.36	9.39	0.14	2.2

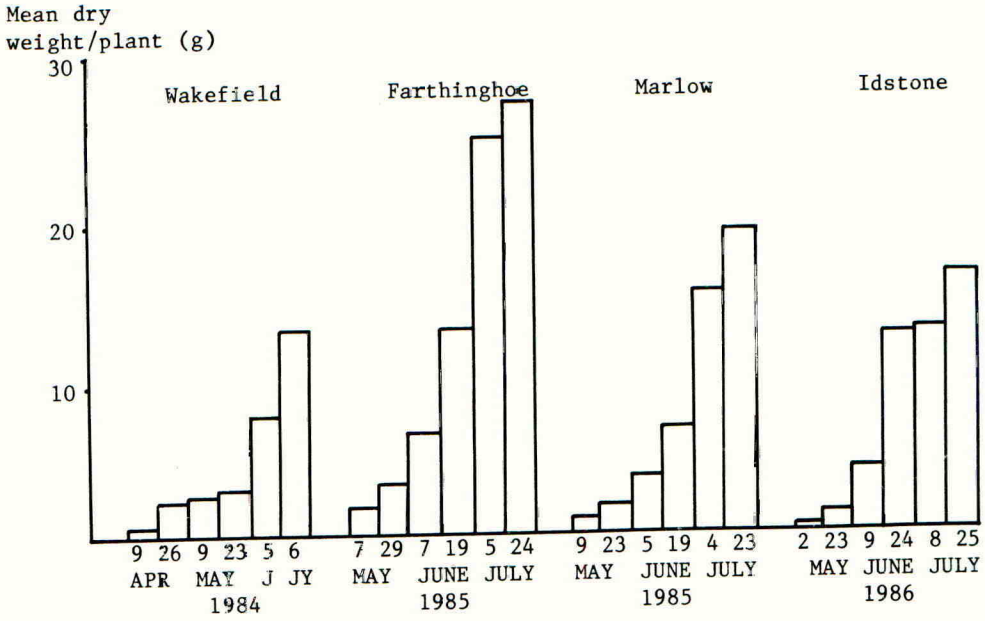

Fig.1. Growth of cleavers at 4 sites.

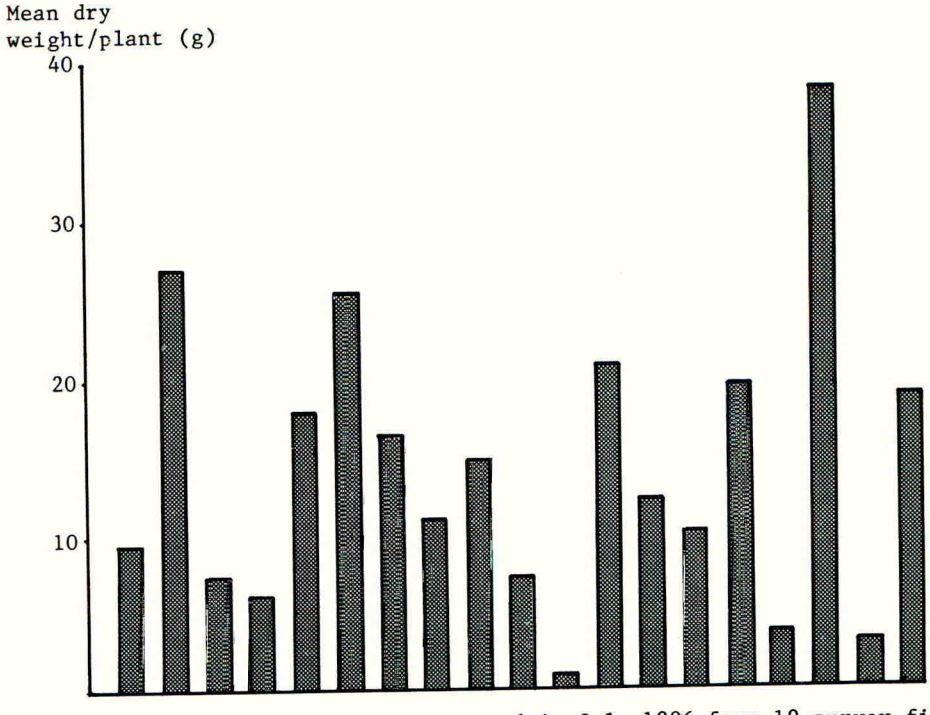

Fig.2. Dry weights of cleavers removed in July 1986 from 19 survey fields.

Survey

In 19 fields, cleavers ranged in size from an average of 0.8 to 38g/plant (Fig.2). Most fields were of medium/heavy soil and variation in plant size could not be linked to soil type or the moisture content of the surface soil. There was slight evidence that the smallest cleavers were associated with the shallowest soils. In 9 fields sampled early, re-sampling showed that cleavers on average doubled in weight between June and July.

TABLE 2

Comparison between predicted and recorded yield losses

Site	Dry wt/plant July		Crop Equivalent	% Yield loss		Crop Eq. needed to predict actual loss
	Crop	Weed		Predicted	Recorded	
1981/82						
Chesterton	(not recorded)				56.8	10.01
1982/83						
Chesterton	6.6	24.2	3.69	10.8	28.9	12.36
Broadleaze	5.2	15.7	3.00	16.3	15.5	2.83
1983/84						
Wakefield	5.2	12.8	2.46	12.0	28.8	7.33
1984/85						
Farthinghoe	6.5	27.4	4.20	41.9	52.0	6.32
Marlow	6.9	19.2	2.77	7.2	12.0	4.91
1985/86						
Idstone	5.5	16.2	2.96	4.1	8.6	6.54
Mean	6.0	19.2	3.18	15.4	28.9	7.19

DISCUSSION

The extreme competitiveness of cleavers and the potential for small populations of this weed to seriously reduce wheat yields are confirmed by the results of these experiments. As few as 4 cleavers plants/m^2 reduced wheat yield by 1 t/ha; in all experiments grain yield losses of between 0.7 and 2.9% were recorded for each cleavers plant/m^2 present.

A system of predicting crop yield losses due to weeds using Crop Equivalent values has been described previously (Wilson 1986). One of the assumptions is that competition is by direct replacement, so that the total dry weight (above ground) of weed plus crop equals that of the weed free crop. The form of response of crop yield to increasing weed density is best described by a rectangular hyperbola (Cousens et al. 1984).

In each experiment where Crop Equivalents were derived, the Crop Equivalent was used to obtain a 'predicted' yield response (Table 2). This was found by expressing the weed Crop Equivalents as a percentage of the total Crop Equivalents (crop + weed) (Wilson 1986). In five of the six experiments, predictions of yield losses were lower than those actually recorded. Under-estimation of yield loss suggests that weed dry weight is an inadequate measure of the competitive effects of cleavers. Additive competition, whereby weed weight does not compensate for the loss of crop weight, could be due to the considerable late growth of cleavers overtopping and shading the crop. Crop Equivalent values which would have been needed to predict the recorded losses have been calculated (Table 2); these average 7.2, compared with the average of 3.2 derived from plant dry weights. The value of 7.2 represents a large increase over the previously used values for prediction but is a more realistic reflection of the considerable competitive powers of this weed. These experiments therefore show that the system of Crop Equivalents or 'Relative Production Indices' (Håkansson 1986) is not applicable to cleavers, although it may be of considerable value in describing relative competitive abilities of many other species.

The cleavers plants at Farthinghoe were individually twice as large as those at Wakefield. Much greater variability (< 1 to 38 g/plant) was found between the cleavers plants from the different survey fields, but the survey was too limited in scope to explain this variability. Cleavers is reported to be favoured by moisture retentive, deep loam and clay soils rich in nutrients (Hanf 1983). Cleavers growing in such soils, because of their natural late phase of growth, can readily exploit wet summers and late nitrogen applications to produce very large and competitive plants. Other broad-leaved weeds, which make most of their growth early in the season and are suppressed more by crop competition, are likely to be less variable in size than cleavers.

It is not easy to define a threshold population for cleavers and allowance has to be made for the variability in competitiveness as described above. With any system of weed management based on threshold weed populations, a small yield loss has to be accepted if low levels of weeds are to be left untreated. The value of such a yield loss would be less than the cost of control. A 1% yield loss might be considered to be acceptable. Using the lowest and highest yield responses of the experiments reported

here, a 1% yield loss would be given by 1.4 and 0.3 cleavers plants/m^2 respectively. In these experiments weights of cleavers plants ranged from 12.8 to 27.4g per plant. In the survey greater variability was found between cleavers plants from the different fields (<1 to 38g per plant). Such variability is too great to be encompassed by a single fixed threshold value. If a single value is used, sufficiently low to allow for the most competitive situation, then the majority of fields containing less competitive cleavers will be sprayed unnecessarily, negating the economic advantage of using thresholds. At present it is not possible to use a range of threshold values, applicable to different competitive situations, because we have insufficient experience to predict variation in the competitive ability of cleavers in wheat.

For the long term management of cleavers a threshold needs to take into account the weed seed return and the potential build-up of the weed if it is not controlled. Although seed production per plant is very dependant on weed density, it is likely that vigorous plants, growing in isolation from each other, could each produce well over 1000 seeds (N.C.B.Peters personal communication). Very large rates of increase have been recorded. When establishing an artificial infestation of cleavers, a 23-fold increase in seedling numbers occurred over 2 years with shallow tine cultivations (R.J.Froud-Williams personal communication). In a natural infestation of cleavers at Kineton (Warwickshire), monitored by the present authors from 1980-83, rates of increase were greater from low to medium population (15-fold) than from medium to high population (3-fold).

Cousens derived economically optimum thresholds for wild-oat (Avena fatua) and black-grass (Alopecurus myosuroides) by taking into account the costs and efficiency of control, seed production and seed death over a 10-year period (Cousens et al. 1986, Doyle et al. 1986). He found that a reasonable estimate of a long term threshold could be obtained by dividing the economic threshold by the rate of increase (Cousens 1986). With cleavers the very large potential increase of a low density infestation means that the optimum threshold has to be extremely low.

Thus, to allow for the variability of cleavers and for the potentially very rapid build-up from low populations, the long-term threshold would have to be an extremely low population, well below 1 plant per $20m^2$. Seedlings are difficult to monitor at such a low density, particularly if distribution is patchy. It is concluded that the concept of a numerical threshold, while relevant to other less competitive weed species, is not realistic for cleavers.

ACKOWLEDGEMENTS

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USING DECISION THRESHOLDS FOR THE CONTROL OF GRASS AND BROAD-LEAVED WEEDS AT THE BOXWORTH E.H.F.

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ABSTRACT

Spray decision thresholds based on weed densities have been in use on the Boxworth EHF as part of the MAFF Boxworth Project for four seasons. Densities were estimated from quadrat surveys of each field. A study of the survey technique indicated that mean weed density values could contain large errors and that increased sampling might be required. The grass weeds, wild-oat (Avena fatua) and common couch (Elymus repens), were adequately managed under the system and fewer herbicide applications were made against them. Broad-leaved weed populations were extremely variable. Difficulties were found in applying the decision threshold for such weeds using densities taken from a single assessment in autumn, particularly when germination was delayed. Field surveys took 15 min/ha. Both theoretical and practical aspects of the use of the thresholds are discussed.

INTRODUCTION

The multidisciplinary project on pest and disease control systems based at the MAFF Experimental Husbandry Farm (EHF) at Boxworth has been in progress for six seasons (Hardy 1986). During the first two years, baseline data were collected. From sowing in 1983, three areas have received different pesticide treatments. The economic and ecological effects of these three levels of pesticide use are being assessed. The treatment regimes are as follows:

1. Full Insurance - receiving pesticides on a prophylactic basis with a pre-determined spray programme.
2. Supervised - receiving pesticides only when pests achieve levels likely to affect yield. Decision thresholds are used.
3. Integrated - receiving pesticides as in the Supervised area, but combined with cultural operations to further reduce pesticide use.

The Supervised and Integrated treatments have been implemented by using weed, pest and disease spray thresholds. While predictive thresholds have been used for pests and diseases for some years, there had been little practical or theoretical consideration of weed thresholds at the outset of the Project, perhaps with the exception of that by Eggars & Niemann (1980).

Wilson & Cussans (unpubl.) developed spray thresholds for use within the Boxworth Project, using information on the competitive effects and population dynamics of weeds. Data on grass weed species, particularly the likely return of viable seed and seedling germination and survival, were used to predict recruitment to the succeeding crop (Cussans & Moss 1982).

Thresholds were set based on grass head densities in the preceeding crop. A different approach was required for dicotyledonous species as the variability of germination precluded the prediction of populations from one season to the next. In addition, weed floras contain a range of species not all of which are equally competitive, but which are treated equally for control purposes. In order to accommodate the competitive effects of a mixed broad-leaved flora, Wilson (1986) has developed the concept of 'crop equivalents' and set a threshold value. If weed populations exceeded the thresholds, the farmer, with whom the final spray decision rested, was advised to apply herbicides. In this paper, the changes in estimates of field populations under a system of threshold use are described.

METHODS

Study site

The site, described by Marshall (1985), comprised four fields within the Integrated area (1,2,3W,3E), three fields in the Supervised area (4,5,6) and four in the Full Insurance area (7,8,9,10). Winter wheat was the main crop, with a break of winter oilseed rape. In the 1985 harvest year, fields 1, 2, 5 and 9, and in the 1987 harvest year, fields 3W, 4 and 10 were sown to rape. The herbicides fluazifop-butyl and propyzamide were used in the oilseed rape crops. The first wheat crops in the Integrated and Supervised areas which followed oilseed rape, did not receive grass herbicides.

The herbicide programme in the winter wheat was based on late-spring application of difenzoquat for the control of wild-oat (*Avena fatua*), pre-harvest glyphosate for common couch, (*Elymus repens*),autumn application of isoproturon for blackgrass (*Alopecurus myosuroides*) control and an autumn sequence of triallate and metoxuron for the control of brome grasses (*Bromus* spp.). Broad-leaved weeds were controlled in autumn and spring with a range of herbicides, notably bromoxynil + ioxynil, mecoprop and fluroxypyr.

Field surveys

Grass populations were estimated in early July in winter wheat crops by counting heads present in 0.25m² quadrats in a sampling grid, based on wheelings through the crop. Assessments were not possible in the rape crops. The sampling intensity, selected in 1982 on the basis of availalble resources, was 6 points/ha with four random quadrats thrown at each sample point off the wheelings. Broad-leaved plants were counted in November and March in 0.1m² quadrats, on the same sampling patterns used for assessing grasses in July.

The accuracy of the survey method was investigated in an intensive study of a single field in July 1984. Quadrat counts were made at an intensity of 35 points/ha, six times that initially adopted, sampling every 25 paces along every wheeling through the crop. Data from the four quadrats taken at each point were bulked and changes in the statistics of the mean with sampling intensity were examined. Different sampling intensities were artificially drawn from the data, by discarding alternate wheelings and by starting from different sides of the field.

Thresholds

Grass species data were expressed as mean head densities/m² for the weediest 25% of each field. If the mean head density exceeded 2.0 heads/m² for any grass species, apart from wild-oat, the farmer was advised to spray

the following year. The threshold for wild-oat was set at 0.5 heads/m². In the case of common couch, the advice given in early July was acted upon before harvest. The treatment has nevertheless been considered as the first herbicide application to the following crop.

The density of each dicotyledonous weed species in November and March was multiplied by a crop equivalent value (Table 1) and summed to give a crop equivalent for the mixed-species weed community. If the mean for summed crop equivalents for the weediest 25% of wheelings in autumn exceeded 5 crop equivalents/m², then the farmer was advised to apply broad-leaved weed herbicides. More recent work by Wilson (1986) has indicated the need to modify some of the crop equivalent values, particularly for cleavers (Galium aparine). However, for the purposes of this experiment, crop equivalent values were not altered.

TABLE 1

Crop equivalent values for different weed species as used in the Boxworth Project.

Species	Crop equivalent
Cleavers (Galium aparine)	1.73
Common poppy (Papaver rhoeas)	0.89
Charlock (Sinapis arvensis)	0.90
Mayweeds (Tripleurospermum spp.)	0.62
Common chickweed (Stellaria media)	0.50
Common field-speedwell (Veronica persica)	0.20
Red dead-nettle (Lamium purpureum)	0.18
Field forget-me-not (Myosotis arvensis)	0.16
Ivy-leaved speedwell (Veronica hederifolia)	0.14
Field pansy (Viola arvensis)	0.13
Venus's-looking-glass (Legousia hybrida)	0.12
Parsley-piert (Aphanes arvensis)	0.08

At the outset of the experiment, it was agreed that the advice given on the basis of thresholds could be overruled by the farm staff, if weed populations appeared to have changed from the time surveys were made. In 1985, a formal system of crop-walking was initiated. This will allow comparison of the decisions made using quadrat surveys to be compared with those from more superficial crop inspection and will be published elsewhere.

RESULTS

Weed density estimation

Three grass species were recorded in the intensive study of field 4 in July 1984. Mean head densities of common couch, barren brome (Bromus sterilis) and meadow brome (Bromus commutatus) were estimated using different sampling intensities and patterns through the crop (Table 2). The accuracy of the estimates varied markedly with sampling intensity. At the intensity used for routine surveys, 6 points/ha, the error for the most abundant species was of the order of 30%, while for the rarest species this was about 80%. A sampling intensity of 18 points/ha gave an error of 20% for barren brome, 25% for common couch and about 50% for meadow brome. Even a sampling intensity of 35 points/ha gave an error of 30% for this species. Variation in the mean values for similar sampling intensity reflected the patchy occurrence of weeds with wide differences between wheelings.

TABLE 2

Head densities per m² of three grass species with different sampling intensities. (SD = standard deviation; SE = standard error as % of mean)

Sampling intensity (points/ha)	Number sample points	Barren brome Mean	SD	SE	Common couch Mean	SD	SE	Meadow brome Mean	SD	SE
35	407	3.79	10.74	14.0	0.92	3.33	17.9	0.16	1.03	31.5
18	205	4.09	11.97	20.5	0.78	3.30	29.5	0.28	1.39	34.3
18	202	3.55	9.40	18.6	1.06	3.37	22.3	0.04	0.40	70.0
13	152	1.08	3.38	25.4	0.88	3.78	34.7	0.17	1.03	48.5
13	130	5.42	10.16	16.5	0.66	2.28	30.2	0.10	0.82	71.0
13	125	5.47	15.59	25.5	1.26	3.67	26.1	0.03	0.36	100.0
9	105	3.64	10.78	28.9	0.77	3.13	39.6	0.29	1.55	52.8
9	103	3.28	7.69	23.1	1.31	4.08	30.7	0.04	0.39	100.0
6	77	0.60	1.89	36.2	1.03	4.05	45.0	0.07	0.47	81.5
6	67	5.72	13.15	29.1	0.54	1.93	43.9	0.13	1.10	100.0
6	63	4.46	11.64	32.9	1.40	4.07	36.7	0.32	0.32	65.6

Grasses

There were five common grass weed species at Boxworth, wild-oat, common couch, blackgrass, barren brome and meadow brome. The average head densities of wild-oat in the worst 25% of each field from 1982 to 1987 are given in Table 3. In succeeding Tables, the application of a herbicide to grasses notated (*), was in response to weed densities in the previous harvest year.

TABLE 3

Mean head densites per m² of wild-oat in July in the weediest 25% of adjacent wheelings at Boxworth EHF, with the harvest years(*) when difenzoquat was applied.

	Baseline year		Treatment year			
Field	1982	1983	1984	1985	1986	1987
Integrated area						
1	0.3*	0	0	R.	0	0
2	0.3*	0*	1.0	R.	0.6	0.4
3W	0*	1.1*	4.3*	0.2*	0	0
3E	0*	0*	0	0	0	R.
Supervised area						
4	0.4*	0*	0	0	0.1	R.
5	0.03*	0*	0	R.	0	0
6	0.9*	0.8*	0.06*	0.06*	0	0
Full Insurance area						
7	-	0*	0*	0*	0*	0*
8	0.08*	0*	0*	0*	0*	0*
9	2.9*	0*	0*	R.	0*	0*
10	R.	0*	0*	0*	0*	R.

R. = winter oilseed rape. No assessment.

During the baseline years of 1982 and 1983, difenzoquat was applied as

a routine measure at half-rate (0.5 kg a.i./ha). Thereafter, the decision threshold of 0.5 heads/m² was imposed in the Integrated and Supervised areas. After 1983, difenzoquat was only used in fields 3W and 6. Using the threshold allowed fewer applications compared with baseline years. On two occasions the farmer overruled the advice given on the basis of thresholds; in field 6 in 1985 when difenzoquat was applied and in 1987 on field 2, when it was not applied following hand-roguing in July 1986. After oilseed rape, some wild-oats survived in this small field (4.6ha), but numbers declined from levels recorded in 1984, before the break crop. In the majority of possible spray occasions (18/20) threshold advice was followed.

Data for common couch (Table 4) show that the threshold system allowed some reduction in the use of pre-harvest applications of glyphosate. Using the threshold permitted a slow increase in populations in several fields before control was recommended. For example, mean densities in field 6 increased from 0.8 heads/m² in 1984 to 6.8 heads/m² in 1986. The field was then sprayed in August 1986 and by July 1987 only 0.3 heads/m² were recorded. There were four occasions when the thresholds were overruled on the basis of likely increases. These were in fields 2, 3E and 6 in 1984 and 3W in 1987. In 21 out of 25 spray occasions threshold advice was followed.

TABLE 4

Mean head densites per m² of common couch in July in the worst 25% of each field at Boxworth EHF. Occasions when glyphosate was applied before harvest in the previous crop are shown thus (*).

	Baseline year		Treatment year			
Field	1982	1983	1984	1985	1986	1987
Integrated area						
1	0.4	8.5*	2.3*	R.*	0	0
2	0.1*	1.3	0*	R.	0	0
3W	1.2	25.0	0.4*	0.9	1.0	R.*
3E	1.4	0.6	0*	0	0	0
Supervised area						
4	0.08*	1.0	0.3	0.9	2.5	R.*
5	2.6	4.8*	0.1*	R.	2.1	0*
6	0.3*	1.4*	0.8*	1.2	6.8	0.3*
Full Insurance area						
7	-	3.8*	0.3	0*	0	0*
8	0.4*	3.9	1.3	0*	0	0*
9	0.3*	4.0*	1.0*	R.*	0	0*
10	R.*	0	0.3	0*	0	R.*

R. = winter oilseed rape.

Blackgrass head numbers fluctuated markedly (Table 5) and populations in 1984 and 1987 increased after poor control with spring applications of isoproturon. There was also little control of blackgrass in oilseed rape grown in 1985, as populations in 1986, were similar to those found in 1984. In 16 out of 20 spray occasions, the threshold advice was followed. However, the results are confounded by the effects on blackgrass of triallate and metoxuron applications for brome control. Fields 3E and 6, apparently unsprayed for blackgrass in 1984, received herbicides for brome control. This also occurred in fields 4 and 6 in 1985 and 1986. Advice was overruled in fields 2 and 3E in 1987, when populations in 1986 were below

the threshold, though subsequent counts were high.

TABLE 5

Mean panicle densites per m² of blackgrass in the weediest 25% of each field at Boxworth with the harvest years when isoproturon was applied (*).

	Baseline year		Treatment year			
Field	1982	1983	1984	1985	1986	1987
Integrated						
1	7.4*	4.1*	33.7*	R.	32.6	73.8*
2	0*	0*	3.3	R.	1.6	7.8*
3W	0*	0.3g	20.7	33.1*	0*	R.
3E	19.3*	81.6g	55.7	10.0*	0*	21.5*
Supervised						
4	0*	0*	0	0	0	R.
5	5.3*	1.5g	0	R.	2.6	6.7*
6	2.4*	2.8*	0.9	0.1	0	0.3
Full Insurance						
7	-	2.7	0.05*	0*	0	0*
8	1.2*	3.8*	0*	0.2	0	0.1*
9	0.5	0	0*	R.	0*	9.5*
10	R.	3.7*	0*	0	0	R.

R.= winter oilseed rape; g=chlorsulfuron+methabenzthiazuron

Dicotyledonous weeds

Using plant counts made in November, the mean crop equivalents/m² for the worst 25% of each field were used to advise on the need for control measures. Crop equivalent values in autumn and spring are given in Table 6, with a record ($) of herbicide applications made in response to the counts in the same season.

TABLE 6

Mean crop equivalent values per m² for the worst 25% of each field in autumn and spring at Boxworth, with the seasons when broad-leaved weed herbicides were applied ($). ^ = headland only

Field	1982/3		1983/4		1984/5		1985/6		1986/7	
	Aut	Spr	Aut	Spr	Aut	Spr	Aut	Spr	Aut	Spr
Integrated area										
1	6.2	4.1$	55.1	9.5	Rape		0	16.9$	47.5	23.7$
2	13.6	27.9$	87.3	31.0$	Rape		0	9.9$	17.1	6.7$
3W	(3W and 3E)$		84.3	36.3$	28.2	1.9$	0	0.6$	Rape	
3E	(144.6	27.8)	92.7	1.8$	22.1	4.6$	0	11.5$	19.5	24.8$
Supervised area										
4	4.8	2.8$	4.9	0.2$	11.1	1.9^	0.1	2.1$	Rape	
5	1.2	0.3$	0.7	0	Rape		0.1	17.3$	21.0	7.8$
6	-	2.9$	5.5	2.3$	28.7	4.9$	0.5	6.4$	24.1	3.0$
Full Insurance area										
7	-	55.7$	16.4	8.2$	5.8	1.6$	0	0.6$	2.5	3.8$
8	10.9	2.1$	2.3	0.4$	13.1	6.6$	0	0.5$	17.7	4.4$
9	7.6	6.5$	2.3	0$	Rape		0	0.3$	1.2	7.3$
10	19.8	6.4$	11.1	2.2$	8.1	2.2$	2.7	2.6$	Rape	

There were large seasonal and field-to-field variations in dicotyledonous weed populations. In the Integrated area there were high crop equivalents in the 1984 harvest year, while in the autumn of 1985 there was very little germination. There were few occasions in which a field remained unsprayed throughout the season, as most fields exceeded the threshold in autumn or spring. Only fields 1 and 5 were unsprayed in 1984 and field 4 in 1985. It has therefore not been possible to assess the crop equivalents threshold in terms of reduced applications of herbicides or the build-up of weeds. There were some difficulties in applying the 5 crop equivalents/m^2 threshold. For example, in 1985/6, no herbicide would have been advised simply on the basis of autumn counts. In addition, there have been occasions when populations have declined over winter without herbicide application. This occurred in field 4 in 1984/85 and no herbicide was applied in spring. In field 6, there were marked declines in broad-leaved weeds over winter to below the threshold in 1984, 1985 and 1987 harvest years, though herbicides were applied.

DISCUSSION

The use of thresholds has been of widespread interest, as it offers the potential of more rational decision-making and hence pesticide use (Cussans et al. 1986). However, there are both practical and theoretical criticisms of current approaches (Cousens 1987). The most serious is that whilst initially developed from population and crop/weed competition data, most thresholds are set with a safety factor which is chosen arbitrarily. Often little consideration has been given to the economics of control and some thresholds may be impractical to implement.

Implementation of most thresholds depends on estimating weed populations on a field scale. In the present study, the sampling intensity of 6 points/ha was selected on the basis of what might be practically achieved. Detailed examination of up to six times this sampling intensity indicated that sampling error could be as great as 100%, depending on the species. The data indicated that for an abundant species, sampling at 6 points/ha gave a mean with a 30% error. For a species with a mean head density close to the threshold level of 2 heads/m^2, a sampling intensity of 18 points/ha would be required to produce means with a similar 30% error. At present, there is no information on how accurate weed density estimates need to be to satisfy particular thresholds, an subject confounded by the introduction of arbitrary safety factors. Investigations into the effects of changing decision thresholds on model populations are required, so that the accuracy of the field estimates of weed populations may be gauged. Computer studies have indicated that changes in the threshold for wild-oat have little impact on the economics of control (Pers. comm. R.D. Cousens). The implication may be that density estimates need not have high accuracy.

In practical terms a sampling intensity for field surveys three times that initially selected would be impossible. The 6 points/ha sampling intensity required approximately 15 min/ha so a 10 ha field could take 2.5 hours for one person. Such surveys were carried out over the 120ha area by between four and eight people; no farmer could expend such effort. It may only be possible for farmers and advisers to assess weed populations by crop inspection and not detailed quadrat counts. In the future, the advice following an independent crop inspection will be compared with that gained from quadrat counting, so the practicality and accuracy of thresholds may be further assessed.

In this experimental study at Boxworth, the use of spray decision thresholds has not led to major weed problems. Among the grasses, use of the thresholds for wild-oat and common couch resulted in fewer applications of herbicides compared with the prophylactic programme, without excessive weed increases in succeeding crops. Populations of blackgrass fluctuated markedly in the Integrated area, with large increases in years following rape when no herbicide was applied or when there was less effective chemical control. The high seed return of this species makes effective control operations essential (Moss 1980).

The use of the crop equivalents threshold for broad-leaved weeds was not adequately tested, as only three fields remained unsprayed for a whole season, perhaps indicating that the threshold erred on the side of safety. Nevertheless, several useful findings have been made. The threshold, based on autumn estimates of seedlings, was of little use in the 1986 harvest season when there was almost no autumn germination. This was followed by considerable spring germination which required control. Some weed species compete early in the life of the crop, while others compete later. There are difficulties in integrating mixed-species assemblages into a single measure, applied on one occasion, for control measures over an entire season. Assessments of weed populations are needed on several occasions.

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THE VALUE AND PRACTICALITY OF USING WEED THRESHOLDS IN THE FIELD

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ABSTRACT

In winter cereals, herbicide costs can account for more than 20% of total variable costs. With professional pride playing a significant role in the decision making process, it is probable that expenditure is well in excess of that justified on strict economic grounds. Field techniques designed to quantify the agronomic and economic effects of weed infestations would be beneficial.

It is argued that spray thresholds are of greatest value when deciding to apply a herbicide or not. They are less important when it comes to herbicide choice.

The time required for weed population assessments will probably preclude the widespread use of spray thresholds.

INTRODUCTION

In the current financial situation it is important that all inputs are assessed for cost effectiveness. Table 1 suggests that herbicides are responsible for a significant proportion of total variable costs when producing either winter wheat or barley. (i.e. an average of 19.2 - 21.6%). In terms of grain yield this would be equivalent to approximately 0.4-0.5t/ha.(i.e. 6% of gross output) With this level of expenditure it is essential that the whole question of weed control is looked at critically.

One function of an agronomist is to decide on the need for a herbicide spray. If a treatment is deemed necessary decisions on the choice of product, and whether a whole or part field application is required must be made. Some means of quantifying the decision making progress would lead to more objective recommendations.

WEED THRESHOLDS

Introduction

In order to introduce a quantitative element in weed control decisions the concept of thresholds has been put forward. For example, safe spray thresholds of 1 and 5 plants /m^2 have been put forward for spring wild oats (Avena fatua) and black-grass (Alopecurus myosuroides) respectively. (Cousens et al., 1985). For mixed broad-leaved weed populations the idea of "crop equivalents" has suggested (Wilson 1987). Here the population densities and competitive abilities of the various weed species present are integrated and expressed in terms of their effect on crop growth. A spray decision threshold of 5 crop equivalents /m^2 has been proposed.

Professional Pride

Although safety thresholds take account of factors such as yield loss through competition, future weed seed production and variable herbicide performance, they do not include an element for professional pride. Many farmers would find the thresholds mentioned above unacceptable because of crop appearance. A weed free crop is aesthetically pleasing.

Although the financial squeeze has led to a moderation in view whereby a few weeds will be tolerated, pride is still an important factor in the decision making process. In consequence herbicide expenditure in many cases could be in excess of that justified on strict economic grounds.

TABLE 1

Herbicide costs expressed as a percentage of gross output and total variable costs for 7 years (1979/80-1985/86); Winter Wheat and Winter Barley.

Season	Winter Wheat		Winter Barley	
	% gross output	% variable costs	% gross output	% variable costs
'79/80	6.6	24.0	6.4	21.6
'80/81	5.5	21.0	6.5	21.8
'81/82	7.2	27.9	6.0	25.5
'82/83	6.0	22.9	5.8	22.0
'83/84	5.6	22.5	5.3	20.7
'84/85	6.2	20.5	6.6	20.5
'85/86	5.0	18.9	5.5	19.1
Average	6.0	19.2	6.0	21.6

Average herbicide cost:

Wheat: £47.35/ha
Barley: £40.16/ha

Variable performance

Poor herbicide performance is not an uncommon problem. Moreover, practical difficulties can preclude a herbicide application. Such circumstances can be helped if the weed situation is "one spray failure away from disaster".

Safe spray thresholds (which are derived on the basis of competition and future weed seed buildup and then arbitrarily reduced to allow for a margin of safety) should take the variable performance risk into account. However, with the degree of unreliability associated with soil applied herbicides, there could be justification in looking at the risk element in spray thresholds more critically. This is especially the case when dealing with a prolific weed species such as black-grass. Concern in this area would encourage many advisors to recommend treatment below the safe black-grass threshold of 5 plants /m^2 mentioned earlier.

Seed Crops

In general, safe spray thresholds are sufficiently low to prevent major problems with harvesting, drying and contamination. (Cousens et al. 1985). Cereal seed crops where weed seed contamination can cause rejection could be an exception. Black-grass in a herbage seed crop can also

cause similar problems.

Wild Oats

Where the eventual aim is to reduce a population of wild oats (Avena spp.) down to roguable levels, rather than to adopt a policy of containment, a spray threshold approach would be inappropriate in the early stages as seed return should be avoided. Theoretically it should be possible to use thresholds in the latter stages to decide whether the population has dropped to a level which is roguable. However, in practice, at very low population levels, it can be difficult to detect the weed during the herbicide application window, and yet there can still be too many to rogue. One solution to the problem is to leave untreated strips across the field. This will give an indication of the problem without putting too much pressure on the roguers.

Weeds that are difficult to control

Some weeds are difficult to control selectively and in consequence farmers are prepared to attack very low infestations to prevent seeding and establishment. Examples include barren brome (Bromus sterilis) and soft brome (Bromus mollis) in cereals. Non selective herbicides are commonly used on small patchy infestations. In such circumstances spray thresholds would be of limited value.

Rotational considerations

Rotational weed control is often practiced; eg. cleavers (Galium aparine) and common poppy (Papaver rhoeas) before oil-seed rape or blackgrass before herbage seed. In such circumstances predictive thresholds would be needed. A threshold for cleavers in the crop prior to rape will be lower than prior to another cereal. The fact that decisions can be taken two or three years before the crop in question will lead to complications. Thresholds would have to be sophisticated to take this into account.

THE USE OF THRESHOLDS IN THE FIELD

Assessments in previous crop

In spite of some residual herbicides having a wide application window (eg. isoproturon) there is merit in applying the products either pre- or early post-emergence of the crop as they tend to be more effective on germinating seeds or small seedlings. Early autumn treatments also allow the farmer to fully utilise the number of spraying periods available (Spackman 1983). A programmed approach will also lead to improvements in labour efficiency. However, early treatment means that spray decisions have to be taken before the full emergence of the weed populations. This is a common problem with annual grass weeds. Decisions are based on assessments made either before harvest in the previous crop or on past history. For pre-harvest measurements, a predictive spray threshold would be appropriate. It has been suggested that such thresholds could be developed for species which have a moderate degree of dormancy and a comparatively short seed life. (Cussans et al. 1986) Obviously a predictive scheme would have to take account of the dynamic interaction between straw disposal, cultivation and weather on seed survival and germination.

To minimise costs, the integration of sequential herbicides is essential. Such integration often has to be planned prior to the anticipated weed problems becoming apparent. Again, if available, predictive thresholds could be of value

Autumn/winter/early spring assessments

Having applied an autumn herbicide programme, the farmer or advisor can record the surviving weed species and population densities over the winter months. So called, safe spray thresholds could be utilised if desired especially on surviving broad-leaved weeds. The recent introduction of broad-leaved weed herbicides which can be applied to cereals up until full flag leaf emergence (eg. fluroxpyr and metsulfuron methyl) and the extension of the application window for some wild oat herbicides has prolonged the period in which weed populations can be assessed.

Pre-harvest assessments

The pre-harvest control of common couch (Elymus repens) using glyphosate provides a good opportunity for plant density assessments as the weed emerges above the crop several weeks before treatment.

Weed population assessments

The major problem when using spray thresholds is that quantitive plant density measurements have to be made. The patchy distribution of weed problems renders meaningful assessments both time consuming and labour intensive. A suggested whole farm survey technique for the spring wild oat (A. fatua) was estimated to take 0.5 man hours/ha. (Wilson 1981) On average an advisor in the Association of Independent Crop Consultants (AICC) is responsible for an area of 3,450 ha (Campbell 1987, personal communication). On the assumption that 40 hours a week are spent in the field making assessments, a hypothetical calculation would suggest that the advisor would complete his work in 43 weeks. Weed assessments on 3 dates in the "Boxworth project" have taken in the region of 45 minutes/ha. (Marshall 1987) Using the same hypothetical basis as before the average AICC member would take 65 weeks to complete the work. Clearly such a work load is impractical and as such could prevent people from using spray thresholds.

Current situation

At present, when looking at a potential weed problem, subjective assessments are made of the population density and area of field infested. A knowledge of the relative competitive abilities of the various weed species (even when spray thresholds are not being used) is of value when judging an overall weed problem. Greater emphasis can be placed on the more competitive species.

CHOICE OF HERBICIDE

The major factors influencing herbicide selection are the crop being grown, weed species present and cost. Weed density and weed size, edaphic and climatic conditions, product compatibility and sequential restrictions and application window will also influence the decision. Moreover, product changes within and between fields should be minimized in order to maintain sprayer output and minimize errors.

It can be seen that weed density is only one of many factors which are taken into account. Moreover to select a herbicide on grounds of cost and efficacy using spray thresholds would require a degree of precision and sensitivity which does not as yet exist. In consequence it is suggested that spray thresholds are of greater value in deciding whether to treat or not, rather than in choosing a herbicide.

SUMMARY

With the increase in financial pressure on British agriculture, there

is a greater need to look at herbicide usage objectively. Quantitative field assessment techniques will help in this respect.

Current attitudes in respect to crop visual appearance and pride will have to change before herbicide applications are based purely on economic grounds. Such changes have been detected over the last two or three seasons, but there is still some way to go.

Spray thresholds must be related to the circumstances in which they are being used. For example, a predictive threshold could be influenced by the rotation or the use to which a subsequent crop will be put. (eg. seed production) Lower threshold values would be more appropriate where the risk of herbicide failure is high. Such flexibility and sophistication would enhance the value of the spray threshold concept.

The major problem with spray thresholds is the time taken to assess the weed population. This above all could restrict the use of thresholds. The logistics of taking on additional competent staff on a seasonal basis, providing the necessary training and transport etc. would make this avenue difficult to follow.

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SESSION 10B

MODE OF ACTION AND METABOLISM OF HERBICIDES: III (continued)

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BEHAVIOUR OF GLUFOSINATE-AMMONIUM IN WEEDS

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ABSTRACT

Glufosinate-ammonium, under the commercial name Basta^{R}, is a non-selective herbicide for post-emergence weed control. Translocation and metabolic pathways were examined in a number of plant species using ^{14}C-labelled active ingredient.

Three days after application, the radioactive substance exported from the treated leaves and detected in the untreated parts of the plants amounted to 2.0 % in Calopogonium mucunoides, 2.2 % in Lathyrus tuberosus, 2.5 % in Convolvulus arvensis, 3.3 % in Trifolium subterraneum, and 4.3 % in Sorghum halepense.

In the three investigated plant species, S. halepense, T. subterraneum and C. arvensis, the ^{14}C-labelled active ingredient was metabolised by formation of non-phytotoxic 3-methyl-phosphinicopropionic acid, and of three other substances, which occurred only in very small quantities. Unchanged active ingredient translocated to the roots amounted to 6.6 % in S. halepense, 3.3 % in T. subterraneum, and only 1.2 % in C. arvensis.

The differences in specific sensitivity could be accounted for by the varying concentrations of unchanged active ingredient transported into the roots. The intensity of basipetal transport also played an important part.

The sensitivity of the investigated plant species to glufosinate-ammonium increased in the following order: C. mucunoides, L. tuberosus, C. arvensis, T. subterraneum and S. halepense.

INTRODUCTION

Glufosinate-ammonium (HOE 039866) is an organophosphorus compound. The free amino acid on which it is based is referred to in the literature as phosphinothricin and is a metabolic product of Streptomycetes viridochromogenes (Gugel 1971, Bayer et al. 1972).

Glufosinate-ammonium is a contact herbicide with a particular systemic mode of action. It has a non-selective action on a wide spectrum of annual and perennial weeds and grasses when applied post-emergence at a rate of 0.3 - 1.5 kg active ingredient/ha. The substance has a rapid effect, causing the plants to wither and die after as little as 2 - 5 days, depending on environmental factors (Hoechst AG 1982).

The substance is degraded within the plant to 3-methyl-phosphinicopropionic acid, which has no further phytotoxic effect (Götz et al. 1983).

Studies with ^{14}C-labelled glufosinate-ammonium after foliar treatment show marked translocation in the leaf, extending from base to tip. Transport from the treated leaf to the other parts of the plant takes place only to a very limited extent (Hoechst AG 1982).

MATERIALS AND METHODS

Plant material

The studies were conducted with the species Sorghum halepense (L.), Lathyrus tuberosus (L.), Convolvulus arvensis (L.), Trifolium subterraneum (L.) and Calopogonium mucunoides (L.). Except for C. mucunoides, all of the test plants were cultivated in greenhouses (17 - 22 °C, 50 - 70 % relative humidity, 15 hours daylight, with additional lighting when required). C. mucunoides was cultivated in the greenhouse of the Institute under tropical conditions (20 - 25 °C, 12 hours additional lighting, 60 - 70 % humidity). As soon as the cotyledons had formed, the seedlings were transferred into water jars containing nutrient solution at half ionic concentration; 3 days later they were further cultivated in a nutrient solution at full ionic concentration.
(Composition of nutrient solution per 1000 ml: 1 330 mg $Ca(No_3)_2 \times 4\ H_2O$; 200 mg KNO_3; 330 mg KH_2PO_4; 170 mg $MgSO_4 \times 7\ H_2O$; 20.00 mg $MnCl_2 \times 4\ H_2O$; 29.00 mg H_3BO_3; 50.00 mg Fe-EDTA (SEQUESTREN 138 Fe); 0.22 mg $ZnSO_4 \times 7\ H_2O$;; 0.08 mg $CuSO_4 \times 5\ H_2O$; 0.03 mg $Na_2MoO_4 \times 2\ H_2O$.)

Radioactive substance

The trials were conducted with ^{14}C-glufosinate-ammonium (Hoe 039866 - ammonium-DL-homoalanin-4-yl-(methyl)-phosphinate-(1,2-^{14}C)) manufactured by Hoechst AG, Frankfurt am Main (spec. activity 24.36 /uCi/mg, 4.87 mCi/mM, 901 mBq/mg)

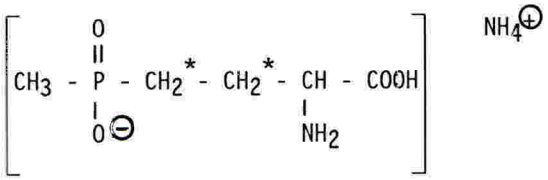

Fig. 1. Structural formula of glufosinate-ammonium

Application

In the translocation studies, foliar treatment of each plant was conducted with 0.5 /uCi in 10 /ul aqueous solution. With reference to active substance content, this amounted to a 0.2 % solution of glufosinate-ammonium. In the metabolism studies with S. halepense, C. arvensis and T. subterraneum, the application rate per plant was 1 /uCi in 20 /ul aqueous solution. The weeds were treated as follows: S. halepense (4 - 5 leaf stage), lower third of second leaf; T. subterraneum (2 - 3 leaf-triplet stage) and C. arvensis (5 -6 leaf stage), first foliage leaf; L. tuberosus (4 -5 leaf stage), second pair of foliage leaves; and C. muconoides (1 - 2 leaf-triplet stage), middle leaf of first leaf-triplet.

Exposure period

The exposure time was 3 days.

Harvesting and processing of test material

For the translocation studies, the plants were placed after harvesting in a climatic cabinet with circulating air at 95 °C and allowed to dry for 2 hours. The treated leaf in each case had been separated from the leaf-stem. After the dry weight had been determined, the plant samples were

combusted in a Packard TRI-CARB 306; the radioactivity was measured with a Packard TRI-CARB 3380 liquid scintillation spectrometer (Packard, Downers Grove, Ill., U.S.A.). For the metabolism studies, the plants were separated into shoots and roots, and the green weights of each were determined. The plant material was then briefly rinsed with methanol and distilled water.

Extraction

The homogenised plant material was first extracted three times with ethyl acetate, after which the same process was repeated with methanol, methanol/water (1 : 1), and distilled water. The centrifugates obtained by each extraction medium were evaporated to dryness and finally dissolved in the same medium.

Thin layer chromatography (TLC)

The extracts were separated in a mixture of 1-pentanol, formic acid and water (48.8/48.8/2.4; v/v/v), on TLC plates manufactured by Merck, Darmstadt, FRG (silica gel 60 F_{254}, thickness of layer 0.25 mm). The TLC plates were evaluated with an LB 2821 TLC LINEAR ANALYSER (manufactured by Berthold, Wildbad, FRG).

Reference substances for identification

Glufosinate-ammonium (Hoe 039866) and 3-methyl-phosphinico propionic acid (Hoe 061517) were identified with ^{14}C-labelled reference substances provided by Hoechst AG.

RESULTS AND DISCUSSION

Translocation

S. halepense, T. subterraneum, C. arvensis, L. tuberosus and C. mucunoides were investigated in order to establish how much radioactive substance is translocated from the treated leaf to the other parts of the shoots and to the root system. Three days after application, the radioactive substance exported from the treated leaves amounted to 2.0 % in Calopogonium mucunoides, 2.2 % in Lathyrus tuberosus, 2.5 % in Convolvulus arvensis, 3.3 % in Trifolium subterraneum, and 4.3 % in Sorghum halepense (Table 1).

Taking the total radioactive substance exported from the leaf as 100 %, the major portion of this, i.e. 64.1 % to 88.7 %, was in the remaining parts of the shoot, except for S. halepense, where it was only 48.1 %. Thus ^{14}C-glufosinate-ammonium applied via the leaf is easily taken up by all 5 plant species. However, marginal exportation from the treated leaf and basipetal translocation down to the root system also took place. Transportation was via the phloem. Thus the translocation of glufosinate-ammonium in plants is partly symplasmatic and partly apoplasmatic. Many other herbicides, e.g. amitrol, are also transported in this way (Crafts and Yamaguchi 1964; Müller 1976). However, transportation via the phloem represents only a minor part of total translocation, a fact which accounts for the limited transportation to the subterranean parts of the plants. In the case of the easily translocatable glyphosate, as much as 33 % of the radioactivity applied e.g. to S. halepense had been transported to other parts of the plant after 6 days (Lolas and Coble 1980). But 3 days after application of glyphosate, only 3.5 % of the applied radioactive substance had been translocated to the untreated parts of C. arvensis (Sandberg et al. 1980).

Taking into account the different plant sizes, S. halepense and T. subterraneum showed the highest concentrations (1 200 and 1 160 dpm/mg dry weight respectively). C. arvensis and L. tuberosus, with 590 and 500 dpm/mg respectively, occupied an intermediate position (Table 1). Based on the

different concentrations of radioactive substance in the roots, it is reasonable to assume that the sensitivity of the studied plant species increased in the following order: C. mucunoides, L. tuberosus, C. arvense, T. subterraneum and S. halepense.

TABLE 1

Translocation of radioactive substance in S. halepense, T. subterraneum, C. arvensis, L. tuberosus and C. mucunoides after foliar treatment with ^{14}C-glufosinate-ammonium. Applied radioactive substance: 0.5 µCi/plant. Exposure time 3 days. Mean values from 6 replicates. Readings: dpm and %.

Plant species	^{14}C-substance exported from treated leaf (dpm)	(%)	^{14}C-substance in shoots (dpm)	roots (dpm)	^{14}C-substance in shoots (dpm/mg dry wt.)	roots (dpm/mg dry wt.)
S. halepense	38 160	4.3	18 360	19 800	690	1 200
	100 %		48.1 %	51.9 %		
T. subterran.	36 220	3.3	24 580	11 640	530	1 160
	100 %		67.9 %	32.1 %		
C. arvensis	26 650	2.5	17 090	9 560	310	590
	100 %		64.1 %	35.9 %		
L. tuberosus	23 190	2.2	14 880	8 310	680	500
	100 %		64.2 %	35.8 %		
C. mucunoides	16 760	2.0	14 870	1 890	380	280
	100 %		88.7 %	11.3 %		

Metabolism

The metabolism of ^{14}C-glufosinate-ammonium was studied in the three plant species S. halepense, T. subterraneum and C. arvensis. The principal interest here was to determine how much unchanged substance reached the roots by basipetal transportation. For this purpose, the plant material after foliar treatment and 3 days exposure time was divided into roots and shoots and extracted with various solvents, ranging from hydrophobic to highly hydrophilic. The results are set out in Table 2.

Table 2 clearly shows a correlation between the total radioactivity measured in dpm/g fresh weight in the shoots and the sensitivity of the three plant species to glufosinate-ammonium. Thus the sensitive species S. halepense, with 4 732 550 dpm/g fresh weight, showed the highest concentration, compared with only 4 162 660 dpm/g fresh weight in the relatively tolerant C. arvensis. T. subterraneum occupied an intermediate position with 4 554 700 dpm/g fresh weight. As in the shoots, the total concentration in the roots also showed a correlation with plant susceptibility to the substance. Thus the highest concentration was 409 830 dpm/g fresh weight in S. halepense, and the lowest 114 840 dpm/g fresh weight in C. arvensis. T. subterraneum was in between, with 192 690 dpm/g fresh weight.

Most of the radioactive substance was extractable from the various parts of the plant with ethyl acetate, methanol, methanol/water, and distilled water. The insoluble portion of the total radioactivity was only 1.3 - 2.7 % in the shoots, and 0.6 - 3.5 % in the roots.

TABLE 2

Extractability of radioactive substances from shoots and roots of S. halepense, T. subterraneum and C. arvensis after foliar treatment with ^{14}C-glufosinate-ammonium (extraction with ethyl acetate, methanol, methanol/water, and distilled water. Exposure time: 3 days. Applied radioactive substance: 30 /uCi. Readings: dpm/g fresh weight and %.

	^{14}C-labelled substances in extracts from shoots					
Plant	Total radio-activity (dpm)	Ethyl acetate (dpm)	Methanol (dpm)	Methanol/ water (dpm)	distilled water (dpm)	non-extractable residues (dpm)
S. halepense (4-5 leaf stage)	4 732 550 100 %	47 330 1.0 %	4 273 490 90.3 %	298 150 6.3 %	52 060 1.1 %	61 520 1.3 %
T. subterraneum (2-3 leaf stage)	4 554 700 100 %	22 770 0.5 %	3 803 180 83.5 %	573 890 12.6 %	31 880 0.7 %	122 980 2.7 %
C. arvensis (5-6 leaf stage)	4 162 660 100 %	37 460 0.9 %	3 700 610 88.9 %	283 060 6.8 %	41 630 1.0 %	99 900 2.4 %
	^{14}C-labelled substances in extracts from roots					
Plant	Total radio-activity (dpm)	Ethyl acetate (dpm)	Methanol (dpm)	Methanol/ water (dpm)	distilled water (dpm)	non-extractable residues (dpm)
S. halepense (4-5 leaf stage)	409 830 100 %	20 900 5.1 %	316 390 77.2 %	55 740 13.6 %	14 340 3.5 %	2 460 0.6 %
T. subterraneum (2-3 leaf stage)	192 690 100 %	12 900 6.7 %	150 520 78.1 %	18 290 9.5 %	4 240 2.2 %	6 740 3.5 %
C. arvensis (5-6 leaf stage)	114 840 100 %	7 810 6.8 %	91 070 79.3 %	9 760 8.5 %	2 640 2.3 %	3 560 3.1 %

Table 3 shows the quantities of radioactivity in the separate extracts and the quantities of radioactive substances in the TLC extracts obtained with methanol and methanol/water from shoots and roots.

The metabolisation of glufosinate-ammonium in the shoots proceeded fairly slowly in all three species. The quantities of glufosinate-ammonium in the shoot material after 3 days exposure were 84.1 % in S. halepense, 82.4 % in T. subterraneum, and 81.8 % in C. arvensis. The portions of unchanged active ingredient were rather lower in the roots. A possible reason for this was that metabolites are more readily transported from the shoot to the roots. As compared with this, approximately the same amount of the systemic herbicide active ingredient glyphosate (82 %) was present after 3 days in weeds such as Convolvulus arvensis, Convolvulus sepium, Cirsium arvensis and Polygonum amphibium (Sandberg et al. 1980).

In the parts of the various plant species investigated in this study, glufosinate-ammonium is metabolised to 3-methyl-phosphinico-propionic acid (Rf value 0.34) and to a very minor extent to other compounds, ranging from hydrophilic to fairly hydrophobic, with Rf values of 0.03, 0.55 and 0.77 (Table 3). According to Götz et al., glufosinate-ammonium is degraded in the plant to 3-methyl-phosphinico-propionic acid, which has no phytotoxic effect.

It becomes possible to form an exact picture of the distribution of radioactive substance in the treated plants only if the various compounds extracted from shoots and roots separately are considered together with the degree of their extractability in the different solvents, based on the data given in Table 3. It emerges that the greatest portion of unchanged active ingredient reaching the roots by basipetal translocation was 6.6 % in the most susceptible species, S. halepense. The portion was 3.3 % glufosinate-ammonium in the less susceptible T. subterraneum, and only 1.2 % in the moderately susceptible C. arvensis.

It is probable that the different sensitivity of the three plant species is due not only to the varying intensity of basipetal transportation, but also to these active ingredient levels.

Additional HPLC separations of the plant extracts confirmed the TLC findings concerning the sensitivity of the three weed species.

TABLE 3

Quantities of radioactive substances after application of ^{14}C-glufosinate-ammonium to shoots of S. halepense, T. subterraneum and C. arvensis; TLC-separation of shoot and root extracts after a 3-day exposure time. Readings: dpm/g fresh weight and %. Solvent system: 1-pentanol/formic acid/water (48.8/48.8/2.4; v/v/v/)

Substance	S. halepense shoots (dpm)	S. halepense roots (dpm)	T. subterraneum shoots (dpm)	T. subterraneum roots (dpm)	C. arvensis shoots (dpm)	C. arvensis roots (dpm)
Ethyl acetate extract *	47 330 1.0 %	20 900 5.1 %	22 770 0.5 %	12 900 6.7 %	37 460 0.9 %	7 810 6.8 %
Methanol and methanol/water extracts:						
Radioactivity at origin (Rf value 0.03)	17 590 0.4 %	-	-	-	7 080 0.2 %	-
Glufosinate-ammonium (Rf value 0.19)	3 981 500 84.1 %	338 270 82.5 %	3 751 310 82.4 %	155 470 80.7 %	3 404 970 81.8 %	91 100 79.3 %
3-methyl-phosphinico-propionic acid (Rf value 0.34)	559 730 11.8 %	31 900 7.8 %	625 770 13.7 %	13 340 6.9 %	565 120 13.6 %	8 240 7.2 %
Substance 4 (Rf value 0.55)	-	-	-	-	6 510 0.2 %	950 0.8 %
Substance 5 (Rf value 0.77)	12 820 0.3 %	1 950 0.5 %	-	-	-	540 0.5 %
Water extract *	52 060 1.1 %	14 340 3.5 %	31 880 0.7 %	4 240 2.2 %	41 630 1.0 %	2 640 2.3 %
Non-extractable residue	61 520 1.3 %	2 460 0.6 %	122 980 2.7 %	6 740 3.5 %	99 900 2.4 %	3 560 3.1 %
Total radio-activity	4 732 550 100 %	409 820 100 %	4 554 710 100 %	192 690 100 %	4 162 670 100 %	114 840 100 %

* could not be detected, due to the lower amount of radioactivity

ACKNOWLEDGEMENTS

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MODE OF CROP TOLERANCE TO PYRIDATE IN CORN AND PEANUTS

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ABSTRACT

Pyridate, O-(6-chloro-3-phenyl-4-pyridazinyl)-S-octyl-carbonothioate, (formerly coded CL 11344), is a new herbicide from the chemical group of the phenylpyridazines developed by Chemie Linz /Austria for post-emergence control of weeds.
Pyridate undergoes rapid hydrolysis after penetration into the plant yielding 6-chloro-3-phenyl-pyridazine-4-ol (coded CL 9673) which has been established as the herbicidal active principle by inhibiting photosynthetic electron transport of photosystem II. Crop tolerance in corn and peanuts is based on metabolic inactivation of CL 9673. Metabolic conversion into conjugates of N- and O-glucosidic types are found to be the first detoxification steps. Formation of N-conjugates was discussed to be a key-reaction for subsequent substitution of chlorine by compounds with sulfhydryl groups. No interference of CL 9673-conjugates of corn and peanuts with the Hill reaction using isolated broken spinach chloroplasts was found indicating that inactivation by conjugation is the basis for selectivity.

INTRODUCTION

Pyridate, O-(6-chloro-3-phenyl-4-pyridazinyl)-S-octyl-carbonothioate, (formerly coded CL 11344), is a new herbicide from the chemical group of the phenylpyridazines developed by Chemie Linz, Austria, for post-emergence control of weeds in corn, peanuts, wheat, alfalfa and cole crops and is marketed under the registered trade names 'LENTAGRAN' and 'TOUGH' (Figure 1).

Figure 1. Structural formula

Herbicidal properties of pyridate were first described by Diskus et al., 1976. Pyridate penetrates easily into the plant tissue due to the lipophilic property of the ester component. After penetration pyridate undergoes rapid hydrolysis yielding 6-chloro-3-phenyl-pyridazine-4-ol (coded CL 9673) which has been established as the herbicidally active principle. Pyridate was shown to be extremely safe for corn and peanuts when used in postemergence weed control. This report outlines the mode of action of pyridate and the mechanism of metabolic detoxification of CL 9673 in corn and peanuts.

MATERIALS AND METHODS

Photosynthesis measurements

The time course of photosynthetic rates of pyridate treated leaves of corn and peanuts (tolerant plants) and of cleavers (Galium aparine, sensitive plant) was measured over a period of four days. Fully developed leaves were treated at intervals of 4, 2, and 1 days and 8, 6, 4, 2, 1 and 0,5 hours by brushing 0,25 % a.i. aqueous pyridate suspension (formulated as wettable powder) on the upper leaf surface. Treated plants were kept under the normal day-night rhythm in a green house.

The photosynthetic rates were evaluated on excised entire leaves of peanuts and cleavers or leaf discs (corn, area approximately 5 cm^2) measuring the uptake of gaseous ^{14}C-carbon dioxide for 30 minutes in a plexiglass box (volume 1 L) under light from fluorescent tubes (distance 20 cm). ^{14}C-radiolabelled carbon dioxide was liberated from 10 μCi sodium-(^{14}C)-bicarbonate solution (specific activity 0,1 mCi/mM) with dilute sulphuric acid. Fixation of ^{14}C-carbon dioxide was terminated by aeration and by shock heating of the leaf discs at 80 °C. Leaves were dried and oxidized in a sample oxidizer (mod. TRI CARB 306, Packard Instr., U.S.A.) . Radioactivity was trapped in CARBOSORB (Packard Instr., U.S.A.) and measured in a PERMAFLUOR V cocktail (Packard Instr., U.S.A.) in a liquid scintillation counter (mod. TRI CARB 300 C, Packard Instr., U.S.A.).

Metabolic conversion of ^{14}C-pyridate in leaves of corn and peanuts.

^{14}C-pyridate (^{14}C-labelled in the 4,5-position of the pyridazine ring) was synthesized by A. Zohner, Chemie Linz with a spec. activity of 5,07 mCi/mM and a radiochemical purity $\geq$ 98 % determined by thin layer chromatography.

Corn (5-leaf stage) and peanuts plants (12 cm diameter) were treated with ^{14}C-pyridate by spraying an aqueous suspension (0,25 % a.i.) of a wettable powder formulation (application rate corresponding to approximately 1,8 kg a.i. /ha). Leaves samples were taken dayly and extracted with cold acetone and acetone/water. Extracts were chromatographed without further

cleaning by thin layer chromatography (tlc) on silicagel 60 plates with a fluorescence indicator in the solvents acetone/water (9:1). The radioactive spots were located and evaluated with a linear analyzer (mod. LB 2842, Ludwig Berthold, FRG). Radioactive spots on the tlc-plates were identified by co-chromatography of standards.

Measurements of photosynthetic electron flow

Metabolites of ^{14}C-pyridate were tested for their ability to inhibit the Hill reaction of isolated broken spinach chloroplasts (thylakoids). The test followed a procedure described by Kovac et al. 1976 and Zohner et al.,1977, using a spray technique with a Hill reagent on freshly developed thin layer chromatograms.

Preparation of the spraying solution:

Chloroplasts were isolated from spinach leaves according a normal procedure (Jacobi et al., 1974). Broken chloroplasts were resuspended in a 10 % glycerol solution at a chlorophyll concentration of about 30 mg/ml (Solution 1). This suspension was prepared and stored at a temperature between 0 - 4 °C.

The Hill reagent solution (Solution 2) was prepared by dissolving 40 mg of 2,6-dichlorophenol indophenol (DPIP, Sigma, FRG) in 100 ml of a 66 mM buffer solution of sodium potassium phosphate of pH 6,5.

Procedure for the Hill reaction test:

Acetone extracts of ^{14}C-pyridate treated leaves were chromatographed on silicagel 60 thin layer plates 20 x 20 cm in the solvents chloroform/methanol (6:4) and dried at room temperature. To avoid unspecific interferences with the Hill reagent, the plates were pre-developed in the same solvents and dried before use. The plates were then sprayed with a mixture of chloroplast homogenate (1 volume of Solution 1) and DPIP solution (2 volumes of Solution 2), covered with a glass plate and exposed to the light of fluorescent tubes at a distance of 20 cm until the blue background color turns to yellow-green due to the photoreduction of DPIP by the chloroplasts (= Hill reaction).

Compounds which inhibit the photosynthetic electron flow were visualized by the blue colour remaining on the sprayed plate after exposure to light.

Pyridate and CL 9673-conjugates could be also visualized by the Hill reaction test if the developed plates were heated at 150 °C for 30 minutes. By this procedure, pyridate and conjugates of CL 9673 were decomposed partly to free CL 9673 which inhibited the Hill reaction.

RESULTS AND DISCUSSION

The photosynthetic rate of pyridate treated leaves of peanuts dropped to approx. 80 % within 3 hours and recovered fully within 8 hours. In treated leaves of corn the rate dropped below 5 %, recovered slowly and reached approx. 80 % after 4 days indicating a less powerfull detoxification capacity than that found in peanuts. The photosynthesis of treated leaves of cleavers was inhibited totally (more than 98%) and irreversbly (Figure 2).

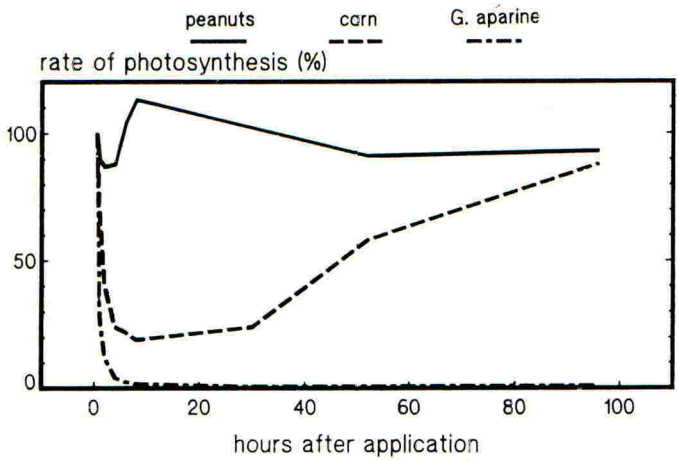


Figure 2. Time course of the photosynthetic rates of leaves of peanuts, corn and cleavers (Galium aparine) after treatment with pyridate.

Time course of metabolic conversion of ^{14}C-pyridate in leaves of peanuts and corn

Fast hydrolization of ^{14}C-pyridate to ^{14}C-CL 9673 was the first metabolic step. Low intermediate concentrations of free ^{14}C-CL 9673 were found in both, peanuts and corn. From the photosynthetic measurements discussed above, it could be derived that concentrations of free CL 9673 in the leaf tissue were too low to inhibit photosynthesis. In leaves of peanuts C-CL 9673 was detoxified mainly to ^{14}C-CL 9673-N-glycoside (Figure 3). In leaves of corn only small amounts of C-CL 9673-N-glycoside and ^{14}C-CL 9673-O-glycoside but higher amounts of more polar ^{14}C-metabolites were detectable at lower Rf-values (Figure 4). Hydrolization of these more polar metabolites did not yield free ^{14}C-CL 9673.

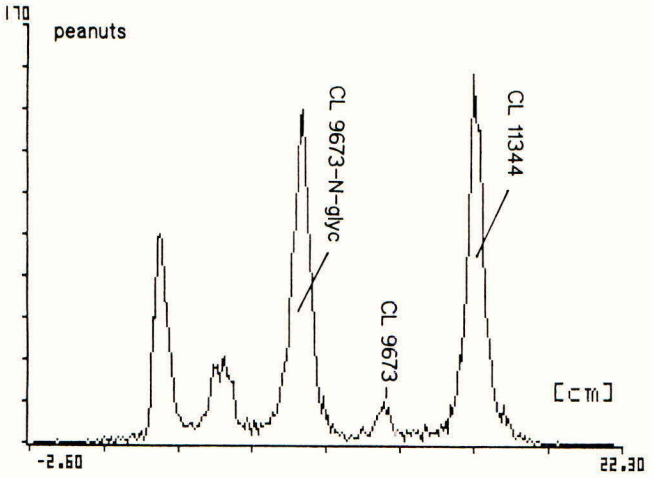


Figure 3. Metabolization of ^{14}C-pyridate in peanuts (3 days after application; tlc-scanning, silicagel; solvents: chloroform/methanol, 10 : 4)

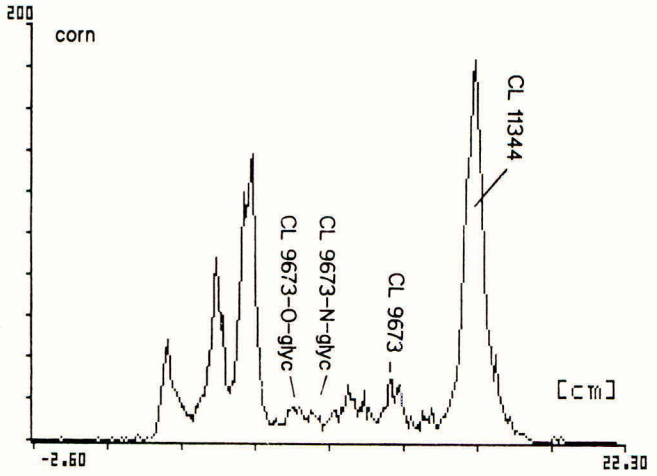
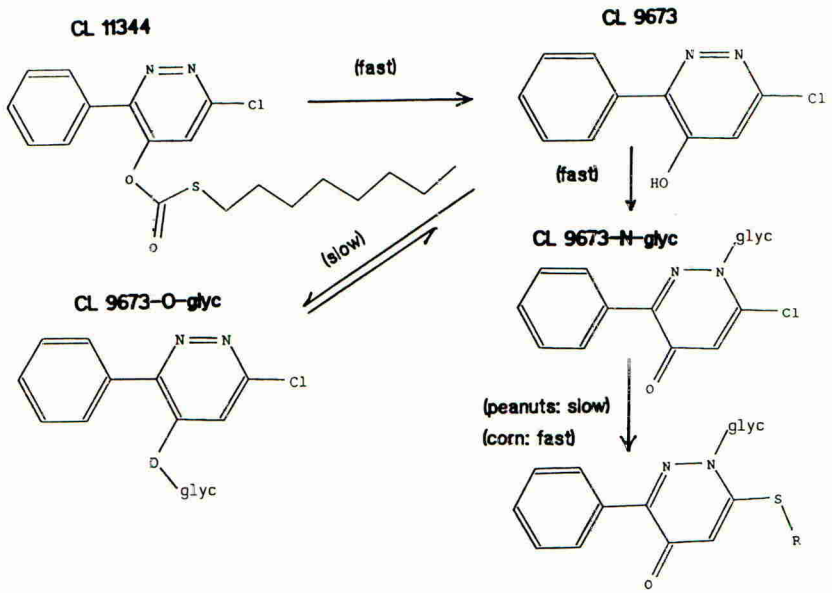


Figure 4. Metabolization of ^{14}C-pyridate in corn (6 days after application; tlc-scanning, silicagel; solvents: chloroform/methanol, 10 : 4)

Investigations of the detoxification mechanism of atrazine in corn leaves have shown that the key step for detoxification

is a conjugation reaction with glutathione by substitution of the chlorine (Lamoureux, G.L. et al.; 1972). It was found by chemical synthesis that CL 9673-N-glucoside reacted readily with SH-compounds like glutathione or cysteine by substitution of the chlorine whereas free CL 9673 and CL 9673-O-glucoside showed no reaction (unpublished). N-glycosylation of CL 9673 appears to be a necessary metabolic step which activates the pyridazine ring for conjugation reactions by substitution of chlorine. Assuming a same reactivity in vivo, the following metabolic pathway for detoxification of pyridate in corn and peanuts is proposed (Figure 5):



Hydrolysis and formation of CL 9673-N-glucoside are the common first metabolic steps. Formation of CL 9673-O-glycoside is not relevant for detoxification in corn and peanuts. CL 9673-N-glycoside is metabolized then to highly polar conjugates, but with low rates for peanuts and high rates for corn. The low intermediate concentrations of CL 9673-N-glycoside in corn could be explained by a fast conjugation with glutathione or other SH-compounds by substitution of the chlorine.

Interference of pyridate metabolites with the Hill reaction.
The phytotoxicity of the main metabolites of pyridate was investigated on broken spinach chloroplasts by studying the Hill reaction on thin layer chromatograms of leaf extracts of peanuts and corn (Figure 6).

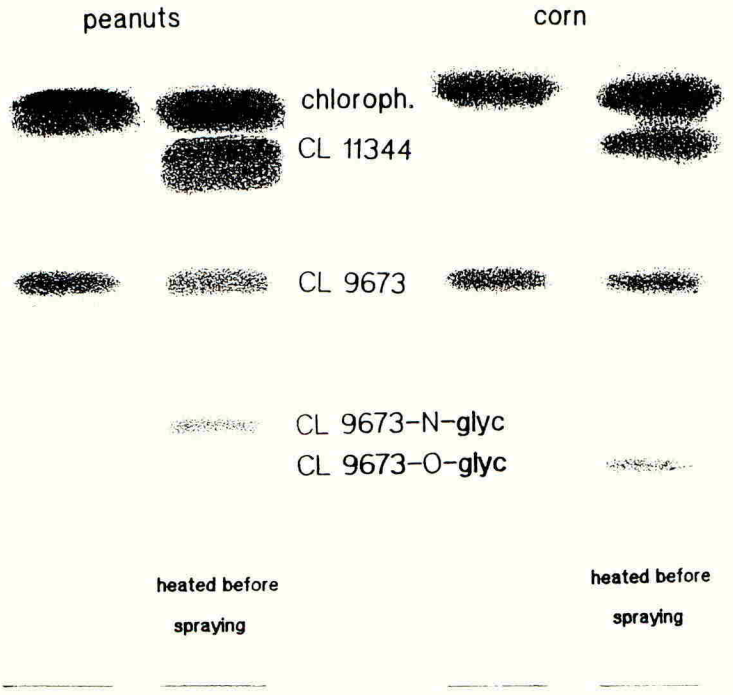


Figure 6. Interference of pyridate metabolites of peanuts (3 days after treatment) and corn (6 days after treatment) with the Hill reaction on spinach chloroplasts (visible spots indicate inhibition of the Hill reaction; tlc: silicagel; solvent: chloroform/methanol, 10:4)

CL 9673, was the only compound which inhibited the photosyntetic electron flow of the chloroplasts (the spot with the highest Rf-value belongs to extracted chlorophyll). Pyridate by itself and the metabolites of CL 9673 showed no inhibition of the Hill reaction. The nature of the CL 9673-metabolites were investigated by studying their interference with the Hill reaction after cleavage on heated chromatograms. Additionally to

CL 9673 also pyridate and CL 9673-N-glucoside became visible in peanuts. Pyridate and even the small amounts of CL 9673-O-glycoside became visible in corn by this method (CL 9673-N-glycoside decomposed not as compleately as CL 9673-O-glycoside and was not detectable by the Hill reaction at low concentrations after heating). The polar metabolites with Rf-values below that of CL-9673-N-glucoside showed no inhibition of the Hill reaction after heating, indicating that these metabolites could not be decomposed to CL 9673.

CONCLUSION

Due to the lipophilic property of the ester component, treated leaves absorb pyridate readily. In the plant pyridate hydrolyzes quickly to a phenolic compound (CL 9673) which has been established as the herbicidally active metabolite. The mode of action of CL 9673 is based on the inhibition of the photosynthetic electron transport. Pyridate tolerant plants such as peanuts and corn detoxify CL 9673 into conjugates. In peanuts a one-step detoxification forming CL 9673-N-glycoside was found. In corn a two-step mechanism of further conjugation of CL 9673-N-glycoside with glutathione is proposed. Formation of CL 9673-N-glycoside was found to be an essential metabolic step for substitution of chlorine by endogenous SH-compounds.

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THE MODE OF ACTION OF THE HERBICIDAL QUINOLINECARBOXYLIC ACID, QUINMERAC[1] (BAS 518 H)

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ABSTRACT

Quinmerac (BAS 518 H, 7-chloro-3-methyl-quinoline-8-carboxylic acid) is a new experimental herbicide for the selective control of cleavers (Galium aparine) in wheat and other crops. Several test systems for the detection of auxin activity have demonstrated the hormone-type character of the quinoline-carboxylic acids. Studies with ^{14}C labelled BAS 518 H revealed that quinmerac is taken up by leaves and roots. It is translocated acro- and basipetally. Further studies demonstrated significant differences between the root growth of wheat and cleavers when these were exposed to quinmerac. These results led to the conclusion that quinmerac controls cleavers by root inhibition and hormone-type effects.

INDRODUCTION

BAS 518 H (quinmerac) (Fig. 1) is a new post-emergence herbicide currently being development by BASF Aktiengesellschaft to control cleavers (Galium aparine), Veronica spp. and Lamium spp. in cereals, rapeseed and sugarbeet. Chemical properties and field trial results are

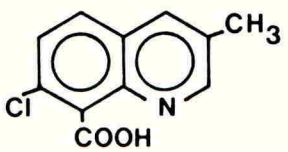

Fig. 1. Chemical structure of BAS 518 H (quinmerac).

published elsewhere (Wuerzer et al. 1985, Nuyken et al. 1985, Haden & Menck 1986). In this paper results covering uptake, translocation and the mode of action are presented.

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[1]proposed common name

MATERIALS AND METHODS

Auxin tests

The cucumber test was performed as described by Sloan and Camper, 1986. Ethylene biosynthesis was determined using soybean (Glycine max. cv. SRF 450 P) leaf discs treated with herbicide solutions for 24 h in sealed test tubes. Ethylene production was then measured by gas chromatography (Packard, model 419).

Uptake and translocation

Plants were grown in controlled growth chambers (22 °C, 400 $\mu E \cdot m^{-2}\ s^{-1}$. For uptake studies, ^{14}C BAS 518 H (spec. radioact. 549,5 MBq/mM) was applied with an adjuvant to the second leaf of wheat (Triticum aestivum cv. Caribo) or the first whorl of cleavers (Galium aparine).

Treated leaf samples taken at different time intervals were washed with water/nekanil (0,05 %) to remove residues that had not penetrated. Samples were then combusted in an oxidizer (Zinser, Oxymat, OX 300) and the evolved $^{14}CO_2$ was absorbed in a LS cocktail and radioassayed in a scintillation counter (Packard, Tricarb, 460 CD). Root uptake was investigated in a hydroponic culture. Samples were combusted and treated as described above.

Root growth

Cleavers and wheat seeds were surface sterilized in a 1 % sodium hypochlorite solution for 30 min and then stored at 4 °C until the primary root emerged. Germinated seeds were transfered to Petri dishes lined with filter paper that had been treated with different herbicide solutions and incubated for 4 days at 25 °C. The root length was then measured and compared with that of seeds incubated in distilled water.

Chemicals

All experiments were performed with analytical grade chemicals. 2,4-D, picloram and dicamba were purchased from Riedel de Haen, Seelze, FR-Germany.

RESULTS

Auxin activity

Cleavers plants treated with BAS 518 H were stunted, and their leaves were reduced in size, twisted and dark green. Phytotoxic symptoms resembled morphological changes that occur after the application of hormone-type herbicides such as benzoic acids or some pyridine compounds.

Experiments with cucumber seedlings (Fig. 2 A) revealed that BAS 518 H inhibited root growth to the same extent as 2,4-D, picloram or dicamba. Visual symptoms were also similar. At high herbicide concentrations, the roots were stunted and resembled a plant cell callus.

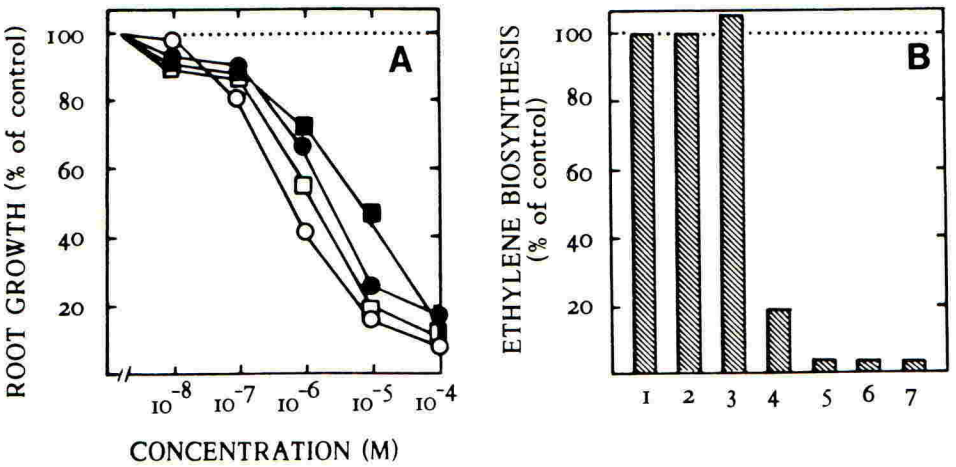

Fig. 2. A: Influence of BAS 518 H (-●-), 2,4-D (-○-), dicamba (-□-) and picloram (-■-) on root growth of cucumber seedlings.
B: Influence of BAS 518 H (4), 2,4-D (1 = control), dicamba (2), picloram (3), sethoxydim (5), bentazon (6) and water (7) on ethylene biosynthesis.

BAS 518 H induced ethylene biosynthesis in soybean leaf discs. Although the activity was lower than that of 2,4-D, dicamba or picloram it nevertheless revealed a distinct auxin activity that was significantly different to that of leaf discs treated with water or herbicides that have a completely different mode of action (Fig. 2 B).

Uptake and translocation

BAS 518 H was rapidly absorbed after a leaf application, particularly under conditions of high humidity and in combination with an adjuvant. Within 24 h, 90 - 100 % of the herbicide had penetrated into the leaf.

In cleavers, the major portion of BAS 518 H was translocated to the green parts of the shoot below the treated leaf during the first 24 h. Similar amounts were transported to the upper parts of the plant and to the root (Fig. 3 A). In wheat, the major part of quinmerac remained in the treated 2nd leaf, and 40 % of the applied substance was transported during the following days mainly to the 3rd and 4th leaf. Only small amounts were found in the roots and the nutrient solution (Fig. 3 B).

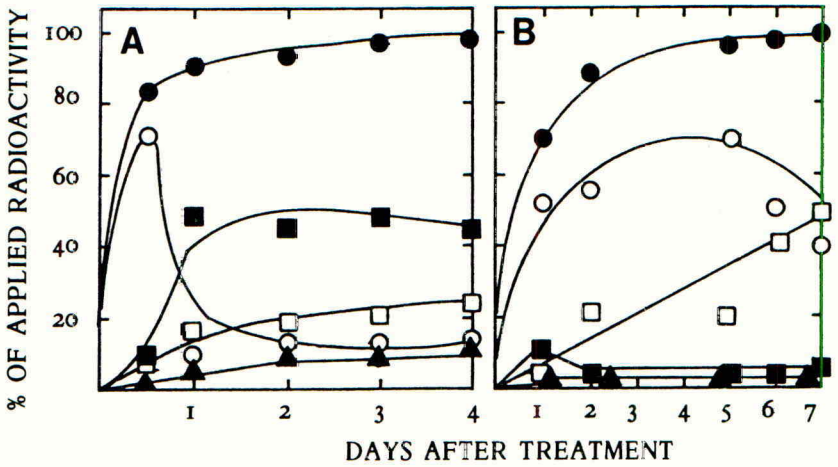

Fig. 3. Uptake and translocation of quinmerac by leaves of cleavers (A) and wheat (B).
-●- total uptake
-○- treated leaf
-■- stem below treated leaf or primary leaf (wheat)
-□- stem above treated leaf or 3rd and 4th leaf (wheat)
-▲- root and nutrient solution

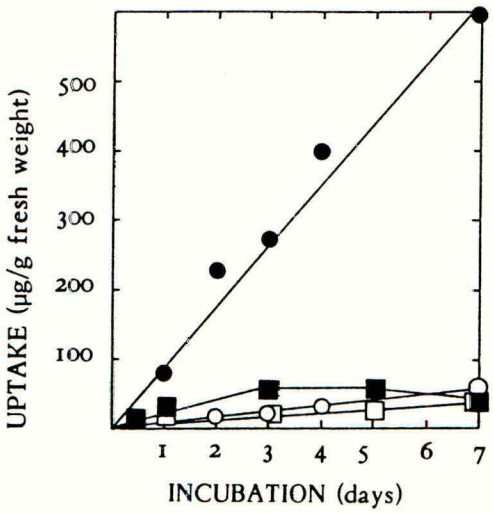

Fig. 4. Uptake and translocation of BAS 518 H by roots of cleavers and wheat.
-●- wheat root
-○- wheat leaves
-■- cleavers root
-□- cleavers shoot

BAS 518 H was initially well absorbed by roots of cleavers and wheat (Fig. 4). Within a few hours, a 10 fold concentration of the active compound could be detected in the roots compared with that in the nutrient solution. After 24 hours, the uptake of quinmerac by cleavers roots had stopped. In contrast, wheat accumulated BAS 518 H during the entire incubation period, which resulted in a 200 fold root concentration compared with that of the nutrient solution. On the other hand, similar radioactivity was measured in the green parts of cleavers and wheat. The differences in root uptake could be considered as an expression of varying sensitivity of cleavers and wheat roots to quinmerac (Fig. 5).

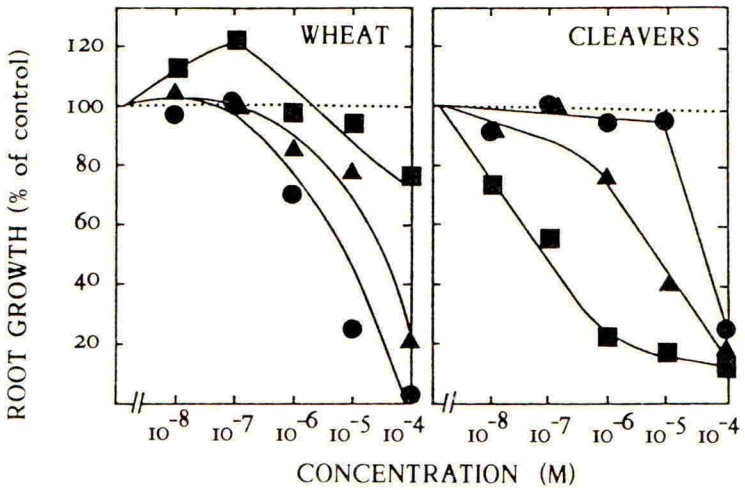

Fig. 5. Root growth of wheat and cleavers seedlings after application of BAS 518 H (-■-), 2,4-D (-●-) and picloram (-▲-).

Whereas wheat roots were almost insensitive to BAS 518 H, the compound was perceptible to cleavers roots at concentrations as low as 10^{-6}M. In contrast, root growth of germinating wheat seedlings was inhibited by 2,4-D and to a lesser extent by picloram. However, both herbicides were only weak inhibitors of cleavers root growth.

DISCUSSION

The visible morphological alterations which followed an application of BAS 518 H, together with the results of auxin test systems clearly demonstrated a quinmerac auxin activity. Consequently, in addition to the pyridine derivatives, benzoic acids and phenoxy compounds, quinolinecarboxylic acids must be regarded as a new form of hormone-type herbicides. In addition, quinolinecarboxylic acids with total different substitution patterns have been known as plant hormones for a long time (SAHASHI 1925, MATSUSHIMA et al. 1973).

The mode of leaf uptake of quinmerac resembled that of fluroxypyr (Sanders & Pallett 1987). Like other weak acids (Lichtner 1984), BAS 518 H was translocated acro- and basipetally. The lower translocation rate of quinmerac in wheat in comparison to cleavers may be due to morphological differences between mono- and dicotyledonous plants (Vanden Born 1966). Hormone-type herbicides are known to lead to an imbalance in the auxin level of the plants, however the exact mechanism for this type of herbicide has not been fully elucidated.

Quinmerac gives excellent control of cleavers and since our test systems have revealed only a rather weak auxin activity, the results indicate an activity at two levels. The high cleavers sensitivity was expressed in a strong inhibition of root growth which in turn disturbed the nutrient and water balance, and caused a cessation of growth or stunting of the plant. Furthermore the auxin activity caused morphological changes by influencing the hormone balance.

Quinmerac is selective in wheat because wheat roots are absolutely insensitive to BAS 518 H and auxin injury is usually weak in monocotyledonous plants. Whether additional metabolic differences between cleavers and wheat may favour the selectivity will be investigated in the future.

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THE SELECTIVITY OF CLOPYRALID IN SUGAR BEET; STUDIES ON ETHYLENE EVOLUTION.

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ABSTRACT

Clopyralid induces rapid, concentration-dependent evolution of ethylene from excised scentless mayweed leaves, which precedes the onset of visible epinastic symptoms, and is comparable to that induced by exogenous auxin. However, incubations using inhibitors (AVG and Co^{++}) and a precursor of ethylene biosynthesis (ACC) have shown symptom development to be independent of ethylene production in this experimental system. Incubation of excised sugar beet leaves with clopyralid does not result in increased ethylene production or the development of visual epinastic symptoms after 24 h. However, an intact ethylene biosynthetic pathway does exist in sugar beet since sensitivity to IAA and 2,4-D was demonstrated. These observations indicate that the selectivity of clopyralid is not solely related to differential ethylene evolution, but is more likely to result from differential binding affinity to an "auxin site".

INTRODUCTION

The herbicide clopyralid, a discovery of The Dow Chemical Company, is recommended for selective weed control in Brassica crops and sugar beet. It is particularly active against weeds of the families Compositae and Leguminosae (Dow Technical Information). Recent studies in this laboratory have shown scentless mayweed (Matricaria perforata Merat.) to be highly susceptible to clopyralid whilst sugar beet (Beta vulgaris cv. Salohill) is tolerant. The basis of this selectivity remains obscure, being independent of differential herbicide uptake, translocation or metabolism (Thompson & Cobb, 1986).

Clopyralid is a synthetic pyridine compound which, like certain benzoic acid and phenoxyalkanoic acid derivatives, is described as an "auxin-type" herbicide. This classification arises from the fact that many effects of such herbicides on plant growth and anatomy are similar to those caused by exogenously applied auxin (Haagsma, 1975; Hall et al, 1985). One common symptom is the rapid induction of young stem and leaf epinasty which has been linked to the production of ethylene gas by treated tissues (Baur & Morgan, 1969; Hall et al, 1985). This association is supported by the findings that auxin increases the rate of ethylene production in vegetative tissue (Imaseki, 1981), and that exposure of whole plants to ethylene at very low concentrations can lead to epinastic symptom development (Crocker, 1948). It is now established that auxin-type herbicides stimulate ethylene production in a number of species, and a recent report suggests that enhanced ethylene production following the application of clopyralid to sunflower (Helianthus annus) is a factor involved in resulting symptom development (Hall et al, 1985). These authors also cite differential ethylene production in clopyralid-tolerant and susceptible species, although no inference is made of the significance of this difference in terms of a possible contribution to clopyralid selectivity.

This paper describes the measurement of ethylene production in response to clopyralid application in excised leaves from tolerant sugar beet and

susceptible mayweed. Its relationship to symptom development and selectivity has also been assessed with reference to our current understanding of ethylene biosynthesis. Ethylene is produced from methionine via intermediates S-adenosylmethionine (SAM) and 1-aminocyclopropane carboxylic acid (ACC) (Yang & Hoffman, 1984). The conversion of SAM to ACC is catalysed by the enzyme ACC synthase and forms the rate-limiting step in the pathway. The production of ACC synthase is auxin sensitive and the enzyme is specifically inhibited by aminoethoxyvinylglycine (AVG) (Imaseki, 1981). The conversion of ACC to ethylene is catalysed by an enzyme complex referred to as the ethylene forming enzyme (EFE) and is not rate-limiting. This final reaction step may be inhibited by cobalt ions (Yang, 1981).

MATERIALS AND METHODS

Plant Material

Plants were propagated in J. Arthur Bowers seed and potting compost in 5 cm deep trays. Sugar beet seeds were sparsely sown at a depth of 5 mm, whilst mayweed seeds were surface sown. Trays were maintained under glasshouse conditions with a 14h day (200-400 µmole photons/m^2/s, photosynthetic photon flux density, PPFD) and 20-25°C. In all experiments plants were used at the three to four leaf stage.

Chemicals

All test solutions were first dissolved in methanol (final concentration (<0.2%), and diluted with 10^{-3} M MES (2-[N-Morpholino]-ethane sulfonic acid) buffer adjusted to pH6 with NaOH. AVG, ACC, 2,4-dichlorophenoxyacetic acid (2,4-D) and indole-3-acetic acid (IAA) were obtained from the Sigma Chemical Company. Cobaltous chloride was obtained from BDH chemicals. Technical grade clopyralid was a gift from The Dow Chemical company.

Incubation system

For consistency with previous work (Thompson & Cobb, 1986), leaf 3 of both species was used. Following excision at the stem, leaves were exposed to test chemicals in gas tight vials as illustrated in figure 1.

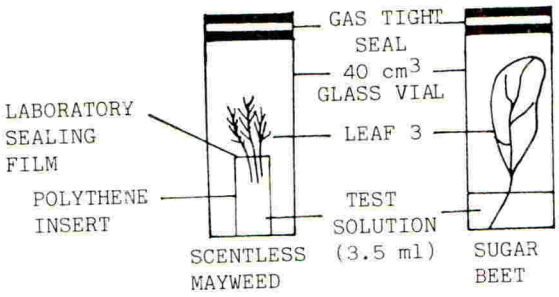

Fig. 1. Experimental system for measurement of ethylene production by excised leaves.

The vials were incubated in a controlled temperature water bath at 25°C and illuminated at 50 µmoles photons/m^2/s (PPFD) using natural white fluorescent tubes (Phillips). One cm^3 gas samples were withdrawn at regular intervals

using a gas-tight syringe. Ethylene concentration was then determined in a gas chromatograph (Sigma 3B-Perkin Elmer) equipped with a Poropack Q. 80-100 mesh column (Perkin Elmer) and a flame ionisation detector. Oven temperature was maintained at 100°C. A laboratory computing integrator (Perkin Elmer LC1-100) was used to calculate the amount of ethylene in each sample with reference to appropriate standards. After each withdrawal, gas volumes in each vial were amended with ambient air and results adjusted to account for dilution during sampling. Data are expressed on a fresh weight basis and are corrected, where necessary, for any ethylene present in blank tubes. Epinastic symptom development was both visually assessed and recorded photographically. Each experiment was fully replicated on 3 or 4 separate occasions and mean values plus or minus standard errors are presented.

RESULTS

Initial studies clearly demonstrated a differential ethylene response in clopyralid - susceptible and tolerant species (Figure 2).

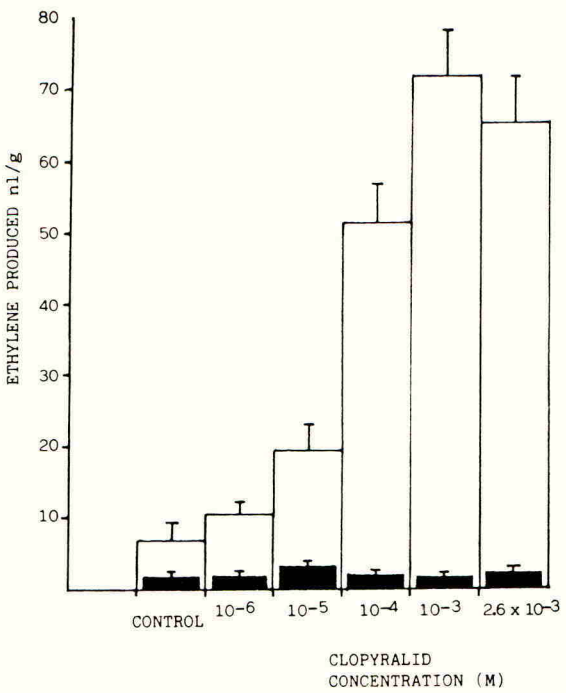

Fig. 2. The effect of clopyralid upon ethylene evolution in mayweed (□) and sugar beet (■) following incubation of excised leaves for 24h. Each point is the mean from 8 separate plants. Bars represent S.E.'s.

After 24h incubation of excised leaves with a range of clopyralid concentrations, tolerant sugar beet showed no increased ethylene production with respect to controls, whilst the identical treatment of scentless mayweed resulted in a concentration-dependent increase in ethylene production of up to 10 fold. This increase was highly significant ($P<0.001$) at 2.6×10^{-3} M and 1×10^{-3} M clopyralid and significant ($P<0.01$) at 10^{-4} M. Sugar beet leaves appeared unaffected by the herbicide after the 24h period, whereas mayweed exhibited classic epinastic symptoms which increased in severity with increasing herbicide dose. Short-term studies revealed that the clopyralid-induced increase in ethylene production in mayweed was an extremely rapid and concentration-dependent response (Figure 3), being measurable after only 1h incubation and sustained for the following 5h.

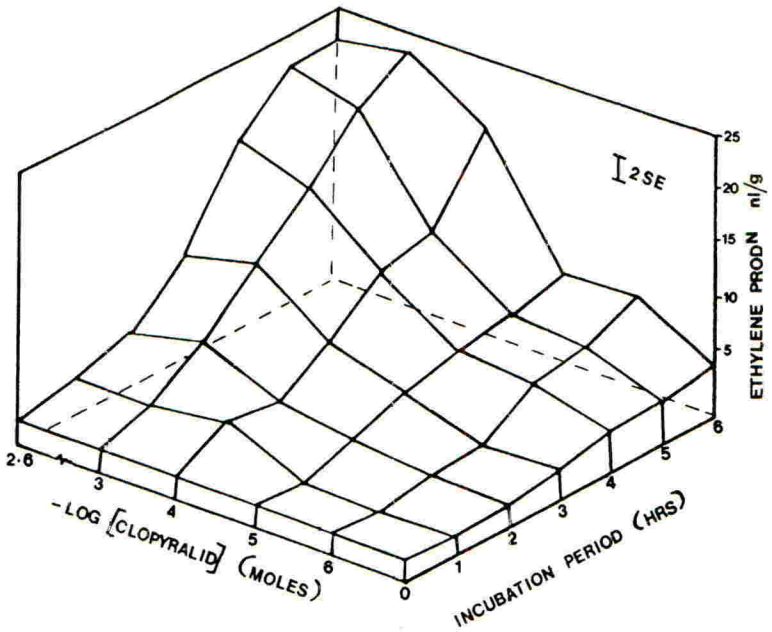

Fig. 3. Effect of clopyralid upon ethylene evolution in scentless mayweed, dose-response and time-course over 6h. Each point is the mean from 8-12 separate plants. Average standard error is indicated.

Increases were statistically significant ($P<0.01$) after only 2h at the two highest clopyralid concentrations and after 3h at 10^{-4} M. Clopyralid concentrations of 10^{-5} M and 10^{-6} M also gave consistently increased ethylene production although values were not statistically different from controls. As found in the 24h experiment, incubation of excised mayweed leaves with 10^{-3} M clopyralid gave the greatest ethylene production with 2.6×10^{-3} M yielding no further increase. Symptom development commenced after 4h at the highest clopyralid doses, and by 6h leaf epinasty was noted at all concentrations. The response of mayweed to 10^{-3} M clopyralid was equivalent to that induced by 10^{-3} M IAA over both 6 and 24h, as illustrated in figure 4.

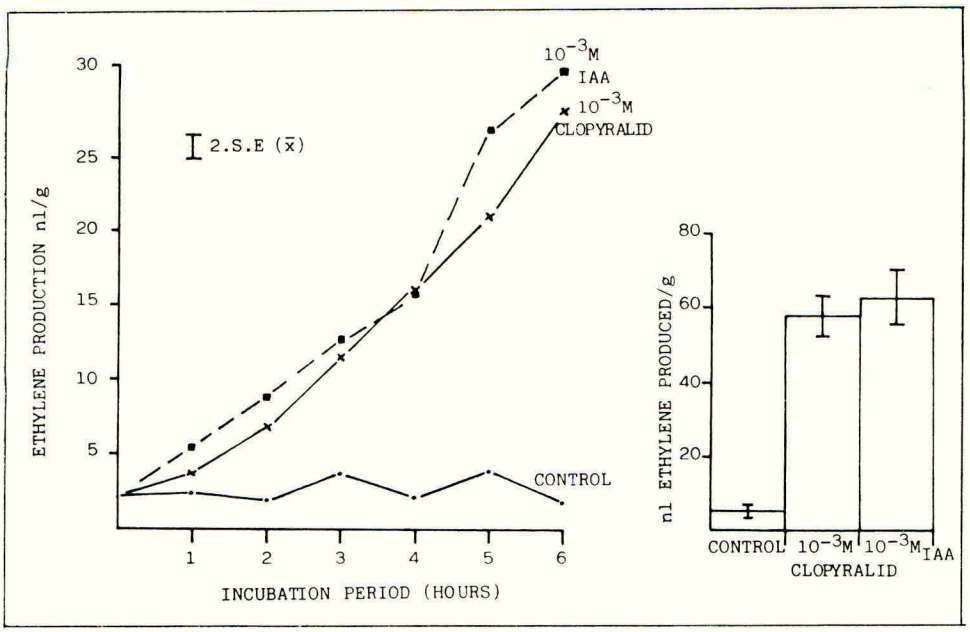

Fig. 4. Effect of clopyralid and IAA on ethylene evolution in scentless mayweed over 6 and 24h. Each point is the mean from 8 separate plants. Bars represent S.E.'s.

The onset and development of visual epinastic symptoms was similar with both clopyralid and IAA. The ethylene biosynthesis inhibitors AVG and Co^{++} reduced mayweed ethylene production in response to 10^{-3}M clopyralid by approximately 50% over 6 and 24h incubations (Figure 5). However, although ethylene production was reduced in the presence of these inhibitors, symptom development was not diminished.

Although sugar beet showed no symptom development or increased ethylene production in response to 10^{-3}M clopyralid, the same concentration of IAA or 2,4-D increased ethylene production by 5 and 12 fold respectively (Figure 6).

These marked increases were accompanied by typical symptom development. However, although sugar beet was insensitive to clopyralid, both species were capable of converting exogenous ACC to ethylene over a 24h period. (Table 1). No symptom development was noted with either species after 24 hours.

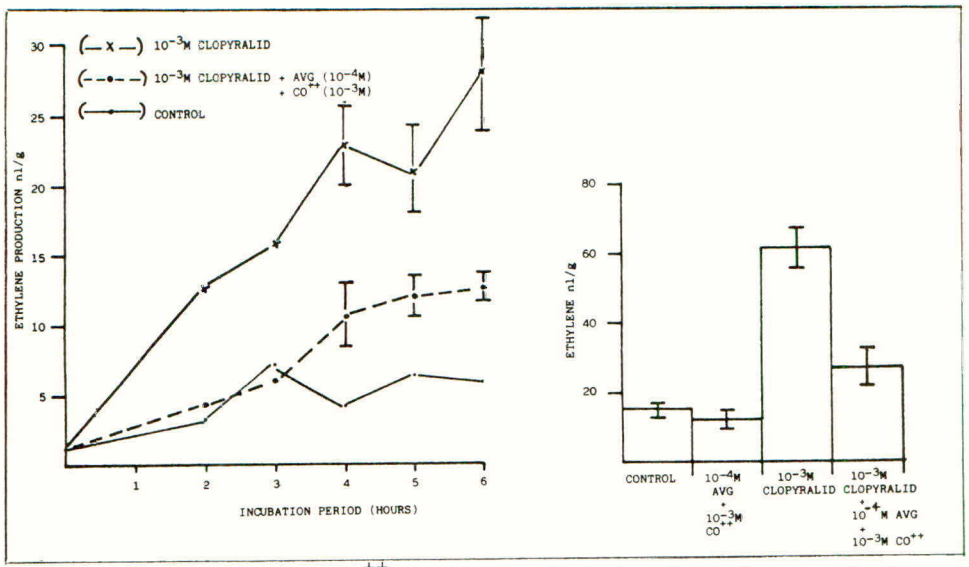

Fig. 5. Effect of AVG and Co^{++} on clopyralid-induced ethylene production in mayweed over 6 and 24h. Data are means of 6-8 replicates. Bars represent S.E.'s.

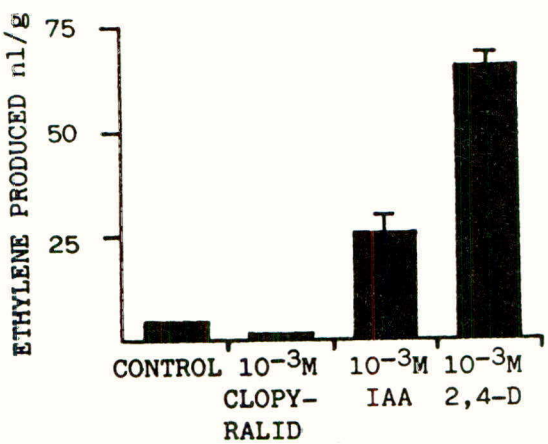

Fig. 6. Effect of clopyralid, IAA and 2,4-D on ethylene evolution in sugar beet after 24h incubation. Each point is the mean from 8-12 separate plants. Bars represent S.E.'s.

Table 1. Production of ethylene from 10^{-3}M ACC in mayweed and sugar beet. Data are means of six replicates plus or minus standard errors.

INCUBATION PERIOD (H)	ETHYLENE PRODUCTION, nl/g Mayweed	Sugar beet
2	20.03 ± 3.92	11.69 ± 1.17
4	38.99 ± 4.52	21.12 ± 1.19
24	163.62 ± 10.90	245.91 ± 32.41

DISCUSSION

Using a relatively simple assay system of incubating excised leaves in herbicide solutions, this study has demonstrated that clopyralid induces a rapid and concentration-dependent production of ethylene from a susceptible species, scentless mayweed, that precedes the onset of epinastic symptom development. Furthermore, no epinastic symptom development was observed in sugar beet as a result of clopyralid application in this system. This observation reveals an apparent inconsistency between our previously reported whole plant data (Thompson & Cobb, 1986) and the current study, in that symptoms were observed in the previous but not the present study. However, it should be borne in mind that the former study used whole plants with foliar application of formulated clopyralid, whilst the current experiments employed the direct, vascular uptake of unformulated clopyralid-acid into leaf explants.

These findings suggested that the observed leaf epinasty is directly caused by ethylene production in mayweed. However, the apparent link is disproven by the further observations that (1) although ethylene evolution was induced by incubation with the precursor ACC alone, leaf epinasty was not observed, (2) exposure to ethylene gas (using prepared standard gases, data not shown) also failed to produce epinastic symptoms in our system, and (3) whilst AVG and Co^{++} inhibited clopyralid-induced ethylene evolution by 50%, symptom development was not affected. Hence, we conclude that ethylene evolution alone is not the sole cause of epinastic symptoms in response to clopyralid incubation in our experimental system.

Neither ethylene production nor epinastic symptom development were observed in excised sugar beet leaves in response to clopyralid incubation. This species is, however, capable of producing ethylene when supplied with the precursor ACC or with IAA and 2,4-D, but symptom development was only observed following incubation with IAA and 2,4-D. Thus, this species has the physiological capacity to produce leaf epinasty in response to herbicides in this experimental system, but is insensitive to clopyralid over 24h. From our present results we conclude that differential ethylene production is not the sole basis of clopyralid selectivity in sugar beet and scentless mayweed.

It is our opinion that ethylene production is a symptom of auxin-type herbicide activity in susceptible species, and we consider it to be one of a cascade of events initiated by binding at an auxin receptor site. There are many published reports of responses to auxin which occur on a time scale from a few minutes to several hours (Evans, 1974). These effects range from almost immediate changes in cell membrane permeability (Fitzsimons et al,

1987), to stimulations in protein synthesis and carbohydrate deposition after 1 to 2 hours, which later appear as measurable growth responses (Evans, 1974). The production of ethylene and visible symptoms therefore appear to be separate measurable responses to the common stimulus of herbicide binding. In this sense, we speculate that clopyralid does not induce epinasty in sugar beet, in this system, due to a relatively poor binding affinity for the auxin binding site in this species.

Finally, the measurement of ethylene production may be a valuable tool in determining potential sensitivity to auxin-type herbicides, and assessing selectivity and structure/activity relationships.

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SYNERGISM AND ANTAGONISM OF HERBICIDES WITH MONOOXYGENASE INHIBITORS

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ABSTRACT

Triazole compounds, known to be inhibitors of monooxygenase enzymes were found to be antagonistic with EPTC in corn but synergistic with EPTC in sorghum. Interactions were also observed with other herbicides (*e. g.* metazachlor, SC-0774), but, the type of interactions varied with different rates of herbicide application. One of the monooxygenase inhibitors, BAS-110, had no apparent influence on rates of EPTC metabolism to water soluble metabolites. However, BAS-110 did significantly reduce EPTC uptake in both corn and sorghum.

INTRODUCTION

Various thiocarbamate and chloroacatamide herbicides can be antagonized and made more selective for use in crops such as corn and sorghum through the use of chemical antidotes such as dichlormid and other chloroacetamide compounds (Pallos & Casida, 1978). The most studied mechanism for these safeners involves the elevation of glutathione levels and their enhancement of herbicide detoxication through conjugation with glutathione (Lay & Casida, 1976, Leavitt & Penner 1979, Lay & Niland, 1985). However, in our own studies it has been difficult to correlate the rates of these processes with the degree of antidote action in different crop species (Ekler and Stephenson, 1987). Thus, we have begun to speculate that the glutathione conjugation system is not the only mechanism involved in the safening of these herbicides. One of the primary herbicides involved in these interactions is EPTC, and its metabolism in plants first involves an oxidation step to a sulfoxide then a subsequent conjugation of the sulfoxide with glutathione (Lamoureux & Rusness, 1987).

Because the metabolic pathway for EPTC in plants may involve an oxidation by monooxygenase enzymes, some investigators have speculated that monooxygenase inhibiting compounds (MOI) could have a role in antidote action (Lay & Casida, 1976, Fedtke and Trebst, 1987). It is difficult to compare the phytotoxicity of EPTC and its sulfoxide metabolite because the sulfoxide is likely to be unstable. Kőmíves and Dutka (1980) observed that a monooxygenase inhibitor (MOI), piperonyl butoxide, was synergistically phytotoxic with EPTC in corn plants. However, they did not examine the effects of piperonyl butoxide on EPTC metabolism, thus the mechanism for that synergism is uncertain. Furthermore, to avoid uptake problems, they had to inject piperonyl butoxide into the plant stems. A family of new triazole plant growth regulators has recently been developed

(Rademacher & Jung, 1986) which retard plant shoot growth without causing obvious malformations. Their mechanism is an inhibition of giberrelic acid synthesis through an inhibition of membrane bound monooxygenases.

The purpose of this study was to examine the possible role of monooxygenase enzymes in regulating the phytotoxicity of herbicides such as EPTC and metazachlor. The triazole compounds appear to have an advantage over other monooxygenase inhibitors like piperonyl butoxide, because they are taken up more easily.

MATERIALS AND METHODS

Common and chemical names and the function of the active ingredients used in the experiments are listed in Table 1. The experimental data were statistically analyzed by descriptive statistics followed by a Duncan's Multiply Range Test (DMRT) with $P < 0.05$.

Interaction of herbicides and monooxygenase inhibitors on plant growth

Seeds of corn (PAG SX-111) and sorghum (King Grain P 508 GB) were soaked overnight in tap water and then planted into premoistened vermiculite in foam cups (350 mL). The chemicals were applied immediately after planting as a treatment solution in 100 mL of water. Two corn or four sorghum plants per cup were grown in a growth room at 26 °C in light for 16 h and at 21 °C in dark for 8 h. The relative humidity in the growth room was 75 %. The plants were watered three times per week with 100 mL of water or half-strength Hoagland's nutrient solution (every third watering was nutrient). The experiments were set up in a completely randomized design. All treatments were applied to a total of four replicates. Corn and sorghum shoot heights were measured and the plants were harvested after three weeks.

Metabolism experiments

Corn and sorghum plants (planted and grown in a growth room as written above) were pretreated with a solution of BAS-110 (80 µg/L) or dichlormid (6.7 µM) 2 and 4 days after planting, respectively. Six days after pretreatment, the shoots were excised with a razor blade and placed into glass scintillation vials, containing propyl-labeled [^{14}C]EPTC (sp. act. 1.32 GBq/mmol) in 1 mL of water. EPTC concentration in the vials for corn was 40 µM: 5 µM (6.3 kBq) [^{14}C] and 35 µM unlabeled compound. The vials for sorghum contained only [^{14}C] labeled EPTC in a concentration of 2 µM (2.6 kBq). Three corn or five sorghum shoots were placed into each vial. One hour later, some of the shoots were weighed, wrapped in a small sheet of plastic film, immersed into liquid nitrogen and stored in a freezer until sample extraction. The remaining shoots were transferred to plastic scintillation vials containing 2 mL of water for 1, 3, 5 and 7 hours and then frozen as described above. To measure the [^{14}C] content of the tissue and the rate of metabolism of the herbicides, the frozen samples (fresh weights were 0.8-1.2 g and 1.5-2.2 g for sorghum and corn, respectively) were cut into small parts and extracted in 4 mL of a mixture of acetone and water (7:3) using a Brinkman homogenizer. After centrifugation (7,500 *g*, 15 min), the supernatants were extracted with 1 mL of dichloromethane to separate water soluble conjugates from the less polar parent herbicides.

The samples were extracted in conical centrifuge tubes (15 mL) with a vortex shaker. To get a proper separation of the liquid phases, the samples were centrifuged at 2,000 *g* for 5 min. The efficiency of the extraction method was 94 ± 3 % for EPTC and 97 ± 4 % for metazachlor. The radioactivity in both the aqueous and the organic phases was determined by liquid scintillation counting (LSC).

TABLE 1

Active ingredients used in the experiments.

Function	Common name	Chemical name
Herbicides	Chlorsulfuron	2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazine-2-yl)-aminocarbonyl]-benzenesulfonamide
	EPTC	S-ethyl dipropylthiocarbamate
	Metazachlor	N-(2,6-dimethyl-phenyl)-N-(1-pyrazolyl-methyl)-chloroacetamide
	SC-0774	not released (Stauffer)
safener	Dichlormid	N,N-diallyl-2,2-dichloroacetamide
mono-oxygenase enzyme inhibitors (MOI)	BAS-110	2-(2,4-dichlorophenyl)-2-hydroxy-3-methoxy-3-(1H-1,2,4-triazole-1-yl)-propane
	BAS-111	1-phenoxy-3-(1H-1,2,4-triazole-1-yl)-4-hydroxy-5,5-dimethylhexane
	Tetcyclacis	5-(4-chlorophenyl)-3,4,.5,9,10-pentaaza-tetracyclo-5,4,1,$0^{2,6}$,$0^{8,11}$-dodeca-3,9-diene

RESULTS

Numerous increases (synergism) or decreases (antagonism) in the phytotoxicity of the various herbicides to corn or sorghum can be elucidated by careful enumeration of the significant differences in Fig. 1 and 2. However, many of these interactions were not consistently repeated

in the various bioassays conducted. The major interactions that were consistently observed are as follows.

Herbicide - MOI interactions in corn

The triazole MOI-s, BAS-110 and BAS-111, were significantly antagonistic with EPTC in corn at all rates of EPTC application (Fig. 1b). BAS-110 and BAS-111 were antagonistic with the new herbicide SC-0774 in corn (Fig. 1d) at the lowest rate, but, BAS-111 had no effect and BAS-110 became synergistic at the higher herbicide rates applied. BAS-110 was antagonistic with metazachlor (Fig 1c) at the middle herbicide rate, but, BAS-111 and tetcyclacis were synergistic at the highest herbicide rate. None of the three MOI compounds had any type of interaction with chlorsulfuron (Fig 1a).

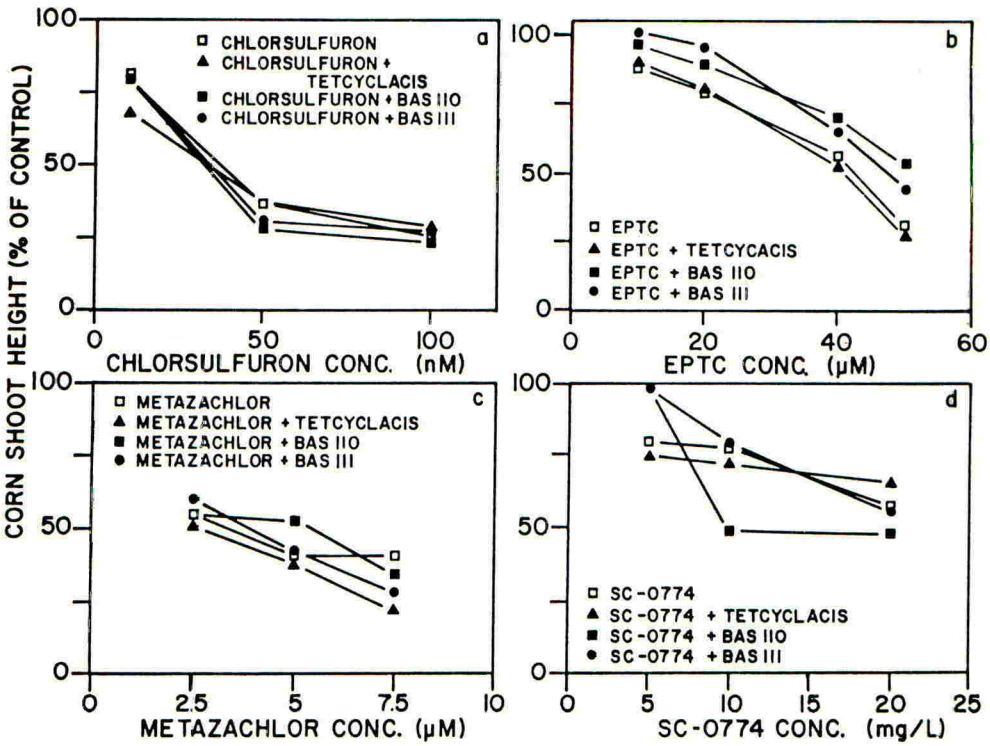

Fig. 1. Herbicide - monooxygenase inhibitor (MOI) interactions in corn. Rates for BAS-110, BAS-111, and tetcylacis were 80, 80, and 15 µg/L, respectively, in combination with the various herbicides at the rates shown.

Herbicide - MOI interactions in sorghum

In contrast to results with corn where antagonistic interactions were observed, all of the MOI-s, particularly BAS-110, were synergistically phytotoxic with EPTC in sorghum (Fig. 2b). Synergistic interaction was also found between BAS-111 and metazachlor (Fig. 2c) at the lowest herbicide rate, but, BAS-110 was antagonistic at the two highest metazachlor rates. Slight synergism was observed between the MOI-s and low rates of SC-0774 (Fig. 2d), but, no significant interactions were observed between the MOI-s and chlorsulfuron (Fig. 2a) in sorghum.

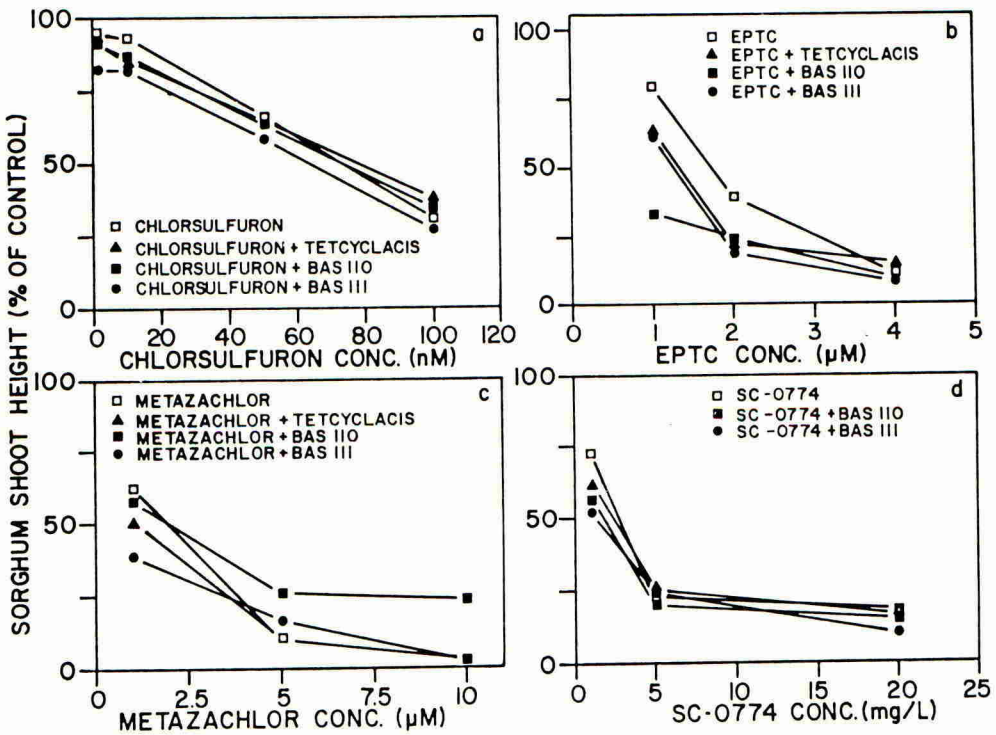

Fig. 2. Herbicide - monooxygenase inhibitor (MOI) interactions in sorghum. Rates for BAS 110, BAS 111 and tetcyclasis were 80, 80 and 15 μg/L, respectively, in combination with the various herbicides at the rates shown.

The effect of BAS-110 and dichlormid on EPTC uptake and metabolism

Of the many different herbicide - MOI interactions observed, those involving BAS-110 or BAS-111 with EPTC were the most interesting. These MOI compounds were antagonistic with EPTC in corn, but, synergistic with EPTC in sorghum. In an attempt to explain the conflicting behaviour in the two species, herbicide metabolism experiments were performed. [^{14}C]EPTC uptake and the rate of the metabolism of the herbicide were compared in plants after pretreating them with BAS-110 for six days, in comparison with EPTC alone. Dichlormid, the most widely used thiocarbamate safener was also included as a pretreatment.

Because of the different sensitivity to the herbicide, corn shoots were treated with 20 times more EPTC than sorghum shoots. Herbicide uptake by corn was 7-9 times higher than by sorghum (Table 2). Sorghum metabolized EPTC much quicker than corn (Fig. 3). There were no significant differences found in the rate of metabolism to water soluble metabolites when the plants were pretreated with either dichlormid or with BAS-110, compared with the unpretreated plants. Nevertheless, both corn and sorghum absorbed significantly less EPTC when the plants were pretreated with BAS-110 (Table 2). Dichlormid pretreatment did not significantly change the uptake of EPTC either by corn or by sorghum.

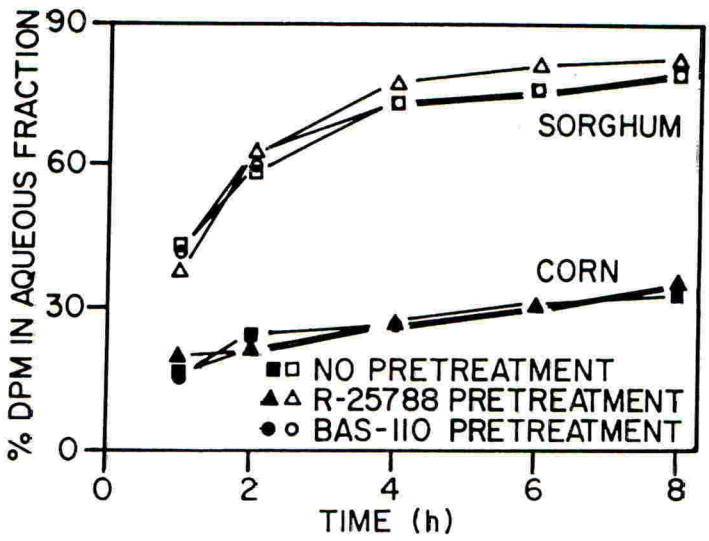

Fig. 3. Influence of 6-day pretreatment with dichlormid (6.7 µM) or BAS 110 (80 µg/L) on the kinetics of [^{14}C]EPTC conversion to water soluble metabolites in corn and sorghum.

TABLE 2

Effect of safener (dichlormid) and monooxygenase inhibitor (BAS-110) pretreatment on EPTC uptake by corn and sorghum shoots[1].

Pretreatment for 6 days	EPTC uptake after 1 h (DPM/g fresh weight) Corn	Sorghum
None	298 ± 13 (100 %)	42.4 ± 3.7 (100 %)
Dichlormid	312 ± 17 (105 %)	39.5 ± 4.2 (93 %)
BAS-110	223 ± 11 (75 %)	24.0 ± 3.4 (57 %)

DISCUSSION

The first step in the metabolic pathway for EPTC is oxidation (Lay & Casida, 1976) to a sulfoxide moiety which in turn may be further oxidized to a sulfone (Horváth and Pulay, 1980) and/or conjugated with glutathione (Lay and Casida, 1976). In studies with acetanilide herbicides, Leavitt and Penner (1979) established that oxidation was not a prerequisite to their conjugation with glutathione. This is likely to be true for metazachlor (a chloroacetamide) as well. The importance of oxidation step(s) in the metabolism of EPTC can have a role in the significant interaction of EPTC with compounds known to inhibit monooxygenase enzymes in plants. Lay and Casida (1974) have indicated that EPTC-sulfoxide is more toxic than EPTC to most plants, but, less toxic than EPTC to corn. They suggest that EPTC-sulfone would not be a good herbicide. Kómives and Dutka (1980) rank the sulfone as the most toxic and EPTC as intermediate in phytotoxicity to corn.

In our studies, BAS-110 was synergistically toxic with EPTC in sorghum, but, antagonistic with EPTC in corn. BAS-110 inhibited uptake of EPTC in both species. However, BAS-110 did not affect the disappearance of the parent EPTC in either corn or sorghum. The various metabolites were not characterized. The differential action of BAS-110 as an EPTC antagonist in corn and as an EPTC synergist in sorghum may eventually help define the selective mechanism for this herbicide in plants. However, a greater understanding of the significance of these interactions in the selective mechanism for EPTC will require a better understanding of the identity and toxicity of the various oxidation products and conjugates of EPTC in corn and sorghum.

[1]The original DPM values found in corn shoots are multiplied by 5 in Table 2 because [^{14}C]EPTC was only 20 % of the total EPTC treatment.

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BIOCHEMICAL ASPECTS OF SAFENER ACTION: EFFECTS ON GLUTATHIONE, GLUTATHIONE -S- TRANSFERASE AND ACETOHYDROXY ACID SYNTHETASE IN MAIZE

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ABSTRACT

The chemical safener N,N-diallyl -2,2 -dichloroacetamide (DDCA) increased the glutathione content of root tissue, and glutathione-S-transferase activity in the root and shoot tissues of the treated maize (Zea mays) plants. Napthalic anhydride (NA) also enhanced glutathione-S-transferase activity in root and shoot, but had no effect upon glutathione content.

Both NA and DDCA enhanced the activity of acetohydroxy acid synthetase (AHAS), the target site of sulfonylureas, in treated plants. However, AHAS extracted from safened plants was more sensitive to chlorsulfuron inhibition. NA in vitro did not alter AHAS inhibition by chlorsulfuron.

INTRODUCTION

The herbicide safeners napthalic anhydride (NA) and N,N-diallyl -2,2 -dichloroacetamide (DDCA) enhance the tolerance of maize (Zea mays) to thiocarbamate herbicides, and to a lesser extent, the sulfonylureas (Hatzios 1983 Parker 1983).

Although much research has been directed towards their mode of action, the biochemical basis for the protective action of these safeners in maize is still not clearly understood.

DDCA enhances the level of glutathione-S-transferases (G-S-T) and reduced glutathione (GSH) in treated tissue (Lay and Casida 1976, Mozer et al 1983, Komives et al 1985, Lay and Niland 1985), and hence the rate of thiocarbamate detoxification via GSH conjugation of their sulfoxides. G-S-T isoenzymes have been identified in maize (Guddewar and Dauterman 1979, Mozer et al 1983). These have different herbicidal specificities, and the induction of isoenzymes particular to certain herbicide molecules, may account for the specificity of safener action. (Edwards and Owen 1986).

Sweetser (1985) reported an enhanced rate of sulfonylurea metabolism in NA/DDCA treated tissue, which was believed to be associated with an induction of the cytochrome P-450 mixed function oxidase system (Fedtke 1987).

Because of the association between safener action and the induction/enhancement of enzyme systems in treated tisue, it seemed appropriate to investigate the effect of these safeners upon acetohydroxy acid synthetase (AHAS) activity, the target site of the sulfonylurea

herbicides (Ray 1984). If either the total amount of AHAS is altered considerably, or isoenzymes are induced with decreased sulfonylurea sensitivity, then this might in part explain the safening action reported by Parker (1983).

MATERIALS AND METHODS

Plant Material

Seeds of maize (var LG 11) were washed in water to remove fungicide dressing, before applying either NA (97% w/w a.i.) or DDCA (20% w/w a.i.) at rates of 0, 0.25, 0.5 and 1.0% by seed weight.

Three seeds were sown per 9 cm pot in Vermiculite and watered thoroughly with 50% Hoaglands solution. Pots were placed in a light cabinet (16 hours light, 390 μ E M^{-2} S^{-1} 27/19^{o}C), and watered every two days with 50 ml of nutrient solution.

GSH and G-S-T assays

Both assays were carried out using root and shoot tissue of 5 day old plants. (2 leaf stage).

GSH was assayed spectrophotometrically by following the reduction of 5-5'-dithiobis-2-nitrobenzoic acid (DTNB) by GSH in the presence of NADPH and glutathione reductase (Low et al 1983, Smith et al 1984).

G-S-T activity was measured spectrophotometrically using 1-chloro-2,4, dinitrobenzene (CDNB) as a substrate. (Mozer et al 1983). Both assays were carried out at 25^{o}C.

AHAS extraction and assay

Root and leaf tissue was used from 7 day old plants (3 leaf stage). The methods used were based upon those of Chaleff and Mauvais (1984) as modified by R. Wallsgrove (personal communication).

(i) Leaf Extraction

Approximately 0.8g of the youngest leaves of plants was homogenised on ice in 5.5 ml of extraction buffer containing; 50 mM KH_2PO_4, 5 mM $MgSO_4$, 10 mM pyruvate, 0.5 mM cocarboxylase, 10 μ M flavin adenine dinucleotide (FAD), 1 mM L-leucine, 1 mM L-valine, 10% ethanediol (v/v), 0.05% Triton X 100, at pH 7.5, with 50% w/w polyvinyl polypyrrolidone (PVPP). The crude extract was strained through 4 layers of muslin, and centrifuged at 11,600g for 8 minutes. 2ml was desalted on a Sephadex G 25 column equilibrated with resuspension buffer containing 50 mM KH_2PO_4 pH 7.5, 5mm $MgSO_4$, 10 mM pyruvate, 30% ethanediol.

(ii) Root Extraction

Approximately 5g of root tissue was ground in a pestle and mortar in 10 ml extraction buffer with 0.5g PVPP and a small amount of washed sand. The resulting brei was strained through 4 layers of muslin, and spun at 4000g for 5 minutes. AHAS was precipated from the supernatant solution using

$(NH_4)_2SO_4$ between 25 and 50% saturation. The pellet collected by centrifuging for 20 minutes at 10,000g was resuspended in 2ml of resuspension buffer and desalted as above.

Assay

In a total of 650 µ l, the assay contained; 50 mM KH_2PO_4 pH 7.5, 50 mM pyruvate, 10 mM $MgSO_4$, 250 µM cocarboxylase, 20 µ M FAD, 125 µ l enzyme, and as required, 125 µ l chlorsulfuron. Samples were incubated at 30°C for 1 hour, when the reaction was stopped by the addition of 125 µ l of 3 M H_2SO_4. After incubation at 60°C for 15 minutes, the acetoin content of samples was determined by the sequential addition of 125 µ l 20% NaOH, 187 µ l 0.5% Creatine, 187 µl 5% ∝napthol. After 1 hour the samples were centrifuged and absorption at 530 nm recorded.

AHAS activity was expressed as a percentage values by comparison with the absorption values of treatments with samples incubated without chlorsulfuron. Where NA was used *in vitro*, it was first dissolved in acetone, before diluting in the phospate assay buffer. Final acetone concentration in the assay mixture was 0.25% v/v.

Protein assay

The protein content of enzyme extracts was determined using the method of Bradford (1976).

RESULTS

Treatment of maize seeds with NA and DDCA enhanced the activity of G-S-T in root and shoot tissue in a dose dependent manner (figure 1). The rsponse to DDCA treatments was greater than with equivalent NA treatments. Root and shoot tissue showed similar G-S-T enhancement with each safener (two to three fold at 1%).

No significant GSH response was found with DDCA in shoot tissue, or with NA treatment in root or shoot. However, DDCA did enhance the GSH content of root tissue in a dose dependent manner. (Figure 2).

At an application rate of 0.5% by seed weight, NA gave an increase in extractable AHAS activity of 1.25 to 1.5 fold, in both root and third leaf after 7 days. (Table 1). The response to DDCA was less however, with an increase of between 1.0 to 1.3 fold. These responses were less than those shown for G-S-T at the 0.5% application rate i.e. approximately a two fold increase in activity for both safeners.

To investigate the possible induction of a chlorsulfuron resistant isoenzyme of AHAS with safener treatment, inhibition curves were plotted for AHAS extracted from safened and unsafened tissue. Figures 3 a and b reveal that both NA and DDCA pretreatments at 0.5% decreased the activity of the extracted AHAS over a range of chlorsulfuron treatments, when compared with non-safened plants.

The addition of NA *in vitro* (10^{-5} -10^{-8} M), to AHAS extracted from unsafened plants, resulted in a 10 - 15% decrease in activity as compared with an NA free control (Figure 4). A similar result was found with the same NA concentrations in the presence of 10 ppb chlorsulfuron.

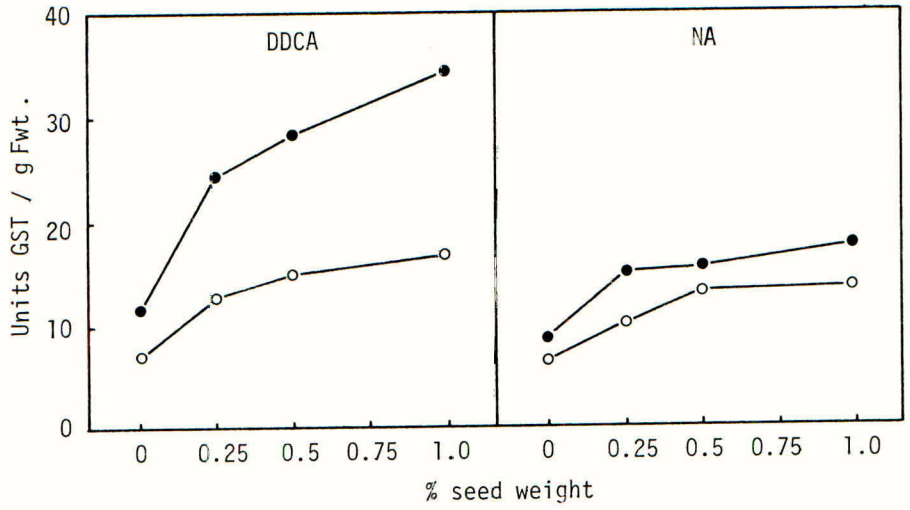

Fig. 1. Effect of DDCA and NA upon G-S-T activity in root (●——●) and shoot (○——○) tissue 5 days after treatment.

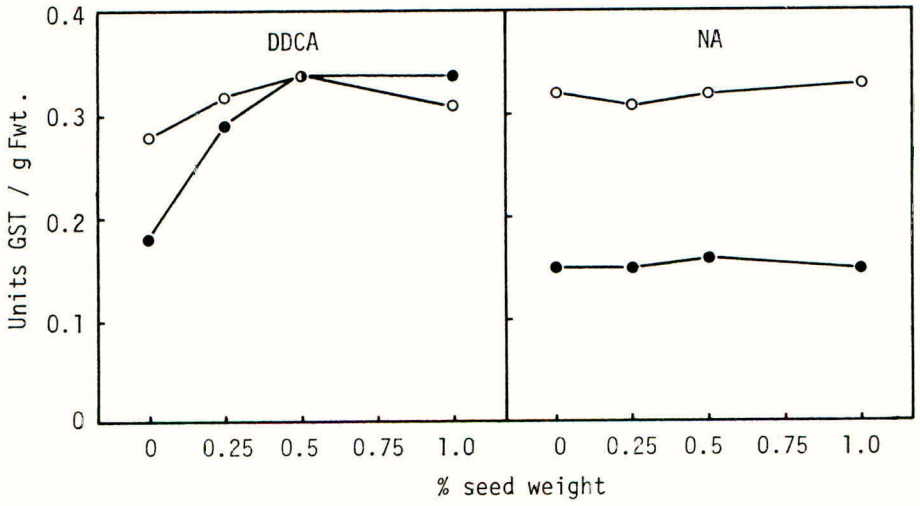

Fig. 2. Effect of DDCA and NA upon GSH levels in root (●——●) and shoot (○——○) tissue 5 days after treatment.

TABLE 1.

Effect of DDCA / NA as seed dressing at 0.5% by seed weight on extractable AHAS activity 7 days after treatment.

Treatment	Leaf tissue		Root tissue	
	Abs./Protein	Abs./Fwt.	Abs./Protein	Abs./Fwt.
Control	7.72	0.66	9.59	10.1
DDCA	8.86	0.71	9.50	13.2
NA	9.91	0.87	12.43	15.2

Abs./Protein = Absorption 530nm / (ug Protein / 0.1ml enzyme extract).

Abs./Fwt. = Absorption 530nm / g fresh weight extracted tissue.

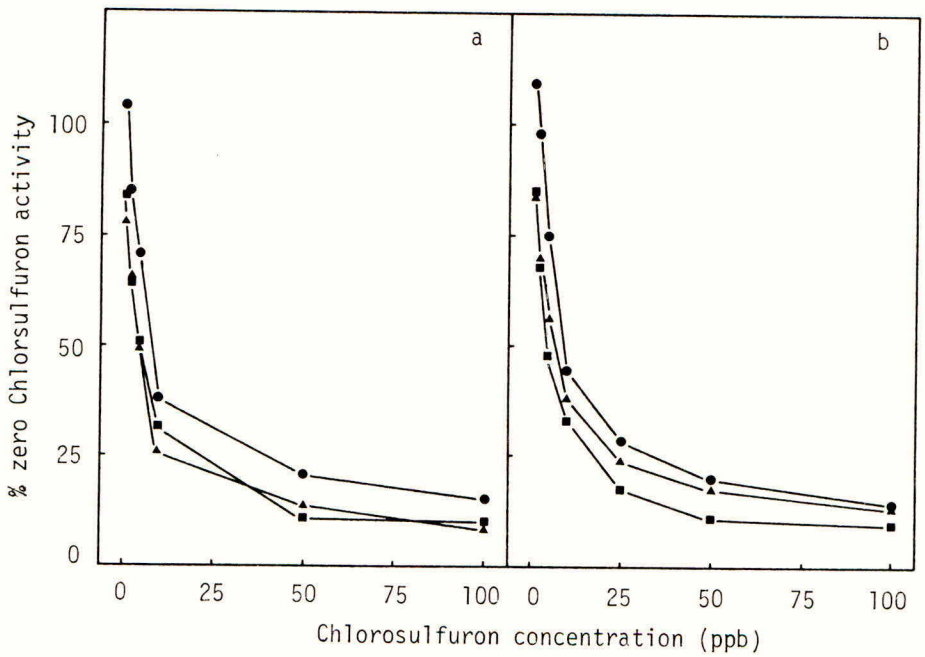

Fig. 3. AHAS / Chlorsulfuron inhibition curves with DDCA / NA pretreatments in a) shoot and b) root. (●——●) control, (■——■) DDCA and (▲——▲) NA.

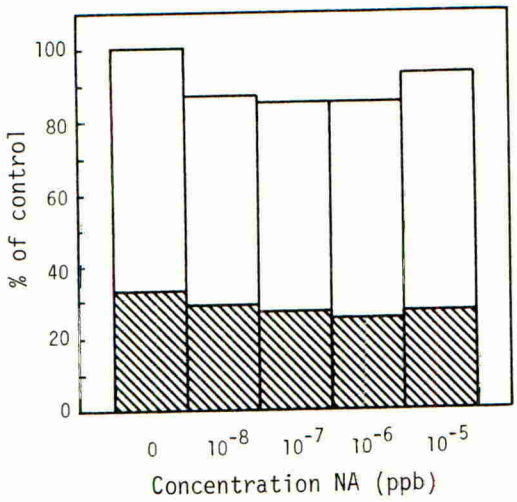

Fig. 4. Effect of NA *in vitro* upon AHAS activity ± Chlorsulfuron.
□ 0 ppb Chlorsulfuron, ▨ 10 ppb Chlorsulfuron.

DISCUSSION

The conjugation of thiocarbamate herbicide sulfoxides with glutathione, mediated by glutathione-S-transferases, is an important detoxification mechanism in maize. (Hatzios 1982). Therefore, any increase in the endogenous levels of glutathione and glutathione-S-transferases is likely to increase the tolerance of maize to these herbicides, by enhancing the rate at which they are metabolised.

It has been reported that DDCA treatment enhanced root GSH content and the activity of G-S-T in root and shoot tissue. (Lay and Casida 1976, Mozer *et al* 1983). This response was confirmed in this work. (figure 1 and 2). NA treatment produced no change in endogenous GSH, but did increase G-S-T activity significantly (figure 1). This was similar to the findings of Mozer (1983), but opposed to those of Lay and Casida (1976), who found no G-S-T enhancement with NA. A possible explaination of these conflicting reports is the choice of substrate used to assay G-S-T activity. CDNB was used in this investigation and also by Mozer (1983), whereas EPTC sulfoxide was chosen as the subtrate by Lay and Casida (1976). G-S-T occurs as at least two isoenzymes (Guddewar and Dauterman 1979), and these have been shown to have different substrate specificities (Edwards and Owen 1986). Hence NA may enhance G-S-T isoenzymes which are active with CDNB, but not EPTC sulfoxide.

The use of other chemicals which modulate GSH levels and G-S-T activity could provide a means of modifying the sensitivity of both crop and weed plants to herbicides such as the thiocarbamates, thus extending their range of use. The herbicide tridiphane which inhibits G-S-T activity (Lamoureux et al 1986) has been used to synergise the activity of EPTC in the grass weed giant foxtail (Setaria faberi) (Ezra et al 1985). Other compounds, such methionine sulphoximine, which inhibit glutathione biosynthesis (Rennenberg and Uthemann 1980) may prove of value.

The safening of maize to sulfonylurea herbicides was reported to be associated with an increased rate of metabolism via the cytochrome P-450 mixed function oxidase system (Sweetser 1985). Since both this enzyme system and G-S-T activity are enhanced by safener treatment, the possibility that AHAS activity is also enhanced should not be overlooked as a possible mechanism of action.

The results presented here (figures 3 a and b, table 1) indicate that although AHAS activity was enhanced in safened plants, the sensitivity of AHAS to Chlorsulfuron was also increased. NA was also shown to have no effect in vitro upon AHAS inhibition by chlorsulfron (figure 4) and thus would be unlikely to alter the binding of the herbicide with the enzyme.

Hence, the safening of maize to chlorsulfuron by NA and DDCA, did not appear to be explained by the changes in AHAS activity observed.

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THE USE OF FLUROCHLORIDONE FOR WEED CONTROL IN THE POTATO IN POLAND

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ABSTRACT

Small plot experiments and commercial trials on weed control with flurochloridone (3-chloro-4-chloromethyl-1-trifluro-tolyl)-2-pyrrolidone were carried out in Poland in the years 1981-1986.Pre-emergent applications of flurochloridone within 10 days after potato planting at the rate 0.5-0.75 kg/ha a.i. had given excellent control of annual broadleaved weeds.Tankmix of flurochloridone at the rate 0.375 kg/ha a.i. with pre-emergent herbicides (linuron or metribuzin) showed similar efficacy.When applied too late (shortly before potato emergence) flurochloridone caused injury to the crop.

INTRODUCTION

Potato yield reductions due to weeds reach 10-40% in Poland.The main weed species are *Chenopodium album, Sinapis arvensis, Viola arvensis, Anthemidae and Elymus repens*. The weeds are controlled on the acreage 150 thousand ha (7% of the total acreage) but the need for herbicide usage is increasing from year to year.

Broadleaved weeds are usually controlled with EPTC, linuron, monolinuron, cyanazine, metribuzin or prometryn. These chamicals show better efficacy when applied to soil with sufficient moisture content.

Flurochloridone (Stauffer Chemical Co.) is a novel herbicide selective for the potato. Commercial product is an emmulsifiable concentration marketed under the name "Racer 25EC".

This paper reports the study on the efficacy of flurochloridone against broadleaved weeds and its safety to the potato in field experiments and commercial trials in Poland.

MATERIAL AND METHODS

Flurochloridone was tested in field experiments at Bonin, Northern Poland, in the years 1981-1983 and in commercial trials located on 217 sites all over the country in the years 1983-1986.

In small plot trials (33 sq.m per plot) the treatments were randomized within 4 replicated blocks. Commercial trials were of 0.5-1 ha in large fields.

Flurochloridone (R-40244) was applied within 10 days after potato planting. The doses used ranged from 0.5 to 0.75 kg/ha a.i., the higher one being appplied on heavy soils. In the case of tankmixes the dose of flurochloridone was 0.375 kg/ha a.i. and the other herbicides were linuron (Afalon 50) at the rate 0.5 kg/ha a.i. or metribuzin (Sencor 70WP) at the rate 0.35 kg/ha a.i. All herbicide combinations were applied in 200-400 l of spray volume. Mechanical cultivation were the standard.

Efficiency of weed control in small plots was estimated by counting weeds in 3 x 1 m squares and in commercial trials - by visual estimation of soil surface covered with weeds.

Crop tolerance was assessed according to the EWRC scale, where 1 = no damage, 9 = plants killed.

RESULTS

In small plot trials good efficacy of weed control with flurochloridone has been confirmed against the following broadleaved weeds: *Gallium aparine, Thlaspi arvense, C.album, Gallinsoga parviflora* and *Stellaria media*.Reduced efficacy was seen against *Cirsium arvense, Convolvulus arvensis, Equisetum arvense* (table 1). Anthemis spp. occured mainly later in blanks (no potato plant), however in closed canopy these weeds were absent.

TABLE 1

Efficiency of the control of some weed species with flurochloridone in small plot trials (Bonin, mean for the years 1981-1983)

Weed species	Flurochloridone 0.5 kg/ha	Flurochloridone 0.375 kg/ha + 0.35 kg/ha metribuzin
Anthemis spp.	xx	xxx
C.arvense	0	0
G.aparine	xxx	xxx
T.arvense	xxx	xxx
Veronica sp.	xx	xx
Viola sp.	x	x
C.album	xxx	xxx
E.arvense	0	0
C.arvensis	0	0
G.parviflora	xxx	xxx
S.media	xxx	xxx

Efficiency: xxx - 95-99%; xx - 90-94%; x - 75-89%; 0 - 0-74%

The results of commercial trials indicate excellent control of *C.album*,*S.arvensis*, *S.media*, *Amaranthus retroflexus* and *V.arvensis* (table 2). Tankmixes of flurochloridone at a decreased rate plus linuron (0.5 kg/ha) or metribuzin (0.35 kg/ha) showed similar efficiency of weed control to the high rate alone (table 3).

TABLE 2

Efficiency of the control of some broadleaved weeds in commercial trials (mean for the years 1983-1986)

Weed species	Frequency	Efficiency of the control/per cent of crops full	good	medium	unsatisfactory
A.retroflexus	37.2	43.6	49.7	4.2	2.5
Anthemis spp.	57.2	32.1	27.6	33.8	6.5
C.album	96.8	64.8	28.8	5.3	3.1
S.arvensis	88.9	50.4	39.2	6.1	4.3
S.media	61.2	71.4	25.1	3.5	0
V.arvensis	45.3	41.6	46.2	10.1	2.1
E.arvense	61.3	18.4	2.6	29.0	5.0
C.arvensis	42.3	20.1	7.3	48.3	24.3
C.arvense	71.1	13.2	19.6	32.1	35.1
Echinochloa crus-galli	43.7	15.7	14.3	37.1	32.9
E.repens	73.4	0	1.7	41.7	56.6

TABLE 3

Efficiency of the control of some weed species with various rates of flurochloridone

Weed species	Dose of flurochloridone/per cent of sites 0.75 kg*	0.5 kg	0.375 kg + other herbicide
G.aparine	100.0	76.7	95.9
Veronica sp.	98.4	90.9	94.3
V.spp.	85.4	71.9	82.4
T.arvense	100.0	96.4	98.1

*heavy soil

Weed control with flurochloridone was fairly stable from year to year which confirms earlier research (Adamczewski *et al.*).

Flurochloridone alone or in mixture with other herbicides applied shortly before potato emergence or post-emergence injured potato plants (4-8 in EWRC scale) and the damage later resulted in a yield decrease (table 4). The herbicide applied 10 days after planting was selective for the potato.

TABLE 4

Influence of the date of flurochloridone application on tuber yield in the years 1983-1986

Days before emergence	No. of sites	Yield difference between treated and untreated - t/ha
0	12	- 0.3
2 - 5	28	+ 0.2
6 - 14	43	+ 9.7

Significant differences in the yields between treated and untreated fields were found. In commercial trials flurochloridone produced yields on average 5.1 t/ha higher. When decreased rates of flurochloridone in mixture with other herbicides were used the average yield was higher by 4.3 t/ha (table 5).

TABLE 5

Comparison of potato yields produced in commercial trials (mean for the years 1984-1985, n = 61)

Treatment	Rate per ha	Tuber yield in t/ha
flurochloridone	0.5-0.75 kg	27.6 a
flurochloridone+ other herbicide	0.375 kg +	26.8 a
control (mechanical cultivation till emergence)		21.5 b

DISCUSSION

The results of the experiments presented have shown that flurochloridone alone (0.5–0.75 kg/ha a.i.) or in mixture with linuron or metribuzin at a decreased rate (0.375 kg/ha a.i.) gives excellent control of broadleaved weeds. The level of the efficiency was not much changed from year to yearr. Some other weed species were controlled less effectively.

When applied early in the season i.e. within 10 days after potato planting the product is selective to the potato. The mechanism of this selectivity seems to be based on mechanical separation of the potato plant from the chemical (Forbes *et al.*1985).

As compared to other pre-emergent herbicides e.g.linuron, metribuzin, EPTC, flurochloridone reduces the need for mechanical cultivation. Sometimes only in heavy soils a need for additional cultivation may arise.

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