Proceedings 7th British Insecticide and Fungicide Conference (1973)

A RISK ANALYSIS OF THE CONTROL OF BARLEY MILDEW WITH FUNGICIDES

J. Gilmour and R.H. Fawcett
Edinburgh School of Agriculture, West Mains Road, Edinburgh, EH9 3JG

Summary In trials in south-east Scotland, Golden Promise spring barley gave an average yield response of 560 kg/ha during 1969-72 (710 kg/ha during 1970-72) when mildew was controlled by ethirimol seed treatment. In trials during 1970-72 with sprayed fungicides for mildew control, Golden Promise gave a mean best response of 565 kg/ha and an average response of 330 kg/ha to all single sprays applied up to the onset of heading. At 1973 prices the break-even response for ethirimol seed treatment was 150 kg/ha, while that for the sprays was 225 kg/ha when the cost of application and a small wheeling loss were included.

Using these data a risk analysis is presented in which the risk (i.e. expected opportunity cost) of a wrong decision (i.e. recommending unprofitable control or not recommending profitable control) can be assessed given an estimate of the yield response and cost data for the available disease control measures. Differences in cultivar response and some other factors influencing mildew incidence are also considered.

Résumé Lors d'essais culturaux dans le Sud-Est d'Ecosse, l'augmentation moyenne du rendement de l'orge cultivar Golden Promise, a été de l'ordre de 560 kg/ha pendant les années 1969-72 (710 kg/ha de 1970-72) après avoir lutté contre le blanc des graminées à l'aide d'un traitement d'ethirimol appliqué aux semences. Lors d'essais durant les années 70 à 72 pendant lesquels on a effectué des pulvérisations de fongicides contre le blanc, l'augmentation maxima du rendement du Golden Promise a été de 565 kg/ha et l'augmentation moyenne de 330 kg/ha pour chaque pulvérisation effectuée jusqu'à l'apparition de l'épiaison. Selon les prix de 1973, on a pu obtenir une augmentation de 150 kg/ha une fois les frais de traitement d'ethirimol applequé aux semances amortis. Tandis qu' une augmentation de l'ordre de 225 kg/ha a été obtenue une fois les frais de pulvérisations amortis y compris le coût de l'application et la perte causée par les roues du tracteur.

En se basant sur ces données, on peut établir une analyse du coût d'opportunité selon laquelle le risque d'une fausse décision (telle que par example la recommandation de moyens de contrôle infructueux ou l'omission de moyens profitables de contrôle) peut être estimée une fois établis l'évaluation de l'augmentation du rendement et le coût des mesures de contrôle de la maladie. On a également étudié les différences d'augmentation de rendement des variétés d'orge et de certains facteurs d'influence sur le blanc.

INTRODUCTION

Many workers have shown that certain xylem-translocated fungicides, applied as seed treatments or as single sprays to the growing crop, can give effective control of powdery mildew (Erisyphe graminis) on barley and consequent yield increases. Within the context of a single trial or a series of trials many of the responses obtained have been considered statistically significant at some chosen probability, while others have not. These results however do not, in themselves, indicate the likely profitability of a proposed disease control measure (Lester 1971). An answer to this common advisory question may be provided by a risk analysis in which the expected opportunity costs of making the wrong decisions, i.e. recommending unprofitable control and not recommending profitable control, can be balanced against each other.

In this paper we illustrate such a risk analysis with data on barley mildew control from south-east Scotland, following the neo-classical approach of Wald (1950). Dillon and Officer (1971) have compared this approach with the classical or sampling theory approach, in which only the possibility of recommending unprofitable control would be assessed and then only at the break-even point.

YIELD, RESPONSE AND COST DATA

Since 1969 trials have been made in south-east Scotland with ethirimol as a seed treatment on barley cultivars of differing susceptibility to mildew to determine their potential response to mildew control, both in small plots, where high dose rates (1.7 - 3.1 kg a.i./ha) were necessary, and in larger evaluation plots when the fungicide was applied at the commercial rate (0.63 kg a.i./ha). The results for Golden Promise in these trials, some of which have already been reported (Gilmour 1971), are summarised in Table 1.

Table 1

Golden Promisegrain yields and responses to ethirimol seed treatment, 1969-72

	number of trials	untreated yield (t/ha at 85% d.m.)	yield response (kg/ha)	percentage response	response range (kg/ha)
1969	5	4.42	120	2.7	-20 to 590
1970	5	4.21	520	12.4	280 to 790
1971	8	4.54	830	18.3	460 to 1460
1972	3	4.78	770	16.1	290 to 1200
1969-72	21	4.49	560	12.5	-20 to 1460
1970-72	16	4.51	710	15.7	280 to 1460

The 1970-72 average untreated yield is close to that estimated for the East Central and South East regions of Scotland, weighted by barley acreage, over the past 3 years, 4.57 t/ha (DAFS 1971, 1972). In 1969 mildew was not at all prevalent owing to generally unfavourable weather and a zero average response was obtained from the four trials located in typical barley growing areas. The response of 590 kg/ha was obtained in a trial on one of the School of Agriculture farms at an altitude of 200 metres which sustained a late attack of mildew. The standard deviation of the

1969-72 average response was 405 kg/ha while that for the 1970-72 average was 320 kg/ha, including variation between sites and between years. These correspond to coefficients of variation of 9 and 7 per cent respectively of the mean untreated yields.

Trials have also been carried out with chloraniformethan, ethirimol, tetrachloroquinoxaline and tridemorph using commercial sprayers on farm crops of Golden Promise. In some trials responses similar to those given by ethirimol seed treatment were obtained from single spray applications, but in general the level of response was lower (Table 2), indicating the benefit of prolonged protection by seed treatment.

Table 2

Golden Promise grain yields and responses to single applications of sprayed fungicides, 1970-72

	number of trials	untreated yield (t/ha at 85% d.m.)	mean best response (kg/ha)	mean response to all sprays up to GS 10.1 (kg/ha)	response range to all sprays up to GS 10.1 (kg/ha)
1970	2	4.63	595	280 (3 occasions)	-310 to 840
1971	2	5.12	530	290 (5 occasions)	50 to 700
1972	4	5.54	570	420 (11 occasions)	190 to 770
1970-72	8	5.10	565	330 (19 occasions)	-310 to 840

The mean untreated yield for 1972 includes a result of 7.31 t/ha obtained on a School of Agriculture farm following weather almost ideally suited to the soil type and altitude. Without this result the means would be 4.95 t/ha for 1972 and 4.90 t/ha over the 3 year period. The response of -310 kg/ha was obtained in a trial affected by drought and in which there was unusually great within-plot variation.

In 1970 and 1971 the greatest responses were obtained from applications made at the onset of heading, even when wheeling damage was taken into account (Gilmour 1971). In 1972 in one trial much earlier applications, at growth stage 6-7 (Feekes scale, Large 1954) when there were only extremely low levels of mildew in the crop, gave the best results; another trial followed the 1970-71 pattern; while in three others the wheeling losses with late applications (GS 10) made them less effective than earlier ones (GS 6-8). In all 8 trials the best responses were obtained from applications made at GS 10.1 or earlier.

In 4 trials sprays were applied at GS 6-7 (the latest stage at which a herbicide can be safely used) and gave a mean response of 225 kg/ha with a standard deviation of 445 kg/ha. This mean includes the loss of 310 kg/ha from one trial in 1970; the mean response from the 3 other trials was 410 kg/ha with a standard deviation of 265 kg/ha. On only one occasion did such an early application give the best response.

Wheeling losses have been assessed in these spraying trials as the difference between the overall crop yield and the yield of untracked strips cut from the sprayed plots. In 1972 losses calculated for a 12m sprayer boom averaged 65 kg/ha for early applications (GS 6-7 on 4 occasions) and 140 kg/ha for later applications (GS 8-10.1 on 10 occasions). This latter figure can probably be reduced to about 70 kg/ha if the fungicide application follows the tracking pattern of an earlier herbicide application. These estimates are comparable with those from earlier trials (Gilmour 1971).

Sieving tests have shown that increase in grain size has been the principal source of yield response to fungicidal control of mildew, 10-20 per cent more grain being retained on a 2.5 mm sieve in some of these trials. This improvement in quality can of itself increase the value of a grain sample, but this factor has not been taken into account in the present analysis.

In 1973 ethirimol seed treatment cost £5.29 per hectare. The most commonly used sprayed fungicides averaged £3.50 per hectare, to which should be added £2.10 per hectare for application when the fungicide is not applied with a herbicide.. The likely price of barley is a major factor. Malting barley is traditionally purchased at harvest in south-east Scotland and the prices have been £28 per tonne in 1970, £25 per tonne in 1971 and £26.50 in 1972. This year an appropriate price would now be £50 per tonne, but this was not forecast at the time when barley growers were having to make decisions about mildew control. Accordingly we have priced barley at £35 per tonne for 1973, this being the price quoted in the London grain market during January 1973 for barley to be harvested in September 1973. The future price during May and early June was similar.

The costs of mildew control in several situations and the corresponding estimated responses are summarised in Table 3. The mean response to the 4 earliest applications (GS 6-7) may be taken as an estimate of the response to a fungicide applied with a herbicide, while the mean best response may give an indication of the likely response when an application is made in accordance with an appropriate forecast.

Table 3

Summary of 1973 costs and estimated responses expressed in kg/ha

	fungicide	application	wheeling loss	break-even response	estimated response	standard deviation
seed treatment						
1969-72	150	-	-	150	560	405
1970-72	150	-	-	150	710	320
sprays						
GS 6-7	100	-	-	100	225	445
low wheeling losses						
mean best	100	60	65	225	565	225
all up to GS 10.1	100	60	65	225	330	275
high wheeling losses						
mean best	100	60	140	300	565	225
all up to GS 10.1	100	60	140	300	330	275

During 1970-72 all responses to ethirimol seed treatment exceeded the break-even response. In 1969 only one response out of 5 exceeded the break-even value. Three of the responses to the 4 earliest sprays exceeded the appropriate break-even response. All of the responses contributing to the mean best response, i.e. the best response in each trial, exceeded the break-even value corresponding to the lower level of wheeling losses, while in 7 of the 8 trials the best response also exceeded that corresponding to the higher level of wheeling losses. Of the 20 sprays applied up to GS 10.1, 5 failed to give responses greater than the lower break-even response, while 7 of the responses did not exceed the higher break-even value. It must be emphasised that the wheeling losses included here are for an effective sprayer boom width of 12 m. Some contractors' sprayers have 16 m booms while many farm sprayers are only 9 m wide.

THE NEO-CLASSICAL RISK ANALYSIS

The risk, i.e. the expected opportunity cost, of making a wrong decision (recommending unprofitable control or not recommending profitable control) may be considered as the product of the actual opportunity cost at a particular level of response and the probability of obtaining that response. The loss curves in Fig. 1 illustrate the opportunity costs for the present problem when the true yield response to treatment, R, is allowed to range from -200 to 800 kg/ha, with a break-even response, Rb, of 225 kg/ha. Because the cost per kilogram is constant the loss curves are linear and symmetric.

The response to treatment can be adequately represented by a normal distribution and so the probability density around any specified response can be obtained from the ordinates of the standard normal distribution, given an estimate of the mean response to treatment, Rt, and the standard deviation of that estimate (Fisher and Yates 1963). The probability curve in Fig. 2 has been drawn for an estimated mean response of 565 kg/ha with a standard deviation of 225 kg/ha, the values for the mean best spray in a low wheeling loss situation. The values plotted were obtained from the "corresponding ordinate" column of Table 4 in which the full calculations are illustrated. In practice, we have used a simple computer program which tabulates values for the risk curves (Fig. 3) directly, together with an estimate of the area under each.

Table 4

Calculation of point expected opportunity costs for an estimated mean response of 565 kg/ha with a standard deviation of 225 kg/ha

true response (kg/ha)	associated opportunity cost (£/ha)	deviation from mean response	normal standard deviate	corresponding ordinate	expected opportunity cost (£/ha)
unprofitable control recommended					
0	7.88	-565	-2.51	0.017	0.13
200	0.88	-365	-1.62	0.107	0.09
225	0	-340	-1.51	0.127	0
profitable control not recommended					
225	0	-340	-1.51	0.127	0
400	6.13	-165	-0.73	0.305	1.87
600	13.13	35	0.16	0.394	5.17
800	20.13	235	1.04	0.231	4.65
1000	27.13	435	1.93	0.062	1.67

point expected opportunity cost = associated opportunity cost x corresponding ordinate

The optimum decisions are to recommend fungicidal control if the true response, R, exceeds the break-even response, Rb, and not to recommend treatment if R is equal to or less than Rb. However, as we do not know the true or population value of R, but have only an estimate of it, Rt, the problem must be analysed in probabilistic terms. Decisions on the basis of the risk curves in Fig. 3 may be made by the minimax criterion of choosing that act which has the smallest maximum risk, following the approach of Wald (1950). Thus for the three situations illustrated in Fig. 3 we

Fig. 1 OPPORTUNITY COST

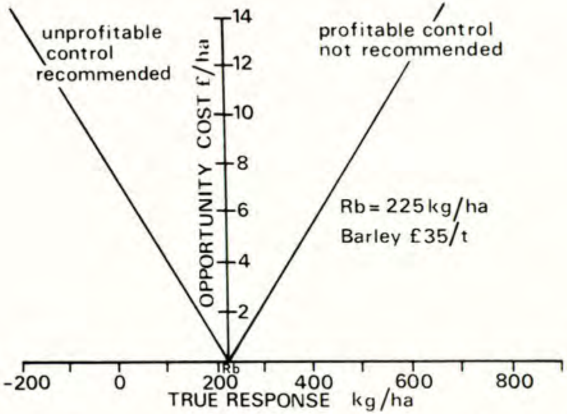


Fig. 2 PROBABILITY DENSITY

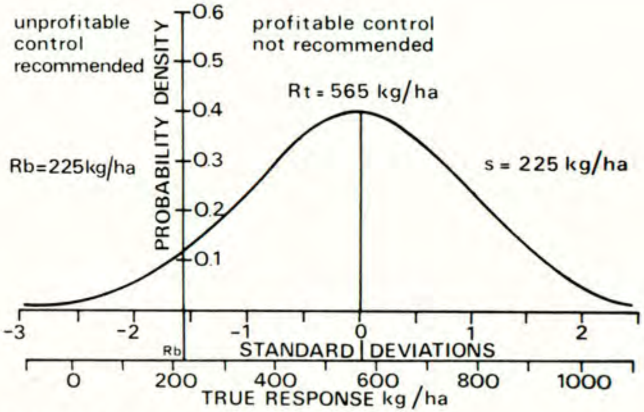
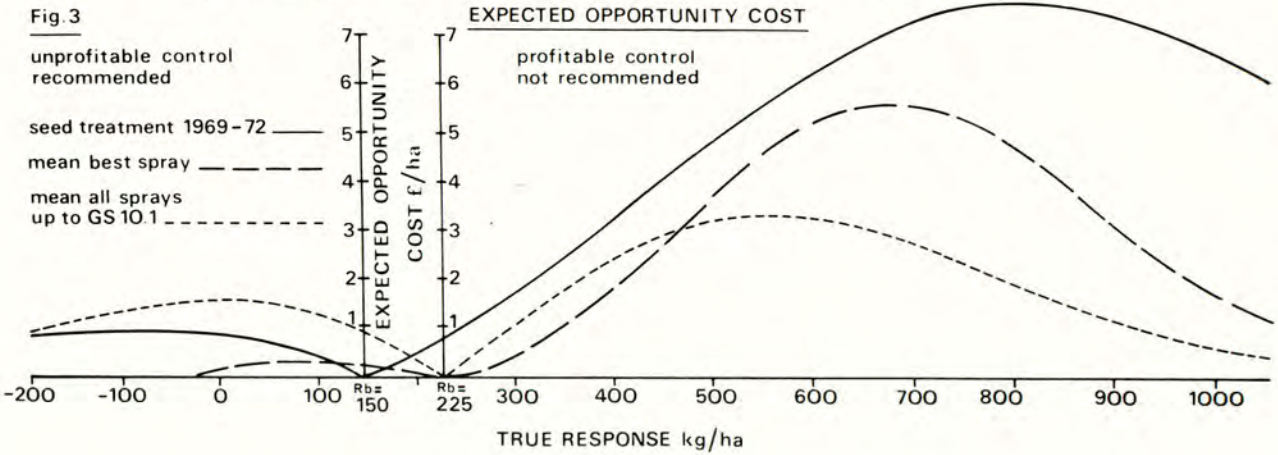


Fig. 3 EXPECTED OPPORTUNITY COST



should recommend disease control because the decision to recommend control when it is not profitable has a smaller maximum risk than the alternative decision of not recommending profitable control in all 3 cases. Another approach is to choose on the basis of minimising the total expected risk, i.e. choosing the act with the smallest area under its risk curve. For the examples in Fig. 3 this criterion would lead to the same decisions as the minimax criterion. The maximum and total risks for the range of situations covered by Table 3 are tabulated in Table 5.

Table 5

Maximum and total risks in various mildew control situations

	unprofitable control recommended		profitable control not recommended	
	maximum risk (£/ha)	total risk (£/ha)	maximum risk (£/ha)	total risk (£/ha)
seed treatment				
1969-72	0.92	1.11	7.61	15.45
1970-72	0.17	0.13	8.88	19.73
sprays				
GS 6-7	2.78	4.28	4.89	8.59
low wheeling losses				
mean best	0.20	0.18	5.57	12.07
all up to GS 10.1	1.52	2.23	3.29	5.91
high wheeling losses				
mean best	0.38	0.42	4.66	9.68
all up to GS 10.1	2.07	3.29	2.58	4.33

The relationship between the maximum risk and the total risk is a reflection of the relative size of the standard deviation of the estimated mean response and serves to emphasise the desirability of using only well-founded estimates of the response to treatment in calculations of this kind. This is especially important when the associated opportunity costs are very high.

While we are, at this stage, principally concerned with the balance of risk between the alternative decisions within a given situation, the differences between the risks associated with the mean best spray response and the response to all preheading sprays, may be taken as an indication of the potential value of a method for forecasting the best time of spray application.

CULTIVAR RESPONSE

Differences in the responses of spring barley cultivars to the control of mildew have been reported by several workers (Doodson and Saunders 1969, Gilmour 1971, Little and Doodson 1971, 1973), and in general these follow the pattern of cultivar susceptibility to mildew. Preliminary trials to determine whether control would alter the yield ranking of cultivars in the list of Varieties of Cereals Recommended for Scotland issued by the Scottish Agricultural Colleges were made in 1972 when single small plots of 8 cultivars were grown with and without ethirimol seed treatment (2.4 kg a.i./ha) alongside barley cultivar trials. The results, based on 5 harvested trials, are given in Table 6.

Table 6

Mean yields and responses to ethirimol treatment of 8 cultivars, 1972

cultivar	resistance score*	untreated yield (t/ha at 85% d.m.)	response (kg/ha)	ranking untreated	ranking treated	ranking change
Julia	6	5.65	45	3	6	-3
Hassan	6	5.52	135	6	8	-2
Midas	5	5.77	345	1	2	-1
Zephyr	5	5.76	325	2	3	-1
Gerkra	4	5.47	280	7	5	+2
Sultan	4	5.64	335	4	4	0
Ymer	2	5.21	475	8	7	+1
Golden Promise	1	5.53	600	5	1	+4

* 1973 Recommend List for Scotland (9 = resistant, 0 = susceptible)

The mean response of Ymer is considerably greater than that recorded in an earlier series of trials (Gilmour 1971), while that for Julia is very much less. The cultivars which were the most susceptible to mildew showed the greatest responses to its control, to such an extent that the ranking order of the cultivars for yield was markedly altered. In contrast, in trials in England and Wales, mildew control had little effect on ranking order (Little and Doodson 1971, 1973). These responses have been used to construct risk curves for mildew control on these cultivars by ethirimol seed treatment, taking a break-even response of 150 kg/ha and a standard deviation of the estimated responses of 320 kg/ha. The maximum and total risks are given in Table 7. These risk analyses show clearly the balance in favour of controlling mildew on all of these cultivars except Julia and Hassan.

Table 7

Maximum and total risks for mildew control on 8 cultivars

cultivar	unprofitable control recommended: maximum risk (£/ha)	unprofitable control recommended: total risk (£/ha)	profitable control not recommended: maximum risk (£/ha)	profitable control not recommended: total risk (£/ha)
Julia	3.66	6.49	1.89	2.82
Hassan	2.83	4.68	2.58	4.16
Midas	1.32	1.81	4.58	8.63
Zephyr	1.44	2.01	4.37	8.13
Gerkra	1.72	2.50	3.91	7.05
Sultan	1.38	1.91	4.47	8.37
Ymer	0.72	0.86	6.02	12.23
Golden Promise	0.36	0.36	7.51	16.10

In the trials reported by Little and Doodson (1971, 1973) the response of the cultivars to mildew control was conditioned by their susceptibility to brown rust (Puccinia hordei) where this disease occurred. For example, in 4 trials where brown rust did not occur the mean response of Midas was 540 kg/ha, while the average response over all 18 trials was 330 kg/ha (Little and Doodson 1973). From these results it may be inferred that the mean response of Midas in the presence of brown rust was only 270 kg/ha and this value should be entered in the risk analysis for this situation. Similar adjustments should be made for other diseases whenever appropriate information is available (Gilmour 1973a).

OTHER FACTORS INFLUENCING THE RESPONSE

The response to mildew control, and hence the risk associated with a particular control measure, is primarily a reflection of the incidence of mildew. Some of the factors influencing this incidence in south-east Scotland have recently been reviewed (Gilmour 1973a). Thus large responses may be expected where spring barley is sown late in relation to nearby spring crops or is grown adjacent to winter barley or when there is a sequence of particularly favourable weather. If the likely disease incidence can be predicted the formula derived by Large and Doling (1962) may give an indication of the expected losses, i.e. potential responses. It must be recognised, however, that 25 per cent leaf area infection was the highest value used by Large and Doling while more severe infections are now common, and that their data related to Proctor, a cultivar that has given a different pattern of response from most other cultivars in recent trials (Little and Doodson 1971, 1973).

Late sowing usually leads to a more severe mildew attack (Last 1957), although this is not invariably the case (James 1969, Melville and Lanham 1972). In one trial in 1971 a response to ethirimol seed treatment of 1460 kg/ha was obtained from Golden Promise which had been sown 3 weeks later than the surrounding farm crop of Golden Promise in which mildew was not controlled (Table 1). The effect of proximity to winter barley is likely to be similar, as in both situations there will be locally high levels of inoculum at a relatively earlier growth stage of the later-sown spring barley crop. Appropriate estimated responses may be entered in the risk analyses for these situations although it is hoped that the winter barley problem in south-east Scotland will soon be greatly reduced (Gilmour 1973b).

The influence of a given sequence of weather on the estimated responses to treatment may be predicted from a knowledge of its likely effect on mildew incidence and a risk analysis made appropriate to that situation. There remains, however, the problem of assessing the likely occurence of such a sequence of weather. Forecasting systems which are currently under development (Polley and King 1973, Smith 1973) may prove satisfactory for predicting the optimal time for spray application, but such systems will not necessarily also indicate whether the resultant yield responses will be profitable as the final level of disease is dependent of the weather after the infection forecast has been made. In addition, these forecasting systems are of no relevance to seed treatment mildew control. We are currently examining the possibility of using a games theory approach to treat predicted weather patterns as a series of events against which the various possible mildew control measures can be considered. The expected opportunity costs obtained from the risk analyses presented here will be used as basic entries in the pay-off matrix of actions against events, to produce disease control strategies which illustrate different approaches to risk.

Acknowledgements

We wish to thank the many colleagues in the School of Agriculture who undertook much of the trials work, the farmers who made available the sites and the chemical manufacturers who provided the fungicides.

References

DAFS (1971) Agricultural Census June 1971. Summary for counties, regions and Scotland. Department of Agriculture and Fisheries for Scotland, Economics and Statistics Unit, Edinburgh.

DAFS (1972) Agricultural Census June 1972. In the press.

DILLON J.L. and OFFICER R.R. (1971) Economic versus statistical significance in agricultural research and extension: a pro-Byesian view. Farm Economist 12, 31-46

DOODSON J.K. and SAUNDERS P.J.W. (1969) Observations on the effects of some systemic chemicals applied to cereals in trials at NIAB. Proceedings 5th British Insecticide and Funicide Conference (1969) 1, 1-7

FISHER R.A. and YATES F. (1963) Statistical Tables, Edinburgh and London, 1963.

GILMOUR J. (1971) Fungicidal control of mildew on spring barley in south-east Scotland. Proceedings 6th British Insecticide and Fungicide Conference (1971) 1, 63-74.

GILMOUR J. (1973a) Decision factors in barley mildew control in south-east Scotland. In: Economic and social values in the assessment of crop protection and pest control measures. Society of Chemical Industry Monograph. In the press.

GILMOUR J. (1973b) Winter barley must go. East of Scotland College of Agriculture Technical Note 20C.

JAMES W.C. (1969) A survey of foliar diseases of spring barley in England and Wales in 1967. Annals of Applied Biology 63, 253-263.

LARGE E.C. (1954) Growth stages in cereals. Plant Pathology 3, 128-129

LAST F.T. (1957) The effect of date of sowing on the incidence of powdery mildew on spring-sown cereals. Annals of Applied Biology 45, 1-10.

LESTER E. (1971) Cereal leaf disease control: practicable and economic considerations. Proceedings 6th British Insecticide and Fungicide Conference (1971) 3, 643-647.

LITTLE R. and DOODSON J.K. (1971) The comparison of yields of some spring barley varieties in the presence of mildew and when treated with a fungicide. Proceedings 6th British Insecticide and Fungicide Conference (1971) 1, 91-97.

LITTLE R. and DOODSON J.K. (1973) The reaction of spring barley cultivars to mildew, their disease resistance rating and an interim report on their yield response to mildew control. Journal of the National Institute of Agricultural Botany 12, 447-448

MELVILLE S.C. and LANHAM C.A. (1972) A survey of leaf diseases of spring barley south-east England. Plant Pathology 21, 59-66.

SMITH L.P. (1973) Meteorological Office Communication.

WALD A. (1950) Statistical Decision Functions, New York 1950.

Proceedings 7th British Insecticide and Fungicide Conference (1973)

THE PROBLEM OF FUNGICIDE TOLERANCE IN THE FIELD

M. S. Wolfe
Plant Breeding Institute, Trumpington, Cambridge CB2 2LQ.
A. Dinoor
Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot, Israel.

Summary Surveys of populations of *Erysiphe graminis* on barley (barley powdery mildew) revealed the presence of various levels of tolerance to the fungicide, ethirimol. The frequency of occurrence of tolerant forms was greatest in crops treated with ethirimol, less in adjacent fields and least in fields isolated from treated crops.

In an attempt to reduce the frequency of tolerant forms in the pathogen population at the beginning of the spring season, a recommendation was made to avoid ethirimol treatment of winter barley. This would reduce the size of the bridge between successive treated spring crops, upon which tolerant forms could survive without competition, and thus delay the onset of spring epidemics on treated crops.

Inefficiency of a fungicide in the field could be due to several factors including high frequency of tolerance in the pathogen population. It is recommended that all fungicide-pathogen combinations should be monitored since the early detection of failures due to tolerance can be determined only in this way.

Resumé Au cours de recensements des populations d'*Erysiphe graminis* contaminant l'orge (mildiou poudreux de l'orge) des niveaux variés de tolérance pour le fongicide éthirimol se révélèrent. La fréquence des formes tolérantes fut la plus importante dans les cultures traitées à l'éthirimol, moindre dans les champs avoisinants et moindre encore dans les champs isolés des cultures traitées.

Dans le but de reduire le nombre des formes tolérantes dans la population atteinte, il est recommandé d'éviter de traiter l'orge d'hiver à l'éthirimol. Ceci devrait reduire la connexion entre les successives cultures de printemps traitées, permettant aux formes tolérantes de survivre sans compétition et par ce fait devrait retarder le début des épidémies de printemps dans les cultures traitées.

L'inéfficacité d'un fongicide dans un champ peut être dû à plusiers facteurs, en particulier à la haute fréquence de

tolérance dans la population atteinte. Il est recommandé que toutes les combinaisons fongicide-maladie soient enregistrées, car c'est seulement de cette façon que la détection précoce des échecs dûs à la tolérance peut être determinée.

INTRODUCTION

Some of the effects of the large-scale introduction of fungicides on pathogen populations were discussed previously (Wolfe, 1971). It was suggested that monitoring for tolerance should follow closely the introduction of the fungicides so that early information could be obtained on the occurrence and rate of increase of tolerant forms of the pathogen involved. Action could than be taken to modify the situation.

It was also pointed out that the early stages of development of tolerance in pathogen populations would be difficult to detect because of the initial low frequencies of tolerant forms. The detection might be particularly difficult in situations involving systemic fungicides where a gradual decline in fungicide levels in the host during the growing season might encourage the build-up of pathogen forms with low levels of tolerance.

Following a consideration of these problems, and the development of appropriate techniques, monitoring of tolerance to ethirimol was included in 1973 in large-scale field surveys of variation in populations of Erysiphe graminis DC., the causal pathogen of barley powdery mildew.

METHODS AND MATERIALS

In the population survey, a total of more than 10,000 single colony isolates was obtained from ten field sites in East Anglia. The fields could be arranged in groups according to whether they were isolated from ethirimol treatment, adjacent to treated crops, or treated. Varieties at the same site were generally sampled from adjacent fields. Minimum field size was 6 ha and most were sampled on two occasions.

Other isolates, obtained from the Physiologic Race Survey, ADAS and other sources, were also investigated.

In the population survey, sets of 50 single colony isolates from upper leaves were collected at each of four points along a central axis across each field. The points were fixed along the axis at increasing distances from the border separating adjacent fields under survey.

Single colony isolates were considered to be stable, genetically pure lines, but there was evidence of mixtures in a small proportion of the colonies. After collection the isolates were maintained on 2 cm seedling leaf segments of either cv. Proctor or cv. Golden Promise, kept in closed polystyrene boxes containing 0.5% agar incorporating 150 ppm benzimidazole. The boxes were kept in controlled environment cabinets at 5-8°C with 8h light, 16h dark.

For testing, spores from the maintenance stocks were inoculated using a paintbrush on to the adaxial surface of the distal two-thirds portion of detached seedling leaves (cv. Proctor) which had been previously treated with 50% a.i. col formulation of ethirimol at either nil, 50, 200 or 400 ppm on the seed. These doses were considerably lower than the recommended commercial field rate of approximately 4000 ppm a.i. Low doses were selected in order to reveal the population size of tolerant forms and to simulate field conditions, i.e. exposure of the pathogen mostly to levels of ethirimol usually less than those experienced immediately after seedling emergence. A small proportion of the tolerant population was capable of growth on leaves derived from seed treated with 4000 ppm a.i. However, the relationship between the seed dose range and the actual amount of effective material in the leaves is unknown.

The detached leaves, which were inoculated simultaneously with three or four fungal isolates along the length of the lamina, were maintained on benzimidazole agar as above, but at 15°C. Mildew development was assessed 10 days after inoculation. Inoculation with several isolates along the leaf allowed comparison between isolates, which gave an indication of the reliability of a particular test, and conserved space.

Assessments were made on a 0-9 scale and the isolates classified as either sensitive (nil, or some growth on leaves from seed treated at 50 ppm ethirimol), intermediate (decreasing growth at 50, 200 and 400 ppm), or tolerant (more or less unrestricted growth at all concentrations).

RESULTS

The overall frequencies of sensitive, intermediate and tolerant isolates in the three groups of fields is shown in Fig. 1.

Fig. 1

Overall frequencies of sensitive, intermediate and tolerant isolates in fields isolated, adjacent to treated, and treated with ethirimol

The pattern indicates that tolerant forms have increased in frequency following the use of ethirimol but that they have not yet become sufficiently frequent or competitive to dominate the pathogen population, even on treated crops.

The overall data indicate a gradient of decreasing frequency of tolerant forms with increasing separation from a treated area. Gradients in frequency of tolerance could be detected within some fields: examples are shown in Table 1.

Table 1

Gradients of ethirimol tolerance within fields: the source of tolerant inoculum was adjacent to sampling spot 1

	Rel. frequency of isolates with tolerance at each sampling spot			
Site, variety and date	1	2	3	4
Fersfield, Julia, 23 May	40	57	25	0
Fersfield, Julia, 14 June	60	33	17	17
Hopton, Julia, 6 June	40	31	14	0
Cockley Cley, Mosane, 17 May	23	15	5	6

The persistence of gradients relatively late into the season indicated competition for space on uninfected leaf area between tolerant and sensitive isolates. The distribution of tolerance could serve as a marker for inoculum of local origin competing with external, sensitive, inoculum. The relative competitive ability of the tolerant and sensitive forms was not determined because no data were available for the relative size of the inoculum sources which contributed to the observed gradients.

By comparing gradients and frequencies between varieties, however, it became evident that there was an interaction between variety and the degree of competitive success of the tolerant forms. This appeared to be related to the relative resistance of varieties to pathogen isolates carrying the Sultan virulence gene. Table 2 illustrates the comparison between the relative frequencies of sensitive, intermediate and tolerant forms at Fersfield on cv. Julia, which supports only a low frequency of Sultan-virulent isolates, and cv. Maris Mink, upon which all isolates are Sultan-virulent.

Table 2

Relative frequencies of ethirimol tolerance and Sultan-virulence on two fields each of cvs. Julia and Maris Mink at Fersfield

	23 May				14 June			
	Sens	Int	Tol	Sult-vir	Sens	Int	Tol	Sult-vir
Julia	70	20	10	18	72	26	2	7
Maris Mink	52	32	16	100	53	27	20	100
		P = .2 - .3					P < .001	

The data in Table 2 indicate that the relationship to the Sultan-virulence gene is closer with the isolates classified as 'tolerant' than with those classified as 'intermediate' since the frequencies of both 'tolerant' and Sultan-virulent isolates declined at about the same rate on Julia, but remained approximately constant on Maris Mink, over the period indicated. The relationship between 'intermediate' and Sultan-virulent isolates is less clear.

The relationship between tolerance and Sultan-virulence was also evident at other sites, e.g. North Creake and Cockley Cley, as shown in Table 3.

Table 3

Relative frequencies of sensitive, intermediate and tolerant forms of the pathogen amongst isolates virulent or avirulent on Sultan

Site and variety	Sultan	Sensitive	Intermediate	Tolerant	
North Creake,	virulent	17	21	8	P <.001
Proctor	avirulent	36	15	3	
Cockley Cley,	virulent	19	11	0	P <.001
Mosane	avirulent	63	7	0	

A similar trend was apparent on cv. Julia in different situations (Table 4) where there was a decline in the frequency of 'tolerant' forms with time, which may have been related to a decline in the frequency of Sultan-virulence. The comparisons, however, are not statistically significant because of scarcity of data.

Table 4

Relative frequencies of ethirimol tolerance in the pathogen population on cv. Julia in fields isolated from, adjacent to, or in ethirimol-treated crops (sampling dates separated by three weeks)

		Sensitive	Intermediate	Tolerant
'Isolated'	Date 1	90	7	3
(Langham)	Date 2	90	10	0
'Adjacent'	Date 1	69	23	8
(Fersfield)	Date 2	73	25	2
'Treated'	Date 1	32	47	21
(Hopton)	Date 2	82	18	0

The distribution of tolerant isolates on the untreated crop of cv. Mosane at Cockley Cley, illustrated in Tables 1 and 3, demonstrated the ability of tolerant isolates to survive and overwinter. The cv. Mosane crop was surrounded by woodland, except on the south side, where the area was being cropped with parsnips. The tolerant isolates followed a gradient from the highest frequency at the south, parsnips, end of the field, to lowest at the north (Table 1), and tolerance was more frequent in the Sultan-virulent fraction of the population than in the Sultan-avirulent fraction (Table 3).

The source of the tolerance in 1973 was not, at first, obvious, but it emerged that the area sown with parsnips in 1973 had been cropped with ethirimol-treated Maris Otter in 1972. A search in the field borders of the parsnips revealed mildew-infected volunteer plants: isolates from these were found to be mainly both ethirimol-tolerant and Sultan-virulent. This suggested that tolerant forms over-wintered successfully on volunteer plants and influenced the epidemic which developed early in cv. Mosane. It is possible, of course, that the mildew infection on the volunteer plants in 1973 came from earlier volunteers, which were responsible for both infections.

The population survey data were obtained mainly from Norfolk and Suffolk, an area in which both winter barley and ethirimol usage are common. However, since isolates exhibiting ethirimol tolerance were obtained from sites as far apart as Cambridge, Rosemaund and Ardglass in County Down, it was evident that the phenomenon of ethirimol tolerance was not uncommon. Isolates from other sites were all ethirimol-sensitive. Insufficient data were available during the summer of 1973 to determine the general distribution of ethirimol tolerance in the United Kingdom.

DISCUSSION

The mechanism of tolerance

The results obtained from the ethirimol tests showed that the populations of isolates were variable in the extent of tolerance. It was evident from the raw data that sub-divisions of tolerance existed within the 'intermediate' grade and probably also within the 'tolerant' grade. There were also different forms of tolerance apparent, for example, some isolates exhibited a gradually decreasing ability to grow on leaves treated at increasingly higher concentrations, whereas others grew successfully at the lower concentrations and not at all at the high concentrations. These observations suggested that more than one mechanism may be involved in ethirimol tolerance, affecting both the kind and degree of tolerance.

It was not possible to subdivide further the classes of tolerance because of variability in the experimental system. Since the test seedlings were glasshouse-grown at weekly intervals over several months, it was impossible to regulate the amount of ethirimol taken up by individual leaves, or the amount available at points along the length of the leaf.

Variation in ethirimol content is, of course, present in the treated crop and probably to a much greater extent. During the course of a growing season ethirimol uptake varies with climatic and edaphic conditions, and distribution within the plant is dependent on the changing volume of the root system and the aerial organs. Variation within and between host plants in the field was probably the major reason for the variability encountered in the pathogen populations.

The frequency of tolerance in populations

Clearly, since the introduction of ethirimol on a commercial scale, the frequency of tolerant forms of the pathogen has increased in the general pathogen population. The rate at which this has occurred, and at which it may occur subsequently, depends on various factors, the most important of which are probably the ability of the different tolerant forms to survive and compete, both with each other and with sensitive forms, and the amount of ethirimol which is used.

The magnitude and importance of the competitive ability of tolerant forms is as yet unknown. Competitive ability seems to be associated, to some extent, with Sultan-virulence, but the reason for this is also unknown. Tolerant forms appeared to accumulate and compete effectively in untreated crops of cv. Maris Mink, a variety which possesses the Sultan resistance gene and which is susceptible, therefore, only to Sultan-virulent isolates. Tolerant forms were less frequent on untreated crops of cv. Julia, and decreased with time; these effects appear to be associated with the low frequency of Sultan-virulence on this variety.

If, however, the pathogen population is subjected to continuous high selection pressure from the fungicide, then it is likely that the tolerant fraction of the population will increase and become better fitted to survival and competition in the population.

The effect of tolerance in the population

The main action of ethirimol is to delay the onset of the mildew epidemic to the extent that the amount of mildew which is able to develop towards the end of the season has little effect on yield and quality. However, if the frequency of tolerant forms increases, as indicated above, then the delay of the epidemic will be gradually lessened until there is no further economic benefit from treatment. The situation is analogous to that of the newly introduced gene for host resistance (Van der Plank, 1968).

In some situations observed in 1973, where tolerant forms were identified, it appeared likely that there would be little or no economic gain from the use of ethirimol. In other situations, following very late epidemic development, it seemed that the effectiveness of ethirimol control would be little impaired, if at all. The difference between these situations was presumably due to differences in local conditions affecting the build-up of tolerance.

Brooks (1972), studying the effect of mildew on yield by using ethirimol, confirmed earlier work in showing that early epidemics had the most severe effect on yield. Thus, preventing orslowing-down the early build-up of inoculum has the dual purpose of containing the multiplication of tolerant forms and thereby holding back the worst influence of mildew on yield.

Tolerance and winter barley

A major feature of barley cultivation which encourages the increase in frequency of tolerant forms is ethirimol treatment of winter barley. Treated winter barley acts as a bridge between successive treated spring crops allowing tolerant forms to survive in a non-competitive situation and increase the amount of tolerant inoculum available in the spring to generate new epidemics on treated spring crops. This effect was particularly noticeable in two separate field situations in Norfolk (Burnham Market and East Harling: data not available) where treated winter and spring crops were grown in adjacent field areas. In each case it was evident that severe mildew on the winter crop, presumably with a high frequency of tolerant forms, had acted as a reservoir of inoculum for the treated spring crop, which then acted as a further screen for the early multiplication of the tolerant forms. Because of the early onset of mildew it was difficult to distinguish either crop from severely infected, untreated crops.

Because of this influence of treated winter barley on the population structure, it was recommended to ICI (Plant Protection Ltd), the manufacturers of ethirimol, that the use of the fungicide on the winter crop should be stopped. By adopting this strategy, of removing the selective medium, i.e. ethirimol-treated barley, at the time when the mildew population is at its smallest size, it was felt that the population structure would be most affected for the least loss in fungicide control and the amount of tolerant inoculum available for epidemic initiation would be reduced.

The effectiveness of the strategy cannot be pre-judged because of lack of knowledge of the stage of development already achieved by the tolerant fraction of the population. Urgent and comprehensive studies are required during the coming months to monitor the situation as it develops further and to determine whether further strategies of use could be employed, for example, varietal recommendations for treatment based on the resistance, or lack of it, of particular varieties to the Sultan virulence gene.

In addition to relieving selection pressure in the winter by not using ethirimol in this period, it is also essential to reduce the size of the over-wintering mildew population by all means possible. Ideally, varieties and fungicides would be used on the winter crop which differed, respectively, in their resistance and mode of action, from those used in the spring crop. For the moment, however, it is necessary to use various methods of crop hygiene and fungicides other than ethirimol, to check the winter barley epidemic. It is important to stress that the role of controlling mildew on winter barley should now pass to a range of fungicides and not to only one. The widespread use of another single fungicide on winter barley, especially if it is also used on the spring crop would provide further selection pressure for the development of tolerant forms of the pathogen. It is also desirable that, where possible, a different fungicide is used on the winter and spring crops.

It seems to us that the level and distribution of tolerance to each fungicide in use should be monitored and that no single fungicide should be recommended for general use over wide areas of both winter and spring barley. From the little we know about the adaptability of fungi to new situations, a countrywide usage of one fungicide renders it very vulnerable to ineffectiveness.

Acknowledgements

The authors wish to acknowledge the technical help of Mrs. S. Wright, Miss L. Baker and Miss K. White, the provision of samples by the Physiologic Race Survey and ADAS, and the cooperation of ICI (Plant Protection Ltd).

References

BROOKS, D.H. (1972) Observations on the effects of mildew, *Erysiphe graminis*, on growth of spring and winter barley. *Annals of Applied Biology*, *70*, 149-156.

VAN DER PLANK, J.E. (1968) *Disease Resistance in Plants*. 206 pp, New York, Academic Press.

WOLFE, M.S. (1971) Fungicides and the fungus population problem. *Proceedings of the 6th British Insecticide and Fungicide Conference*, *3*, 724-734.

NOTES

Proceedings 7th British Insecticide and Fungicide Conference (1973)

THE EFFECTS OF SOME FUNGICIDES ON MILDEW OF SPRING BARLEY IN THE SOUTH-WEST OF SCOTLAND

A. G. Channon and A. G. Boyd

The West of Scotland Agricultural College, Auchincruive, Ayr KA6 5HW.

Summary In field trials in south-west Scotland, mildew infection of spring barley was slight in 1969 and 1970, but severe in 1971. In all three years, however, reductions in mildew and associated increases in yield were recorded in crops of the variety Golden Promise grown from seed treated with ethirimol. Several other less susceptible varieties also showed less mildew when grown from treated seed, but the yield gains were less than in Golden Promise.

Single foliar sprays with ethirimol, tridemorph or chloraniformethan reduced infection about equally, and generally increased yields of Golden Promise. Results suggest that in practice, in the presence of mildew, a fungicidal spray at Feekes Growth Stage 7-9 might be expected to give a worthwhile yield response.

Résumé Par suite des essais du champ dans le sud ouest d'Écosse, on constata que l'orge de printemps était legèrement atteinte par le mildiou en 1969 et 1970 mais sévèrement en 1971. Cependant en tous les trois ans des réductions du mildiou et par suite des augmentations du rendement se présentèrent dans des moissons de la varieté Golden Promise cultivées de graines traitées par ethirimol. Plusieurs d'autres varietés qui étaient moins sensibles avaient moins du mildiou quand elles étaient cultivées de graines traitées, mais les augmentations du rendement étaient moins de celui de Golden Promise.

Tous les coups de vaporisateur de feuilles simples avec ethirimol, tridemorph ou chloraniformethan reduisirent également l'infection et augmentèrent les rendements de Golden Promise. Les résultats suggèrent qu 'en présence du mildiou un vaporisateur de fongicide employé à l'Étape de Croissance 7-9 (Feekes) doit donner le plus grand rendement.

INTRODUCTION

Earlier advisory experience (Grainger, 1969) suggested that mildew of cereals (*Erysiphe graminis*) was a problem of relatively little importance in the south-west of Scotland. More recently, widespread cropping with the mildew-susceptible variety Golden Promise suggested that the use of the newly available systemic fungicides might be justified. Trials were therefore made at Auchincruive and at two other sites in 1969-71 to test their effects on mildew and grain yields.

METHOD AND MATERIALS

The trials were of randomized block design, with three to six replicates; plots were 40 x 2 m, unless otherwise stated.

All the seed was pre-treated with an organo-mercurial fungicide, over which ethirimol was applied, using a rotating drum. In 1969 and 1970 a wettable powder formulation of ethirimol (80% w.p.) was used, at a rate of approx. 12 g a.i./kg seed, while in 1971 a liquid 'col' formulation (500 g a.i./l) was applied at 4.5 ml a.i./kg seed (9 ml a.i./kg seed in Trial 3). At a sowing rate of approx. 188 kg/ha these represented applications of 2.24 and 0.84 kg ethirimol/ha.

Foliar applications of the various fungicides (as emulsifiable concentrate or 'col' formulations) were made with a Drake and Fletcher sprayer with six 000 Bray jets 30 cm apart, at a rate of approx. 450 l/ha.

Mildew was assessed as percentage leaf area infected (Anon., 1970; Anon., 1971) at Feekes Growth Stages 10.5 and/or 11.1 (Large, 1954), on the top three leaves, though in one trial assessment at G.S. 10.5 was made on leaf 3 only. Analysis of arithmetic and angular transformed values gave similar results, so only the former are quoted.

Harvesting was by combine harvester and yields were corrected to 15% moisture content.

RESULTS

Seed dressing/variety trials

Several trials were made to study the effect of ethirimol seed-dressings on different barley varieties, in which the seed was sown during late March to early April, and harvesting was in late August to early September.

Table 1

Effect of ethirimol seed dressings on mildew and yield of spring barley. Trials 1-4 1969-71

Variety	% mildew on top three leaves at G.S. 11.1		Grain yield (t/ha)	
	Nil	Ethirimol	Nil	Ethirimol
Trial 1. Auchincruive 1969				
Golden Promise	5.9	0.1	6.25	6.62
Ymer	1.0	0	5.23	5.27
Julia	0.1	0	5.65	5.46
S.E. (difference)	± 0.7		± 0.18	

Table 1 (cont'd)

Variety	% mildew on top three leaves at G.S. 11.1		Grain yield (t/ha)	
	Nil	Ethirimol	Nil	Ethirimol
Trial 2. Stranraer, Wigtownshire, 1970				
Golden Promise	3.0	1.1	5.30	5.45
Midas	1.6	0.9	5.15	5.38
S.E. (difference)	± 1.5		± 0.27	
Trial 3. Barbush, Renfrewshire, 1971				
Golden Promise	31.7	3.9	4.31	4.70
Ymer	42.1	9.4	3.99	4.19
S.E. (difference)	± 2.6		± 0.18	
Trial 4. Auchincruive, 1971				
Golden Promise	65.8	13.7	4.10	5.18
Ymer	54.9	11.6	4.28	4.88
Zephyr	44.2	3.5	5.16	5.46
Julia	39.6	2.7	5.00	5.17
Midas	34.3	6.2	4.85	5.60
Sultan	33.1	2.0	4.92	5.25
S.E. (difference)	± 4.7		± 0.20	

In 1969 and 1970 (Table 1) mildew infection was low, but in 1971 the disease was severe. In all these trials, however, and in another at Stranraer in 1969, the use of an ethirimol seed-dressing reduced the amount of disease on the treated crops. Disease control was associated with yield increase but the correlation was not close. There appeared to be variation in varietal response to mildew reduction; this may have been due to host-fungicide interaction, limitations in the method of mildew assessment, or some other factor.

Seed dressing/foliar spray trials

1970 trials

Two trials were made in 1970, in which crops grown from seed treated or untreated with ethirimol either received no further treatment or were subsequently sprayed with ethirimol (80% w.p.) at a rate of 2.24 kg a.i. in 450 l/ha. Trial 5 was sown on 24 April, sprayed on 18 June (at G.S. 9), assessed for mildew at G.S. 11.1 on 20-21 July and harvested on 13 September. The comparable dates for Trial 6 (plot size 7 x 2.6 m) were 3 April, 16 June (G.S. 8), 1-2 July and 29 August. The results are shown in Table 2.

Table 2

Effect of seed-dressing and foliar sprays on mildew and yield of spring barley. Trials 5 and 6. 1970

Variety and foliar spray	% mildew on top three leaves at G.S. 11.1		Grain yield (t/ha)	
	No seed dressing	Ethirimol seed-dressing	No seed dressing	Ethirimol seed-dressing
Trial 5. Auchincruive 1970				
Golden Promise				
Nil. Control	6.2	1.4	5.49	5.13
Ethirimol	1.2	1.2	4.89	5.04
Midas				
Nil. Control	2.2	1.3	5.13	5.23
Ethirimol	1.1	1.8	4.87	5.13
S.E. (difference)	± 1.3		± 0.19	
Trial 6. Auchincruive 1970				
Golden Promise				
Nil. Control	8.3	1.1	4.39	5.83
Ethirimol	1.7	0.8	4.69	5.68
S.E. (difference)	± 1.2		± 1.08	

Although, as indicated earlier, the level of mildew was low in 1970, seed-dressing and foliar spray both reduced infection but there was no extra benefit from the combined treatment.

Yield response was greater following seed-dressing than after the foliar spray, the combined treatment again offering no advantage. Indeed, in five comparisons out of six there was a yield increase after seed-dressing, whereas in the alternate six comparisons involving the foliar spray there was only one increase and five decreases in yield.

1971 trials

In the 1971 trials the combination of seed-dressing and foliar spray was abandoned and comparisons were only made between the two treatments separately. The range of fungicides tested was extended to include tridemorph and chloraniformethan.

In the first trial (No. 7), plots of Golden Promise (7 x 2.6 m) were sown on 1 April with seed treated or untreated with ethirimol. Subsequently the untreated plots were sprayed either with ethirimol (25% col) or with tridemorph (75% e.c.) each at 0.52 l a.i. in 450 l/ha at G.S. 4-5, 6-7, 9-10, 10-10.1 (11 May, 1, 14 and 18 June respectively). Mildew was assessed on leaf 3 at G.S. 10.5 as well as on the top three leaves at G.S. 11.1, and the grain was harvested on 18 Aug. (Table 3).

Table 3

Effect of seed-dressing and foliar sprays on mildew and yield of spring barley. Trial 7. Auchincruive 1971

Spray application at G.S.	% mildew on:				Grain yield (t/ha)	
	Leaf 3 at G.S. 10.5		Top three leaves at G.S. 11.1			
	Ethirimol	Tridemorph	Ethirimol	Tridemorph	Ethirimol	Tridemorph
4-5	20.9	18.1	40.3	37.0	5.79	6.80
6-7	15.8	7.7	35.6	26.6	5.68	7.27
9-10	15.1	20.3	26.6	23.5	6.08	6.29
10-10.1	12.8	20.0	29.5	21.1	6.11	6.56
Ethirimol seed-dressing	10.1		20.8		6.64	
Nil. Control	26.0		45.5		5.66	
S.E. (difference)						
Control v seed-dressing	± 3.0		± 3.2		± 0.43	
Control or seed-dressing v sprays	± 3.6		± 3.9		± 0.52	
Between sprays and/or growth stages	± 4.2		± 4.5		± 0.60	

The results above show that, as sprays, both fungicides caused some reduction in mildew. Although no close relationship between mildew and yield was apparent it is evident that the crop yields reflected to some extent the mildew recorded at G.S. 10.5. Thus the highest yields with each fungicide correspond to the lowest mildew figures at this growth stage (ethirimol sprayed at G.S. 9-10 and 10-10.1; tridemorph at G.S. 4-5 and 6-7).

Foliar applications of ethirimol gave lower yields than tridemorph, suggesting possible phytotoxicity of the former treatment; this gains support from the data in Table 2. The ethirimol seed dressing controlled mildew and this was associated with increased grain yield.

In the second trial (No. 8) on Golden Promise, similar treatments were made, but chloraniformethan (25% e.c.) at 0.26 l a.i. in 450 l/ha was also included. The seed was sown on 7 April, the sprays were applied at G.S. 4-5, 7-8, 9-10 and 10.1 (20 May, 10, 23 and 28 June respectively), mildew was assessed at G.S. 10.5 and 11.1, and the crop was harvested on 9 Sept. The infection at these stages and the resultant yields are shown in Tables 4 and 5 respectively.

Table 4

Effect of seed-dressing and foliar sprays on mildew of spring barley. Trial 8. Auchincruive 1971

Spray application at G.S.	% mildew on top three leaves at:					
	G.S. 10.5			G.S. 11.1		
	Ethirimol	Tridemorph	Chloraniformethan	Ethirimol	Tridemorph	Chloraniformethan
4-5	24.6	28.1	31.0	54.4	44.9	48.3
7-8	15.4	17.3	13.4	31.9	38.3	21.0
9-10	11.0	7.2	13.9	18.5	12.8	19.3
10.1	17.3	20.1	23.9	22.1	19.0	29.4
Ethirimol seed-dressing		10.6			23.2	
Nil. Control		34.6			60.4	
S.E. (difference)		± 4.3			± 6.2	

In this trial, as in Trial 7, both ethirimol and tridemorph reduced mildew about equally at G.S. 10.5, and the latter fungicide was on the average slightly more successful at G.S. 11.1. In contrast to Trial 7, however, the greatest yield responses were obtained with the sprays applied at G.S. 7-8 and 9-10. It can be seen that these correspond with the lowest mildew figures for each fungicide at G.S. 10.5. The responses to chloraniformethan were essentially similar to those of the other two chemicals.

The level of mildew control obtained with the ethirimol seed-dressing paralleled that recorded in the earlier trials. On this occasion it resulted in the highest yield of the trial, out-yielding the control by more than 1 t/ha.

Table 5

Effect of seed-dressing and foliar sprays on grain yield (t/ha)
Trial 8. Auchincruive 1971

Spray application at G.S.	Ethirimol	Tridemorph	Chloraniformethan
4-5	4.42	4.33	4.37
7-8	4.72	5.02	4.86
9-10	4.70	4.84	4.95
10.1	4.63	4.64	4.62
Ethirimol seed-dressing		5.38	
Nil. Control		4.32	
S.E. (difference)		± 0.20	

DISCUSSION

The results show that in the first and second years the mildew levels were generally low, while in the third year considerable infection occurred. It is also evident that both the mildew and yield responses showed remarkable consistency throughout the experiments. In all three years the ethirimol seed treatment reduced the amount of disease in the crop, the effect persisting to the "milky ripe" stage of development (G.S. 11.1). Reductions in mildew were also obtained with foliar sprays of ethirimol, tridemorph and chloraniformethan, there being little to choose in fungicidal efficiency between them.

The ethirimol seed treatment generally resulted in increased yields, though the magnitude did not necessarily reflect the level of disease reduction. In general the sprays also resulted in yield gains though there was a suggestion in some trials of a depression in yield associated with the ethirimol sprays.

At current prices an increase in grain yield of approx. 0.12 t/ha would pay for the cost of the various fungicide treatments (including the ethirimol seed-dressing), and it is apparent, particularly in 1971, that the yield benefit often exceeded treatment costs.

Advisory experience has indicated that mildew occurrence is sporadic in the south-west of Scotland. In situations favourable to the disease, however, and especially where a mildew-susceptible variety is being grown, the use of ethirimol seed treatment as a precaution against possible mildew build-up is justified. In other situations reliance should perhaps be placed on the use of a single spray with any of the three systemic fungicides tested.

The timing of the single spray clearly depends upon the likelihood of an upsurge of the disease, and work is in progress elsewhere to find a satisfactory forecasting system. Meantime, if mildew has begun to establish in the crop, yield benefits are likely from sprays applied at almost any stage of development up to G.S. 10. Progressive delay in spraying, however, reduces the potential yield benefit and this is exacerbated by increasing wheel damage. In practice therefore it may be inadvisable to enter the crop after about G.S. 8 or 9 unless there is imminent likelihood of a severe flare-up of the disease. It is not anticipated that such a situation will occur frequently in the south-west of Scotland.

Acknowledgements

Our thanks are due to our colleagues in the Crop Husbandry and Plant Pathology Departments and in the Area offices of the College for technical assistance, and to the various firms who have provided the fungicides used in the trials. The co-operation of the farmers on whose premises the trials were made, and the analyses provided by the ARC Unit of Statistics, Edinburgh, is gratefully acknowledged.

References

ANON., (1970) Provisional guide for the assessment of cereal diseases. MAFF Plant Pathology Laboratory Publication 1970.

ANON., (1971) Provisional guide for the assessment of cereal diseases. MAFF Plant Pathology Laboratory Publication 1971.

GRAINGER, J. (1969) The reduction of crop disease losses in West Scotland. Research Bulletin No. 43 of the West of Scotland Agricultural College, Auchincruive, 1-80.

LARGE, E. C. (1954) Growth stages in cereals. Plant Pathology, 3, 128-129.

Proceedings 7th British Insecticide and Fungicide Conference (1973)

THE EFFECTS OF MILDEW (ERYSIPHE GRAMINIS) ON LEAF GROWTH AND YIELD OF SPRING BARLEY

B.L. Rea
The Boots Company Ltd., Lenton Research Station, Nottingham
R.K. Scott
School of Agriculture, University of Nottingham

Summary Experiments in mildew infected spring barley crops in the East Midlands during 1971 and 1972 tested the effects of applying a systemic fungicide sequentially and at different growth stages. During the growth analysis the area of leaf laminae and the areas affected by disease were measured.

On a heavily infected crop in 1972 yields of sequentially treated, early treated and untreated crops (5620, 4265 and 3588 kg/ha respectively) were directly proportional to Leaf Area Duration (LAD). Although increases in LAD from early treatment were similar on less severely infected crops in 1971, yields from untreated crops were much better and responses to treatment not as substantial. Treatment at different growth stages had consistent effects on the development of the leaf surface, but relationships between grain yield and LAD were not so straightforward.

Résumé Des essais effectués en 1971 et 1972 sur les cultures d'orge de printemps infestées de mildiou dans l'Est des comtés centraux de l'Angleterre ont permis d'étudier les effets d'un fongicide systémique appliqué en série et à différents stades du développement. La surface des limbes des feuilles et les zônes infestées par la maladie furent mesurées au cours de l'analyse de croissance.

Sur une culture de 1972 fort infestée, les récoltes de cultures traitées en série, traitées à un premier stade et non traitées (5620, 4265 et 3588 kg/ha respectivement) étaient directement proportionnelles à la Durée de la Surface de la Feuille (LAD). Bien que les accroissements de la LAD dûs à un premier traitement étaient semblables sur des cultures moins infestées en 1971, les récoltes de cultures non traitées se sont avérées bien meilleures et la réponse au traitement ne fut pas si favorable. Le traitement appliqué à différents stades du développement avait des effets évidents sur le développement de la surface de la feuille mais le rapport entre la récolte de grains et la LAD n'était pas si évident.

INTRODUCTION

Field experiments on 13 spring barley crops in the East Midlands between 1970-72 tested the effect of applying a systemic fungicide to the foliage, sequentially or at different growth stages (Rea and Brown, 1971). In general a single application gave the greater part of the yield improvement of sequential treatment and there was no clearly defined best time for a single dose. The response to treatment at the onset of mildew infection, just prior to stem extension (G.S.4-5 on the Feekes Scale - Large 1954), was usually similar to, sometimes slightly less than, that from treatment during early stem extension (G.S.6-8). The response to a later application, between the emergence of the flag leaf (G.S.9) and partial heading out (G.S. 10.3) was more variable, sometimes as great, on other occasions less than for earlier applications.

In 1971 and 1972 opportunities arose to investigate the effects of the various fungicidal treatments on the development of the leaf surface and the relationship with grain yield. Studies in the glasshouse (Last, 1962; Paulech, 1969) revealed that with pot grown plants mildew restricted the development of the root system more than the shoot, and lamina development least of all. Mildew inhibited photosynthesis and stimulated respiration i.e. diminished Net Assimilation Rate. Brooks (1972) confirmed effects on root and shoot growth in field experiments. Large and Doling (1962) were able to relate the percentage loss of grain yield to the extent of mildew infection after the completion of heading and before the onset of ripening. The effect was not directly proportional, there being more severe effects with low levels of infection and less severe effects with heavy infection. In disease free crops Thorne (1966) has shown that grain yield is directly related to Leaf Area Duration i.e. the integrated Leaf Area Index from ear emergence until harvest. In the experiments reported here it was possible to test how grain yields were related to the extent and duration of the leaf surface both including and excluding the proportion covered by pustules or yellowed by the disease.

METHOD AND MATERIALS

Growth analysis was done on two experiments in 1971 and one in 1972, details of which are given in Table 1. Each experiment was in a randomised block design, replicated sixfold in 1971 and fivefold in 1972. The fungicide was triarimol((2,4-dichlorophenyl)α-phenyl-5-pyrimidine methanol) formulated as a 4.5% e.c., applied at 1.8 l/ha in 225 l water by the Lenton small plot sprayer. Plots were 44 m long and 2.7 m wide allowing areas for growth analysis samples with appropriate discards at either end. Final yields were estimated on the central strip 37 m long and 1.8m wide harvested by combine.

The first treatment coincided with the onset of rapid infection. Samples for growth analysis were taken when treatments were first applied and at two weekly intervals thereafter. The sampling unit was one metre of row from each plot. Plants were dug, roots discarded since an unknown proportion was recovered, and weights determined. Leaf laminae from 10 randomly selected tillers were traced, the disease covered regions marked in, then areas were determined using a planimeter. In addition estimates of lamina area covered by mildew were made by reference to the mildew key (ADAS 1971). The total lamina area in the harvest unit was estimated by simple proportionality knowing the number of tillers in the whole sample and expressed as an index of the ground from which the sample came (LAI). Values for Leaf Area Duration were obtained by measuring the area under the LAI curves and expressing them in weeks, one week's LAD being equivalent to the area encompassed by unit Leaf Area Index persisting for one week.

Table 1

Details of the experiments.

LOCATION	CALVERTON 1972	BARTON 1971	THRUMPTON 1971
Variety	Sultan	Zephyr	Sultan
Sowing date	8.4.72	15.3.71	11.3.71
Sowing rate (kg/ha)	140	160	160
Fertiliser $N:P_2O_5:K_2O$(kg/ha)	43:17:17	67:34:34	47:39:78
Treatments			
1. Untreated			
2. Application at G.S.5	8.6.72	12.5.71	12.5.71
3. Application at G.S.7	20.6.72	19.5.71	19.5.71
4. Application at G.S.9	28.6.72	1.6.71	1.6.71
5. Sequential application	8.6.72) 20.6.72) 12.7.72)		
Grain Harvest date	1.9.72	17.8.71	18-19.8.71

RESULTS

Comparison of untreated, sequentially treated and early treated crops, 1972.

Mildew was first seen in the late drilled, rather sparsely tillered, Calverton crop at the beginning of June and the first spray was applied on 8 June when 2.5% of the leaf surface was affected by mildew. The infection then spread very rapidly to reach 40% in the untreated crop by mid July (Fig. 1). Sequential treatment prolonged the phase of expansion of the leaf surface and increased maximum Leaf Area Index (LAI) by 30%. Throughout treated plants had at least one additional active leaf. The overall Leaf Area Duration (LAD) was increased by 46% and Mildew Free Leaf Area Duration (MFLAD) by 70% (Table 2). The single early application maintained an extra leaf active during late June, but the leaf surface became reinfected and both in terms of LAD and MFLAD the benefit was slightly less than half that of the sequential treatment. Grain yields were equally well correlated (r = 0.99) and virtually directly proportional to LAD whether or not infected areas were included, but the regression line for MFLAD more nearly passes through the origin (Table 3). Yields were also very well correlated (r = 0.99) with LADs measured post ear emergence i.e. during grain filling, but the direct proportionality no longer held, e.g. sequential treatment increased LAD post ear emergence nearly fourfold but grain yield by only 60%.

Sequential treatment increased all components of yield as did treatment at G.S.7, although to a lesser extent. Early treatment increased ear number and grains per ear but not grain weight, and later treatments increased ear number, rather surprisingly, and grain weight. In this heavily infected crop even the best treatment at G.S.7 gave slightly less than half the yield improvement of sequential spraying.

Table 2

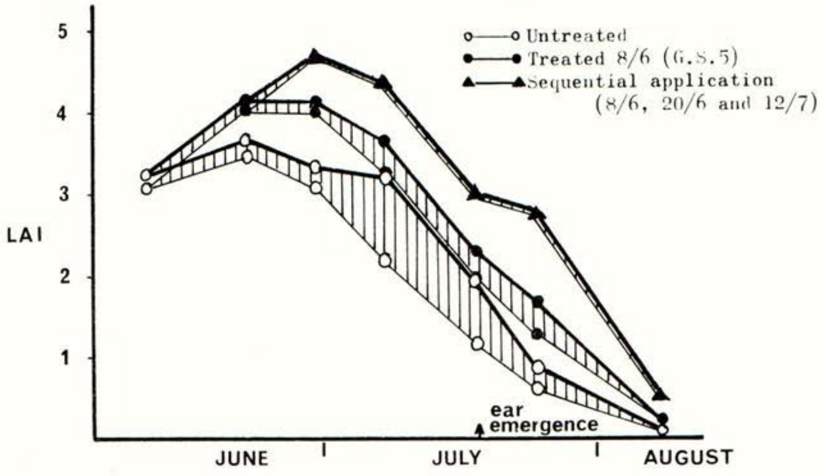
Grain yield, its components and the relationship with Leaf Area Duration.

Site Location & Treatments	Grain Yield kg/ha	LAD overall(wks)		LAD post ear emergence(wks)		Ear No. per m^2	Grain No. per ear	1000 Grain wt. (g)
		Total	Mildew Free	Total	Mildew Free			
CALVERTON 1972								
1. Untreated	3588	19.8	16.6	2.5	1.6	626	17.6	34.4
2. Application at GS5	4265	23.9	21.7	3.9	3.0	673	19.2	34.9
3. Application at GS7	4541					716	18.9	38.3
4. Application at GS9	4114					696	17.4	37.5
5. Sequential treatment	5620	28.9	28.2	6.6	6.3	745	19.5	39.2
Standard error	70.3					22.0	0.33	0.56
BARTON 1971								
1. Untreated	5369	20.4	18.9	5.3	4.9			39.4
2. Application at GS5	5946	29.2	28.3	7.0	6.9			40.5
3. Application at GS7	5745	23.2	21.8	6.6	6.4			40.4
4. Application at GS9	5921	25.5	24.8	8.9	8.8			39.1
Standard error	69.0							0.39
THRUMPTON 1971								
1. Untreated	4880	19.1	17.2	3.1	2.8			31.5
2. Application at GS5	5143	26.7	25.1	5.5	4.9			32.5
3. Application at GS7	5469	24.0	22.3	5.4	5.0			31.8
4. Application at GS9	5369	25.5	23.7	8.1	7.4			30.7
Standard error	90.3							0.59

Fig. 1

Effect of fungicide on total lamina area index (TLAI), area covered by mildew pustules, and mildew free area index (MFAI) on spring barley at Calverton 1972.

TLAI ——, MFAI ——, Area covered by mildew pustules ||||



In 1971 growth analysis data were available from the untreated crop and from crops treated at different growth stages.

The effect of treatment at different growth stages.

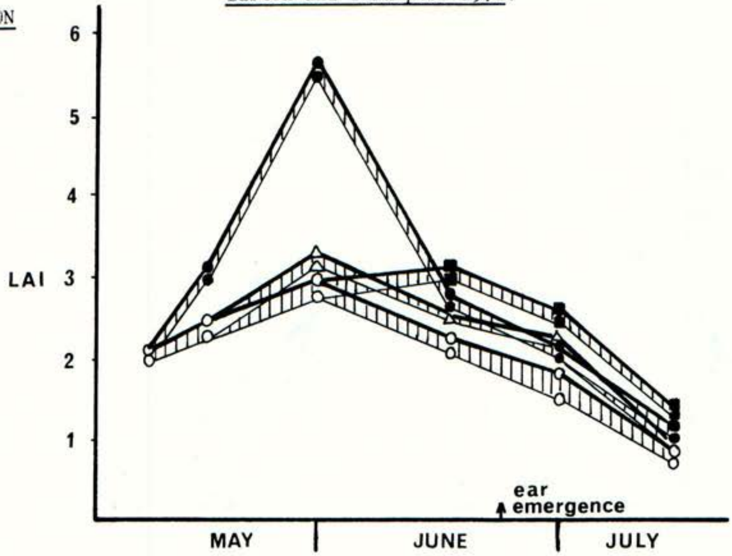
In 1971 crops were drilled about a month earlier than in 1972 and events occurred about one month earlier throughout. Mildew began to spread during early May and by 12 May, when the first treatment was applied, mildew infection was 5% at Barton and 10% at Thrumpton. The infection was always more severe at Thrumpton but never so severe as at Calverton in 1972. Both 1971 crops had at least five and sometimes six leaves partially green during June. At the end of the month there were still four or five active leaves at Barton, but only four on treated plants and three on untreated plants at Thrumpton. By mid July there were three or four active leaves at Barton and two or three at Thrumpton, with late treated plants noticeably greener.

The differential timing resulted in very similar patterns of leaf development at both sites. Early treatment (G.S.5) increased leaf area primarily in June and later treatment (G.S.9) increased it during July (Figs 2a and 2b). The mid treatment (G.S.7) increased leaf area from late May onwards but to a lesser extent than the other treatments. The overall LADs of untreated crops were similar at all three sites, but yields in 1971 were much heavier (Table 2). Although early spraying increased LAD more at both 1971 sites than at Calverton yields were not so much improved. At both sites LAD and MFLAD were most extensive from early treatment and more extensive from the late than the middle application yet yields were similar; neither effects of timing nor the interaction between timing and dose rate were significant at either site (Rea and Brown, 1971). In general yield was slightly

Fig. 2

Effect of fungicide on total lamina area index (TLAI), area covered by mildew pustules, and mildew free area index (MFAI) on spring barley at Barton and Thrumpton 1971.

a) BARTON



TLAI ——, MFAI——, Area covered by mildew pustules ||||

○—○ Untreated
●—● Treated 12/5 (G.S.5)
△—△ Treated 19/5 (G.S.7)
■—■ Treated 1/6 (G.S.9)

b) THRUMPTON

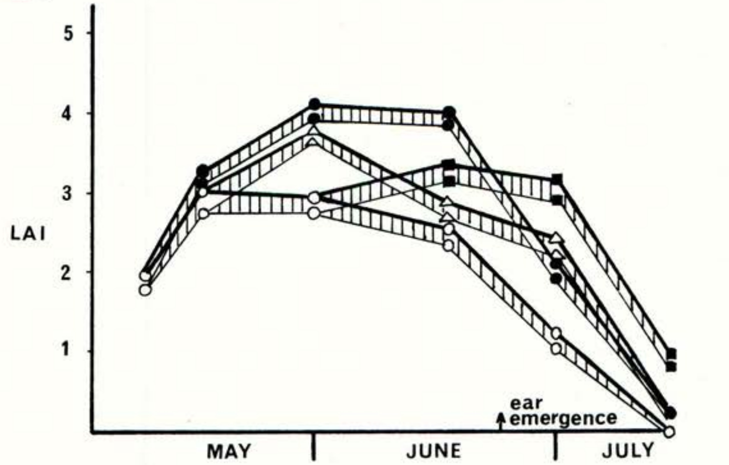


Table 3

The relationship between Leaf Area Duration (LAD) and grain yield.

	Overall	Correlation Coefficient	Post ear emergence	Correlation Coefficient
CALVERTON 1972				
LAD:	yield = -965.9(± 757.66) + 225.8(±31.36)	0.99	yield = 2370.8(±273.46)+489.2(±6.27)	0.99
MFLAD:	yield = 577.0(± 442.40) + 175.6(±21.33)	0.99	yield = 2935.3(± 79.03)+426.5(±18.82)	0.99
BARTON 1971				
LAD:	yield = 4164.6(± 553.19) + 62.7(±22.58)	0.90	yield = 4741.6(±505.52)+135.5(±71.50)	0.82
MFLAD:	yield = 4352.8(± 461.62) + 62.7(±20.07)	0.91	yield = 4791.8(±437.79)+138.0(±63.97)	0.84
THRUMPTON 1971				
LAD:	yield = 4039.2(±1016.66) + 50.2(±42.65)	0.64	yield = 4764.6(±363.78)+100.4(±62.72)	0.73
MFLAD:	yield = 4114.4(± 978.43) + 50.2(±43.90)	0.63	yield = 4791.8(±437.79)+138.0(±63.97)	0.84

better correlated with MFLAD than total LAD at both sites and agreement was closer at Barton (r = 0.82 - 0.91) than at Thrumpton (r = 0.63 - 0.84) (Table 3). In the main the origin of the poorer correlation at Thrumpton was the small increases in yield from increased LAD associated with early treatment. By measuring LAD post ear emergence this effect was minimised and correlations were improved. Not all yield components were determined in 1971 but the evidence available indicates that yield increases occurred via effects on grain number rather than grain size. There was a striking difference in the effect of the fungicide treatment on the thousand grain weight of Sultan at Thrumpton in 1971 and Calverton in 1972. At Calverton thousand grain weight was significantly increased by all treatments except application at G.S.5, whereas there was no significant change at Thrumpton.

DISCUSSION

Evidence for the existence of a direct relationship between the extent of mildew infection and yield loss is rather contradictory. On the one hand greenhouse experiments show that the effects of mildew are likely to be more severe than the degree of restriction of the leaf surface would suggest (Last, 1962, and Paulech, 1969). Root and shoot development can be severely affected as mildew reduces Net Assimilation Rate. Yet Last (1963) and Large and Doling (1962) show that while this holds true with relatively mild infections, effects on photosynthesis and yield from heavier infections are less than would be expected from examination of the leaf surface.

Mildewicides which are the usual agency for testing these relationships have a dual role; they restrict the incidence of disease and permit extra leaf development thereby. Comparisons using the Mildew Key (Large and Doling, 1962) do not directly take account of the second effect. In our experiments we found that estimates of the extent of infection using the Mildew Key in 200 comparisons agreed well with measured values when infection was light, but over-estimated the level when leaves were heavily infected. This may be one of the reasons for the over-estimation of yield losses using the Key or may simply reflect the precision with which it was used.

In the present work relationships between the extent of a healthy leaf surface and yield were very close in a heavily infected crop but not in less severely infected crops. The lack of a sequential treatment at Barton and Thrumpton makes it more difficult to interpret the information which does exist. One possible reason for the breakdown of the relationship between leaf area and yield improvement in some situations may be that leaves which are most affected are least capable of phytosynthesis since being lower leaves they are more heavily shaded. Parallel studies in barley crops monitored the contribution of individual organs to grain yield and measurements of mildew infection on individual leaves will be combined with this data to explore these relationships more fully.

Acknowledgements

We thank Christine Shaw and Anthony Bown for their growth analysis, Professor J.D. Ivins, and colleagues at The Boots Company for their continued interest. Our thanks are also due to Elanco Products Ltd. for supplying the fungicide.

References

ADAS. (1971) Guide for the assessment of cereal diseases. Ministry of Agriculture, Fisheries & Food, Plant Pathology Laboratory, Harpenden.

BROOKS, D.H. (1972) Observations on the effects on mildew, Erysiphe graminis on growth of spring and winter barley. Annals of Applied Biology 70 149-156.

LARGE, E.C. (1954) Growth Stages in cereals. Illustration of the Feekes Scale. Plant Pathology 3 128-9.

LARGE, E.C. and DOLING, D.A. (1962) The measurement of cereal mildew and its effect on yield. Plant Pathology 11 47-57.

LAST, F.T. (1962) Analysis of the effects of Erysiphe graminis on the growth of barley. Annals of Botany 26 279-289.

LAST, F.T. (1963) Metabolism of barley leaves inoculated with Erysiphe graminis Annals of Botany 27 685-690.

PAULECH, C. (1969) Influence of Erysiphe graminis on the quantity of dry substance and on the growth of the vegetative organs. Biologia, Bratislava 24, 10, 709-719.

REA, B.L. and BROWN, P.A. (1971) Control of barley powdery mildew using triarimol. 6th British Insecticide & Fungicide Conference 1 52-62.

THORNE, G.N. (1966) Physiological aspects of grain yield in cereals. In: The Growth of Cereals and Grasses. Edited by F.L. Milthorpe and J.D. Ivins, Butterworths, London.

NOTES

Proceedings 7th British Insecticide and Fungicide Conference (1973)

DEVELOPMENT OF THE CHLORQUINOX RECOMMENDATIONS FOR MILDEW CONTROL OF SPRING BARLEY

E. Evans, J. Marshall and M.H. Rickard

Fisons Ltd., Agrochemical Division, Chesterford Park Research Station,
Nr. Saffron Walden, Essex

Summary Chlorquinox is a new fungicide of low mammalian toxicity developed specifically for use on cereals. The product has been tested extensively over the past four years in small plot and farmer trials in the East and South of England. The curative aspects of the compound have been stressed but data are provided to show that curative treatments at low levels of disease are more effective in stimulating yield increases than similar treatments made later in the epidemic. This product is a reliable treatment for mildew on spring barley in the U.K. as long as applications are made prior to the logarithmic phase of epidemic development, whether this occurs prior to or during the stem extension phase of the crop.

INTRODUCTION

Tetrachloroquinoxaline is a general mildew fungicide which has been developed over the past four years for the control of cereal mildew (*Erysiphe graminis*). A product of low toxicity to mammals with acute oral LD50 values in the range of 100 to 400 mg/kg leaving very low residues in both straw and grain at harvest, it has been tested extensively on spring barley throughout northern Europe and found to be non-phytotoxic under a wide variety of climatic, edaphic and cultural conditions. This report is confined to studies in the United Kingdom but the pattern of results recorded has been confirmed in trials in other areas.

METHOD AND MATERIALS

In the work reported here a 25% wettable powder formulation of chlorquinox was used at a rate of 3.36 kg/ha in 225 l/ha of water. In small plot trials the product was applied to 9 x 2 m plots with 8 replicates/site using a knapsack sprayer. "Grower" trials were applied with farmers' sprayers on large plots not less than 30 m wide. As yielding potential and disease levels may vary considerably from one end of a field to another, unsprayed areas were left between each treated plot in farmer trials. Yield responses of treated plots were calculated relative to the yield of adjacent unsprayed plots.

In large-plot yield trials four strips of 30 metres were harvested with a Hege 125 Miniature Combine and weighed separately; in small plot trials the harvested strip was 9 m long. Disease assessment was based on the key devised by Large and Doling (1962) being an estimate of the mean percentage of leaf surface covered by mycelium and leaf yellowing associated with mildew on the top three leaves at GS 10.5 on the Feekes scale (Large 1954). Percentage disease control was calculated using the formula,

$$\% \text{ disease control} = \frac{D_2 - D_1}{D_2} \times 100$$

where D_1 = disease assessment on treated crop and D_2 = disease assessment on untreated crop. The data reported here are confined to trials on spring barley carried out primarily in the east and south of England on farmer crops irrespective of nearness or otherwise to overwintering sources of mildew.

Statistical analysis of all data has been carried out where practical but the variation shown throughout these yield trials rarely allowed multiple comparison tests to show significant differences between treatments within individual experiments. These tests were therefore avoided and preference given to non-parametric tests (Dunnett 1970) comparing data from simple comparative treatments on a large number of trial sites.

As yield response is dependent on the severity of the epidemic as well as the efficiency of a given treatment, multisite data were considered as frequency/response histograms when sufficient data had accumulated.

RESULTS

Timing recommendation

Since the incidence, distribution and intensity of mildew varies from site to site and from season to season it was necessary to repeat replicated timing trials as frequently as possible and to confirm the overall pattern by reference to the performance of the product under a wide variety of field conditions. This was achieved by carrying out 7 small plot trials in 1970 and again in 1971. Disease and yield records were taken from a series of plots sprayed at weekly intervals and related to the growth stage at the moment of application. The pattern of disease control and yield response was fairly consistent and is summarised as mean values of the 7 trials in 1971 (Table 1).

Table 1

Disease control and yield response to variously timed sprays of chlorquinox on spring barley in East England (mean of 7 sites)

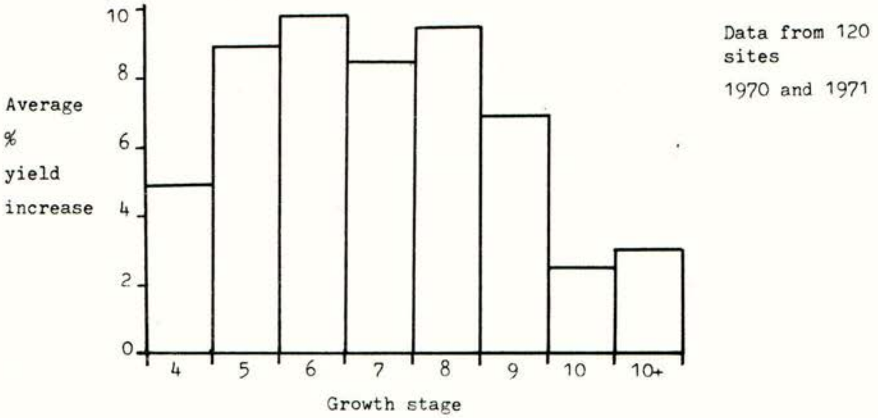
GS at application	Mean % disease at GS 10.5	Mean % disease control at GS 10.5	% yield increase over control
4	16.1	12	3.2
5	14.8	19	7.4
6	11.5	37	6.3
7	9.3	49	6.0
8	9.2	50	5.5
9	5.7	69	1.8
10	6.9	62	1.9
Untreated	18.3	0	0

The data given in Table 1 show that the overall yield benefit of a given spray treatment is not necessarily related to the level of disease at GS 10.5; being largely a reflection of the nearness of the spray treatment to the time of assessment.

The relationship between yield and growth stage at application suggested in Table 1 is further confirmed by the data in Fig 1 which summarises the results from 120 trial sites over a 2 year period, 1970-1971. This pattern probably reflects the increased susceptibility of barley during extension, i.e. GS 6-8.

Fig 1

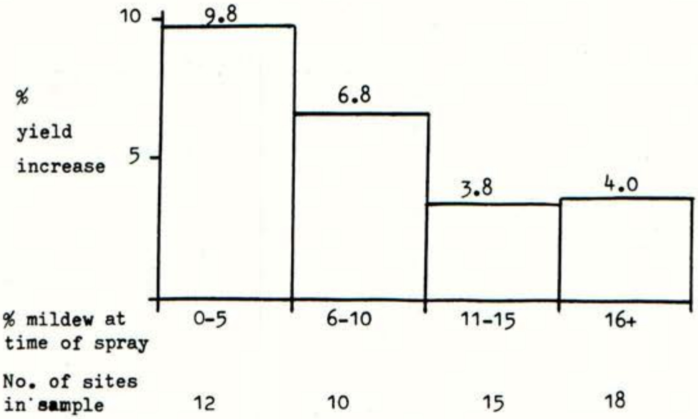
The relationship between time of application of chlorquinox and increases in yield of spring barley



The use of antimildew sprays on cereals often depends on the curative aspects of these products, but their performance can vary according to the level of disease on the crop at the time of spray application regardless of the stage of growth. Fig 2 shows the yield increase from a single chlorquinox treatment made at different stages in the early part of the epidemic.

Fig. 2

Yield data of spring barley correlated with level of mildew at time of spray application (1972 and 1973)



The yield benefit of early spraying in terms of mildew levels are obvious from the data in Fig 2 but it must be stressed that premature treatment in terms of growth stage may prove wasteful.

To avoid premature application the chlorquinox timing recommendation relates to level of disease and growth stage of the crop as follows:

> "Chlorquinox should be applied to crops during tillering i.e. GS 3-5 only if mildew is present on the younger leaves. Later, during the stem extension period GS 6-9 chlorquinox should be applied immediately mildew is seen in the crop".

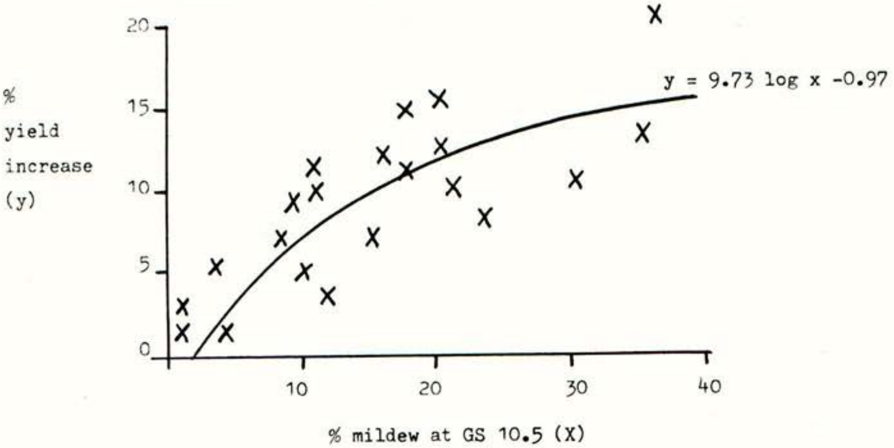
These observations on spray timing apply solely to spring barley; the recommended time to spray wheat would be later, probably during heading.

Disease levels and yield

Large and Doling (1962) found a close correlation between the percentage loss in yield and the amount of mildew recorded on the crop at GS 10.5. Increments of a similar order should follow efficient disease control with a non-phytotoxic fungicide. Fig 3 shows the relationship between yield gain and disease levels on the top three leaves of the crop assessed on 20 different trial sites at GS 10.5 in 1970. This is very close to that of Large and Doling, i.e. yield loss = $2.5\sqrt{\%\text{ mildew}}$ at GS 10.5. This formula proved invalid when comparing applications (Table 1) made at different times or late in the epidemic and must therefore be used with caution. Some variation in response was noted depending on the relative protectant and curative requirement for a given situation; treatments made with low infection levels at the time of application depend largely on protection while those made at a later stage in the epidemic are more dependent on curative activity.

Fig 3

Relationship between mildew levels at GS 10.5 in untreated crop and yield increases following chlorquinox treatment at GS 6-8

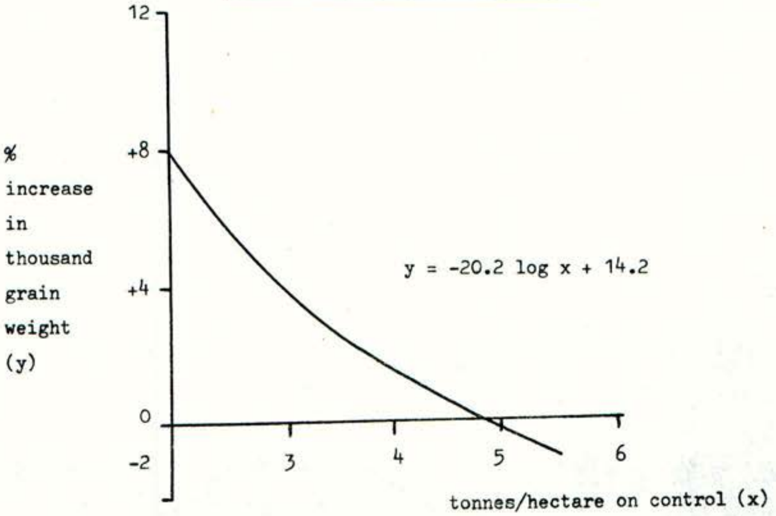


Although chlorquinox sprays gave consistent yield increases over untreated attempts to correlate yield increases with 1000 grain weight failed on samples from 25 sites in 1971 (Spearman coefficient of ranked correlation). The only significant correlation observed was that between increase in grain weight following treatment and yield in untreated crops (Fig 4).

It is concluded that whilst the 1000 grain weight of low yielding crops can be increased substantially by treatment, in heavier crops increased yields would appear to be due to more, not heavier grain. These observations reinforce those made in experiments with benomyl in 1968 (Evans et al).

Fig 4

Correlation between yields in untreated crops and thousand grain weights after treatment with chlorquinox



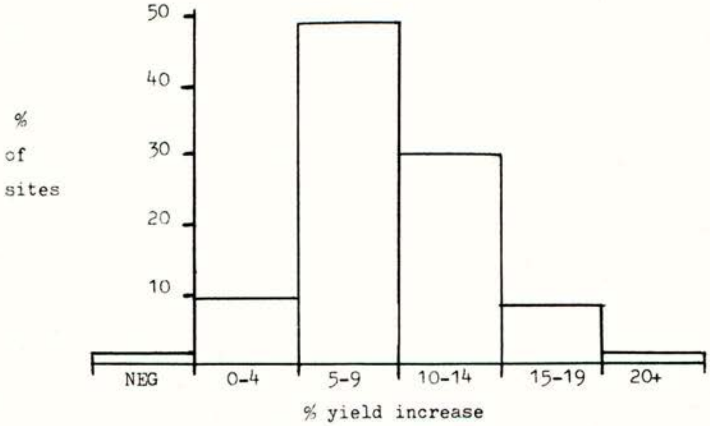
Reliability Trials

Interplot variables may be exaggerated by factors other than the efficiency of the treatments concerned and any valid assessment of the reliability of a given recommendation must be based on a large number of experiments, the data from which may then be plotted as frequency response histograms (Fig 5).

Fig. 5

Reliability of chlorquinox as a mildew fungicide on spring barley, 1971

(frequency/response histogram) (45 sites)



The information in Fig 5 summarises the overall reliability of chlorquinox as a mildew fungicide when used according to recommendation at the beginning of the epidemic and has been confirmed in further experiments.

DISCUSSION

Cereal mildew (Erysiphe graminis) is now recognised as the most important disease of spring barley in the U.K. (James 1969 and King 1972) and northern Europe with the result that several fungicides are commercially available for its control (Pommer & Kradel, 1969 and Brooks, 1970). Chlorquinox was first tested in the U.K. in 1967 and was introduced on a large acreage in 1971 as a safe and reliable product intended for application soon after the start of the epidemic. This usually occurs sometime during stem extension when the crop is particularly susceptible to mildew (Last 1957) although earlier spread of the disease sometimes occurs and requires immediate treatment.

The data in Fig 3 show a very close correlation between level of disease recorded on the untreated crop, i.e. the severity of the epidemic, and the percentage increase in yield afforded by spraying. This is in close agreement with Large and Doling (1962) who estimated potential yield losses due to mildew from disease records taken from the top four leaves at GS 10.5 on the Feeke's scale. The index used in these trials was based on the top three leaves only. In making this statement however it is stressed that this relationship is applicable only when comparing treatments made at one appropriate time and the conversion factor postulated by Large and Doling cannot be used to forecast the comparative yielding potential of treatments made at different times. At present the only method of comparing treatments made at different times is by direct measurement of yields in field experiments.

The main characteristic of all field experiments with cereal mildew appears to be the extreme variability of the data recorded. This may be due to (a) limitations of the technique of application, (b) differences associated with crop structure, or (c) specific aspects of the biology of the pathogen. It is possible to exercise only very limited control over these factors so that field data may give conflicting and confusing results. However these effects can be minimised where data from a large number of trials are jointly examined as frequency response histograms.

Acknowledgements

The authors wish to thank the Directors of Fisons Agrochemical Division for permission to publish this information and would also like to thank their many colleagues who contributed so much to the execution of this work. Thanks are also due to the very many farmers upon whose fields these trials were carried out often at considerable inconvenience.

References

BROOKS, D.H. (1970) Powdery mildew of barley and its control. Outlook on Agriculture, 6, (2), 122-127

DUNNETT, C.W. (1970) Queries and notes - Multiple Comparison Tests. Biometrics, 26, (1), 139-141

EVANS, E., RICKARD, M.H. and WHITEHEAD, R. (1969) Effects of Benomyl on some diseases of spring barley. Proc. 5th Br. Insectic. & Fungic. Conf., 3, 610-619

JAMES, W.C. (1969) A survey of foliage diseases of spring barley in England and Wales in 1967. Annals of Applied Biology, 63, 253-263

KING, J.E. (1972) Surveys of foliar diseases of spring barley in England and Wales, 1969-70. Plant Pathology, 3, 23-35

LARGE, E.C. (1954) Growth stages of cereals. Plant Pathology, 3, 129-130

LARGE, E.C. and DOLING, D.A. (1962) The measurement of cereal mildew and its effect on yield. Plant Pathology, 11, 47-57

LAST, F.T. (1957) The effect of date of sowing on the incidence of powdery mildew on spring sown cereals. Annals of Applied Biology, 45, (1), 1-10

POMMER, E.H. and KRADEL, J. (1967) Substituierte Dimethylmorpholin-Derivate als neue Fungizide zur Bekämpfung echter Mehtaupilze. Negentiende Internationaal Symposium over Fytofarmacie en Fytiatrie, Gent, Belgie, 735

Proceedings 7th British Insecticide and Fungicide Conference (1973)

DEGRADATION OF ETHIRIMOL IN SOIL

I.R. Hill and D.J. Arnold
ICI Plant Protection Limited, Jealott's Hill Research Station, Bracknell, Berkshire.

Summary Pyrimidine ring-C^{14}-labelled ethirimol was incorporated in biologically active and in sterile soils, and incubated for up to 45 weeks. Degradation occurred, but at very different rates in all of the soils studied; half the ethirimol being degraded in from less than one to a maximum of twenty weeks in non-sterile soils. Analysis for volatile and non-volatile degradation products showed that the fungicide was degraded by both chemical and biological mechanisms. Cleavage of the pyrimidine ring (observed as $C^{14}O_2$ evolution) was most probably caused by biological action. The relative rates of ring opening in soils treated by uniform incorporation and by localised high concentrations of ethirimol were very different; in one case localised concentrations decreasing the rate and in two others increasing it. The presence of plants, growing from ethirimol dressed seed, in the one soil studied considerably enhanced the rate of fungicide degradation. After two weeks, less than 3% of the applied activity was found associated with plant material. Pyrimidine ring cleavage was almost entirely restricted to the soil-root environment.

Resumé De l'éthirimol dont l'anneau pyrimidine était marqué au Carbone^{14} a été incorporé à des sols biologiquement actifs ainsi qu'à des sols stériles, et incubé pendant une periode allant jusqu'à 45 semaines. Une dégradation s'est produite, mais à des degrés tres différents dans tous les sols etudiés, la moitié de l'éthirimol étant dégradeé durant une période allant de moins d'une semaine à un maximum de vingt semaines. Les analyses pour détérminer les produits de dégradation volatils et non-volatils ont montré que le fongicide était dégradé par des mechanismes chimiques aussi bien que biologiques. L'éclatement de l'anneau pyrimidine a probablement été l'effet d'une action biologique. Les degrés relatifs d'éclatement de l'anneau dans les sols traités par une incorporation uniforme et par des fortes concentrations localisées d'éthirimol étaient très différentes; dans un cas les concentrations localisées diminuant le degré d'éclatement et dans deux autres cas l'augmentant. La présence de plantes croissant à partir de semences traitées à l'éthirimol, dans le seul sol étudié, a augmenté considérablement le degré de dégradation fongicide. Après deux semaines, moins de 3% de l'activité appliquée était présente dans le tissu des plantes et l'éclatement de l'anneau était presqu'entièrement confiné au système sol/racines.

INTRODUCTION

Ethirimol, 5-n-butyl-2-ethylamino-4-hydroxy-6-methyl pyrimidine (trade names 'Milstem' and 'Milgo'), a systemic fungicide effective in controlling powdery mildew (*Erysiphe graminis*) on cereals, particularly barley (Bebbington et al, 1969; Geoghegan, 1969), is marketed both for seed dressing and spraying of the vegetation. Metabolism of the fungicide in plants has previously been reported (Cavell et al., 1971).

In soil, ethirimol is moderately strongly adsorbed compared with many other pesticides (Graham-Bryce and Coutts, 1971). Adsorption is greatest in soils containing much organic matter and in acid soils, where a large proportion of the chemical is present in the protonated form. Desorption occurs but at a slower rate than adsorption. Thus the physical interaction of the fungicide with the soil may well influence the rate of its degration. The present paper discusses the degradation of ethirimol incorporated both uniformly in soil and as point sources of high concentrations, as occurs with 'dressed' seeds.

METHODS

Chemicals. Ethirimol and its fungicidally inactive 2-amino analogue (Fig.1), labelled at the C-2 in the pyrimidine ring, were 99·2% and 98·9% pure (by 2-dimensional chromatography) respectively, as applied to soil in aqueous solution.

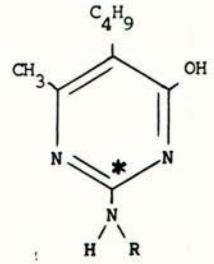


Fig.1

R = C_2H_5 , ethirimol

R = H , 2-amino analogue of ethirimol

✱ indicates position of C^{14} in molecule

Labelled chemical was diluted with unlabelled to a specific activity of 1µCi/100µg and sterilised, in aqueous solution, by filtration through pre-washed Millipore mixed cellulose ester filters, mean pore size 0·22µm. The fungicide was either uniformly incorporated into soil to a final concentration of 10 ppmw or prepared as an aqueous 'col' of particle size 1µm and used to coat barley seeds or acid washed ground glass beads. The final weight of ethirimol per seed or bead was approximately 120µg.

Soils. Field samples, taken no deeper than 9 inches, were returned to the laboratory within one day of collection, carriage being in unsealed polythene bags. Prior to treatment with fungicide, soils were passed through a 3mm sieve (after part air drying where necessary). Sterile soils were prepared by γ-radiation (5·0 Mrads) in sealed polystyrene containers.

Measurements of moisture content and moisture holding capacity (MHC) per weight of dry soil, using Keen-Raczkowski boxes (Keen and Raczkowski, 1921; Coutts, 1930), were carried out immediately to enable soils to be prepared and incubated at 40% of MHC (30% of MHC for peat soils) within 3 days of the original sampling. Other physical and chemical characteristics determined included pH by glass electrode (Peech, 1965); organic matter content by oxidation and titration of excess oxidant (Walkley and Black, 1934; Walkley, 1935); particle size analysis by sequential sedimentation and specific gravity estimations (Day, 1965); cation-exchange capacity (CEC) by sodium saturation (Chapman, 1965); and a distribution coefficient (Kd) indicating the extent to which ethirimol is adsorbed by soil (Graham-Bryce and Coutts, 1971).

Incubation. Soils were dispensed in 30g quantities in small glass crystallising dishes for laboratory incubation and in approximately 200g quantities for greenhouse incubation with coated barley seeds or glass beads. All soils were incubated under passage of moist CO_2-free air, the effluent air being passed through tubes containing, in order, glass wool, 0·1N sulphuric acid (to trap volatile bases), glass wool, 2-methoxyethanol (to trap organic soluble volatiles), glass wool and ethanolamine (to trap $C^{14}O_2$). Sterility of air passing over irradiated soil was maintained using miniature air line filters (Microflow, Fleet).

Soil pots supporting barley plants were incubated in a horizontally partitioned box; the plants growing up through holes in the partition were sealed at soil level using a silastomer (Silastic 9161 RTV, Hopkins and Williams), effectively creating 2 separate environments, one for the soil-root system and one for the foliage. The effluent air from each was monitored separately.

During laboratory incubation the temperature was kept at $19^{\circ} \pm 1^{\circ}$ and the soil moisture content maintained by addition of sterile water at approximately ten week intervals. In the greenhouse the air temperature fluctuated between 18° and 22°, and incubation was continued until mature 'ears' were formed on the barley.

Soil analysis. All quantitative measurements of radioactivity were made using a Packard liquid scintillation counter (Model 3390 with Absolute Activity Analyser 544).

Volatile degradation products were detected by measuring the radioactivity in the trapping solutions, initially at weekly intervals, later every three weeks. Non-volatile products were extracted from soil using a cold acetone wash followed by a hot methanolic soxhlet extraction. The combined extracts were analysed by tlc on silica gel F_{254} plates using 12% methanol in chloroform as solvent, and autoradiographs prepared using Kodak no screen X-ray film. Quantitation of defined degradation products, or groups of products, was made by scraping the appropriate areas of silica from the plates, and shaking with methanol prior to the addition of a fluor and scintillation counting. Extracted soil was dried, ground to a powder and residual activity determined by combustion of sub-samples in a Packard sample oxidiser, Model 306.

RESULTS

Soils. Table 1 shows the properties of soils used in the studies.

Table 1

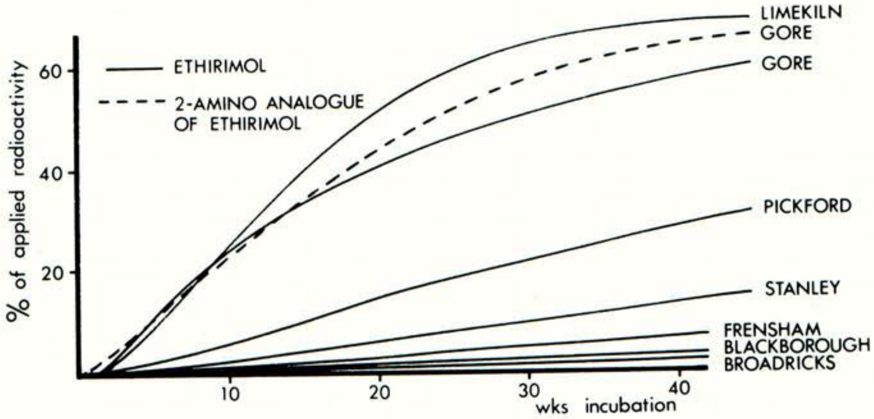
	Sand %	Silt %	Clay %	O.m. %	pH	CEC (m.equiv/100g)	MHC %	Kd
Gore	39	34	27	8·7	8·1	25·9	80·0	17·9
Limekiln	57	20	23	8·5	7·8	22·5	83·7	15·5
Pickford	46	21	33	4·5	7·5	31·5	59·2	26·3
Stanley	74	5	21	2·6	7·6	26·3	48·3	28·7
Frensham	74	15	11	2·1	6·5	9·2	31·7	4·8
Broadricks	65	14	21	4·0	5·5	23·7	57·3	16·9
Blackborough		peat		51·9	5·5	124·1	207·1	not done

ETHIRIMOL UNIFORMLY INCORPORATED INTO SOIL

Degradation products in trapping solutions. Throughout incubation radioactivity was only detected in the ethanolamine traps. Since this radioactivity had passed through organic solvent and acid 'traps' it was assumed to be $C^{14}O_2$, a product of pyrimidine ring cleavage. Fig. 2 shows that ring cleavage occurred in all the non-sterile soils, although rates differed widely. From the sterile soils studied, less than 0·13% of the applied pesticide was recovered as $C^{14}O_2$. Incubation of the 2-amino analogue with Gore soil resulted in pyrimidine ring cleavage of a rate slightly greater than that of ethirimol.

Fig. 2

$C^{14}O_2$ from pyrimidine ring cleavage in biologically active soils



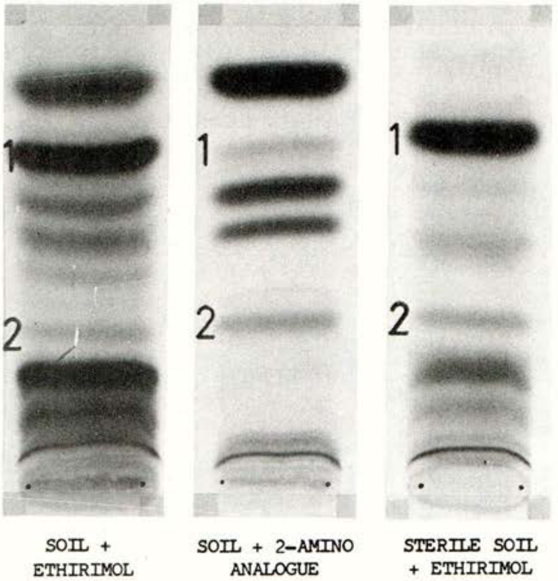
Soil analysis. Solvent extraction initially gave high recoveries of radioactivity, but with the increasing time of incubation of soils treated with ethirimol and its 2-amino analogue recoveries were reduced (Fig. 5). Attempts to exchange the non-extractable ('bound') radioactivity by refluxing extracted soils with high concentrations of unlabelled ethirimol and three analogues failed to displace more than 2% of the activity present. Use of ultrasonication in aqueous and organic systems was similarly unsuccessful. $C^{14}O_2$ was not released by hydrochloric acid digestion, thus 'bound' material was not present as carbonates. The site of 'binding' could be on soil particles, on or in microorganisms or the C^{14} might be incorporated in soil organic matter. It is well known that pyrimidines of nucleic acid origin are incorporated in the humus fraction of soils (Alexander, 1961). Total recoveries of radioactivity from soil extraction and combustion, and from trapping solutions consistently accounted for 90-110% of applied fungicide, thus it was assumed that all the original application of radioactivity was being recovered.

Autoradiographs of chromatographed extracts of Gore soil after 45 weeks incubation are shown in Fig. 3. The amount of extractable residual ethirimol in the soils is shown in Fig. 4, and a more detailed balance of distribution of activity for Gore and Frensham soils in Fig. 5. Although rates of degradation in the various non-sterile soils were very different, the products were essentially the same, differing only in their relative proportions.

After 2 hours soil exposure only 50-80% of the radioactivity could be identified as ethirimol, the remainder being more polar than the 2-amino analogue, and mostly co-chromatographing with degradation products extracted from sterile soils (fig. 3). However, on several occasions and within one week, a reversal of some of this 'degradation' had occurred. This phenomenon was possibly due to the formation and rapid dissolution of complexed or polymerised ethirimol. Initially, the amount of residual extractable ethirimol decreased rapidly (Fig. 4), due partly to formation of these polar products and partly to 'binding'. After this initial period, the amount of 'bound' activity remained relatively constant. The 2-amino analogue degraded somewhat faster than ethirimol (Fig. 4) and with the production of only small amounts of polar degradation products, this possibly implicating the 2-ethylamino group of ethirimol in the rapid formation of these polar products.

Fig. 3

Extracts of Gore soil after 45 weeks incubation

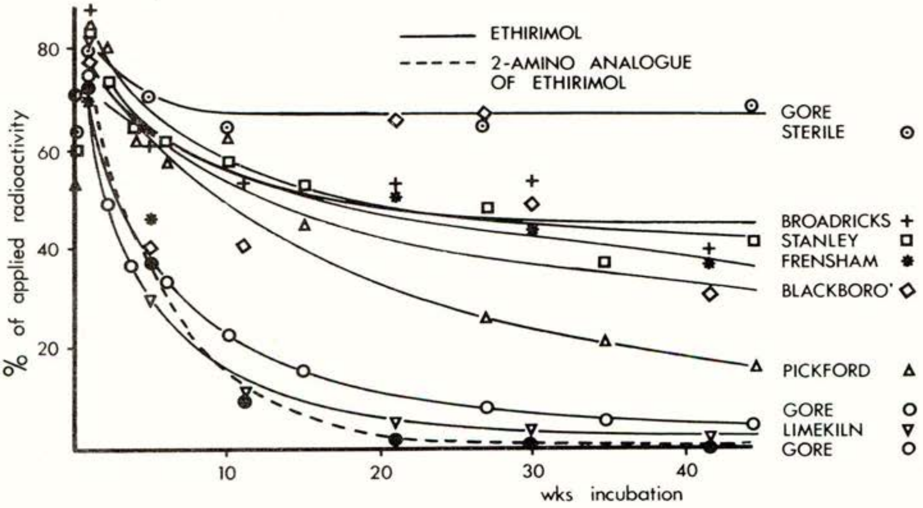


ORIGIN

1. ETHIRIMOL
2. 2-AMINO ANALOGUE OF ETHIRIMOL

Fig. 4

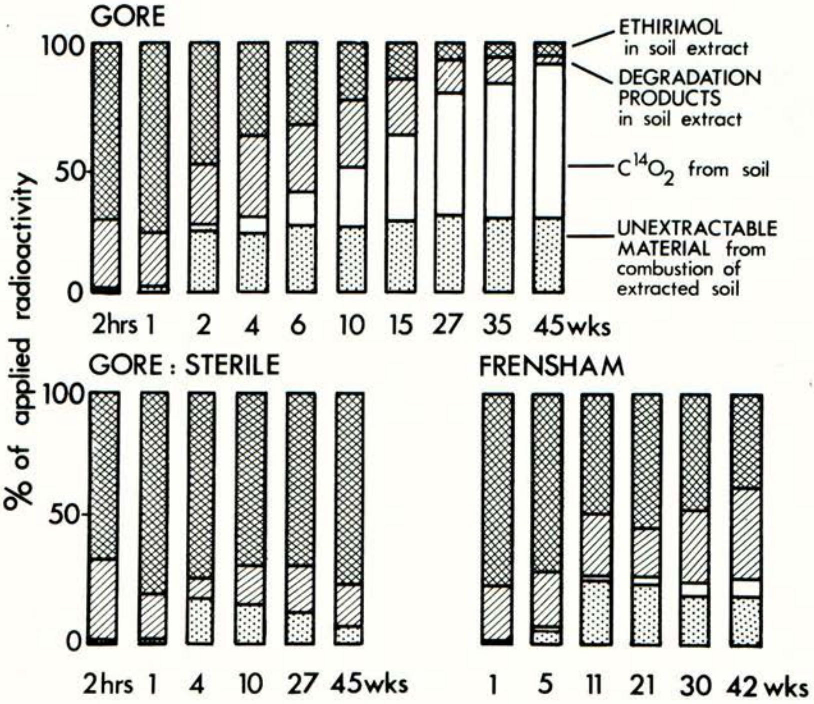
Residual, extractable ethirimol in incubated soils



Two-dimensional chromatography showed as many as 20 degradation products to be present in soil, comparative data from sterile and biologically active soils suggesting that several of these may have resulted from soil chemical activity. All the extractable products of degradation were themselves degraded further with eventual ring cleavage and release of $C^{14}O_2$ (Fig. 5). Ring cleavage was most probably biologically mediated. A sample of Gore soil sterilised by γ-radiation after 5 weeks incubation with ethirimol failed to evolve any further $C^{14}O_2$ with continued incubation.

Fig. 5

Distribution of radioactivity in soil during 45 weeks incubation



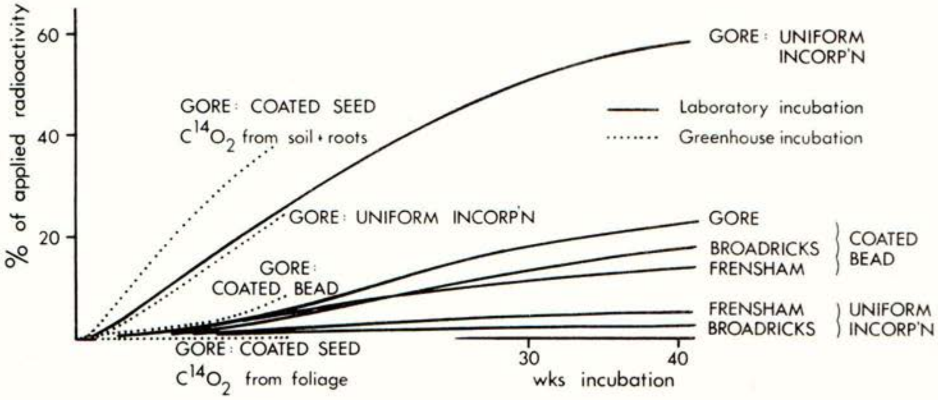
No clear correlation was evident between either total ethirimol degradation or pyrimidine ring opening and any of the physical or chemical soil characteristics measured (Table 1). However, the number of soils studied was relatively limited.

ETHIRIMOL APPLIED AS LOCALISED CONCENTRATIONS IN SOIL

Degradation products in trapping solutions. Radioactivity was again only present in the ethanolamine and assumed to be due to $C^{14}O_2$ from ring cleavage. Fig. 6 shows rates of pyrimidine ring cleavage in soils incubated with glass beads and barley seeds coated with ethirimol 'col', and also with uniformly incorporated fungicide.

Fig. 6

$C^{14}O_2$ from soils incubated with uniformly applied ethirimol, and with ethirimol coated seeds and glass beads



Relative rates of $C^{14}O_2$ evolution from laboratory incubated soils with ethirimol uniformly mixed and as localised concentration on glass beads were different in the three soils studied. With Gore soil, where the local high concentration on a glass bead reduced the rate of ring cleavage, incubation of the coated barley seed resulted in a highly enhanced rate of $C^{14}O_2$ evolution, greater even than the rate observed with the fungicide uniformly incorporated in soil. $C^{14}O_2$ from the incubation of soil with coated barley seeds was almost entirely from the soil-root environment, with less than 0·3% of the originally applied activity being evolved as $C^{14}O_2$ from the foliage. Graham-Bryce and Coutts (1971) have shown the distribution of radioactive ethirimol from localised seed sources into the soil to be considerably limited, under leaching and non-leaching conditions. The same is true for glass beads and germinating seeds (Graham-Bryce and Coutts, pers. comm.). Thus the concentration in these localised zones will be considerably greater than in soil with informly incorporated pesticide.

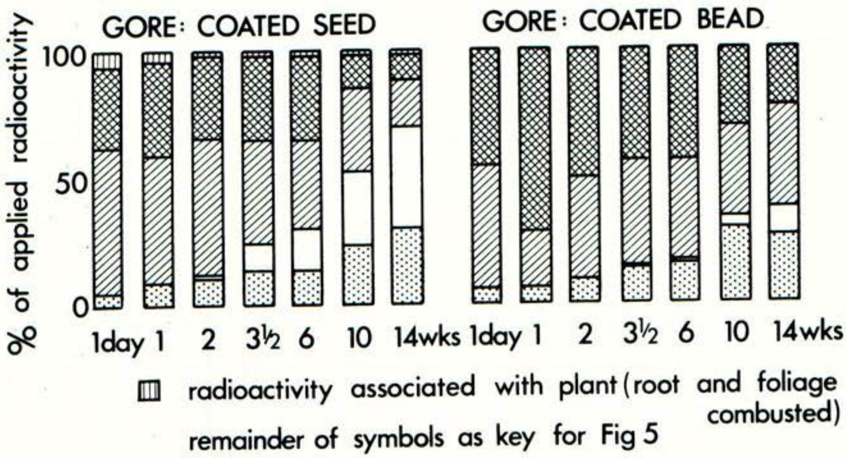
In Gore soil it seems likely that the ethirimol concentration in soil around the glass bead to some extent inhibited metabolism of the fungicide, resulting in a long lag phase, and reduced rate of ring cleavage. Around the seed and roots the rapid degradation may possibly be attributable to the difference in the soil environment, the presence of a different microflora, exudates from roots or a combination of these factors.

Soil analyses. The distribution of radioactivity in Gore soil incubated with coated beads and seeds is shown in Fig. 7.

Degradation of ethirimol was greatest in the presence of germinating barley. In the presence of plants the extractable degradation products were themselves further degraded with release of $C^{14}O_2$, whilst in soil with coated glass beads the amount of extractable breakdown products remained fairly constant. 'Bound' material increased over the incubation period. After the initial week radioactivity associated with plant material (from combustion data) accounted for less than 3% of the original ethirimol seed application; from the sixth week the majority of this being in the foliage.

Fig. 7

Distribution of radioactivity in soils incubated with localised high concentrations of ethirimol



It must be remembered, however, that although the degradation products in the laboratory and the field are likely to be the same, the rates of breakdown of ethirimol may well be different due to the variations in the field environmental conditions and hence in microbial activity.

Acknowledgements

The valuable technical assistance of Mrs M E Perry, Mrs V Hepworth and Miss S L Black is gratefully acknowledged.

References

ALEXANDER, M. (1961). Introduction to Soil Microbiology. John Wiley and Sons, Inc., NY.

BEBBINGTON, R.M., BROOKS, D.H., GEOGHEGAN, M.J. and SNELL, B.K. (1969). Ethirimol: a new systemic fungicide for the control of cereal powdery mildews. Chem.Ind., 1512

CAVELL, B.D., HEMINGWAY, R.J. and TEAL, G. (1971). Some aspects of the metabolism and translocation of the pyrimidine fungicides, in Proc. 6th Br.Insectic.Fungic. Conf., 431-437.

CHAPMAN, H.D. (1965). Cation-exchange capacity, in Methods of Soil Analysis, part 2, 891-901.

COUTTS, J.R.H. (1930). "Single value" soil properties. A study of the significance of certain soil constants. III. Note on the technique of the Keen-Raczkowski box experiment. J.Agric.Sci., 20, 407-413.

DAY, P.R. (1965). Particle fractionation and particle size analysis, in Methods of Soil Analysis, part 1, 545-567.

GEOGHEGAN, M.J. (1969). Pyrimidine fungicides, in Proc. 5th Br. Insectic.Fungic. Conf., 333-339.

GRAHAM-BRYCE, I.J. and COUTTS, J. (1971). Interactions of pyrimidine fungicides with soil and their influence on uptake by plants, in Proc. 6th Br. Insectic.Fungic. Conf., 419-430.

KEEN, B.A. and RACZKOWSKI, M. (1921). The relation between the clay content and certain physical properties of soil. J.Agric.Sci., 11, 441-449.

PEECH, M. (1965). Hydrogen ion activity, in Methods of Soil Analysis, part 2, 914-926.

WALKLEY, A. (1935). An examination of methods for determining organic carbon and nitrogen in soils. J.Agric.Sci., 25, 598-609.

WALKLEY, A. and BLACK, I.A. (1934). An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science, 37, 29-38.

NOTES

Proceedings 7th British Insecticide and Fungicide Conference (1973)

THE BEHAVIOUR OF TRIDEMORPH IN SOIL

S. Otto, N. Drescher
BASF Aktiengesellschaft, Landwirtschaftliche Versuchsstation, Limburgerhof

Summary The adsorption, mobility and degradation of tridemorph in soil were investigated.

The results indicate that, due to the strong adsorption of the fungicide by the soil, there is no danger from leaching with drainage water.

Investigations on the degradation of tridemorph have shown that it is non-persistent in soil, but evidently can be completely degraded biologically.

Degradation is probably initiated by the formation of tridemorph-N-oxide. As additional products of degradation, 2,6-dimethylmorpholine and CO_2 were detected.

Résumé La présente étude concerne l'adsorption, le comportement au lessivage et la dégradation de la matière active dans le sol.

Les résultats des essais montrent, étant donné la forte adsorption de la matière active par le sol, qu'il n'existe pas de risque de lessivage par les eaux d'infiltration.

L'étude de la dégradation de la matière active prouve que cette dernière ne persiste pas dans le sol et qu'elle semble être soumise à une dégradation biologique complète.

Le processus de dégradation débute probablement par une formation de tridémorphe-N-oxide. D'autres produits de dégradation ont pu être decelés: la 2,6-diméthylmorpholine et le CO_2.

INTRODUCTION

The fungicide Calixin$^{(R)}$-active principle tridemorph (N-tridecyl-2,6-dimethylmorpholine) has been in use for a number of years for controlling mildew in cereals. The systemic behaviour of tridemorph in plants has already been reported (Pommer et al., 1969). The results presented at that time have since been substantiated and supplemented by work using radioactive labelled tridemorph (Otto and Pommer, 1973).

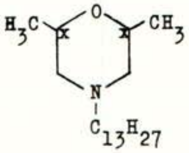
Using the ^{14}C-labelled preparation synthesised by Dr. Burger in the BASF Isotope Laboratory, investigations on the behaviour of tridemorph in soil could also be carried out. Investigations covered adsorption of the fungicide in two types of soil, and its degradation in the loamy-sand soil of our research station.

The results of these investigations will be briefly reported in this paper:

(R) = registered trade mark of BASF AG

EXPERIMENTAL and RESULTS

The material used in the radiotracer experiments was labelled at the C-atoms in positions 2 and 6 in the morpholine ring:



N-tridecyl-2,6-dimethylmorpholine (2,6-^{14}C)
Specific activity: 1.70 ± 0,12 milli-Curie/mMol.

1. Adsorption

The adsorption of the active substance was investigated on two different types of soil. The characteristics of the soils used were:

Limburgerhof soil:	Loamy-sand pH (KCl) 6.7 Organic matter content 1.4 % silt and clay 19.5 %
Bruchfeld soil :	Peaty soil pH (KCl) 7.5 Organic matter content 5.3 % silt and clay 46 %

The adsorption isotherms of the active substance were determined as follows:

To aqueous solutions (100 ml) with fungicide concentrations from 0.2 - 5 ppm 20 g of the appropriate soil was added, and the whole shaken for 10 hours on a mechanical shaker. The suspension was then centrifuged, and the concentration of fungicide in the sediment and in the supernatant aqueous liquor, was determined by measuring the radioactivity. The concentrations measured in the solid and in the liquid phase are plotted against each other in Fig. 1. As may be seen from the graph, the slope of the adsorption isotherm is very steep. At the concentrations investigated the fungicide is completely adsorbed from the aqueous solution by the soil. Fig. 1 shows results for Limburgerhof soil; for Bruchfeld soil practically the same curve is obtained.

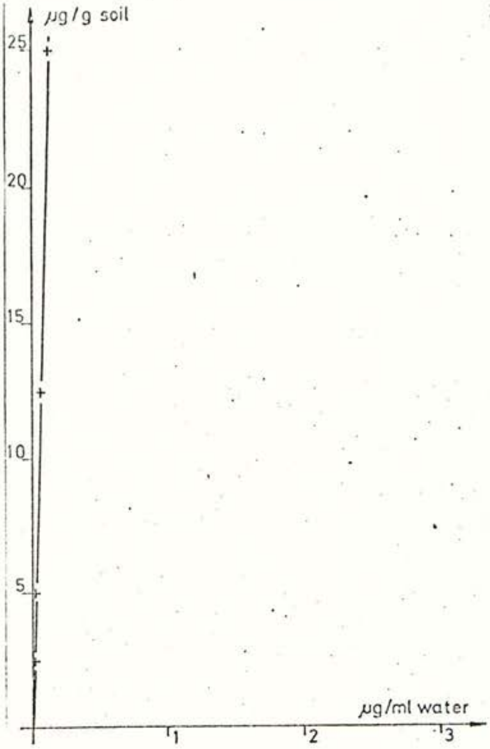


Fig. 1

Adsorption isotherm of tridemorph on Limburgerhof soil

2. Leaching

Due to the strong adsorption of the fungicide, there could hardly be any grounds for fear that tridemorph in the soil would be transported by water to any significant degree, or even that it would be leaching into the subsoil water. Nevertheless, leaching was examined in a model experiment.

Samples of soil were packed into glass columns (inside diameter 5 cm, length 30 cm), and, prior to the start of the test, the samples were moistened to maximum water retention. A small amount of labelled tridemorph was formulated, and applied, at a dosage equivalent to 0.75 litre/hectare Calixin, to the soil. Corresponding to the approx. 20 cm^2 cross section of column, 114 μg fungicide was applied. The columns of soil were eluated with completely desalinated water, using 200 ml per day for 2 days - this being equivalent to extremely heavy rainfall of 100 mm/day.

Radioactive measurements in the soil and the leachate showed that the fungicide, even with extremely heavy rainfall of 100 mm/day, was only washed into the soil to a slight degree. 98 % of recovered radioactivity was detected in the uppermost layer 5 cm of soil. The leachate was completely free of radioactivity.

3. Degradation in the soil

Experimental methods:

Investigations on the degradation of tridemorph were carried out using Limburgerhof soil. The labelled fungicide was dissolved in the volume of water required to moisten the soil, and was applied evenly to the soil (2 kg soil; 10 mg = 5 ppm).

The water content of the soil was adjusted to 12 % (approx. 43 % of maximum water capacity), and the moistened soil was then transferred into a 2 l-glass cylinder. A gentle stream of air (about 1.2 litres air per hour) was passed through the vessel containing the soil throughout the test. The air, on entry, was scrubbed with 2 N potassium hydroxide solution and 2 % sodium carbonate solution, and was simultaneously humidified; on leaving the apparatus, the air passed through 1 N sulphuric acid, to remove any escaping basic substances, followed by passage into a mixture of ß-phenylethylamine and methanol (1:1), to absorb $^{14}CO_2$ which had been formed. The experiment was carried out at ambient temperatures of 22 ± 3 °C.

Sampling and Analysis:

25 g samples were removed from the soil at intervals 6 weeks, and were analysed as follows: The soil was extracted with a mixture of 100 ml methanol and 25 ml 1 N HCl. After filtration of the extract, the methanol was removed in a vacuum rotary evaporator, and the aqueous hydrochloric acid phase which remained was shaken thoroughly with chloroform. The chloroform extract and the aqueous phase were evaporated, the residues were dissolved in a known volume of methanol and aliquots thereof were removed for measurement of radioactivity and for thin-layer chromatography.

To determine the residual activity retained by the soil after the extraction, fractions of the extracted soil were combusted in a combustion automat (Oxymat, Intertechnique), the resulting $^{14}CO_2$ being absorbed in a scintillation cocktail containing phenylethylamine, and the activity was measured in the scintillation spectrometer (TRI-CARB 4322, Packard).

Results:

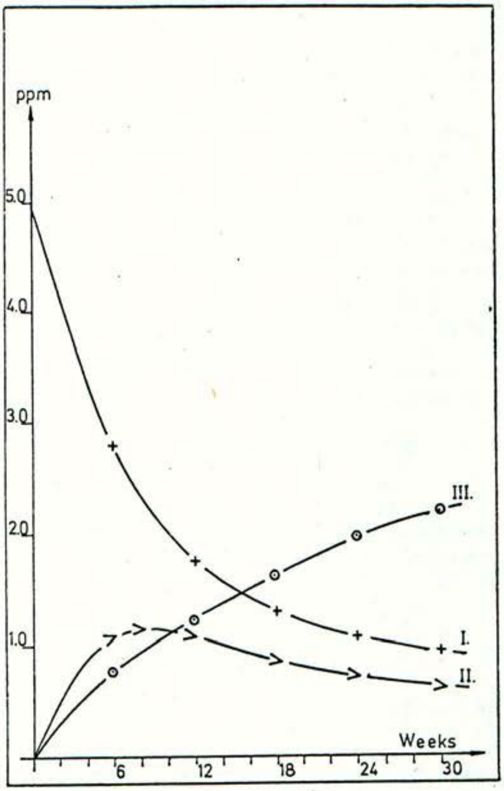
By plotting, for each sample, the activities measured in the chloroform and hydrochloric acid phases, and the residual activity remaining in the soil against time, we obtain the curves shown in Fig. 2.

Fig. 2

Curve I shows the activity extractable with chloroform at the different sampling times and so essentially describes the degradation of the parent compound as this (as shown later) is always the main component in the chloroform extract.

Curve II shows the residual activity in the hydrochloric acid phase in the chloroform/ hydrochloric acid separation. The shape of the curve indicates the formation of one (or several) polar metabolite(s), as the parent compound, always, passes quantitatively into the chloroform phase.

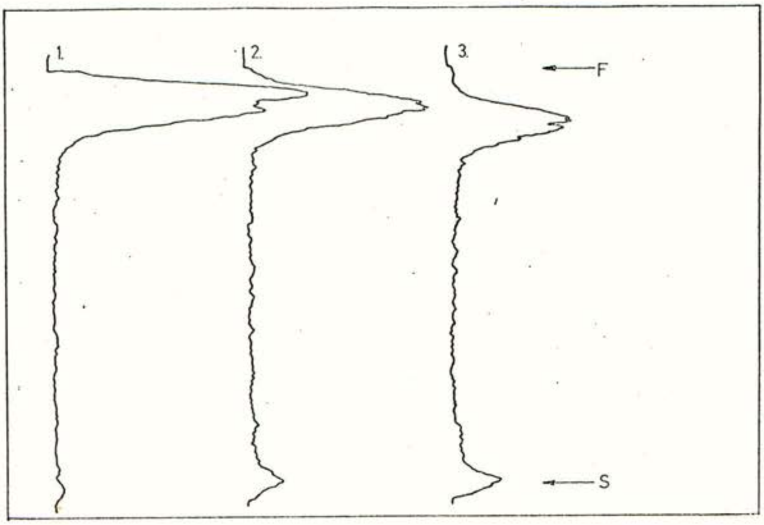
Curve III shows the progressive increase of the residual activity remaining after the methanol/HCl extraction. The shape of the curve indicates that, on degradation of the parent compound, either polar degradation products result, which are very strongly adsorbed onto the soil components, or that certain molecular fragments are built into the organic substance (humic acids) of the soil.



The radioactive materials found in the chloroform and hydrochloric acid phase were investigated further.

The ratemeter curves given by thin-layer chromatograms of the chloroform extracts obtained after 6, 12 and 18 weeks, show that the parent compound (high Rf value component), is broken down (Fig. 3), but that at the time a more polar component is formed, this remaining at the origin. We considered it to be possible that this polar degradation product, whilst still soluble in chloroform, could be the N-oxide of tridemorph. In confirmation of this, the oxidation product obtained from tridemorph and hydrogen peroxide or sodium periodate, when compared by thin-layer chromatography in suitable eluents, exhibited the same behaviour as the polar soil metabolite in the chloroform phase.

Fig. 3



Ratemeter curves taken from the thin-layer chromatogram of chloroform extracts

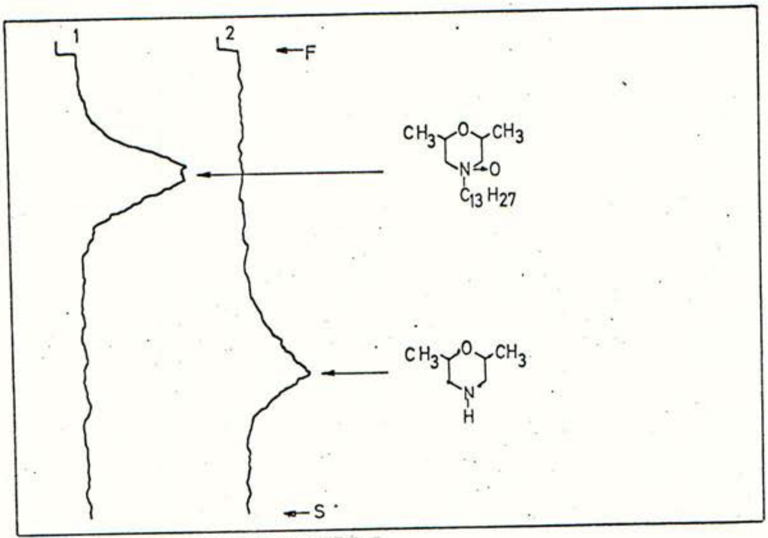
1 = 6 weeks, 2 = 12 weeks, 3 = 18 weeks after start

Silica gel: 0,25 mm; solvent system: hexane:acetone = 8:2

The structure of the tridemorph-N-oxide could be established by infra-red spectroscopy from comparison with material obtained by oxidising tridemorph. The fact that the soil metabolite found in the chloroform phase can be reconverted into tridemorph by means of reducing agents (e.g., Dewarda's alloy in alkaline solution), provides further evidence for its structure.

The residual activity remaining in the aqueous hydrochloric acid phase was investigated by thin-layer chromatography (0.25 mm silica gel; eluent system: benzene/ethanol conc./NH_3 = 55:40:5). The ratemeter curve of the thin-layer chromatogram (Fig. 4) shows that the radioactive component present in the aqueous phase (2) is not identical with tridemorph-N-oxide, but is a very polar substance.

Fig. 4



Thin-layer chromatographie comparison of the slightly polar material in the chloroform phase and the more polar metabolite in the aqueous phase

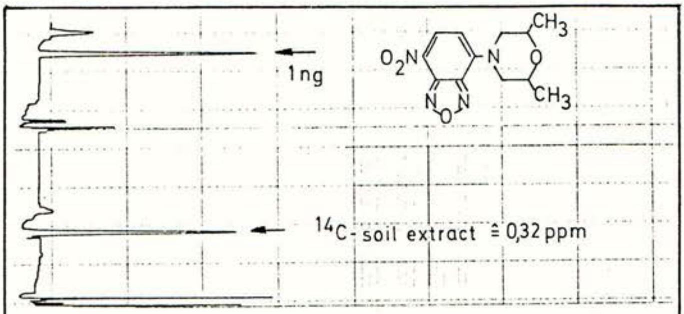
Silica gel: 0.25 mm; solvent system: benzene:ethanol conc.:NH_3
55 : 40 : 5

We established that the polar radioactive component in the aqueous phase could be reacted with reagents such as 2,6-dinitrofluorobenzene, 7-chloro-4-nitrobenzofurazan, as well as with 2,4-dichlorobenzoyl chloride. It is therefore not a tertiary, but most likely a secondary, amino compound. There were grounds to suspect that by cleavage of the tridecyl residue from the parent molecule, 2,6-dimethylmorpholine was formed as a metabolite in the soil.

By comparison with thin-layer chromatograms of the standard substances as well as by IR-spectroscopic and gas chromatographic comparison of the corresponding derivatives, the degradation product found in the aqueous phase could be unambiguously identified as 2,6-dimethylmorpholine.

Fig. 5 illustrates the corresponding gas chromatograms. Here, the component isolated from the soil was compared, after forming the derivative, with the analogous derivative of 2,6-dimethylmorpholine.

Fig. 5



Gas Chromatogram data :
Column: 1.7m long, 2.5mm internal diameter, 2% QF1 on Gas Chrom Q (100-200 mesh)
Temp. of column: 210 °C, Temp. of injector: 230 °C
Detector: (^{83}Ni-ECD) pulse width 1 μ sec pulse frequency 200 μ sec
Temp. of detector: 250 °C, Carrier gas 40 ml/min (N_2)
Purge gas 30 ml/min (N_2)

2,6-dimethylmorpholine is, however, not the final degradation product of tridemorph in soil. We were able to establish that, during the entire duration of the test, $^{14}CO_2$ was evolved at a constant rate. 30 weeks after the commencement of the test, about 20 % of the activity initially added to the soil as labelled tridemorph, had been liberated as CO_2. Since the molecule is labelled in the morpholine ring, this means that even the very stable morpholine ring is cleaved in the soil and is oxidatively broken down. As can be seen from Fig. 6, the decrease in the total activity in the soil (upper curve) corresponds exactly to the amount of $^{14}CO_2$ liberated (lower ascending curve).

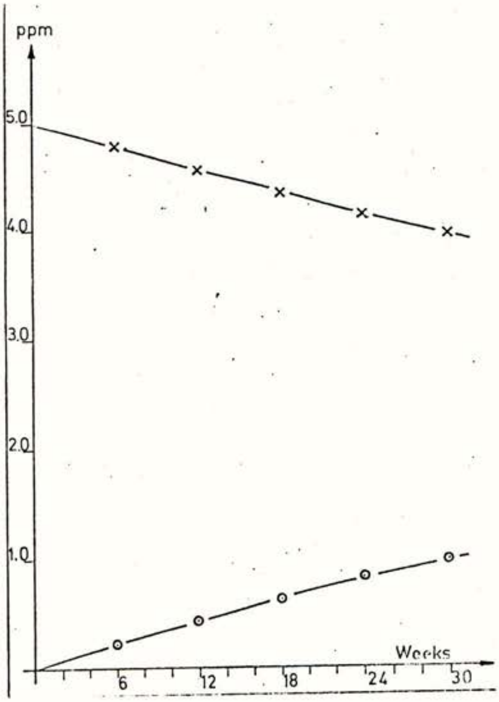
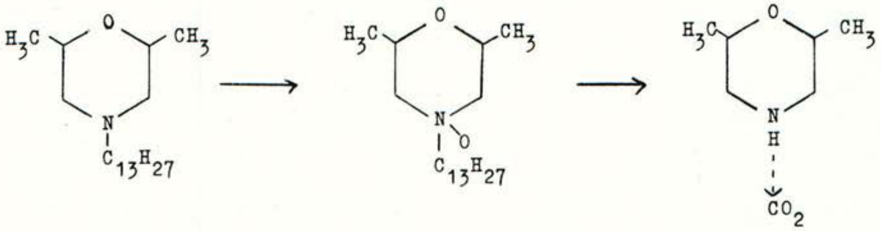


Fig. 6

The decrease of total activity in the soil (upper curve) corresponds to the amount of $^{14}CO_2$ liberated (lower ascending curve)

As the rate of formation of $^{14}CO_2$ remains constant, it can be seen, by extrapolation, that in about 3 years the total activity, which was introduced into the soil as 5 ppm ^{14}C-tridemorph, would be converted into $^{14}CO_2$, provided that the experimental conditions remained constant.

On the basis of the degradation stages thus far established, the decomposition of tridemorph in the soil can be outlined as follows:



DISCUSSION

Taken as a whole, a very favourable picture of the behaviour of tridemorph in the soil, can be drawn. The strong adsorption of the active substance on the soil prevents it from being washed down into the deeper layers of the soil. Therefore, the risk of contamination of surface water by residues does not arise. Investigations into the break down in soil show that the parent compound is non-persistent in the soil, and can obviously be completely broken down biologically. Degradation is initiated by the formation of tridemorph-N-oxide. As further products of degradation, 2,6-dimethylmorpholine and CO_2 have been detected.

Acknowledgements

We thank Dr. P. Beutel, Landwirtschaftliche Versuchsstation, BASF A.G., Limburgerhof, for determining and discussing IR spectra.

References

E.H. Pommer, S. Otto, J. Kradel
Proc. 5th Br. Insec. Fungic. Conf. (1969), 347

S. Otto, E.H. Pommer
XXV International Symposium on Crop Protection, Gent (1973)

Proceedings 7th British Insecticide and Fungicide Conference (1973)

POSSIBLE RELATIONSHIPS BETWEEN STRUCTURE AND MECHANISM OF DEGRADATION OF ORGANOPHOSPHORUS INSECTICIDES IN THE SOIL ENVIRONMENT

J. Adamson and T.D. Inch

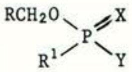
Chemical Defence Establishment, Porton Down, Salisbury, Wiltshire, SP4 0JQ

Summary Rates of hydrolysis and dealkylation of some organophosphorus compounds have been measured in solution. Methyl parathion was hydrolysed and dealkylated 3 and 40 times respectively faster than parathion. The pH values of a natural alkaline soil, the same soil heat sterilised, and an ion exchange resin were varied by the addition of suitable acid or alkaline solutions and the rates of decomposition of methyl parathion and parathion measured at field capacity. The decomposition rates decreased in the order resin, normal soil, sterilised soil, aqueous solution at similar pH values. Since methyl parathion was degraded only 3 times faster than parathion in soil, and there was no evidence for a lag phase or for decomposition in soil washings, and hydrolysis was faster on an ion exchange resin, it was concluded that the rates observed result from surface catalysis and that microbial degradation plays very little part in the removal of these insecticides from soil.

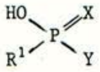
Résumé On a mesuré les vitesses d'hydrolyse et de déalcoylation de quelques composés organophosphores. SNP méthylique a été hydrolysé et déalcoylé respectivement 3 et 40 fois plus vite que SNP. Les mesures de pH d'un sol natural alcalin, le même sol sterilisé par la chaleur, et une résine ionique ont été variées par l'addition des solutions acides ou alcalines convenables, et les vitesses de la décomposition de SNP méthylique et de SNP ont été mesurées à "field capacity". Les vitesses de décomposition ont diminué par ordre: résine, sol normal, sol sterilisé, solution débarrassée du sol, aux mesures de pH semblables. Puisque SNP méthylique a été dégradé dans le sol seulement 3 fois plus vite que SNP, sans une période d'incubation pour la décomposition, et sans aucune décomposition dans les liquides de lavage du sol, quoique la vitesse d'hydrolyse sur une résine ionique a été augmentée, on a conclu que les vitesses observées resultaient de l'action catalytique des surfaces, et que la dégradation microbienne n'a guère d'importance en l'enlèvement de ces insecticides du sol.

INTRODUCTION

In the heterogeneous reaction medium provided by soil, organophosphorus insecticides can be degraded by most of the chemically or enzymatically induced reactions that have been described for organophosphorus compounds. Unfortunately, because widely different mechanisms can lead to the same products, product analysis often provides little insight into the nature of the preponderant modes of degradation. For example, an organophosphorus compound of general formula 1 can be converted into 2 by nucleophilic attack at phosphorus by OH^- (hydrolysis), by nucleophilic attack at carbon (dealkylation) (Hilgetag & Teichmann, 1965; Miller, 1962; Noller & Dutton, 1933) and by oxidative attack at carbon (Donniger et al., 1972).



1



2

R = H, alkyl
R^1 = alkoxy, alkyl, phenyl
X = O, S

One possibility for distinguishing between different mechanisms of degradation is to compare kinetic data from quite distinct chemical reactions in soil free systems with rates of decomposition of the organophosphorus compounds in soil, provided that the rates of the mechanistically distinct reactions differ sufficiently. To investigate the utility of this approach, rates of hydrolysis (nucleophilic attack at phosphorus) and rates of dealkylation with thiosulphate (nucleophilic attack at carbon) of organophosphorus insecticides and analogues prepared by modification of the alkyl substituent (methyl, ethyl, isopropyl) have been measured (Table 1) and the results compared both with previously reported rates of decomposition of organophosphorus insecticides in soil and with the results of soil studies in this laboratory.

Additionally an attempt has been made to distinguish between the relative importance of the pH of the soil solution and other soil effects, on the rate of decomposition of organophosphorus compounds on soil.

METHODS AND RESULTS

Hydrolyses were performed on 10^{-5}M solutions of insecticides in 5% isopropanol in water at a constant ionic strength of 0.1M. Phenol production was followed by U.V. spectroscopy at 400 mμ for p-nitrophenol and 245 mμ for 2,4-dichlorophenol. Dealkylations were performed similarly, utilising the competition between hydroxide and thiosulphate ions at various pH's to determine both rates.

Gas chromatography was used for the estimation of intact starting materials extracted from aqueous solutions with benzene. 1 and 5 ft. columns of SE30 (5%) on Supasorb, with an electron capture detector in the pulse mode enabled direct analyses without prior clean-up. A flame photometric detector and a 10 ft. carbowax (5%) on Supasorb column enabled methylated reaction products to be identified and estimated.

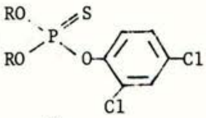
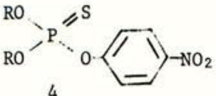
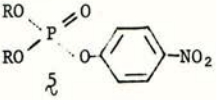
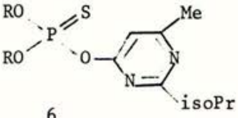
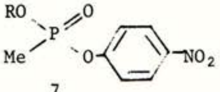
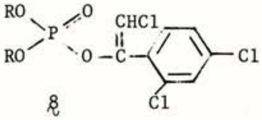
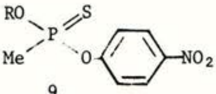
The results of the above experiments are recorded in Table 1.

Soil experiments followed the procedure of Getzin & Rosefield (1958). The insecticide (0.12 mg) was added to soil (5 g) in water, and the soil brought to field capacity. The resin experiments were performed similarly, with Amberlite resin CG 400 (Cl) Type II 200 mesh. The pH of the soil and resin were adjusted with $FeCl_3$ or NaOH solution to the required values. The pH was measured again at the end of each experiment to check for changes in pH.

Soils were extracted with acetone - benzene (1:1) and analysed by glc as described. Recovery rates were shown to be better than 90%. Results are shown in Figure 1. The soil used in this laboratory was from Porton Down with the characteristics shown in Table 2.

TABLE 1

Second Order Kinetic Constants for Hydrolysis and Dealkylation of some Organophosphorus Compounds

R =	Me	Et	isoPr	
 3	8.2	2.6	0.14	$K/[OH^-]$
	1.8	0.035	0.02	$K/[thio^-]$
 4	12.95	3.6	0.37	$K/[OH^-]$
	2.2	0.05	0.01	$K/[thio^-]$
	0.012			K_2 (ethanol)*
 5	143	44.1	3.2	$K/[OH^-]$
	2.3	0.05	0.01	$K/[thio^-]$
 6		10.2	1.26	$K/[OH^-]$
 7		3000		$K/[OH^-]$
		0.87		$K/[thio^-]$
 8	48.7	9.2	0.6	$K/[OH^-]$
 9		400		$K/[OH^-]$
		0.24		$K/[thio^-]$

* Rate of dealkylation in 95% ethanol with 10^{-2}M NaOH.

TABLE 2

Characteristics of Porton Down Soil

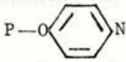
pH_{H_2O}	7.8
pH_{CaCl_2}	7.1
Organic Carbon	7%
Cationic Exchange Capacity	40 me/100 g
Clay	6%
Sand	60%
Moisture Content at Field Capacity	70%

DISCUSSION

The hydrolysis date in Table 1 were measured in order to obtain directly comparable results under standard conditions. The results confirm the well established general trends that methyl esters are hydrolysed faster than ethyl esters, that phosphoryl derivatives are hydrolysed faster than the corresponding thiono derivatives and that phosphonates are hydrolysed faster than phosphates.

Relative rates of nucleophilic attack at carbon were measured using thiosulphate as nucleophile because thiosulphate reacts more rapidly than the soft bases such as iodide, amines, thiols etc. which are more likely to occur in biological systems. The rates of dealkylation were essentially independent of pH, and of the nature of the leaving group and did not vary when P=S was changed to P=O. However, rates of dealkylation are possibly faster in phosphonates than in phosphates.

The most potentially useful diagnostic information relevant to distinguishing between modes of decomposition in soil was that whereas methyl esters are hydrolysed only 2 - 3 times faster than ethyl esters they are dealkylated about 40 times more rapidly.

To date, hydrolysis and dealkylation data have only been obtained in aqueous solution. More meaningful data may possibly be obtained from similar studies in non-aqueous solution since in soil reactions of strongly absorbed organophosphorus compounds may in fact be occurring in a non-aqueous environment. One example of the difference between an aqueous and non-aqueous reaction concerns the hydrolysis of methyl parathion (4, R = Me) and parathion (4, R = Et) in water containing 5% isopropanol and in 95% aqueous ethanol. In the former case hydrolysis occurs with essentially only cleavage of the  bond, whereas in the latter case appreciable P-O-alkyl cleavage was observed (Plapp & Casida, 1958). The kinetic data indicated that demethylation was about 30 - 40 times faster than deethylation, suggesting nucleophilic attack at carbon rather than phosphorus.

In Figure 1 comparative decomposition rates for parathion and methyl parathion are shown. Parathion was degraded about 3 times more slowly than methyl parathion in aqueous solutions and in non-sterile soil. This data suggests that decomposition was preponderantly by a hydrolytic mechanism since a greater difference in rates would have been expected if a reaction involving attack at carbon was involved (cf. Getzin & Rosefield, 1968). Product analysis was consistent with this conclusion as are results from other studies of the decomposition of organophosphorus insecticides in soil loaded to field capacity with water. It remains to be seen, however, whether any indication of mechanism of degradation will be provided by decomposition rate data obtained under field conditions where the water content of the soil is much less than the field capacity. It is under these latter conditions that desalkyl insecticides are usually detected. (Beynon & Wright, 1967, 1969).

The differences in decomposition rates of methyl parathion on sterile and non-sterile soils at field capacity of water, in water in the presence of ion exchange resin, and in aqueous solutions (Figure 1) provides a clear indication of a catalytic surface effect for the hydrolysis mechanism. Since microbial decomposition may be precluded because of lack of an induction period (cf. Konrad, Chesters & Armstrong, 1969) and because soil washings did not enhance degradation rates (cf. Lichenstein & Schulz, 1964; Konrad, Armstrong & Chesters, 1967) it is probable that the effect of soil sterilisation is to change the surface characteristics rather than to reduce the importance of microbial decomposition (Konrad & Chesters, 1969).

The available data for the breakdown of diazinon (Getzin, 1968) (6, R = Et) (Figure 2) parathion and methyl parathion (Figure 1) makes an interesting comparison. Between pH 4 and 9, methyl parathion and parathion are hydrolysed more rapidly in soil than diazinon, whereas in aqueous solution diazinon is hydrolysed more rapidly than either.

Some indication of the importance of surface effects on these hydrolysis rates has been provided by Konrad et al. It was shown (Konrad & Chesters, 1969) that Ciodrin was degraded much faster in non-sterile than sterile soils. However, irradiation sterilisation reduced the adsorption capacity of the soil such that the ratio of degradation rate to adsorption remained constant. Malathion was degraded faster in a sterilised soil, Ella Is, than in the same soil unsterilised (Konrad, Chesters & Armstrong, 1969) because in this case, adsorption was increased by sterilisation.

If it is assumed that the observed rate (K_{obs}) equals the aqueous rate (K_{aq}) plus the surface rate (K_s) then the variation of rate of degradation with soil pH may be explained. For diazinon, K_s is seen to be constant (Fig.2) in the pH range studied ($4.5 \times 10^{-4} h^{-1}$ for sterile and $8.2 \times 10^{-4} h^{-1}$ for non-sterile soil). These experiments of Getzin (1968) were all performed on Sultan Silt loam, and the surface rate applies to this soil only.

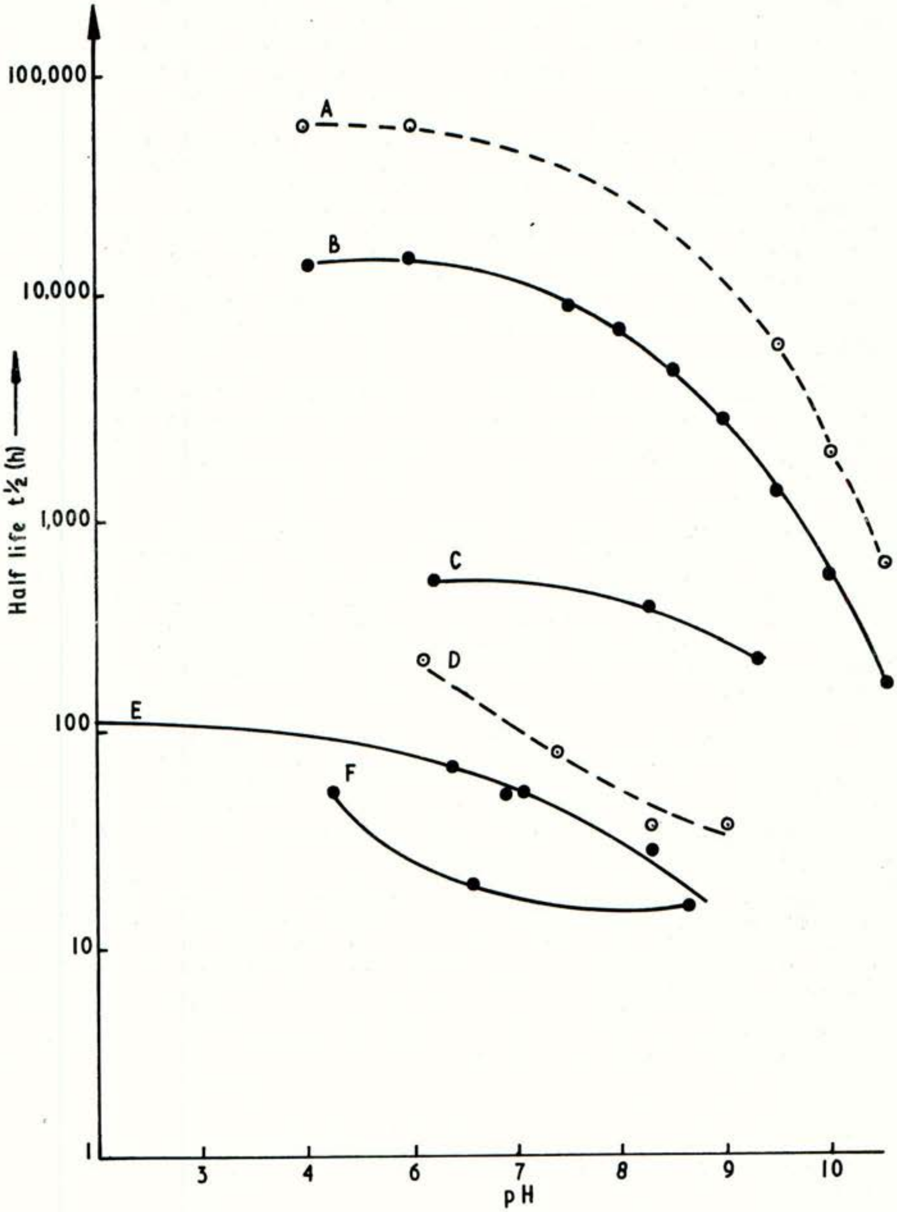
Our experiments with methyl parathion and parathion on soil and ion exchange resin (Figure 1) demonstrate that the surface rate may also be pH dependent. These results may be rationalised if it is assumed that the surface effect has acid and basis catalytic components. Thus for diazinon which is readily hydrolysed in acid and base, the apparent constant surface effect results from the fact that the acidic catalysis decreases as the basic catalysis increases with increasing pH. Parathion and methyl parathion are only hydrolysed under basic conditions so the surface effect increases with increasing pH.

Acknowledgements

One of us (J.A.) thanks the Agricultural Research Council for financial support.

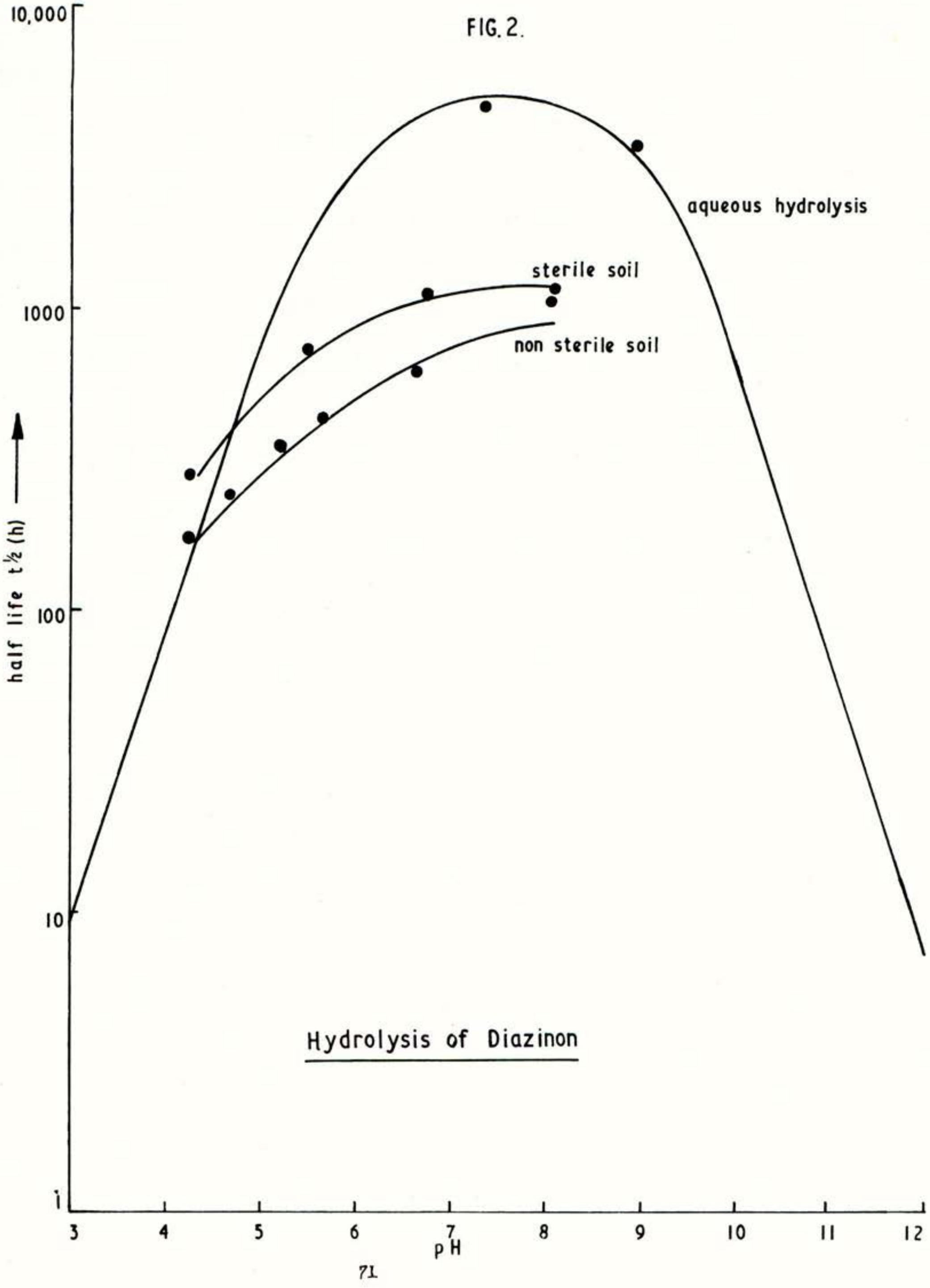
FIG.1

Hydrolysis of Methyl Parathion ●—● and Parathion ⊙--⊙



A and B, aqueous hydrolysis. C. hydrolysis on sterile soil.
D and E, hydrolysis on non sterile soil
F. hydrolysis on ion-exchange resin.

FIG. 2.



Hydrolysis of Diazinon

References

1. BEYNON, K.I. & WRIGHT, A.N. (1967). The breakdown of ^{14}C-chlorfenvinphos in soils and in crops grown in the soils. Journal of the Science of Food and Agriculture, 18, 143 - 150.

2. BEYNON, K.I. & WRIGHT, A.N. (1969). Breakdown of the insecticide 'Gardona' on plants and in soils. Journal of the Science of Food and Agriculture, 20, 250 - 256.

3. DONNINGER, C., HUTSON, D.H. & PICKERING, B.A. (1972). The oxidative dealkylation of insecticidal phosphoric acid triesters by mammalian liver enzymes, Biochemical Journal, 126, 701 - 707.

4. GETZIN, L.W. (1968). Persistence of Diazinon and Zinophos in Soil: Effects of autoclaving, temperature, moisture and acidity. Journal of Economic Entomology, 61, 1560 - 1565.

5. GETZIN, L.W. & ROSEFIELD, I. (1968). Organophosphorus insecticide degradation by heat-labile substances in soil. Journal of Agricultural and Food Chemistry, 16, 598 - 601.

6. HILGETAG, G. & TEICHMANN, H. (1965). The alkylating properties of alkyl thiophosphates, Angewandte Chemie International Edition, 4, 914 - 922.

7. KENNER, G.W. & MATHER, J. (1956). Studies on phosphorylation, Part XIV. The solvolysis by phenols of benzyl phosphates. Journal of the Chemical Society, 3524 - 3531.

8. KONRAD, J.G., ARMSTRONG, D.E. & CHESTERS, G. (1967). Soil degradation of diazinon, a phosphorothioate insecticide. Agronomy Journal, 59, 591 - 594.

9. KONRAD, J.G. & CHESTERS, G. (1969). Degradation in soils of Ciodrin, an organophosphate insecticide, Journal of Agricultural and Food Chemistry, 17, 226 - 230.

10. KONRAD, J.G., CHESTERS, G., & ARMSTRONG, D.E. (1969). Soil degradation of malathion, a phosphorodithioate insecticide, Soil Science Society of America Proceedings, 33, 259 - 262.

11. LICHTENSTEIN, E.P. & SCHULZ, K.R. (1964). The effects of moisture and microorganisms on the persistence and metabolism of some organophosphorus insecticides in soils, with special emphasis on parathion. Journal of Economic Entomology, 57, 618 - 627.

12. MILLER, B. (1962). Ease of displacement of thiol and oxide anions from methyl groups: Carbon basicities of anions of oxygen and sulphur. Proceedings of the Chemical Society, 303.

13. NOLLER, C.R. & DUTTON, G.R. (1933). Note on the preparation of trialkyl phosphates and their use as alkylating agents. Journal of the American Chemical Society, 55, 424 - 425.

14. PLAPP, F.W. & CASIDA, J.E. (1958). Hydrolysis of the alkylphosphate bond in certain dialkyl aryl phosphorothioate insecticides by rats, cockroaches and alkali. Journal of Economic Entomology, 51, 800 - 803.

Proceedings 7th British Insecticide and Fungicide Conference (1973)

ADSORPTION OF ORGANOPHOSPHORUS COMPOUNDS BY SOIL CONSTITUENTS AND BY SOILS

R.E. Grice, M.B.H.Hayes and P.R.Lundie *

Chemistry Department, University of Birmingham, Birmingham, B15 2TT

Summary Isotherms are presented for the adsorption of dimefox by four soils, by humic acid, and by clays, and for the adsorption of menazon by preparations of montmorillonite. Three additional model compounds and a variety of techniques were used to investigate the mechanisms by which certain classes of organophosphorus compounds interact with clays. The practical implications of the results are briefly discussed.

Résumé On présente des isothermes pour l'adsorption du dimefox par quatre sols, par l'acide humique, et par des argiles, ainsi que pour l'adsorption du menazon par des préparations de montmorillonite. Trois autres substances modeles et une variété de techniques furent employées pour la recherche des mécanismes qui entrent en jeu dans l'interaction entre les argiles et certaines classes de composés organophosphorés. On discute brièvement les incidences pratiques de ces résultats.

INTRODUCTION

Adsorption and desorption are among the most important processes determining the effectiveness of soil applied pesticides. It is generally agreed that the organic and inorganic colloidal materials are the most active of the soil constituents for binding (and hence inactivating) such pesticides.

Although application of physico-chemical techniques can yield much information on the overall interactions between any particular soil and pesticide, they fail, because of the complexity of the soil system, to show clearly the adsorption mechanisms involved. The modern approach therefore studies interactions between pesticides and pure clay and extracted humic preparations. In extrapolating this approach to soil systems it should be remembered that soils contain a mixture of clays many of which are interstratified, often coated with humic materials and with oxides and hydroxides of iron and aluminium. Therefore increased emphasis is being placed on studies on soil clay (with and without associated coatings) - organic chemicals interactions.

Organophosphorus compound-soil interactions have not been extensively studied. However, data by Graham-Bryce (1967) on the adsorption of disulfoton by a wide range of soils, and by MacNamara and Toth (1970) for the adsorption of malathion on soils, clay minerals, and organic matter have significantly advanced understanding in this field and have provided many ideas for the research described here.

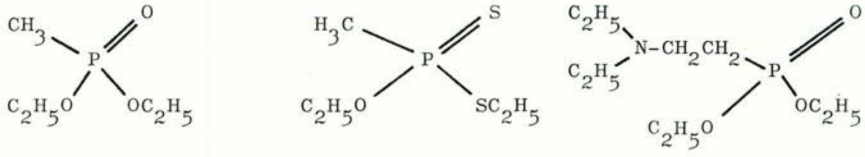
---

* Present address: English Clays, Lovering Pochin & Co Ltd., St. Austel.

This paper will describe the adsorption of dimefox (N N N'N'-tetramethylphosphorodiamidic fluoride) by selected soils, by clay isolated from one of these soils, by humic acid, and by well defined clay preparations. Data from a variety of techniques will give indications about the mechanisms of interactions between clays and menazon (S-[4,6-diamino-1,3,5-triazin-2-ylmethyl]dimethyl phosphorothiolothionate), three model compounds which are not used as pesticides, and, by inference, organophosphorus compounds in general.

METHOD AND MATERIALS

Dimefox and menazon were supplied by the Murphy Chemical Co. Ltd., and by I.C.I., Plant Protection Ltd., respectively. The three model compounds used, O,O-diethylmethylphosphonate (I), O-ethyl-S-ethyl methylphosphonothionate (II), and O,O-diethyl-2-(diethylamino) ethylphosphonate (III) are illustrated in Fig. 1.



I II III

Fig. 1
Model organophosphorus compounds used in research.

Soils and clay and humic acid preparations

Some characteristics of the soils used, as supplied by the Rothamsted Experimental Station, are presented in Table 1.

Table 1

Soils used in adsorption studies

Soil	Clay Content (%)	Organic Carbon (%)	CEC (meq $100g^{-1}$)	pH
Adventurers	28	40·0	170·0	7·3
Brome Pin	12	1·2	10·7	7·7
Prick Willow	68	13·7	85·4	6·5
YNYS	36	1·0	7·1	4·3

In cases of montmorillonite (No. 26, Clay Spur, Wyoming) and vermiculite (near Libby, Montana) clays, samples of <2μm equivalent spherical diameter were used. Homoionic clays were generally prepared by using the appropriate chloride salts, as described by Barshad (1969). Hydrogen ion saturated montmorillonite (hereafter called H^+/Al^{3+}-montmorillonite) was obtained by passing the ground clay suspension (20 g in 1·01) down a column (160 cm^3) of Amberlite 1R-120 (H^+-form) resin. The eluate was freeze dried.

Humic acids were extracted with 0·5N sodium hydroxide from H$^+$-saturated Fenland soil (80% organic matter), and precipitated by stepwise addition of concentrated HCl to pH 1·0. This precipitate was washed with distilled water, then dialyzed against distilled water at 4°C for five days, and the retentates were freeze-dried.

Adsorption experiments

Adsorption from aqueous solution was determined by the conventional slurry technique, or by the Continuous Flow (Diaflo) procedure described by Grice and Hayes (1972) and by Grice *et al.* (1973). Previously (Grice and Hayes, 1972), it had been noted that sedimentation of adsorbent on the membrane of the Continuous Flow cell caused some anomalies in the adsorption isotherms obtained from this technique. For the experiments described here, a very fine stainless steel wire mesh was placed over, and in intimate contact with, the Pellicon PSAC membrane [supplied by Millipore (U.K.) Ltd., Millipore House, Abbey Road, Park Royal, London, NW10 7SP], and the magnet which rested on this mesh was vigorously agitated to prevent adsorbent sedimentation and concentration polarisation at the membrane surface.

Analytical procedures

Aqueous solutions of dimefox (2μℓ aliquots) were analysed by gas liquid chromatography (g.l.c.). A 5ft x 0.25in glass column, packed with S.E. 30 (10%) liquid phase on a Universal Support material (60-85 mesh size), was used in a Perkin Elmer F11 Chromatograph equipped with a series 900, dual, flame ionisation/halogen - phosphorus specific detector. An oven temperature of 110°C was standardised and employed with the appropriate (Perkin-Elmer setting $2\frac{1}{2}$) injection block temperature. Hydrogen (17 lb in^2) and air (25 lb in^2) pressures were as recommended by the makers. There was a gradual variation in response of the detector, and this was overcome by taking into account the response for standard samples injected after every third application of unknown.

Polarography was used to measure the equilibrium solution concentrations of menazon. Samples (0.5 cm^3), in Britton Robinson buffer (5.0 cm^3, pH 6·0), gave a sharp peak at a reduction potential of 1·217 mV. In order to measure decomposition in solution, an aqueous menazon solution was monitored over a 16 day period and hydrolysis was estimated at less than 2% per day.

Thermoanalytical investigations

Adsorbate-clay complexes were formed by adsorbing the appropriate organophosphorus compound from the vapour phase or from solution in *n*-hexane. Thermogravimetry (TG) and derivative thermogravimetry (DTG) studies employed the Perkin-Elmer TGS-1 Thermobalence, and a Perkin-Elmer DSC-1B Calorimeter was used in differential scanning calorimetry (DSC) work. Experimental details are given by Lundie (1971), and will also be presented in later publications.

X-ray diffraction studies

Dimefox was adsorbed from aqueous solution on Na$^+$-montmorillonite and the sample was then formed into an orientated disc. Compounds I, II, and III were adsorbed from the vapour phase or from solution in n-hexane onto the appropriate montmorillonite preparations. Diffraction data were obtained by means of a Picker Powder Diffractometer with smoothed KV and mA stabilized CuKα-radiation (36KV and 20 mA).

Infrared spectroscopy

Spectra of liquid samples were obtained from thin films (between sodium chloride discs) by use of a Perkin-Elmer, Model 457, Infrared Spectrophotometer. For spectra of powdered samples (20mg, prepared by adsorbing compound I from solution in n-hexane onto the appropriate clay preparation) the Model TR 25 Multiple Attenuated Total Reflection (MATR) attachment was used. All spectra were scanned from 4000 - 600 cm^{-1}.

Calorimetric studies

Adsorbents (250-1000mg) were suspended in 25 cm^3 of n-hexane at 30°C. Solutions of I, II, and III in n-hexane (0·5 cm^3 of ca. 1 x 10^{-1}M solution in each case) were sealed separately in 1·0 cm^3 glass vials. The separate reactions were initiated by breaking the vials, and allowing adsorbent and adsorbate to equilibrate in an LKB Precision Calorimeter. Experimental and calculation procedures, as described by Wadsö (1966), were followed. In separate experiments, the amounts of adsorbate adsorbed were measured by g.l.c. Enthalpy changes were expressed in terms of heat evolved $mole^{-1}$ of compound adsorbed. An LKB Batch Microcalorimeter was used in some experiments, and the technique employed was similar to that of Hayes et al. (1972).

RESULTS AND DISCUSSION

Adsorption of dimefox by humic acid, soils and montmorillonite clay

Isotherms for the adsorption of dimefox by nine adsorbents are presented in Fig. 2. Some of these data have been presented already (Grice and Hayes, 1972), but the new stirring procedure used in the Diaflow cell has made it possible to produce more accurate data for the isotherms in this instance. Isotherms 1,4,5,6,7,8, and 9 (Fig. 2) were obtained by the Diaflow procedure, and the solid lines are extrapolated, as broken lines, to the vertical axis. The procedure used to obtain the data involved the collection of 2cm^3 fractions by means of a syphon device, and analysis of each fraction was carried out by g.l.c. Volumes of the fractions were not sufficiently reproducible to allow confident analysis of the data below an equilibrium solution concentration of 0·005g $100g^{-1}$. Taking into consideration the data available, and the errors inherent in it at the low concentrations, we feel that the extrapolations indicated (broken lines, Fig. 2) give a fair representation of the shapes of the isotherms for the adsorption of dimefox on the different adsorbents. Continuous monitoring of the eluent concentrations would largely offset the difficulties experienced. There is good reason to believe that the isotherms presented are of the high affinity type, except perhaps in the cases of 1,2, and 3 (isotherms 2 and 3 were obtained by slurry technique).

It can be seen that the most extensive adsorption took place on H^+-humic acid. Langmuir and inverse Langmuir plots, using the data available from the solid line of isotherm 9 (Fig. 2) gave adsorption capacities of 30·7 and 33·3g, respectively for monolayer coverage per 100g of humic acid. A Courtauld atomic model of the dimefox molecule indicated that it occupied a space with dimensions 5·75 x 6·76 x 8·50Å. The maximum area occupied by the dimefox molecule was thus 57·375$Å^2$.

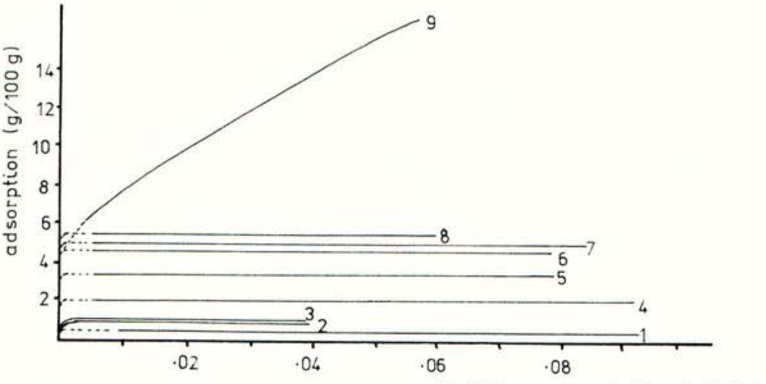


Fig. 2

Isotherms for the adsorption of dimefox by:

1, Brome Pin soil; 2, Na^+-kaolinite; 3, Na^+-illite; 4, Adventurers soil; 5, Prick Willow soil; 6, Clay extracted from YNYS soil; 7, Na^+-montmorillonite; 8, YNYS soil; 9, H^+-humic acid.

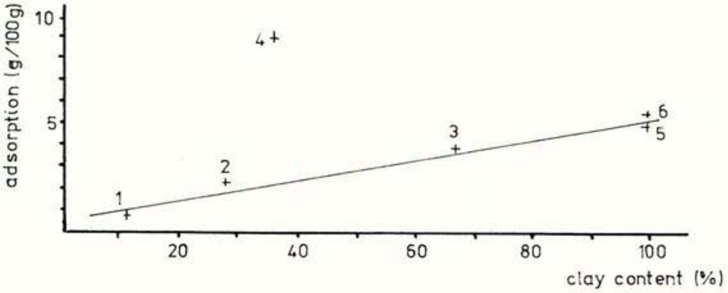


Fig. 3

Correlation with clay contents for the adsorption of dimefox on 1, Brome Pin; 2, Adventurers; 3, Prick Willow; 4, YNYS, 5, Clay extracted from YNYS; 6, Na^+-montmorillonite.

Combination of this value with that of 33.3g $100g^{-1}$ gives a surface coverage area of $747m^2g^{-1}$ for dimefox on H^+-humic acid. This agrees with quoted data for the surface area of such compounds. Because dimefox could be expected to be protonated at the pH of the medium (pH 3·5) it is reasonable to expect that ion-exchange was responsible for some of the adsorption observed. This aspect is being researched further.

There was, however, no correlation between adsorption of dimefox and the organic matter contents of the soils studied. Extents of adsorption, as shown in Fig. 3, correlated with soil clay contents (except in the case of YNYS), and also with adsorption by Na^+-montmorillonite.

It can be seen that the adsorption of dimefox by the clay extracted from the YNYS soil was of the order which might be predicted from the correlation data in Fig. 3. No satisfactory explanation is available at this time for the anomalous adsorption behaviour of the YNYS soil although the high adsorption capacity of this soil might be attributable to the presence of colloidal amorphous materials such as allophanes.

DSC failed to differentiate between the desorption of water and dimefox adsorbed on Na^+-montmorillonite. From adsorption isotherm and microcalorimetry data the ΔH of adsorption was estimated at -15·0 to -17·5 Kj $mole^{-1}$ of dimefox adsorbed, and this range agreed well with the value of -14.6 Kj $mole^{-1}$ obtained from treatment of the isotherm data by the method of Joyner et al. (1947). These data point to the involvement of weak attraction forces, such as van der Waals forces, in the adsorption mechanisms.

Adsorption of menazon by montmorillonite preparations

Isotherms for the adsorption of menazon by Na^+-montmorillonite and by H^+/Al^{3+}-montmorillonite are presented in Fig. 4. It is clearly evident that the latter adsorbent had the higher affinity for the adsorbate.

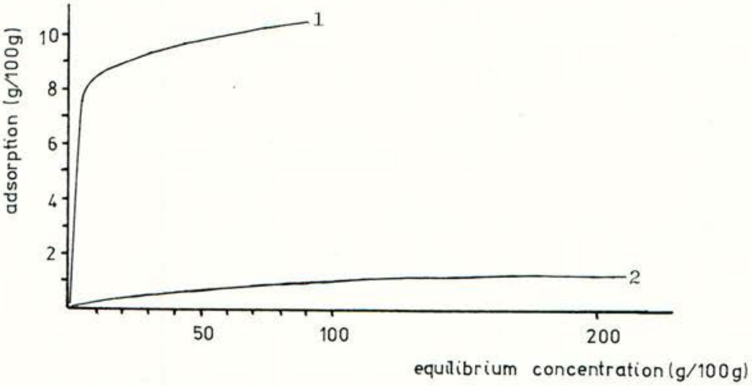


Fig. 4

Adsorption of menazon by: 1, H^+/Al^{3+}-montmorillonite; 2, Na^+-montmorillonite.

A pK_a value of 4·4 was determined polarographically for menazon, and the pH values for Na^+- and H^+/Al^{3+}-montmorillonite suspensions (0·1g in 25 cm^3 water) were 8·7 and 4·1, respectively. These data suggest that the triazine ring in the menazon molecule was protonated in the more acid medium, and was then adsorbed as the charged species.

Microcalorimetry studies gave ΔH adsorption = -11·3 Kj $mole^{-1}$ adsorbed for the menazon-Na^+-montmorillonite system. The enthalpy of adsorption was constant for all points on the isotherm. This indicates that the same mechanism of adsorption operated over the entire range, and points to the involvement of weak specific attraction forces, such as van der Waals forces, between adsorbent and adsorbate. In the case of the menazon-H^+/Al^{3+}-montmorillonite system the enthalpy of adsorption was constant (ΔH = -25·9 Kj $mole^{-1}$ adsorbed) for amounts of menazon adsorbed greater than 2g $100g^{-1}$ of clay. However, the average enthalpy value $mole^{-1}$ adsorbed for amounts less than 1g $100g^{-1}$ was -81 Kj. Thus highly energetic attraction forces were involved at organophosphorus compound with iron and aluminium impurities, or chemical interaction with any iron and aluminium oxides and hydroxides deposited on the clay surface. The value $\Delta H = -25{\cdot}9$ Kj mole^{-1} adsorbed, for the higher levels of adsorption, suggests the involvement of processes such as ion exchange, hydrogen bonding, or non-specific charge-dipole interactions in the adsorption mechanism.

Interactions of model organophosphorus compounds with clays

Compound I was chosen because it is aliphatic, has a relatively low molecular weight, and because it does not contain readily ionizable functional groups. Compound II was chosen in order to examine the effects of the thiophosphoryl and ethylthio functional groups on adsorption, and compound III was chosen because it contains a triethylamine group instead of the methyl group in I.

DSC, TG, and DTG techniques were used to study the desorption of I from powdered glass, K^+-kaolinite, pyrophyllite, Ca^{2+}- and H^+/Al^{3+}-montmorillonite preparations. DTG indicated that desorption from glass powder was of zero-order, and that the attractive forces between glass and adsorbate were no stronger than the organophosphate-organophosphate interactions. Materials desorbed at less than 128°C from K^+-kaolinite were held at energies approximately equivalent to that held by glass. This indicates that some multilayer adsorption had taken place. From results with DSC it was calculated that the energy required to desorb the surface layer of I from K^+-kaolinite was 50 Kj mole^{-1} adsorbed. By the same technique the activation energy for desorption of this compound from pyrophyllite was estimated at 54 Kj mole^{-1}.

Thermoanalytical analysis indicated the presence of three different sites for the adsorption on I on Ca^{2+}-montmorillonite. The energy required to desorb the compound from the external surface was the same as that for pyrophyllite, and an activation energy of 67 Kj mole^{-1} was calculated for desorption from the interlammellar surfaces. However, the strongest interaction was shown to take place between the phosphoryl group of I and the calcium ion located in the interlamellar spaces. From DTG analysis it was shown that the amount of I desorbed in the 300-400°C range corresponded to two molecules of organophosphate per interlamellar calcium ion, and the energy required to remove the adsorbate, co-ordinated in this way, amounted to 137 ± 10 Kj mole^{-1}. A lowering, by 25 cm^{-1}, of the frequency of the infrared absorption band of the phosphoryl group (when I was adsorbed on clay) had indicated the involvement of dipole-charge interactions between this group and the exchangeable calcium ions on the clay. X-ray diffraction data had shown that adsorption of I caused an interlattice expansion of 12·7Å. This indicates that a bimolecular layer of adsorbate, "sandwiching" Ca^{2+} ions, was formed in the interlamellar regions.

The DTG curve for the I-H^+/Al^{3+}-montmorillonite complex had similar features to that for the Ca^{2+}-montmorillonite system.

Reaction calorimetry was used to compare the integral enthalpy (ΔH_i) values for the adsorption, from solution in n-hexane, of the three model compounds (5×10^{-5} moles in each case) by Ca^{2+}-montmorillonite (0·5g, containing 19% by weight of water). The Gibbs free energy change was calculated from the equation $\Delta G = -RT \ln C_s f_s / C f_1$, where C and C_s were the concentrations in solution and on the adsorbate surface, respectively, and f_1 and f_s were the respective activity coefficients, assumed to be unity. Entropy changes (ΔS) were obtained from: $\Delta G = \Delta H_i - T\Delta S$; where T is the absolute temperature.

Results are summarised in Table 2. It should be remembered that the integral enthalpy values take into account the total energy changes in the system, and the ΔH value for the adsorption of the appropriate organophosphorus (OP) compound will be lowered by the amount of energy required to displace the water initially present on the adsorbent surface.

Table 2

ΔG, ΔH_i and ΔS values for adsorption of model compounds by Ca^{2+}-montmorillonite

Compound Formula	No.	ΔH_i, Kj $mole^{-1}$	ΔG, Kj $mole^{-1}$	ΔS, $j°K^{-1}$
$(EtO)_2P(O)CH_3$	I	-34·0	-23.5	-34·4
$(EtO)(EtS)P(S)CH_3$	II	- 3·2	-13·4	+40·3
$(EtO)_2P(O)CH_2CH_2NEt_2$	III	-50·0	-23·5	-75·6
$(EtO)(EtS)P(O)CH_3$	IV	-27·7	-10·1	-58·8

Compound IV is introduced in Table 2 because the thermodynamic data given for it, when compared with those for compound I, provide an evaluation of the effects on the thermodynamics of adsorption of substituting the -SEt for the -OEt group. It can be seen that the $-\Delta H_i$ values for II and IV are less than that for I. The larger value for the phosphoryl (compared with the thiophosphoryl) compound can be attributed to the greater polarity of the phosphoryl group which allows stronger coordination between this compound and the interlamellar calcium ions. Similarly, the polarity of the phosphoryl group was lowered by substituting -OEt for -SEt (compare I and IV), and the effect of this is reflected in the appropriate $-\Delta H_i$ values for adsorption.

Substitution of $\geqslant$PS for $\geqslant$PO and of one -SEt for one -OEt group gave striking differences between the ΔS values for the adsorption of I and II by Ca^{2+}-montmorillonite. These changes reflect the differences in the interactions between the two compounds and the solvent, n-hexane. The less polar molecule will have the higher affinity for the solvent.

X-ray diffraction showed that monomolecular layers of compounds I, II and IV were interlamellarly adsorbed. In the case of III, however, three molecular layers were adsorbed in the interlamellar spaces of Ca^{2+}-montmorillonite. The increased reactivity of this compound can be attributed to the co-operative contribution to adsorption of the tertiary amine group. Co-ordination between the phosphoryl groups and interlamellar calcium ions is almost certainly involved in the adsorption mechanism. A simple model of the conformations adopted by this compound in the interlamellar spaces would have two of the three molecular layers lying on the internal surfaces of the clay lattice, and co-ordinated with calcium through the phosphoryl groups (pointing outwards into the interlamellar space). The middle layer need only share co-ordination with a calcium ion already co-ordinated with one of the surface adsorbed molecular layers.

GENERAL CONCLUSIONS

H^+-humic acid was shown to have a high affinity for dimefox, and the mechanisms of adsorption in this instance were thought to include a contribution from cation-exchange processes. At the pH of agricultural soils, however, the dimefox molecule would not be expected to be protonated, and clays were shown to have a greater affinity for this molecule than did the organic matter content of the soils used. In general it would appear that soil clays will have a higher affinity than soil organic matter for polar aliphatic OP compounds. However, the possibility of strong interactions between these compounds and mineral oxides and hydroxides in soils is recognised. Co-ordination of the $>$PO and $>$PS functional groups with interlattice mineral cations will provide the strongest binding mechanism for soil clay-OP interactions (in the absence of oxide and hydroxide coatings on the clay surfaces). S containing OP compounds will be less strongly bound by soil clays than their O containing analogues.

References

BARSHAD, I. (1969) Preparation of Na saturated montmorillonites. Soil Sci., 107, 337-342.

GRAHAM-BRYCE, I. J. (1967) Adsorption of disulfoton by soil. J. Sci. Fd. Agric., 18, 72-77.

GRICE, R. E. and HAYES, M.H.B. (1972) A continuous flow method for studying adsorption and desorption of pesticides in soils and in soil colloid systems. Proc. 11th Br. Weed Control Conf., 2, 784-791.

GRICE, R. E., HAYES, M.H.B., LUNDIE, P. R. and CARDEW, M. H. (1973) Continuous flow method for studying adsorption of organic chemicals by a humic acid preparation. Chem. and Ind. (London), 233-234.

HAYES, M.H.B., PICK, M. E., and TOMS, B. A. (1972) Application of microcalorimetry to the study of interactions between organic chemicals and soil constituents. Science Tools, 19, 9-12.

JOYNER, L. G., WEINBERGER, E. B. and MONTGOMERY, C. W. (1945) Surface area measurements of activated carbons, silica gel and other adsorbents. J. Am. Chem. Soc., 67, 2182-2188.

LUNDIE, P. R. (1971) Adsorption of organophosphorus compounds by soils and soil colloids. Ph.D. Thesis, University of Birmingham.

WADSÖ, I. (1966) Calculation methods in reaction calorimetry. Science Tools, 13, 33-39.

NOTES

Proceedings 7th British Insecticide and Fungicide Conference(1973)

A SIMPLE RELATIONSHIP BETWEEN SOIL ADSORPTION OF ORGANIC CHEMICALS AND THEIR OCTANOL/WATER PARTITION COEFFICIENTS

G.G. Briggs

Rothamsted Experimental Station. Harpenden. Herts.

Summary The adsorption of un-ionised organic chemical by four Rothamsted soils, expressed as soil organic matter/water partition coefficients (Q), is related to their octanol/water partition coefficients (P) by log Q= 0.524 log P + 0.618. A similar relationship exists between P and Rf values on soil thin layers. Good prediction of soil behaviour of organic chemicals can be obtained using mobility classes based on octanol/water partition coefficients.

Résumé L'adsorption des produits chimiques organiques non-ionisés par quatre sols de Rothamsted, exprimée par les coefficients de partage entre la matière organique du sol et l'eau (Q), a rapport à leurs coefficients de partage entre l'octanol et l'eau (P) par l'expression log Q = 0.524 log P+ 0.618. Un rapport semblable existe entre P et les valeurs Rf sur des couches minces du sol. On peut obtenir une bonne prédiction du comportement des produits chimiques organiques dans le sol en utilisant des classes de mobilité fondées sur les coefficients de partage entre l'octanol et l'eau.

INTRODUCTION

The correlation between adsorption of un-ionised organic compounds by soil and the soil's organic matter content is well established. Several attempts have been made to predict adsorption by soils by relating the organic matter/water partition coefficient. (Q), to the parachor (Lambert 1967, Hance 1969) or free energy substituent constants within related series of compounds (Briggs 1969). In practice, these relationships need to be determined for each series of compounds and they cannot be simply applied to a wide range of chemical structures. This paper reports a relationship between Q and octanol/water partition coefficients that can be easily and widely used.

Previous work has shown that adsorption by soils can be related systematically to chemical structure and supports the hypothesis that organic matter behaves like an organic 'solvent' (Lambert 1967). If it does then equation 1, found by Collander (1950), which relates partition between one organic solvent and water and a second organic solvent and water should hold, as Lambert (1968) has suggested, when organic matter/water is one of the solvent pairs.

$$\log P_1 = a \log P_2 + b \qquad (1)$$

RESULTS

This hypothesis was tested using average values of Q determined as previously described (Briggs 1969) on four Rothamsted soils for 30 chemicals with a wide range of polarities and values of P, the octanol/water partition coefficient, either taken from the literature (Leo et al 1971) or determined spectrophotometrically (Fujita et al 1964) Octanol/water was chosen because of its wide use as a model for biophases in correlations between biological activity and chemical structure. Equation 2 gave a good fit to the data ($r^2 = 0.84$).

$$\log Q = 0.524\ (\pm.048) \log P + 0.618\ (\pm.113) \qquad (2)$$

A similar test of the hypothesis can be applied to results obtained from soil thin-layer chromatography if it is assumed that relative mobility on the plates is governed only by adsorption on soil organic matter as the stationary phase. The Martin and Synge (1941) equation may then be written:

$$\log (1/R_f - 1) = \log Q + \log A_s / A_m \qquad (3)$$

For a given soil A_s and A_m, the cross-sectional areas of the organic phase and water phase, are constants and substituting for log Q from equation 1 gives equation 4 where P is again the octanol/water partition coefficient.

$$\log (1/R_f - 1) = a \log P + \text{constant} \qquad (4)$$

Frontal Rf values for 25 un-ionised pesticides on Hagerstown soil from Maryland U.S.A. were taken from Helling and Turner (1968), Helling (1971 a,b,c) and Helling et al (1971). The regression line is:

$$\log (1/R_f - 1) = 0.517\ (\pm.022) \log P - .951\ (\pm.075) \qquad (5)$$

The slope is almost the same as that in equation 2 as it should be if organic matter from different soils behaves relatively uniformly as an adsorbing surface. Where comparable values of Q are available they are similar for soils from England, Europe and North America despite obviously different origins and probable differences in the detailed chemical structure of the soil organic matter. The close similarity of the slopes of equations 2 and 5 indicates that log P and equation 2 can be used to give an estimate of Q that is widely applicable. It is interesting that the slopes are similar to the average value (0.55) found by Leo et al (1971) in equations relating log P to the partition of organic compounds onto a number of biological macromolecules.

DISCUSSION

Helling and Turner (1968) divided pesticides into five mobility classes based on Rf values on Hagerstown soil (2.5 per cent organic matter) and Helling(1971c) concluded that this classification adequately described behaviour in 14 soils containing 1-8 per cent organic matter, a range common to most agricultural soils. Rf values for a given compound decreased as organic matter content increased; however the range of Rf used to define the mobility class on Hagerstown soil was wide enough to cover most of the soils examined. Using equation 5 these mobility classes can be defined in terms of log P and Q.

Class	Rf	log P	Q
Immobile	0 - 0.09	> 3.78	> 398
Low	0.10 - 0.34	3.78 - 2.39	398 - 74
Intermediate	0.35 - 0.64	2.39 - 1.36	74 - 29
Mobile	0.65 - 0.89	1.36 - 0.08	29 - 4.5
Very mobile	0.90 - 1.00	<0.08	< 4.5

The mobility class from the above table or the approximate values of Q from equation 2 can readily be obtained for new compounds or possible soil metabolites either using log P values from the review by Leo et al (1971), calculated using Hansch's π constant, (Fujita et al 1964) or a simple experimental determination. Many π constants in the literature are derived from the phenoxyacetic acid series where electronic effects of substituents are small; in a series such as the phenylureas where P is very sensitive to electronic effects, experimental values of P, which include these effects, predict Q much better than calculated values.

Three examples illustrate the use of the octanol/water partition coefficient to predict behaviour of three different types of compound in soil.

The herbicide 2,6-dichlorothiobenzamide is converted in soils to 2,6-dichlorobenzonitrile which in turn is hydrolysed to 2,6-dichlorobenzamide (Beynon and Wright, 1972). Using the value for an aliphatic amide (because of steric inhibition of resonance), calculated values of log P are 2.3, 3.0 and 1.3 and indicate mobility classes of 'intermediate', 'low' and 'mobile' for the thioamide, nitrile and amide. This agrees well with field observations. Calculated values of Q are 66, 155 and 20 and experimental values are 57, 135-165 and 5-15 (Beynon and Wright, 1972; Briggs 1968).

The oxime carbamate insecticide aldicarb is rapidly oxidised in soil to the sulphoxide. Experimental values of log P of 0.8 and -0.7 classify the parent compound as 'mobile' and the metabolite as 'very mobile' which is the leaching behaviour observed in practice (Goring, 1972).

The polychlorinated biphenyls, important environmental contaminants are a third example of a different structural type, whose behaviour in soil has not been closely examined. The experimental value (Leo etal, 1971) of log P for biphenyl itself is 4.0 so that it would be classed as immobile in soil. The bulk of the material in commercial PCB samples contains more than 3 chlorine atoms per molecule and has a calculated log P > 6. Extensive metabolism would have to occur before any leaching in soil would be expected.

Octanol/water partition coefficients provide a good prediction of soil behaviour using equation 2 and the mobility classes derived from it. Organic matter in soils has similar properties to the humic materials in lakes, streams and rivers so that a good indication of the likely redistribution in the environment by water transport of un-ionised compounds from any source can also be obtained from a single measurement or calculation.

Acknowledgements

I thank Drs. N. F. Janes, K. A. Lord and I. J. Graham-Bryce for helpful discussions and criticism.

References

BEYNON, K.I. and WRIGHT, A.N. (1972) The fates of the herbicides chlorthiamid and dichlobenil in relation to residues in crops, soils and animals. Residue Reviews, 43, 23-53.

BRIGGS, G.G. (1968) Unpublished results.

BRIGGS, G.G. (1969) Molecular structure of herbicides and their sorption by soils. Nature , 223, 1288

COLLANDER, R. (1950) The distribution of organic compounds between iso-butanol and water. Acta Chemica Scandinavica, 4, 1085-1098.

FUJITA, T., IWASA, J. and HANSCH, C. (1964) A new substituent constant, pi, derived from partition coefficients. Journal of the American Chemical Society, 86, 5175-5180.

GORING, C.A.I. (1972) in ' Organic Chemicals in the soil environment' edited by GORING, C.A.I. and HAMAKER, J.W. (Marcel Dekker, New York), 604.

HANCE, R.J. (1969) An empirical relationship between chemical structure and the sorption of some herbicides by soils. Journal of Agricultural and Food Chemistry, 17 667-668.

HELLING, C.S. and TURNER, B.C. (1968) Pesticide Mobility: Determination by soil thin-layer chromatography. Science, 162, 562-563.

HELLING, C.S. (1971a) Pesticide mobility in soils I. Parameters of Thin layer chromatography. Soil Science Society of America Proceedings, 35, 732-737.

HELLING, C.S. (1971b) Pesticide mobility in soils II. Applications of soil thin-layer chromatography. Soil Science Society of America Proceedings, 35, 737-743.

HELLING, C.S. (1971c) Pesticide Mobility in soils III. Influence of soil properties. Soil Science Society of America Proceedings, 35, 743-748.

HELLING, C.S., KAUFMAN, D.D. and DIETER, C.T. (1971) Algae bioassay detection of pesticide mobility in soils. Weed Science, 19 685-690.

LAMBERT, S.M. (1967) Functional relationship between sorption in soil and chemical structure. Journal of Agricultural and Food Chemistry, 15, 572-576.

LAMBERT, S.M. (1968) Omega, a useful index of soil sorption equilibria. Journal of Agricultural and Food Chemistry, 16, 340-343.

LEO, A., HANSCH, C. and ELKINS, D. (1971) Partition coefficients and their uses. Chemical Reviews, 71, 525-616.

MARTIN, A.J.P. and SYNGE, R.L.M. (1941) A new form of chromatogram employing two liquid phases. Biochemical Journal, 35, 1358-1368.