Proceedings 1977 British Crop Protection Conference - Pests and Diseases

THE ACTIVITY OF THE FUNGICIDE NUARIMOL AGAINST DISEASES OF BARLEY AND OTHER CEREALS

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Summary Nuarimol (α-(2-chlorophenyl)-α-(4-fluorophenyl)-5-pyrimidine methanol) was tested as a fungicide for seed dressing of, and foliar application to, cereals in Europe, particularly barley. Greenhouse and field experiments showed that the compound, at a low rate of use, can control the major seed-borne diseases of barley, namely Calonectria nivalis, Pyrenophora graminea and Ustilago spp. It also has activity at low rates of use as a seed dressing against foliar and soil-borne diseases, for example, Erysiphe graminis and Typhula incarnata. Diseases of other cereals such as seed-borne infections of Leptosphaeria nodorum and Ustilago tritici of wheat are controlled by treatment of seed with the compound.

Foliar applications of nuarimol also effectively control mildew (Erysiphe graminis) on barley. Yield increases are obtained following both foliar and seed treatment applications of the compound.

INTRODUCTION

A series of broad spectrum systemic fungicides, the substituted 5-pyrimidine methanols, was discovered and developed by workers at the Lilly Research Laboratories, Greenfield, Indiana, U.S.A. (Brown et al 1970, 1971, 1975). One member of the series, nuarimol (α-(2-chlorophenyl)-α-(4-fluorophenyl)-5-pyrimidine methanol) was found to show a high level of apoplastic systemic movement in plants (Brown, I.F., in preparation). Nuarimol was therefore investigated in Europe as a seed treatment to control seed and soil-borne diseases and foliar diseases in cereals. At the same time the compound was evaluated as a foliar spray for use on cereals.

METHODS AND MATERIALS

Healthy seed or seed infected with Calonectria nivalis, Leptosphaeria nodorum, Pyrenophora graminea, and P. avenae (barley, wheat and oats) was treated with nuarimol and grown 1.5 cm deep in soil in 10 cm pots under glass for greenhouse efficacy evaluation. Seed was soaked for 1 h in a nuarimol emulsion made by diluting an emulsifiable concentrate to give final concentrations of 10, 20 and 40 ppm. Alternatively, the seed was treated with a wettable powder (w.p.) of nuarimol to give final rates of 0.1 and 0.2 g fungicide/kg seed. In either case control seed was treated with blank formulation. Healthy seed was grown in the greenhouse at 17-20°C with additional artificial light (16 h days). Seedlings (6-8 days old) were inoculated with conidia of Erysiphe graminis. Diseased seed was grown as described by Holmes & Colhoun (1973), and Muskett (1938) in order to obtain expression of symptoms. Disease was assessed as % leaf cover in the case of mildew and % infected plants in the case of seed-borne diseases. Growth retarding effect was assessed 13

days after planting by measuring seedling height and the results were expressed as % of the height of control plants. Statistical significance was evaluated following analysis of variance ($P<0.05$).

For field trials, wheat, barley or oat seed was treated using a variety of methods. In France the methods given by the Commission des Essais Biologiques of the Société Française de Phytiatrie et de Phytopharmacie were used (Bourdin et al, 1971). In the U.K. and Germany, seed was treated by mixing 2 g of a 10% w.p. of nuarimol with 6 ml water and spraying onto 1 kg seed in a rotating vessel. Trials were laid in a randomised block design (4 replications) with plot sizes from 5 m^2 up to 100 m^2. Seed infected with Ustilago avenae, U. hordei, U. nuda, U. tritici, Pyrenophora graminea, P. avenae and Tilletia caries was used for the trials.

Foliar applications on barley were made using, typically, an Azo propane plot sprayer fitted with Teejet 8003 nozzles and operating at 3 bars pressure. Nuarimol formulated as a 9% e.c. was diluted to give an application rate of 40.5 g a.i./ha (spray volume 400 l/ha). Assessments of mildew cover were made 10-12 days after application and subsequently at 10 day intervals by estimating the percent mildew cover on the lowest green leaf or on leaf 3.

RESULTS

Seed treatment - greenhouse tests

Preliminary greenhouse tests were conducted in Europe with nuarimol applied as a seed treatment or as a seed soak. In the tests nuarimol showed activity against a range of seed-borne diseases - Calonectria nivalis, Leptosphaeria nodorum, Pyrenophora graminea and P. avenae. In addition it showed activity against Erysiphe graminis (Table 1). Good activity against wheat pathogens was observed at 0.1 g nuarimol/kg seed, whilst activity against barley and oat pathogens required 0.2 g/kg.

Table 1

The control of a number of seed-borne and foliar pathogens of cereals by seed application of nuarimol (greenhouse tests)

Rate of use	Type of treatment[a]	% disease control					
		Wheat pathogens[a]			Barley pathogens[a]		Oat pathogen[a]
		EG	CN	LN	EG	PG	PA
10 ppm	SS	43	-	-	-	-	-
20 ppm	SS	75	-	-	-	-	-
40 ppm	SS	87	-	-	-	-	-
0.1 g/kg seed	ST	-	100	98	-	-	-
0.2 g/kg seed	ST	-	-	-	77	92	79
Untreated[b]	-	(80)	(13)	(12)	(65)	(3)	(25)

[a]SS = seed soak. ST = seed treatment. EG = Erysiphe graminis. CN = Calonectria nivalis. LN = Leptosphaeria nodorum. PG = Pyrenophora graminea. PA = Pyrenophora avenae. Results (means of two determinations) were taken at the 2-3 leaf stage. For further details see Methods and Materials.

[b]Figures given are disease incidence in plants grown from untreated seed.

In the greenhouse tests, treatment of seeds with nuarimol had little effect on the growth of barley and oat seedlings (Table 2). The majority of field trials conducted to date has been on barley at a use rate of 0.2 g/kg seed. The greenhouse work indicates that this rate of use is safe and effective against a full range of pathogens.

Table 2

The growth retarding effect of nuarimol applied to seed grown in the greenhouse

	No. of varieties tested	No. of varieties showing statistically significant (P<0.05) height reduction after nuarimol treatment[a]		Mean height (% of control height)[a]	
		0.2 g/kg seed	0.4 g/kg seed	0.2 g/kg seed	0.4 g/kg seed
Barley	9	0	1	95	88
Oats	1	0	0	96	96

[a]Observed 11-13 days after planting.

Seed treatment - field trials

Small-plot field trials conducted with infected seed showed that nuarimol gave 90-100% control of Ustilago spp. and interesting but sometimes insufficient control of Tilletia caries on wheat. Pyrenophora graminea was controlled well in spring barley, but inadequately in winter barley (Table 3). In limited additional trials on oats, 100% control of Ustilago avenae and 60% control of Pyrenophora avenae was obtained at 0.2 g/kg.

Control of Erysiphe graminis was obtained following treatment of barley seed with nuarimol at 0.2 g/kg. With spring drilling the control exceeded 75% for a period 2-2½ months from planting. The period of control was found to be similar on a range of varieties showing different susceptibility to mildew (Fig. 1). With winter barley the control of mildew remained in excess of 75% until 130 days after planting, i.e. G.S. 4-5 on the Feekes-Large scale (Large 1954). In some trials substantial control was observed as late as G.S. 9 (250 days after planting).

Nuarimol was also found to have activity against Typhula incarnata in three winter barley seed treatment trials in Northern Germany (1975-76 season). The results are shown in Table 4. The yield responses obtained, although substantial, do not correlate with the level of Typhula or the extent of Typhula control: this is probably due to different amounts of mildew at the trial sites.

During the 1975 and 1976 field trial seasons in France, Germany and the U.K., eight large-plot spring barley seed treatment trials where nuarimol had been tested at 0.2 g/kg were harvested under commercial conditions with a combine. The mean yield increase resulting from treatment was 7.2%. This represents on average 223 kg of additional grain/ha. The corresponding figures for winter barley (eight trials

commencing in 1974 and 1975) are 6.0% and 288 kg. Preliminary results indicate that the thousand grain weight (corrected to 86% dry matter) is slightly increased following nuarimol seed treatment of spring barley.

Table 3

The efficacy of nuarimol seed treatment against seed-borne diseases of barley (0.2 g/kg nuarimol) and wheat (0.1 g/kg nuarimol) in field trials

Pathogen (host)	No. of trials	Mean % infected plants grown from untreated seed	% disease control
Pyrenophora graminea			
(spring barley)	6	41	99
(winter barley)	5	54	46
Ustilago hordei (barley)	5	22	91
Ustilago nuda (barley)	12	18	95
Tilletia caries (wheat)	3	23	65
Ustilago tritici (wheat)	3	6	99

Data are means from number of trials shown.

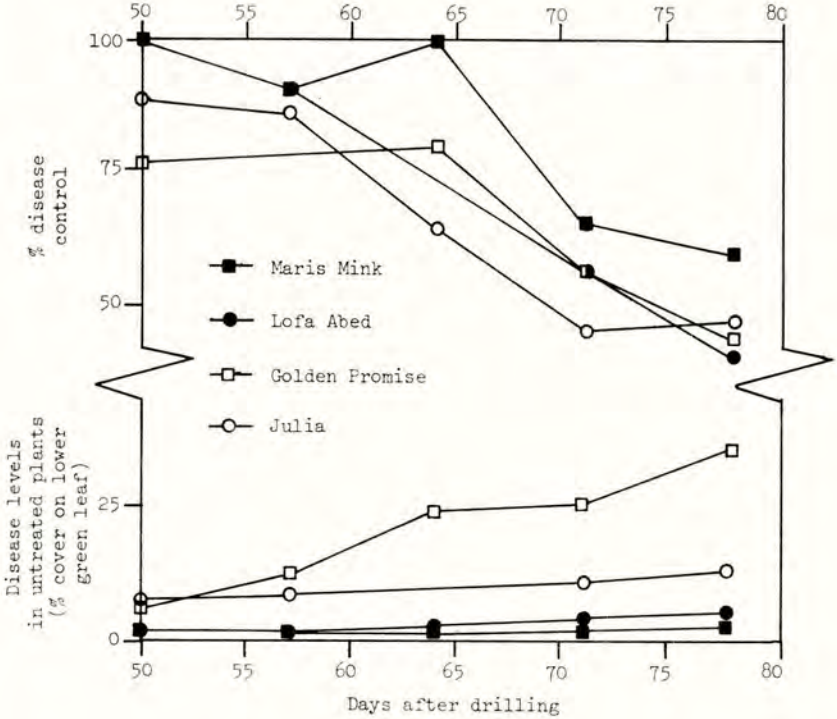
Table 4

The efficacy of nuarimol seed treatment (0.2 g/kg) against Typhula incarnata in field trials (1975-76) on winter barley, cv. Majo

	Site 1	Site 2	Site 3	Mean
Percent infected plants grown from untreated seed	30	50	15	32
Percent disease control following nuarimol treatment	18.1	42.8	89.5	50.1
Yield increase, kg/ha (%)	1050(21.0)	338(6.3)	a	694(13.7)

[a]Not harvested

Fig. 1 The control of Erysiphe graminis following treatment of spring barley seed with nuarimol (1977 trial - Berkshire, U.K.)



Foliar spray - field trials

Activity against Erysiphe graminis was also observed when nuarimol was applied as a foliar spray. In six 1976 field trials in the U.K. on spring barley, where large plots were used and the crop harvested with a combine, a mean yield increase of 11.9% (380 kg grain per ha) was obtained following application of 40.5 g/ha of nuarimol between growth stages 6 and 8. Eradication of existing mildew attacks and 90% to 100% control of new infection was obtained during the period 3-5 weeks after spraying.

The yield response obtained following application was found to be related to the severity of the mildew attack. Severity was assessed in terms of the area of lowest green leaf or leaf 3 covered by mildew in unsprayed plots 10-12 days after nuarimol application. In trials where the attack was light, small yield responses (mean 3.7%) were found. Larger responses in the range 9.8 - 17.2% were obtained where moderate and heavy attacks of mildew were encountered (Table 5). Thousand grain weight was unchanged or slightly increased (1-2% after correction to 86% dry matter) in these trials.

Table 5

Yield response following nuarimol foliar application to spring barley crops carrying various levels of mildew attack (1976 trials)

Percent mildew cover on lowest green leaf or leaf 3 (unsprayed plots)†	No. of sites	Yield increase following nuarimol spray Mean ± S.E.
0-15 (light attack)	5	3.72% ± 2.3
15-40 (moderate attack)	3	12.3 % ± 1.8
>40 (heavy attack)	4	14.8 % ± 2.1

†Assessed 12-14 days after nuarimol application to sprayed plots.

DISCUSSION

The greenhouse and field data in Tables 1-3 and Fig. 1 indicate that nuarimol can be safely applied to spring barley seed at a rate of 0.2 g a.i./kg and will control the important seed-borne diseases, Calonectria nivalis, Pyrenophora graminea and Ustilago spp. In addition, 2-2½ months control of Erysiphe graminis is obtained. Nuarimol thus offers a single chemical seed treatment which at a low rate of use can control the most important pathogens of spring barley.

On winter barley, control of Pyrenophora fell to unacceptable levels (Table 3). The difference between spring and winter barley indicates that nuarimol acts against the fungus as the plant develops rather than by disinfecting the seed. Hence different rates of decline of tissue concentrations of the compound may account for the effect of drilling time on activity against the disease. Combinations of nuarimol with compounds active against Pyrenophora are being developed for use on the winter crop.

The activity of nuarimol seed treatment on winter barley against mildew lasts on average until the middle of stem extension. There is evidence that control of the disease during this period may be beneficial since it can enhance root development and fertile tiller formation, particularly following early drilling (Finney & Hall 1972, Jenkyn 1976). Yield increases are in fact observed following nuarimol treatment of winter barley.

Nuarimol has a high level of activity against barley mildew as a foliar spray applied at 40.5 g/ha (Table 5). Preliminary work, not reported here, has shown that the yield increases obtained from seed treatment followed by foliar spray are additive. Further work is in progress.

The data in Tables 1 and 3 show that nuarimol seed treatment has interesting activity on a number of pathogens of cereals other than barley, namely wheat and oats. On wheat 0.1 g/kg of the compound is effective against Calonectria nivalis, Leptosphaeria nodorum and Ustilago tritici. The activity against Tilletia caries may be improved by combination with other products and further investigation is in progress.

Acknowledgements

The authors acknowledge with thanks the work of Elanco field trials R & D personnel in France, Germany and the U.K. in conducting the field trials reported in this paper. The work of W.R. Smith in the greenhouse and of R. Wiley on formulation development is also gratefully acknowledged.

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NOTES

THE DEVELOPMENT OF THE SYSTEMIC FUNGICIDE, TRIADIMEFON, FOR THE CONTROL OF FOLIAR DISEASES IN SPRING AND WINTER BARLEY IN THE U.K.

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Summary In field trials during 1975-1977, triadimefon used at a rate of 125g a.i./ha gave excellent control of powdery mildew, Erysiphe graminis, in the barley crop. The optimal application timing was just prior to stem extention, although in winter barley additional applications in the autumn may be warranted. Leaf blotch, Rhynchosporium secalis, was well controlled in spring barley with applications made before stem extention, but two applications are likely to be required in a wet season, and in the case of winter barley. Against the rusts, Puccinia spp., triadimefon was very effective when applied on disease appearance, but also gave a good eradicative effect when applied to established infections.

The use of a two spray programme of triadimefon provided season long protection against the main cereal foliar diseases.

Résumé Pendant les épreuves en plein champ de 1975-1977, triadimefon appliqué à la dose 125g a.i./ha a montré une lutte excellente contre Erysiphe graminis dans l'orge. Le temps optimal pour l'applicage était immédiatement avant la montaison, mais dans le cas de l'orge d'hiver, les applications supplémentaires en automne peuvent être justifiés. Rhynchosporium secalis était bien controllé dans le cas de l'orge de printemps avec applications avant la montaison, mais deux applications seront nécessaire dans une saison pluvieuse, et aussi dans le cas de l'orge d'hiver. Contre Puccinia spp., triadimefon a produit son effet après un applicage à l'apparition de la maladie, mais il a aussi montré un effet de déracinement.

L'utilisation d'une programme de deux applications de triadimefon a produit une protection contre les maladies principales foliaires de les céréales pendant toute la saison.

INTRODUCTION

In recent years the availability of effective fungicides for the control of cereal mildew Erysiphe graminis has contributed towards the yield of cereals, our nationally most important arable crop. The higher economic returns being obtained and the greater awareness of the importance of other cereal diseases, however, increases the demands for effective control measures. The specific treatment of disease problems involves complex decision making which requires special expertise, and consequently, in practise, it is difficult to maximise cereal yields (Cock, 1975).

This report attempts to show the contribution towards cereal disease control that can be made by a new active ingredient with the common name triadimefon. A member of the triazole group, with the chemical name 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl) butan -2-one, it was discovered in the laboratories of Bayer AG, Leverkusen, West Germany (Kaspers et al, 1975). The compound has a low mammalian toxicity and is active against a large number of fungi.

Triadimefon has marked systemic activity and has been shown to be translocated in plants in both acropetal and basipetal directions. This basipetal translocation, although of less importance than acropetal movement, is a unique property of the compound (Scheinpflug et al, 1977). Triadimefon is highly active against cereal mildew, it being effective at concentrations of approximately 1.5 ppm, apparently through the prevention of haustorial development (Buchenauer, 1976). In common with other mildewicides triadimefon also exhibits activity in the vapour phase; however with triadimefon this activity is very strong, control of mildew being possible on untreated plants adjacent to treated plants (Scheinpflug and Paul, 1976). In addition to good protective activity triadimefon also shows a strong curative effect (Scheinpflug et al, 1977). Protective and curative properties have also been demonstrated against the rust diseases, Puccinia spp., but relatively higher dose levels are required (Buchenauer, 1976). Against leaf blotch, Rhynchosporium secalis, triadimefon has been shown to reduce considerably the sporulation from infected debris and established lesions. It does not however, appear to prevent the formation of lesions on treated plants but these lesions are apparently unable to sporulate (Jordan, 1977).

The performance of triadimefon in field trials from 1975 to 1977 against the main foliar diseases of barley is covered by this report. Trials work on wheat and oats is reported separately at this conference, whilst on other crops, including top fruit and hops, work is continuing.

METHODS AND MATERIALS

Triadimefon was used as a 25% wettable powder, coded BAY 6681, in all the work on cereals. The standard materials used for comparison were tridemorph (75% e.c.) and fluotrimazole (12.5%e.c.) in mildew trials, captafol (50% col) and tridemorph plus carbendazim (50% w.p.) in leaf blotch trials and benodanil (50% w.p.), or tridemorph plus Polyram (80% w.p.) in rust trials.

A total of 88 trials have been carried out from 1975 to 1977 of which 69 were on spring and 19 on winter barley. Small plot replicated trials of a randomised block design with plots of 2-3m by 15m were conducted to compare treatment rates and application timings using susceptible cultivars such as Maris Mink for leaf blotch and Midas for brown rust. All treatments were applied by means of pressurised knapsack sprayers using fan nozzles, volumes of 300 l/ha and pressures of 2 bars. Crop compatibility was tested by spraying 1m wide strips across a range of cultivars. Grower usage trials, where treatments were applied by the farmer's sprayer to areas of about 0.5 ha, were also executed.

In the majority of trials, applications were timed according to crop growth stage recorded on a decimal scale (Zadoks et al, 1974), irrespective of disease level. In specific trials with leaf blotch and rust diseases, treatments were also applied either when disease lesions were first apparent or when the disease had reached an infection level on leaf 3 of about 5-10%. Disease assessments were carried out normally at growth stage 75-85 by grading the percentage leaf area infected on the uppermost 3 leaves. Results were expressed as percentage control based on the mean level of infection on the untreated. Green leaf area assessments were carried out at the same time and are expressed as a percentage of the untreated.

Yield measurements were obtained by harvesting about $30m^2$ from each plot in replicated trials, using a Claas Compact 25 combine and about $300m^2$ areas in grower usage trials using the farmer's combine. Grain was sub-sampled from each plot and, after cleaning, measurements were made of 1000 grain weights and grain size >2.8mm and >2.2mm. The yields were corrected to 14% moisture and both yields and grain quality were expressed as a percentage of the untreated.

In the tables, results on each disease are reported from individual trials in which meaningful infection levels occurred. The Bayer trials centres are indicated by the trial numbers as follows; A - Elm Farm Trials Station, Suffolk, ER - Eastern, MR - Midland, NR - Northern, SC - Scottish, SR - Southern and WR - Western. The overall performance of triadimefon, measured by various parameters which are not necessarily related, is summarised (Table 5) using medians of results from all trials with >2% infection. The comparison treatment referred to as the standard was tridemorph in the majority of trials. The reliability of each median was analysed statistically using Wilcoxon's signed rank test in addition to analyses carried out on all data from individual replicated trials.

RESULTS

Spring Barley Mildew levels were not particularly high during 1975, and in 1976 the hot dry summer inhibited the spread of early infections to the upper leaves. Results from trials in which adequate disease levels occurred (Table 1) demonstrate the excellent control obtained with triadimefon at all rates and application timings.

Table 1

Spring barley - % control mildew (mean top 3 leaves) at crop stage 75-85 in individual trials 1975-76

Treatments	Rate a.i. g/ha	Crop stage	Replicated Trials A/1 1975	NR/1 1975	SR/2 1975	WR/3 1976	Grower Trials ER/4 1976	SC/1 1976	SC/2 1976	SR/3 1976	SR/4 1976	WR/1 1976
Single applications												
triadimefon	125	30-32	100	92	89	94	99	-	-	100	99	82
triadimefon	250	30-32	97	98	97	-	-	-	-	-	-	-
tridemorph	525	30-32	90	68	66	-	76	-	46	67	0	-
triadimefon	125	32-45	89	84	99	-	28	98	99	-	-	-
triadimefon	250	32-45	62	92	97	-	-	-	-	-	-	-
tridemorph	525	32-45	-	-	-	-	-	89	93	-	-	63
Double applications												
triadimefon + triadimefon	62.5 + 62.5	30-32 32-45	93	100	99	94	-	-	-	-	-	-
triadimefon + triadimefon	125 + 125	30-32 32-45	-	-	-	94	98	100	100	100	100	99
fluotrimazole + fluotrimazole	94 + 94	30-32 32-45	100	97	99	79	92	-	-	-	-	-
Untreated control												
% Infection			4.7	12.6	11.8	5.6	10.7	27.4	14.5	13.2	24.6	4.8

In trials on the cultivar Maris Mink, the incidence of leaf blotch (Table 2) was higher in 1977. The results show that applications made at crop stage 30-31, which largely coincided with disease appearance, gave effective control.

Table 2

Spring barley - % control of leaf blotch (mean top 3 leaves) at crop stage 75-85 in individual trials 1976-77

Treatments	Rate a.i. g/ha	Crop stage	Replicated Trials WR/2 1976	A/4 1977	WR/5 1977	WR/6 1977	WR/8 1977	WR/9 1977	Grower Trial SC/3 1976
Single applications									
triadimefon	125	12-22	-	55	89	65	62	85	-
captafol	1400	12-22	-	18	68	91	44	69	-
Single applications									
triadimefon	125	30	-	-	99	97	89	97	-
Disease appearance applications									
(Crop Stage)			(31)	(31)	(31)	(31)	(30)	(30)	(32)
triadimefon	125		76	98	29	76	92	79	95
tridemorph + carbendazim	525 125		72	85	88	72	53	76	21
Disease established applications									
(Crop Stage)				(60)		(60)	(58)		
triadimefon	125		-	67	-	81	78	-	-
triadimefon	250		-	54	-	67	76	-	-
Double applications									
triadimefon + triadimefon	125 125	30 32-40	95	100	100	99	94	99	-
Untreated control									
% Infection			3.7	13.1	17.1	13.6	29.8	6.8	13.5

The degree of infection with brown rust was higher in 1977 (Table 3) although attacks occurred rather late. Triadimefon whether applied on first appearance of the disease or later, gave good control; some response to increased rate being apparent. The incidence of yellow rust was low but control of this pathogen was also indicated.

A summary of disease results together with yield and grain quality data from 1975 and 1976 trials is given in Table 5. The good disease control given by triadimefon is reflected in both yield response and grain quality.

Winter barley During the autumn of 1975 and early spring of 1976 considerable mildew infection occurred which was well controlled by autumn applications of triadimefon (Table 4) resulting in increased crop vigour. In most trials the disease did not spread to the upper leaves of the crop.

In 1977 the control of leaf blotch, which was present from the early spring, was rather variable and generally not very high (Table 4).

A summary of disease results together with yield and grain quality data from 1976 trials is given in Table 5.

Table 3

Spring barley - % control of brown and yellow rust (mean top 3 leaves) at crop stage 75-85 in individual replicated trials 1975-77

Treatments	Rate a.i. g/ha	Crop stage	Brown Rust SR/1 1975	A/3 1976	A/5 1977	MR/3 1977	SR/6 1977	SR/7 1977	SR/9 1977	Yellow Rust NR/1 1975	NR/3 1977
Single applications											
triadimefon	125	30-32	39	15	-	-	-	-	93	71	-
Disease appearance applications											
(Crop Stage)			(32)	(39)	(39)	(54)	(45)	(45)		(37)	(80)
triadimefon	125		73	31	84	100	94	81	-	75	67
triadimefon	250		85	64	92	-	-	-	-	100	-
benodanil	1100		-	13	83	84	83	47	-	-	87
Disease established applications											
(Crop Stage)				(50)	(75)	(58)	(83)	(83)	(58)		
triadimefon	125		-	36	74	98	49	100	100	-	-
triadimefon	250		-	31	85	100	100	100	-	-	-
tridemorph + Polyram	525 2200		-	-	28	84	0	47	-	-	-
Double applications											
triadimefon + triadimefon	125 125	30-32 32-55	-	-	78	100	94	32	100	-	98
Untreated control											
% Infection			2.1	2.8	9.3	4.6	12.3	4.6	29.4	1.9	1.7

Crop compatibility No phytotoxicity or adverse effects on crop yields were recorded even when multiple applications of triadimefon at 250g a.i./ha were applied. Triadimefon was sprayed on a total of 38 spring barley cultivars and 11 winter barley cultivars at Elm Farm Trials Station over a period of 3 years without any problems.

DISCUSSION

Despite the relatively low levels of mildew in many trials, control achieved by triadimefon was impressive and resulted in virtual disease elimination (Table 1). Single applications made just prior to stem extention were usually sufficient to keep the crop free from disease although in winter barley autumn applications were needed to provide control of overwintering infections. The high activity and strong vapour action of triadimefon (Scheinpflug et al, 1977) caused problems in replicated cereal mildew trials; less disease was found on untreated plots within the trial than in the surrounding crop and this probably reduced yield differences. Greater yield responses from large plot grower usage trials supports these suggestions. Overall, however, a considerable benefit in terms of yield and grain quality resulted from triadimefon treatments (Table 5).

The good control of leaf blotch in spring barley (Table 2) was not reflected in the winter barley crop (Table 4). This discrepancy may in part be explained by the different times of year during which the disease develops; the high rainfall in the early spring of 1977 encouraging the rapid spread of the pathogen in the winter crop.

Table 4

Winter barley - % control of mildew, leaf blotch and rusts (mean top 3 leaves) at crop stage 26-30 (E) and 75-85 in individual trials 1976-77

Treatments	Rate a.i. g/ha	Crop stage	Mildew						Leaf Blotch								Brown Rust		Yellow Rust
			Replicated Trials				Grower Trials		Replicated Trials					Grower Trials			Grower Trials		
			A/2 1976	ER/1 1976		MR/1 1976	ER/3 1976	SR/3 1977	ER/2 1976	ER/6 1977	SR/5 1977	WR/4 1977	WR/7 1977	ER/3 1976	ER/5 1977	MR/2 1977	ER/5 1977	SR/8 1977	SR/8 1977
Autumn applications			(E)	(E)		(E)													
triadimefon	125	21-26	70	48	29	68	-	-	18	-	15	7	7	-	-	-	-	-	-
triadimefon	250	21-26	69	69	94	95	-	-	23	-	-	-	-	-	-	-	-	-	-
Spring applications																			
triadimefon	125	24-32	-	-	100	-	99	100	28	87	32	46	30	59	87	-	69	72	100
tridemorph + carbendazim	525 125	24-32	-	-	69	-	98	-	39	-	-	-	-	35	-	-	-	-	-
triadimefon	125	32-55	-	-	-	-	-	99	-	88	82	34	25	-	52	-	63	99	100
Double applications																			
triadimefon + triadimefon	125 125	21-26 24-32	-	-	100	-	-	-	55	-	74	18	41	-	-	68	-	-	-
triadimefon + triadimefon	125 125	24-32 32-55	-	-	-	-	-	-	-	93	78	50	34	-	89	21	85	99	100
Untreated control																			
% Infection			23.1	6.4	20.0	9.2	6.7	6.7	6.8	7.0	8.6	32.1	5.9	6.4	22.2	20.4	3.9	3.8	4.1

(E) - Overwintering infection assessed in early spring

Table 5

Summary of results, expressed as medians, from trials in which infection levels exceeded 2% and yields from all trials harvested, in parenthesis

Treatments	Rate a.i. g/ha	Crop stage	% mildew control	% leaf blotch control	% brown rust control	Relative green leaf area	Relative yield	Relative grain weight	Relative grain >2.8mm	Relative grain >2.2mm
SPRING BARLEY										
Single applications										
triadimefon	125	30-32	99**	97**	69**	131**	116** (109**)	102**	114**	101*
standard		30-32	70**	74**	22	105**	108** (108**)	103**	107	101
triadimefon	125	32-55	94**	-	81**	130**	107** (106**)	103*	126**	101*
Double applications										
triadimefon	125	30-32								
+ triadimefon	125	32-55	100**	99**	89**	138**	115** (109**)	104**	129**	102*
Untreated control										
Level on untreated			10.5% Infection	11.6% Infection	7.8% Infection	55.5% Green leaf	3706 kg/ha (4252) kg/ha	33.1g /1000	27.8%	87.0%
Maximum no. trials			22	10	18	39	26 (38)	26	18	26
WINTER BARLEY										
Spring applications										
triadimefon	125	24-32	93**	53**	71	118*	110*	102	112	102
standard		24-32	81	39	-	112*	102	102*	115	100
Double applications										
triadimefon	125	21-26								
+ triadimefon	125	24-32	99	58**	-	114*	112	101	98	102
triadimefon	125	24-32								
+ triadimefon	125	32-55	96	69**	98	112**	-	-	-	-
Untreated control										
Level on untreated			3.6% Infection	6.9% Infection	3.9% Infection	65.1% Green leaf	4437 kg/ha	29.1g /1000	3.0%	86.4%
Maximum no. trials			8	12	3	10	6	5	5	5

Medians significantly different from the untreated control, * at P <0.05 and ** at P <0.01

Since triadimefon controls the disease largely by preventing or reducing sporulation (Jordan, 1977) the poor results are probably due to high infection pressure from untreated areas in replicated trials. Indications from a survey of commercial usage are that effectiveness improves when large areas are treated. It is likely however, that a 2 spray programme is required to provide consistently good results against this disease.

Control of brown rust in barley is difficult since it usually builds up rapidly late in the season when control measures are impractical and often result in little yield benefit. Good results were obtained in trials with a 2 spray programme (Table 3) although in one trial, SR/7, control was poor due to the second application being applied too early. On the other hand, when considerably larger areas were treated, as in SR/9, excellent control was obtained from a single early application.

Triadimefon provides the barley grower the means by which he can control the major cereal foliar diseases. To apply any material for the specific control of individual diseases requires great care but in the case of mildew the routine use of a fungicide can be justified (Cock, 1975). Triadimefon used as a 2 spray programme during stem extention can provide the barley grower with season-long protection. This approach may alleviate the necessity for careful disease monitoring and enable optimal yields to be obtained.

Acknowledgements

The authors wish to thank the staff of Bayer U.K. Limited who participated in the development work and the farmers who co-operated with trials.

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Proceedings 1977 British Crop Protection Conference - Pests and Diseases

THE DEVELOPMENT OF THE SYSTEMIC FUNGICIDE, TRIADIMEFON, FOR THE CONTROL OF FOLIAR DISEASES IN WHEAT AND OATS IN THE U.K.

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Summary Winter wheat field trials conducted in 1975-77 showed triadimefon at 125g a.i./ha to be effective against powdery mildew (Erysiphe graminis), yellow rust (Puccinia striiformis), and brown rust (P. recondita). Suppression of foliar infections of Septoria spp. was also noted. Applications made during stem elongation provided protection against mildew and yellow rust on the foliage. Sprays at ear emergence were required for the control of ear mildew and late occurring foliar mildew and rusts. A programme of two sprays afforded optimal control throughout the disease susceptible period.

In winter and spring oats single and double sprays applied during stem elongation gave excellent control of powdery mildew (E. graminis).

Résumé Les essais en plein champ de 1975-77 ont démontré que triadimefon appliqué à la dose 125g a.i./ha au blé d'hiver est efficace contre l'oidium des céréales (Erysiphe graminis), la rouille jaune (Puccinia striiformis) et la rouille brune du blé (P. recondita). Une réduction des maladies foliaires de Septoria spp. est aussi digne de remarque. Les applications appliquées pendant la montaison a produit une lutte contre l'oidium et la rouille jaune du blé sur le feuillage. Les applications au temps d'épiage étaient nécessaires pour la lutte contre l'oidium des épis, et aussi l'oidium foliaire et les rouilles qui se produisent plus tard dans la saison. Une programme de deux applications a fourni une protection optimale pendant toute la période prédisposée aux maladies.

Dans le cas de l'avoine de printemps et d'hiver, une seule application et aussi deux applications appliquées pendant la montaison ont donné une protection excellente contre l'oidium des céréales (E. graminis).

INTRODUCTION

National disease surveys (King, 1977) show that high levels of disease occur on winter wheat in association with particular cultivars. Although the average disease levels are not severe, estimated yield losses are between 2 and 10%. Fungicide usage on the wheat crop has been low, but the current value of the crop and the potential for disease control warrant the investigation of broad spectrum materials.

Triadimefon is the common name for 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl) butan -2-one, a compound of low mammalian toxicity with fungicidal activity against a wide range of agricultural and horticultural diseases (Kaspers

et al, 1975). The compound shows marked systemic properties in both acropetal and basipetal directions (Kaspers et al, 1975) and also exhibits pronounced vapour phase activity (Scheinpflug and Paul, 1976). Greenhouse experiments have shown excellent curative and protective effects against cereal mildew and brown rust at concentrations of 1.5 and 147 ppm. respectively (Buchenauer, 1976). In 17 German field trials, rates of 125g a.i./ha gave average effectiveness of 87% against yellow rust, and resulted in an average yield increase of 13% (Siebert, 1976). Triadimefon was found to be the most effective yellow rust fungicide in trials in Schleswig-Holstein (Bauers, 1976).

Field trials evaluating triadimefon for disease control in winter wheat and oats are reported here. Development of the compound on barley is reported in a separate paper at this conference.

METHODS AND MATERIALS

During 1975-1977 a 25% w/w wettable powder formulation of triadimefon was used in 76 trials, of which 53 were on wheat and 23 on oats. Replicated trials were carried out using randomised block designs with four replicates and plot size of 2-3m x 15m. Applications were made using pressurised knapsack sprayers with fan nozzles, water volumes of 300 l/ha and pressures of 2-3 bar. Unreplicated grower usage trials compared triadimefon with the farmer's standard fungicide using farm sprayers on plots of 0.2-0.5 ha. Comparison treatments were usually tridemorph (70% e.c.) in mildew trials, and tridemorph plus Polyram (80% w.p.) or tridemorph followed by benodanil (50% w.p.) in rust trials. These standards were inappropriate where Septoria infections were assessed.

Single sprays were compared with double sprays on both crops, applications being at crop stages 31-39 and 45-60 (Zadoks et al, 1974) on wheat and at 30-31 and 32-39 on oats. Sites were chosen by selecting susceptible cultivars to encourage specific diseases. In addition to disease evaluation trials, several crop tolerance trials were conducted in which swathes of 1m were sprayed across a range of wheat and oat cultivars.

Disease control was measured by assessing the percentage infected area on the uppermost three leaves or ear. Green leaf area was similarly assessed, recording the percentage green tissue. Results relative to untreated values were tabulated.

Yields in replicated trials were harvested from 2m x 15m per plot using a Claas Compact 25 combine harvester. In grower usage trials farm machinery was used to sample areas of 300m^2. In all trials yield data were corrected to 14% moisture content and expressed relative to untreated (Table 3). Grain samples were taken to assess quality in terms of 1000 grain weight and grain size. All data, in replicated trials, were statistically analysed, the median yield values being tested for reliability using Wilcoxon's signed rank test.

Trials are numbered using a prefix to indicate the Bayer region in which they were conducted; ER - Eastern, MR - Midland, NR - Northern, SC - Scotland, SR - Southern, WR - Western.

RESULTS

Winter wheat Of the trials carried out between 1975-77, only 19 developed levels of foliar disease >4% at the final disease assessment (GS 75-85). Results for these trials are tabulated in Tables 1, 2 and 3. Yield data for 1977 trials were not available at the time of writing.

Table 1

Winter wheat - % control of mildew on leaves (mean top 3 leaves) and ear at crop stage 75-85 on individual trials 1975-77

Treatments	Rate a.i. g/ha	Crop stage	Leaf Mildew: Replicated Trials ER/1 1975	ER/3 1977	MR/4 1977	Grower Trials ER/2 1976	MR/3 1976	NR/2 1976	Ear Mildew: Replicated Trials ER/1 1975	MR/1 1976	SR/2 1976	WR/1 1976	ER/3 1977	MR/4 1977	Grower Trials NR/2 1976	MR/2 1976
Single sprays																
triadimefon	125	31-39	21	94				95	27	80		72	69		52	
	125	45-58	48	81	82	88			100		99		67	99		100
standard		31-39		0a									0a			
		45-58				68a					74b					
Double sprays																
triadimefon	125 and	31-39 45-58	44	100			99		100	100		87	84			
standard	and	31-39 45-58	17b						57b	91b		72b				
% mildew on untreated			18.9*	25.4	27.9	9.9	5.4*	4.5	10.3	10.1	16.9	7.1	9.4	26.8	5.8	4.6

* % infection on top 2 leaves only

a = tridemorph 525g a.i./ha
b = tridemorph 525g a.i./ha + 'Polyram' 1760g a.i./ha

Table 2

Winter wheat - % control of yellow rust, brown rust and leaf septoria (mean top 3 leaves) at crop stage 75-85 on individual trials 1975-77

Treatments	Rate a.i. g/ha	Crop stage	Yellow Rust			Brown Rust					Leaf Septoria			
			Replicated Trials			Rep. Trial	Grower Trials				Replicated Trials			Grower trial
			ER/5 1975	NR/1 1975	SR/1 1975	SR/2 1976	SR/3 1977	SR/4 1977	SR/5 1977	SR/6 1977	ER/1 1975	ER/5 1975	SC/1 1976	SC/2 1976
Single sprays														
triadimefon	125	31-39		69	82						5		48	40
	125	45-60		91		68	63	87	69	49	14			
standard		31-39			54b								38b	
		58				29b								
Double sprays														
triadimefon	125	31-39 and 45-58	99	91							1	44		
standard		31-39 and 45-58	26a	91b							6b	15a		
% infection on untreated			8.1	10.9*	17.7	4.0**	47.7	38.3	39.6	4.2	39.3*	4.7	13.0	14.8

* % infection on top 2 leaves only
** % infection on top leaf only

a = first spray tridemorph 525g a.i./ha; later spray benodanil 1120g a.i./ha
b = tridemorph 525g a.i./ha + 'Polyram' 1760g a.i./ha

Table 3

Winter wheat - Relative yield for trials reported and medians of all trials harvested 1975-76

Treatments	Rate a.i. g/ha	Crop stage	Replicated Trials								Grower Trials				Overall median (all trials harvested)
			ER/1 1975	ER/5 1975	NR/1 1975	SR/1 1975	SR/2 1976	MR/1 1976	WR/1 1976	SC/1 1976	MR/2 1976	MR/3 1976	NR/2 1976	SC/2 1976	
Single sprays															
triadimefon	125	31-39	105		99	109*		98	104	100			110	99	102** (13)
	125	45-58	112**		102	107*	116**				98				107* (10)
standard		31-39								97					97 (6)
		45-58					112**								112 (2)
Double sprays															
triadimefon	125 and	31-39 45-58	111**	109	100	117**		97	99			106			102* (19)
standard	and	31-39 45-58	103	113	101	107*		101	96						102* (12)
Yield on untreated (kg/ha)			5707	7390	7291	5148	5816	3777	3289	6983	5575	3529	4809	7050	4720 (28)

Yield significantly greater than untreated at P = 0.05*
P = 0.01**

( ) = No. of observations

Oats Table 4 includes details of winter and spring oat trials where the level of mildew in untreated plots exceeded 4%.

Table 4

Winter and spring oats - % control of mildew (mean top 3 leaves) at crop stage 75-85, and relative yields for individual trials 1976-77

Treatments	Rate a.i. g/ha	Crop stage	Winter Oats Rep. Trials ER/4 1976	Winter Oats Rep. Trials WR/2 1976	Winter Oats Grower Trials MR/5 1976	Winter Oats Grower Trials WR/3 1976	Spring Oats Rep. Trials SC/3 1976	Spring Oats Rep. Trials SC/4 1976	Spring Oats Grower Trials SC/5 1976	Spring Oats Grower Trials SR/7 1977
			% CONTROL OF MILDEW							
Single sprays										
triadimefon	125	30-31	79	46	82	73		100	96	100
		32-39	96	98			100			
standard		30-31			59a	64b			77a	0c
Double sprays										
triadimefon	125 and	30-31 32-39	97	96	99	100		100	99	
Untreated										
% mildew			6.3+	31.0	16.1	6.8	49.4	4.6	20.5	38.2+
			RELATIVE YIELD							
Single sprays										
triadimefon	125	30-31	106**	112*	111	97		104	114	
		32-39	101	110			108			
standard		30-31			98	92			107	
Double sprays										
triadimefon	125 and	30-31 32-39	105**	122**	113	95		108	111	
Untreated										
Yield (kg/ha)			5553	2943	3907	3344	4515	5261	5168	-

Yields significantly greater than untreated at P = 0.05*
P = 0.01**

+ Means of top 2 leaves only
a = tridemorph 525g a.i./ha
b = triforine 266g a.i./ha
c = ethirimol 350g a.i./ha

In both winter wheat and oat trials all treatments prolonged the presence of green leaf tissue. This effect was most pronounced with triadimefon especially following applications at the later timing.

Response to triadimefon in terms of grain quality was slight in both wheat and oats. Individual trials generally showed non-significant differences, although exceptions showed clear improvements in grain quality which related yield increases as in SR/2 (Table 3).

Crop Tolerance Applications of triadimefon at 250g a.i./ha caused no symptoms of phytotoxicity in 43 spring and winter wheat cultivars and 18 spring and winter oat cultivars.

DISCUSSION

Winter Wheat In mildew trials (Table 1) single applications of triadimefon were effective against leaf infection at both timings. In two trials (ER/2 and ER/3) single early applications gave foliar protection until ripening. Excellent control of ear mildew was achieved with the late spray at crop stage 45-58, although early applications often gave adequate control. A programme of both applications was found to be optimal, giving the most reliable protection from the beginning of stem elongation up to ripening.

Three trials incurred levels of yellow rust greater than 4% (Table 2), but disease was present in only one trial, SR/1, at the time of the early application. In this trial the triadimefon treatment applied at 5% infection continued to exert a strong effect five weeks after application when infection rose to 18% on untreated plots. The remaining two trials had low levels of yellow rust present at the late application timing. Here the late application and the programme gave excellent control. In NR/1, the lower level of control given by the early treatment, six weeks after spraying, substantiates the work of Bauers (1976) who reported that triadimefon remains effective against yellow rust for up to four weeks after application.

Brown rust figures for 1977 trials (Table 2) are results of single late sprays of triadimefon applied on appearance of disease between crop stage 50 and 60. Good control of high infection levels was achieved with triadimefon. In trial SR/2, where brown rust developed after the application date, triadimefon afforded good control of a moderate infection when assessed four weeks after spraying.

Septoria leaf symptoms were recorded in 4 trials (Table 2). Results were variable but single early applications of triadimefon and the programme gave 40-50% suppression where infection was light or moderate (ER/5, SC/1 and SC/2).

Yield data for individual trials (Table 3) were often inconclusive, but statistical significance was found in three trials. In ER/1 and SR/2 late applications of triadimefon were correlated with high levels of ear mildew control. Full correlation of yellow rust and yield data is not possible for trial SR/1 as disease results following applications at 45-58 were not obtained. Rust control with the early application, however, resulted in a 9% increase in yield.

Oats All applications of triadimefon gave effective control of mildew (Table 4). In winter oats, the two spray programme was optimal where high levels of infection occurred over a protracted period as in WR/2. In the majority of trials, however, early applications were effective for 7-9 weeks, this protection resulting in yields almost equal to those achieved with 2 sprays. Late applications gave good mildew control but produced lower yield response, possibly due to lack of early protection.

In the spring oat crop where trials were assessed earlier (3-5 weeks after first application), all triadimefon treatments gave 96-100% control of mildew.

Yields suggested that treatments had been equally effective.

Acknowledgements

The authors wish to thank colleagues and farmers for their co-operation.

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Proceedings 1977 British Crop Protection Conference - Pests and Diseases

FIELD TRIALS ON CEREAL MILDEW WITH BTS 40 542, A NEW BROAD SPECTRUM FUNGICIDE

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Summary Results of detailed field trials demonstrating the efficacy of BTS 40 542 against cereal powdery mildew in the United Kingdom during 1976 and 1977 seasons are presented. Mildew control studies have been conducted in autumn and spring sown barley. Phytotoxicity tests have been carried out also on autumn and spring sown wheat and oats. Comparison of different formulation types has shown emulsifiable concentrates and a water dispersible concentrate to be superior to dispersible powders. Yield increases associated with the control of mildew have been demonstrated.

Résumé Les résultats d'essais sur place très poussés démontrant l'efficacité de BTS 40 542 contre le blanc des graminées dans le Royaume Uni pendant les saisons de 1976-1977, sont présentés. Des études pour maîtriser la rouille ont été effectuées avec des semis d'orge d'automne et de printemps. Des essais de thytotoxicité ont été effectués également avec du blé et de l'avoine semés en automne et au printemps. La comparaison de différents types de formules a démontré que les produits concentrés émulsionnables, et un produit concentré dispersable à l'eau, sont supérieurs aux poudres dispersables. Des accroissements de rendement ayant un rapport direct avec la maîtrise de la rouille ont été démontrés.

INTRODUCTION

Following promising results in glasshouse tests (Birchmore, R. J. et al 1977) in which a high level of both eradicant and protectant activity against cereal powdery mildew and other pathogens were demonstrated, a series of detailed (i.e. replicated, small plot) field trials was laid down, starting winter 1975 (1976 season) to confirm the efficacy of BTS 40 542 against certain cereal diseases. A further series of trials was conducted in 1977. This paper reports the results of representative trials, from both seasons, on cereal powdery mildew control in barley.

METHODS AND MATERIALS

The work reported was carried out over an area extending from Yorkshire to Oxfordshire, the majority being concentrated in the East Midlands. It was possible to commence the 1976 season of trials with application to winter barley in late 1975 as a forerunner to the main spring trials. Such application was not practicable in autumn/winter 1976 due to lack of disease. In spring 1976 the first application was made on 18th May whilst in the following year, the effect of climatic conditions on disease incidence delayed application until 20th June.

Trials of randomised block design were planned for three distinct purposes a) for observation and measurement of disease control, b) for determination of crop yield and c) for checking phytotoxicity in a range of cereal cultivars. In the observation trials plot dimensions were 3 m x 20 m, size being increased to $3\frac{1}{2}$ m x 40 m in the case of yield trials. In 1976 replication of treatments was threefold in the case of the former and sixfold with the latter. In 1977 fourfold replication was adopted throughout, the large number of treatments in the yield trials providing further replication of the major treatment factors. Spraying was carried out using the Lenton Small Plot Sprayer (Lush, G. B., Mayes, A. J., 1972) at a volume of 225 l/ha and pressure of 2 bars. One application was made in all cases. Cereal growth stage was defined according to the Feekes/Large scale (Large, E. C. 1954).

Clearance under the Pesticides Safety Precautions Scheme having been obtained, trials were situated in farmers' fields, the remaining area around each trial being treated as necessary, with proprietary mildewicides. In each case the same husbandry practices obtained in the trial area as in the rest of the field.

Assessment of infection levels was made on ten plants taken at random from each plot using the A.D.A.S. cereal mildew key, leaves being numbered from the top downwards. Assessments of mildew infection were also made using a 0-10 visual scoring system where 0 represents no mildew and 10 indicates complete cover. In all cases the figures for mildew infection shown in the tables are the means of replicate values.

Yields were determined using a Claas Comet 1.8 m cut, combine harvester modified for weighing small plot yields. Thousand grain weights were determined using a King Tablet Counter.

In the field trials reported, a number of 25% formulations of BTS 40 542 were compared, including a dispersible powder, water dispersible concentrate (w.d.c.) and an emulsifiable concentrate. Rates ranging between 200 g-1 kg/ha in terms of active ingredient were evaluated. The 75% commercial formulation of tridemorph was included as a standard in both seasons' trials. In 1977 the 25% commercial product based on triadimefon was also included.

RESULTS

Representative results from the two seasons' work are presented in the tables.

In autumn/winter 1975, conditions were conducive to cereal powdery mildew in some localities and trials were carried out. Table 1 gives the results of such a trial at Barnsley, where after the drought of 1975, the field was lightly cultivated before being sown with winter barley following a spring barley crop. There was considerable residual trash which will have provided the inoculum leading to the consequent heavy infection in the winter crop. Whilst the mildew was active, the trial demonstrated the comparable activity of an early type emulsifiable concentrate formulation of BTS 40 542 with the commercial formulation of tridemorph. Some weeks after the final assessment shown in the table, severe frosts and an exceptionally dry spring severely reduced the infection which never significantly re-established.

By the middle of May 1976 mildew infections were present in most barley crops. At Wellesbourne in spring barley (Table 2) a moderate infection at the time of spraying when the air temperature was 21°C, decreased after a period of sudden low temperature and cool winds. By a month after spraying the infection level increased dramatically in the untreated areas, the BTS 40 542 treated plots providing very significant protectant activity. The results in this trial compare very favourably with those of the standard tridemorph formulation.

At another site, Calverton, on spring barley (Table 3) the eradicant properties of BTS 40 542 were well demonstrated on a heavy mildew infection, well controlled by low rates of the compound.

In the winter of 1976, a number of trials was started in winter barley but the infections never seriously developed at that time. In the following spring, infections in these trials increased to a moderate level before being reduced by low temperatures. In spite of this, useful yield increases were recorded at harvest (Table 4).

In the spring of 1977, cold rainy conditions were not conducive to mildew infection and it was not until mid-June that moderate levels began to build up in spring barley. Infection levels never achieved the intensity of 1976 but they were sufficient to confirm earlier results.

Table 5 shows results of a comparison between a dispersible powder, an emulsifiable concentrate and a water dispersible concentrate. Here again eradicant effect is demonstrated. The two latter types of formulation are clearly much more effective than the dispersible powder and compare very favourably with the two standard products. Because of the late season, the readings of the second percentage leaf infection assessment were not available for inclusion in this paper and two visual score assessments are given to round off the picture.

The trial represented in Table 6 developed the highest infection level of the 1977 trials reported. Against this background, BTS 40 542 as an emulsifiable concentrate continued to show very favourably in comparison with commercial standards.

A further indication of the persistence of BTS 40 542 in controlling cereal mildew is shown in Table 7 where there is an indication that at the higher rates of use, the compound was maintaining control after at least one of the standards had started to lose effectiveness.

Special attention was paid to the question of the crop safety of BTS 40 542. In two spring cereal variety trials, the compound was found to be completely without adverse effect at rates up to 1 kg a.i./ha on the nineteen barley, four wheat and five oat varieties tested. In a winter cereal variety trial a similar margin of safety was found in the six barley, thirteen wheat and four oat varieties included.

Residue analysis of field samples from seven 1976 trial sites revealed quantities of less than 0.01 ppm in wheat and barley grain and wheat straw. This figure represents the limit of sensitivity for the method. In barley straw a low residue of 0.025 ppm was detected.

Compatibility tests carried out with a number of phenoxyalkanoic acid herbicides revealed no intereaction. This work is being expanded to cover all relevant potential tank mix situations.

Table 1

The % leaf area infected with mildew in winter barley treated on 6.12.75. at G.S. 3-4

Date	17.12.75.			19.1.76.		
Leaf	3	4	Mean	3	4	Mean
Treatments						
1. BTS 40 542 e.c. 400 g a.i./ha	4.87	10.83	7.55	9.97	6.65	8.30
2. BTS 40 542 e.c. 500 g a.i./ha	3.73	7.14	5.43	7.33	6.10	6.70
3. Tridemorph 525 g a.i./ha	5.10	10.94	8.02	6.13	7.87	7.00
4. Untreated	9.53	23.50	16.50	30.20	34.79	32.50

Location: Barnsley Cultivar: Malta

% mildew infection at application: leaf 3, 2.9 leaf 4, 14.0

Table 2

The % leaf area infected with mildew in spring barley treated on 18.5.76. at G.S. 5-6

Date	3.6.76.			21.6.76.		
Leaf	3	4	Mean	3	4	Mean
Treatments						
1. BTS 40 542 e.c. 400 g a.i./ha	Nil	0.17	0.085	2.4	32.8	17.6
2. BTS 40 542 e.c. 500 g a.i./ha	Nil	0.07	0.035	4.9	22.4	13.6
3. BTS 40 542 e.c. 1000 g a.i./ha	Nil	0.80	0.40	0.8	10.5	5.6
4. Tridemorph 525 g a.i./ha	0.1	0.33	0.17	8.4	49.8	29.1
5. Untreated	0.83	4.03	6.16	32.1	80.0	56.0

Location: Wellesbourne Cultivar: Mazurka

% mildew infection at application: leaf 3, 9.5 leaf 4, 5.6

Table 3

The % leaf area infected with mildew in spring barley treated on 21.5.76. at G.S. 4-5

Date		3.6.76.	
Leaf	3	4	Mean
Treatments			
1. BTS 40 542 200 e.c. g a.i./ha	0.9	6.3	3.6
2. BTS 40 542 300 e.c. g a.i./ha	0.2	3.8	2.0
3. Tridemorph 525 g a.i./ha	0.2	4.4	2.3
4. Untreated	7.0	49.5	25.1

Location: Calverton Cultivar: Julia

% mildew infection at application: leaf 3, 40.5 leaf 4, 59.7

Eradicant activity particularly on leaf 4 should be noted.

Table 4

Grain yield and thousand grain weights, winter barley treated on 16.5.77. at G.S. 7-8

			Thousand grain weights	
	Mean % infection leaf 3 & 4	Yield as % untreated	Mean 4 reps. in g	Mean as % untreated
Treatments				
1. BTS 40 542 e.c. 300 g a.i./ha	0.48	107.7	56.9	102.3
2. BTS 40 542 e.c. 500 g a.i./ha	0.32	106.7	57.2	102.7
3. Tridemorph 525 g a.i./ha	0.34	101.9	56.7	102.1
4. Triadimefon 125 g a.i./ha	0.52	100.5	55.5	99.7
5. Untreated	1.10	100.0	55.6	100.0

Location: Long Benington Cultivar: Malta

% mildew infection at application: leaf 3, 4.0 leaf 4, 8.0

Untreated yield/ha 6345.8 kg

Table 5

Powdery mildew control, spring barley

treated on 20.6.77. at G.S. 6-7

(comparison of formulations)

	percentage leaf area infected					visual score	
Date	14.7.77.					19.7.77.	5.8.77.
Leaf	Flag	2	3	4	Mean		
Treatments							
1. BTS 40 542 d.p. 300 g a.i./ha	0.08	1.25	5.63	11.56	4.63	4.75	5.25
2. BTS 40 542 d.p. 400 g a.i./ha	0.13	2.00	6.43	7.00	3.89	4.25	5.75
3. BTS 40 542 e.c. 300 g a.i./ha	0.08	0.20	3.21	3.55	1.76	2.25	3.50
4. BTS 40 542 e.c. 400 g a.i./ha	0.05	Nil	0.40	3.04	0.87	2.50	4.00
5. BTS 40 542 w.d.c. 300 g a.i./ha	0.08	0.50	0.95	2.93	1.11	2.25	3.50
6. BTS 40 542 w.d.c. 400 g a.i./ha	0.08	0.23	0.85	3.36	1.13	2.50	3.50
7. Tridemorph 525 g a.i./ha	Nil	0.08	0.48	2.60	0.77	2.25	3.75
8. Triadimefon 125 g a.i./ha	Nil	0.03	1.13	4.00	1.29	2.50	3.00
9. Untreated	0.03	2.85	10.50	20.79	8.55	7.50	6.00

Location: Keyworth Cultivar: Aramir

% mildew infection at application: leaf 3, 8

Eradicant activity on leaf 3 should be noted.

Table 6

The % leaf area infected with mildew in spring barley

treated on 17.6.77. at G.S. 6-7

Date	4.7.77.					20.7.77.				
Leaf	Flag	2	3	4	Mean	Flag	2	3	4	Mean
Treatments										
1. BTS 40 542 e.c. 300 g a.i./ha	0.02	0.47	3.35	6.13	2.5	0.53	1.63	3.23	9.0	3.59
2. BTS 40 542 e.c. 400 g a.i./ha	Nil	0.10	2.03	5.45	1.9	0.08	1.76	2.40	2.0	1.76
3. Tridemorph 525 g a.i./ha	Nil	0.22	2.42	7.08	4.2	0.15	3.09	3.91	5.6	3.18
4. Triadimefon 125 g a.i./ha	Nil	0.12	2.40	9.53	3.0	Nil	0.2	2.32	12.59	3.77
5. Untreated	0.02	2.30	11.07	12.65	6.5	4.95	19.95	28.16	67.58	30.16

Location: Calverton Cultivar: Aramir

% mildew infection at application: leaf 3, 7

Table 7

Powdery mildew control, spring barley
treated on 27.6.77. at G.S. 6-7

	Assessment in terms of visual score			
Date	4.7.77.	14.7.77.	28.7.77.	5.8.77.
Treatments				
1. BTS 40 542 e.c. 300 g a.i./ha	3.00	1.25	2.75	4.75
2. BTS 40 542 e.c. 400 g a.i./ha	2.25	2.00	3.00	5.00
3. BTS 40 542 e.c. 450 g a.i./ha	2.25	2.25	3.25	3.00
4. BTS 40 542 e.c. 500 g a.i./ha	2.75	1.50	2.75	2.50
5. Tridemorph 525 g a.i./ha	1.75	1.50	2.00	5.00
6. Triadimefon 125 g a.i./ha	2.50	1.75	1.75	2.75
7. Untreated	8.75	9.25	9.75	10.00

Location: Swarkestone Cultivar: Maris Mink

% mildew infection at application: leaf 3, 6

DISCUSSION

The results reported demonstrate the efficacy of BTS 40 542 in two widely different seasons. The heavier initial infection levels of the 1976 season as exemplified by the trial at Calverton (1976), presented opportunities to demonstrate the eradicant activity of the compound, thus confirming the findings of the glasshouse tests (Birchmore, R. J. et al 1977). This was further confirmed in 1977 trials e.g. at Keyworth. This type of activity is of great benefit to the user particularly where application has to be delayed and when combined with protectant activity, leads to a characteristic healthy green appearance of the crop. The photosynthetic benefit consequent upon the rapid greening is, it is suggested, reflected in increased yields

The activity of BTS 40 542 against powdery mildew has been shown to be amenable to improvement by formulation. In particular, large differences have consistently been shown between dispersible powders on the one hand and emulsifiable and water dispersible concentrates on the other. It is by no means certain that the possibilities of improving activity by formulation have been exhausted.

In most trials, including the special variety trials the rate of 1 kg a.i./ha was included and in all cases was found to be without phytotoxic effect. This safety factor is further demonstrated by the limited yield data so far available.

The overall results as exemplified in this paper indicate BTS 40 542 as being a fungicide of great potential in cereal crops.

Acknowledgements

The authors wish to acknowledge their many colleagues in the company especially those involved in formulating trial material and in carrying out the trials. Particular thanks are also due to Mr. G. B. Lush for assistance with the text.

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Proceedings 1977 British Crop Protection Conference - Pests and Diseases

OBSERVATIONS OF SEPTORIA NODORUM INFECTION IN WINTER WHEAT TO IDENTIFY COMPONENTS OF YIELD LOSS

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Summary Inoculations of field plots of winter wheat with glume blotch (Septoria nodorum) resulted in significant reduction in yields. Sequential inoculations at growth stages (G.S.) 20–29 and 30–31 (Zadoks) reduced yields by 22%; later inoculations at G.S. 45 and 71 increased losses to 50%. Similar results were observed with single inoculations; at G.S. 32 the yield reduction was 28% at G.S. 37–42%, at G.S. 39–58% and at G.S. 58–41%.

Observations were made to identify the components of yield loss. Grain size was greatly reduced by infection and in the most severely affected treatment 75% of the grain passed a 2.4 mm sieve. Also, the numbers and weight of grain per ear were severely reduced on main and secondary tillers but the greatest effect was loss of weight per ear in the secondary tillers.

On a site adjacent to the yield experiment, Septoria development and sporulation in relation to rainfall and humidity were measured following inoculation at G.S. 32 (1 May). The disease established under wet conditions and a subsequent dry period did not inhibit the development of a severe infection by mid-July.

INTRODUCTION

Septoria disease (S. nodorum and S. triticci) is of world wide importance in both winter and spring wheats. Severe losses in yield have been reported by many research workers from naturally and artificially produced infections (Jones and Odebunmi 1971). The disease is important in the United Kingdom in some seasons and is held to be a factor limiting wheat growing in the west where yield losses of 35% and more have been recorded (Jenkins and Morgan 1969). Determination of the effect of Septoria disease in winter wheat has frequently involved the use of fungicides to maintain plots relatively free of the disease for comparison with unsprayed naturally infected plots. This technique is suspect because the fungicide can affect diseases other than Septoria and has also been known to produce yield responses in the absence of obvious disease. In the Trawsgoed work, plots were inoculated at different growth stages, cross inoculation being limited by interplot separation of winter oats. In this way plots with different incidence and duration of Septoria infection were available for direct comparison without the use of fungicides, and opportunity created for an assessment of the disease on the components of yield.

The field inoculation technique was successful in all but one instance (treatment 2 GS 20–29). Despite a dry June established infection progressed to give moderate to high infection on heads at harvest time. Despite the use of oat barrier plots some infection was recorded on unsprayed control plots.

To examine disease progress throughout the season, a further set of plots at the same site were inoculated at an early growth stage and observed thereafter at weekly intervals. Rainfall, temperature and humidity were recorded on the site in order to relate simple meteorological data to disease pattern.

Spore traps in the plots were examined on each occasion when measurable rainfall had been recorded. Data of this nature might eventually provide a rational basis for the use of fungicide applications to commercial crops.

METHODS AND MATERIALS

(i) The trial to determine the effect of S. nodorum inoculations at different growth stages was drilled on the 20 October 1975 with the cultivar Bouquet at 150 kg/ha. Prior to sowing a sample of the Bouquet seed was tested for the presence of S. nodorum but no infection was observed. Treatments were arranged in a 7 x 7 Latin square design, each plot measuring 1.52 m x 2.58 m. To avoid cross infection the wheat plots were separated in each direction with plots of winter oats cv. Peniarth of similar area.

Spores for field inoculation were prepared on Czapecks Dox V8 agar medium, (Lee and Jones 1974), the original culture being supplied by Dr King at Plant Pathology Laboratory Harpenden. Each plot was inoculated with 500 ml of a pycnospore suspension at a concentration of 10^6 spores/ml in water. Each treatment was inoculated at different growth stages (table 1). The plots were completely enclosed in a polythene cage prior to treatment and the covers were left in situ for 96 hours to maintain humidity and ensure spore germination and infection.

The trial was harvested on 5 and 6 August at GS 91 and threshed with a static mini thresher to ensure all the grain was retained. In each treatment 70 plants selected at random were marked and individually assessed for disease throughout, these plants were separately harvested and threshed with a single head thresher. This operation was done with great care so that yield, grain counts, and grain weights were accurately obtained from both main and secondary tillers.

Table 1

The growth stages of the crop and dates at the time of inoculation

Treatments	Growth Stages	Inoculation dates
1	Uninoculated control	
2	20–29, 30–31	23 Feb, 20 April
3	32	5 May
4	37	18 May
5	39	25 May
6	58	14 June
7	45, 71	2 June, 22 June

Disease assessments were carried out at weekly intervals from GS 37 (20 May) using ADAS disease assessment key.

(ii) To examine disease progress through the season, a further plot of cv. Bouquet was sown adjacent to the yield trial described above. The plot area measured 4 x 4 m and divided to give 4 subplots suitable for enclosure under polythene structures as used in the yield trial. The subplots were inoculated with Septoria using the same technique as described above, the polythene structures being left for 96 hours to give ideal disease establishment conditions. The plots were inoculated on two occasions to ensure successful disease establishment, at Growth Stages 30 and 32 on the 20 April and 5 May respectively. Both inoculations were successful and infection maintained under field conditions without any additional treatment.

Two spore traps, in polythene, in the form of a standard meteorological rain gauge were incorporated with the plots at the beginning of May. Great care was taken to ensure that the area in their vicinity was kept bare to ensure minimum contamination by soil splash, and eliminate the possibility of rain washing spores from adjacent wheat plants into the funnels. The traps were changed and examined in the laboratory for the presence and quantity of S. nodorum spores using a lacto phenol filtration technique perfected by Price and Wiggell (Unpublished). Spores were counted by haemocytometer and expressed in terms of spores/ml of rainwater.

RESULTS

The month of May was ideal for the establishment of S. nodorum following inoculations originally carried out on the 20 April GS 30 and repeated on the 5 May GS 32. By the 20 May visible disease symptoms were present on the fifth and sixth leaves. Minimal amount of rain from the 4 May ensured a relative humidity in excess of 70%. Rain water on the 9 May produced a spore catch of 40 spores/ml. Cooke and Jones (1970) observed that 4 days were necessary for infection establishment, and a further 14 days for disease expression on plants, a total of 18 days which suggests that the original inoculation of the 20 April had been successful. The wet period between 17 and 22 May with a maximum spore catch of 5788 spores/ml in one trap, ensured the firm establishment of the disease producing a 40% infection on leaf 5 with symptoms also present on leaves 2, 3 and 4.

Despite the dry weather in June relative humidity remained fairly high on this sheltered site, the minimal rain recorded on 5 days, enabled four spore catches to be recorded, suggesting that glume blotch once firmly established needs only a high r.h. as opposed to actual precipitation for sustained activity. July weather was also favourable to the disease which reached epidemic proportions by the 22nd with 37% infection on the heads. Spore catches during this period reached extremely high levels - 77,000 spores/ml in one trap.

It could be argued that S. nodorum established as early as Growth Stage 30 and remaining untreated by fungicide, can persist throughout the season. Given suitable weather conditions at "flowering time" the disease may assume epidemic proportions producing the "shrivelled grain" condition with consequent high loss in yield, recognised as a feature of glume blotch disease in wheat.

The effect of inoculations at different growth stages, total yields, thousand grain weights, and effects on plant population were illustrated in the following tables (tables 2, 3, 4 and 5).

Table 2

Percentage head and leaf infection at GS 75 (8 July) and total yield of grain (means of 7 plots)

Treatment (Different times of inoculation)	Percentage infection with *S. nodorum*			Total Yield
	Head	Leaf 1	Leaf 2	Tonnes/ha
1. Uninoculated	2.7	20.2	60.9	4.77
2. GS 20–29, 30–31	12.9	61.0	Dead	3.72
3. GS 32	9.9	62.8	"	3.45
4. GS 37	30.4	80.9	"	2.75
5. GS 39	48.9	90.5	"	2.15
6. GS 58	50.7	83.4	"	2.77
7. GS 45, 71	46.8	Dead	"	2.39
				SE ± 0.44

Table 3

The effect of *S. nodorum* inoculation on total yield of grain, 1000 grain weight and grain size

Treatment (GS at inoculation)	Total Yield Tonne/ha	1000 grain (weight (gm)	Sieve tests % grain in each fraction				Total yield t/ha assuming combine harvesting*
			> 3.25 mm	3.25– 2.80 mm	2.80– 2.40 mm	< 2.40 mm	
1. Uninoculated	4.77	31.85	0.53	7.19	67.08	25.27	3.57
2. GS 20–29, 30–31	3.72	25.98	0.35	1.46	47.93	50.17	1.86
3. GS 32	3.45	25.59	0.28	1.93	45.80	52.08	1.65
4. GS 37	2.75	22.62	0.11	1.23	32.72	65.96	0.94
5. GS 39	2.15	21.47	0.08	1.39	23.66	75.14	0.53
6. GS 58	2.77	29.21	0.59	8.91	56.62	33.74	1.84
7. GS 45 and 71	2.39	22.51	0.12	1.61	27.85	70.29	0.71
	SE ± 0.44	SE ± 2.12				SE ± 2.94	

* Assuming loss of grain < 2.40 mm

Table 4

The effect of *S. nodorum* inoculation on plant populations and the grain yield of main and secondary tillers

(from counts of 5 x ½ m rows/plot - means of 7 replicates)

Treatment (GS at inoculation)	Plant Population	Main tiller		Secondary Tillers		Total
	per plot at GS 39	Grains per ear	Grain wt per ear (g)	Tillers per plant	Grain wt per plant (g)	Grain wt/plant
1. Uninoculated	1770	48	1.53	1.82	2.12	3.65
2. GS 20/29 and 30/31	1743	42	1.05	1.30	0.84	1.89
3. GS 32	1824	44	1.17	1.73	1.57	2.74
4. GS 37	1843	37	0.85	1.64	1.03	1.88
5. GS 39	1885	31	0.68	1.60	0.75	1.43
6. GS 58	1945	25	0.79	1.40	0.92	1.71
7. GS 45 and 71	1855	39	0.85	1.45	0.89	1.74
	N/S					

Table 5

Comparison of tiller numbers at GS 32 and fertile tillers at harvest together with yields at harvest

Treatment	Tiller Number at GS 32	Tiller number at harvest	Harvested Yield Tonnes/ha
1. Uninoculated	218	127	4.8
2. GS 20-29 and 30-31	193	91	3.7
3. GS 32	227	121	3.5
4. GS 37	209	115	2.8
5. GS 39	208	112	2.2
6. GS 58	181	98	2.8
7. GS 45 and 71	215	102	2.4

DISCUSSION

Total yield of grain (table 2) of all but the very first of the inoculations were significantly lower than that of the control plots, late inoculations causing the greatest reduction. This agrees with previous work eg Bronnimann (1968a) and Cooke and Jones (1971). The only unexplained result is that obtained with treatment 6 which had the highest infection level on the head ot harvest time but did not have the lowest yield. In general terms the results obtained confirm that the view held by fungicide manufacturers that materials with activity against Septoria diseases might be profitably applied at about GS 39 when Septoria spores in rain-water could be channelled down the flag leaf and sheath thus affecting the emerging ear.

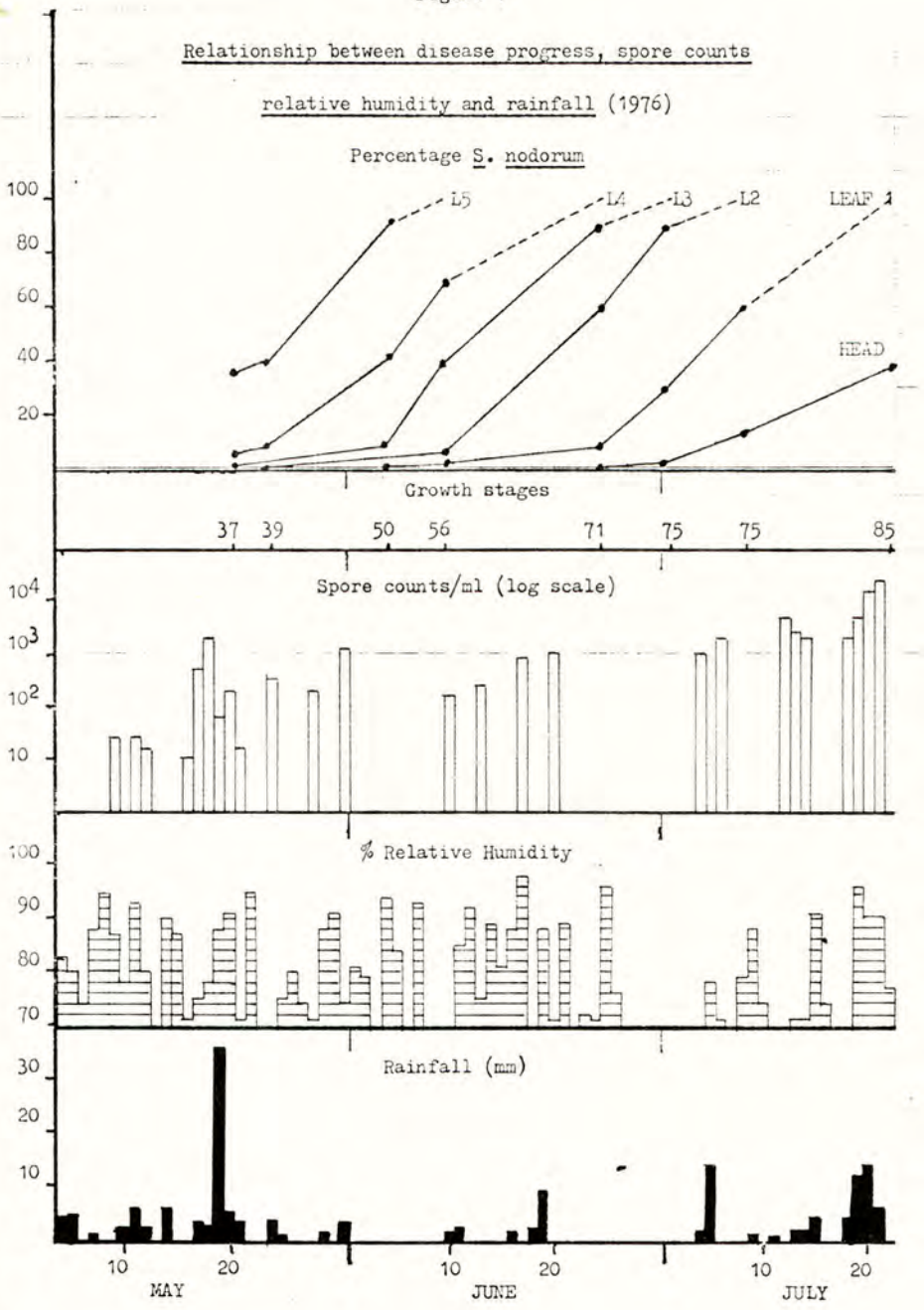
The components of grain yield were assessed in terms of grain size thousand grain weight, also the number of grains per ear and grain weight per ear were separately assessed on the main and secondary tillers.

Grain size (see table 3), as was the case with total yield, was most adversely effected by inoculations at GS 39. If it is assumed that under commercial conditions most of the grain below 2.4 mm would be lost over the back of the combine, then the effect of S. nodorum would be very significant indeed, comparable with the 85% reduction found in some ADAS trials (1972) using fungicide control as opposed to inoculations.

Separate assessment of the main and secondary tillers (table 4) showed S. nodorum reduced the weight of grain borne by both. It appears that the major component loss is from the secondary tillers although tiller number per plot seems little affected on this occasion. In table 5 the 50% mortality of total tiller numbers from GS 32 to harvest and possible relationship to yield warrants further observation.

The spore counts obtained, together with measurements of rainfall, relative humidity as at 0900 hours daily are illustrated in the following charts; the contents of the two traps have been averaged, although there was considerable variation in the numbers of spores in each trap on some occasions - usually attributed to the prevailing wind/rain direction. Percentage Septoria infection as recorded in the corresponding plots in the yield trial are plotted to give an indication of disease levels on the site, pressure of work on the project prevented a further set of values being obtained for the actual plots in use, neither was it possible to obtain yield values for the four plots under discussion.

Figure 1


Relationship between disease progress, spore counts
relative humidity and rainfall (1976)
Percentage S. nodorum
100
80
60
40
20
L5
L4
L3
L2
LEAF 1
HEAD
Growth stages
37 39 50 56 71 75 75 85
Spore counts/ml (log scale)
10⁴
10³
10²
10
% Relative Humidity
100
90
80
70
Rainfall (mm)
30
20
10
10 20 10 20 10 20
MAY JUNE JULY

Acknowledgements

The authors wish to recognise their debt to the ADAS Septoria Working Party and in particular to Mr P. Wiggell and Dr J. King for producing and launching this particular project. Thanks are also due to the staff of the Plant Pathology Department, Trawsgoed, for their assistance with the technical work, and to Mr L. J. Cock for his helpful comment and suggestions.

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Proceedings 1977 British Crop Protection Conference - Pests and Diseases

A PROCEDURE FOR MAKING RECOMMENDATIONS FOR SPRAYING WINTER WHEAT AGAINST SEPTORIA

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Summary This paper suggests a procedure for farmers and advisers for deriving recommendations for spraying winter wheat against Septoria. It uses judgemental mean responses to spray conditional upon certain field observations as defined by a plant pathologist, plus estimates of cost for the individual farm. The procedure takes the form of a questionnaire.

Résumé Cet article propose un procédé pour les fermiers et les consulteurs pour faire deriver des recommendations pour utiliser les fongicides sur le blé tendre d'hiver contre le Septoria. Le procédé utilise des résponses moyen jugeable envers le fongicide, determine par un pathologiste des plantes et conditionelle sur certaine observations aux champs, et en plus des estimations des côuts pour la ferme individuelle. Le procédé prend la forme d'une questionnaire.

INTRODUCTION

The application of fungicides to control diseases on the flag leaves and ears of winter wheat is now a widely-adopted practice. However, evidence from France (Lescar, Bouchet and Faivre Dupaigre, 1973), and the U.K. (Anon 1976) suggests that returns from spraying do not always exceed the costs of application. It is therefore necessary to distinguish between those situations where spraying is likely to be profitable and those where profitability is unlikely. One approach to the prediction of likely yield response involves the analysis of large numbers of experiments using the classical statistical procedures. But a difficulty is that many of the important factors, e.g. weather and genetic changes within the pathogen, are impossible to control adequately enough for the required levels of significance to be obtained. An alternative approach is to use Bayesian concepts of judgemental probability to estimate yield response to sprays under a number of different field circumstances (Webster and Cook, 1978).

Using the results of such an investigation a procedure was developed to aid the non-specialist adviser or informed farmer to take account of some of the many factors involved in the spraying decision.

METHOD

The results of experiments performed by the Agricultural Development and Advisory Service (ADAS) on the application of benomyl or carbendazim, either alone or mixed with maneb or mancozeb were drawn up and reviewed. Table 1 illustrates the variability of the results relative to the year of spraying and the incidence

of disease at growth stage 75 (Zadoks et al 1974). These results formed the basis for the specification in February 1977 of 32 judgemental probability distributions of yield response (Webster and Cook, 1978) for Kent and Sussex for the 1977 Season. The distributions referred to the predicted yield response for each combination of five field observations. Each field observation was defined as a binary variable e.g. either the cultivar was regarded as "susceptible" or it was not. The farmer or adviser is asked to identify the set of field observations which apply to the particular crop under consideration.

As well as identifying the likely yield response under his conditions, the farmer must also estimate the various costs of spraying including the costs of the fungicide, its application and any possible wheeling damage. Since the last two items may well differ considerably from farm to farm a simple budgeting process was devised to identify the cost of treatment measured in terms of yield (the breakeven response).

Table 1

Percentage of experimental results falling in stated yield response intervals

Yield response interval (tonnes/ha)	1973	1974	1975	1976	Incidence of Septoria >5%	Incidence of Septoria <5%
1.3 to 1.5						
1.1 to 1.3						
0.9 to 1.1						
0.7 to 0.9		7	4			5
0.5 to 0.7	21	7	2		20	5
0.3 to 0.5	28	22	0		23	13
0.1 to 0.3	28	15	36	21	28	25
-0.1 to 0.1	18	26	43	21	12	41
-0.3 to -0.1	5	15	11	44	15	9
-0.5 to -0.3		8	2	14	2	1
-0.7 to -0.5			2			1
Number of results*	39	27	46	14	41	85

*each result represents the mean of 4 replicates.

A questionnaire was designed with the object of providing a simple and logical approach to finding the breakeven point and the estimated yield response. The farmer could then make up his mind whether or not to spray on the basis of his own attitudes to risk. But it has been found (Webster, 1977) that many farmers approximate to risk indifference for this decision and thus a straight comparison of the expected mean yield response with the breakeven level is generally sufficient. This approach is adopted here and has the advantage of directness in that it gives an unequivocal 'spray' or 'don't spray' recommendation.

PROCEDURE

A. Estimation of the breakeven yield response needed

Fill in the answers to the questions on the dotted lines, and then carry out the sum as indicated under 6 below.

1. How much (£ per hectare) will the spray material cost? ..... P

2. How much (£ per hectare) will it cost to apply the spray? ..... Q

3. What is the likely % wheeling damage to the crop? ..... R

   Estimate this from Table 2.

4. If the crop stayed healthy, what would you estimate as the likely yield in tonnes per hectare? ..... S

5. What price would you expect to get for the crop, in £ per tonne? ..... T

6. Now calculate $B = \frac{P + Q}{T} + (\frac{R}{100} \times S)$, by filling in the blanks;

$$= \frac{+}{\quad} + (\frac{}{100} \times \quad) = \ldots\ldots\ldots\ldots\ldots\ldots \quad B$$

Table 2

Estimated percentage losses due to wheeling damage

	Spray Boom Width 10m 15m 20m
Aerial Spraying / 'Tramlines' Available	0 0 0
Wheelmarks only (Flag Leaf Stage)	1.7 1.3 .8
(Flowering)	3.5 2.6 1.7
No previous wheelmarks (Flag Leaf Stage)	3.5 2.6 1.7
(Flowering)	7 5.3 3.5

(Sources: Hubbard (1976), Unpublished A.D.A.S. material)

B. Identification of the likely yield response to spray

The expected mean response to spray may be identified from Table 3 by answering the following questions about the crop under investigation. Using each answer in turn, start at the left hand side of Table 3 and move across.

1. Is the flag leaf emerging (FLG) or is the crop flowering (FLO)?

2. Is the wheat cultivar susceptible (CS) or not (CNS)?

"susceptible": Atou, Maris Freeman, Bouquet, Flinor, Maris Ranger, Hobbit, Sportsman, Flanders, Maris Nimrod.

"non-susceptible": Maris Huntsman, Kinsman, Kador, Mega, Maris Widgeon, Champlein.

3. Is the topography of the crop area favourable to the disease (TF) or not (TNF)?

"topography favourable": moist or humid areas, sheltered valleys, areas subject to mists, close to sea.

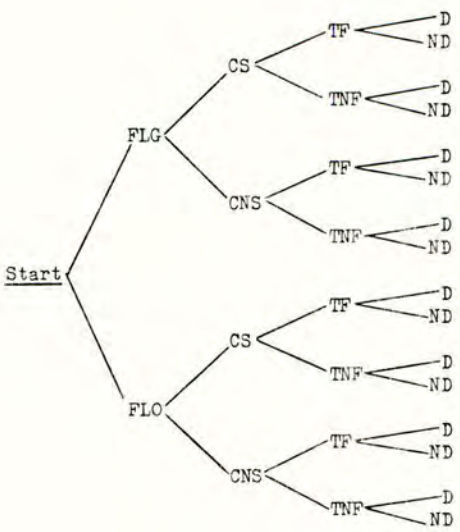
"topography not favourable": not as above, open aspects.

4. Do Septoria lesions cover more than 5% of the second and third leaves (D), or not (ND)?

5. Has a Septoria infection period (wet weather on successive days; Cook, 1977) taken place over the past few days or is one imminent (IP), or not (NIP)?

Table 3

Judgemental mean yield response to spray, Kent and Sussex, 1977 Season, (t/ha)



	IP	NIP
FLG, CS, TF, D	.422	.327
FLG, CS, TF, ND	.282	.037
FLG, CS, TNF, D	.282	.157
FLG, CS, TNF, ND	.117	-.038
FLG, CNS, TF, D	.202	.093
FLG, CNS, TF, ND	.023	-.107
FLG, CNS, TNF, D	.051	.001
FLG, CNS, TNF, ND	-.091	-.168
FLO, CS, TF, D	.321	.171
FLO, CS, TF, ND	.094	.016
FLO, CS, TNF, D	.169	.052
FLO, CS, TNF, ND	-.018	-.067
FLO, CNS, TF, D	.118	-.028
FLO, CNS, TF, ND	-.092	-.173
FLO, CNS, TNF, D	.039	-.115
FLO, CNS, TNF, ND	-.158	-.252

C. The recommendation

If the expected response from Table 3 is greater than the breakeven response, B, the odds are that spraying would be worth while. Conversely if the breakeven

response is greater than the expected response the odds are that spraying would not be worth while.

DISCUSSION

The ordering of the questions under section B was designed so that the more difficult answers were required later in the procedure. Hence if it were found that the calculated breakeven level is less (or greater) than each of the mean responses along the remainder of the branch then spraying (or not) may be recommended without answering further questions. Likewise it is possible to use Table 3 as a basis for making generalizations such as "if you have a non-susceptible variety (CNS) growing in dryish open conditions (TNF) it is more unlikely that spraying would be worth while", since under these conditions the maximum mean response is only .051 t/ha.

The existence of negative responses (indicating toxic effects) clearly shows the non-routine nature of this spraying decision. Indeed when the costs of application etc. are taken into account the losses involved by spraying in incorrect circumstances may be quite as large as the gains involved by spraying correctly.

The data contained in Table 3 are difficult to validate in any objective sense because they are predictions for a coming season. Even if, with hindsight, distributions of experimental results for the same season were available, any similarity between them and the subjective distributions would be coincidental because the actual weather occurring after spraying is uncontrollable. Furthermore any similarity with the long-run distributions from experimental data would also be coincidental because some of the factors in the situation - the host-pathogen relationship, for instance - may change over time and may render the results not strictly comparable. Objective data are, by definition, not available at the time the decision must be taken. The aim is to make the necessary judgements in as logical a manner as possible.

Furthermore the data refer to a particular region using particular types of spray material with existing cultivars, all at a given level of experimental knowledge. If these or any other important factors in the system were to change, the judgemental data would need to be revised.

Finally it should be remembered that the procedure provides a best-bet type of recommendation. In some years farmers may still expect to be unlucky. But in attempting to formalize the experience of the specialist the aim is that the decision is correct more often.

Acknowledgements

The authors are grateful to Mr. S. Evans and Mr. P. James of A.D.A.S., for helpful comments on an earlier draft.

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Proceedings 1977 British Crop Protection Conference - Pests and Diseases

YIELD RESPONSES OF WINTER BARLEY TO LATE FUNGICIDE TREATMENTS

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Summary In experiments over several years on winter barley it was observed that sprays with carbendazim generating fungicides, alone and in mixtures with certain other fungicides at growth stage 10.1-10.5 (Large, 1954) resulted in economically interesting yield increases which could not always be correlated with the control of leaf diseases. This observation is illustrated from 2 trials in 1976 in which applications of the fungicides BAS 325 00 F and BAS 397 00 F were made separately at growth stage 5-7 and 10.1-10.5 on winter barley.

A study in 1977 of the effect of sprays at growth stage 10.1-10.5 on the microflora of flag leaves, glumes and awns of winter barley, undertaken to find a possible explanation for the yield increases, is described.

Résumé Après des essais de plusieurs années sur l'orge d'hiver, on a observé que des pulvérisations de fongicides, à base de carbendazim seuls ou mélangés avec d'autres fongicides, appliquees à l'époque du stade de croissance 10.1-10.5 (Large, 1954), ont abouti à des augmentations de rendement d'intérêt economique qu'on ne pouvait toujours pas attribuer au contrôle des maladies des feuilles. Cette observation a été illustrée par 2 essais en 1976 dans lesquels on a fait des applications de fongicides BAS 325 00 F et BAS 397 00 F séparément au stade de croissance 5-7 et 10.1-10.5.

Dans une étude effectuée en 1977 afin de trouver une explication possible des augmentations de rendement, les effets de pulvérisations tardives, (stade de croissance 10.1-10.5) sur la microflora des dernières feuilles, glumes et barbes, sont décrits.

INTRODUCTION

Although common in wheat, late fungicide applications (after ear emergence) are generally not carried out in barley. Certainly attacks by mildew and rust at heading can result in yield reductions, but control measures are usually carried out earlier, at the first appearance of disease in the crop. Fraselle (1974) reported improved yields of winter barley with carbendazim generating treatments at the beginning and end of ear emergence, and this he put down to control of the above-mentioned diseases. In our experiments on spring and winter barley, however, late applications of methyl thiophanate alone and in combination with maneb repeatedly resulted in yield increases which could not satisfactorily be linked with visible fungal attack (Hampel, 1975). Several workers have studied the effects of fungicides on the microflora of cereals (Dickinson, 1973 a & b; Dickinson & Wallace, 1976; Jenkyn & Prew, 1973), but so far no direct link between changes in the microflora and yield has been traced. The following study was undertaken in an attempt

to determine the factors involved in yield increase in the absence of visible disease symptoms.

In 1976 in 2 observation trials at Limburgerhof the effects of early and late sprays on yield, foot rots and leaf diseases were recorded, and in 1977 follow-up experiments were set up in separate geographical areas to study, in addition, any alterations in the leaf and ear microflora.

METHODS AND MATERIALS

The trials reported were laid down on winter barley in two widely differing areas of Western Germany - Limburgerhof in the Palatinate, southern Germany, and Kiel in northern Germany. Trials in Limburgerhof were on the variety Dura and that in Kiel on Dunja.

Treatments were BAS 325 00 F (70% methyl thiophanate w.p.) and BAS 397 00 F (11.7% methyl thiophanate, 26.7% maneb, 33.3% captafol). BAS 325 00 F was applied at a rate of 0.5 kg/ha and BAS 397 00 F at 3 kg/ha. Both were applied in 400 l. water/ha using a precision knapsack sprayer fitted with Lechler SS 8002 fan jet nozzles, operating at a pressure of 4.5 bar.

The trials were designed as 4 times replicated randomised blocks; the plots were 15 m^2.

In the 1976 experiments early (G.S. 5-7) and late (G.S. 10.1-10.5) sprays were applied, whilst late treatments only were made in 1977.

Tridemorph 75% e.c. (Calixin) at 0.75 l/ha was included in the trials as a "control" known to affect the microflora less than benzimidazoles, dithiocarbamates and captafol (Dickinson, 1973 a; Jenkyn and Prew, 1973).

Microflora studies were carried out on the 1977 trials according to methods described by Dickinson (1973 b), modified for awns and glumes as well as leaves. Twenty flag leaves per plot were sampled, and from each of these two 5 mm^2 leaf sections were cut, bulked and washed in 100 ml water. Glumes and awns from 10 ears per plot were similarly treated. The medium used was 2% malt extract agar containing 50 ppm streptomycin sulphate to inhibit bacterial growth. Samples were taken on two occasions - one week after spraying (G.S. 10.1-10.5) and at ripening (G.S. 11).

RESULTS

Yield data for the 1976 trials are summarised in Table 1 and those for 1977 in Fig. 1. The mean frequency of foot rots on untreated plants in trials at Limburgerhof in 1976 was 70%, almost entirely due to eyespot (Cercosporella herpotrichoides). This was reduced to 30-40% by early sprays, but virtually unaffected by late sprays. Mildew (Erysiphe graminis f. sp. hordei) was present at moderate levels (5%) and late sprays reduced infection only slightly.

Foot rot levels were low in both 1977 trials (approx. 15% in Limburgerhof and 30% in Kiel at G.S. 11). Mildew attack was negligible, but in Kiel 25% brown rust was recorded which was unaffected by fungicide sprays.

The treatments applied had considerable influence on the main microflora components in 1977, as can be seen from Fig. 2. Data for leaf washings are presented, as they gave a more quantitative comparison for all plant parts. Difficulties were experienced in obtaining quantitative results with direct plating of glumes and awns.

The microflora of the awns was so abundant at the second sampling that accurate colony counts of yeasts and Cladosporium were not always possible at the dilutions used. The final counts of these organisms in untreated and BAS 325 00 F treated samples were more than 70,000 colonies obtained from the awns of 40 ears. Data from assessment of the tridemorph treatment on the microflora are not included due to lack of space. The results of this treatment showed tridemorph to have only minor effects on the microflora, confirming the findings of Dixon (1973 a).

The main components of the microflora on all plant organs were Cladosporium spp., pink yeasts (Sporobolomyces spp.), white and cream yeasts (Tilletiopsis and Bullera), Alternaria spp., Epicoccum purpurascens, Aureobasidium pullulans and Mucor spp. Smaller amounts of Fusarium spp., Botrytis cinerea, Helminthosporium gramineum, Septoria nodorum, Rhizopus nigricans and Stemphylium botryosum were also recorded. Results agreed with those of other workers (Skidmore & Dickinson, 1973; Diem, 1974; Mishra & Srivastava, 1974). As noted by Last (1955) the white yeast population increased later in the season.

Table 1

Yield data for two winter barley trials at Limburgerhof, 1976

Treatment	Time of application (G.S.)	Yield of grain, dt/ha Trial A	Trial B
Untreated		39.68	61.99
BAS 325 00 F	5-7	43.16	67.86
BAS 397 00 F	5-7	40.61	65.89
BAS 325 00 F	10.1-10.5	42.12	65.67
BAS 397 00 F	10.1-10.5	44.47	66.20
SD 1%		3.39	5.45
SD 5%		2.52	4.04

Fig. 1

Yield data for 1977 winter barley trials in Kiel and Limburgerhof

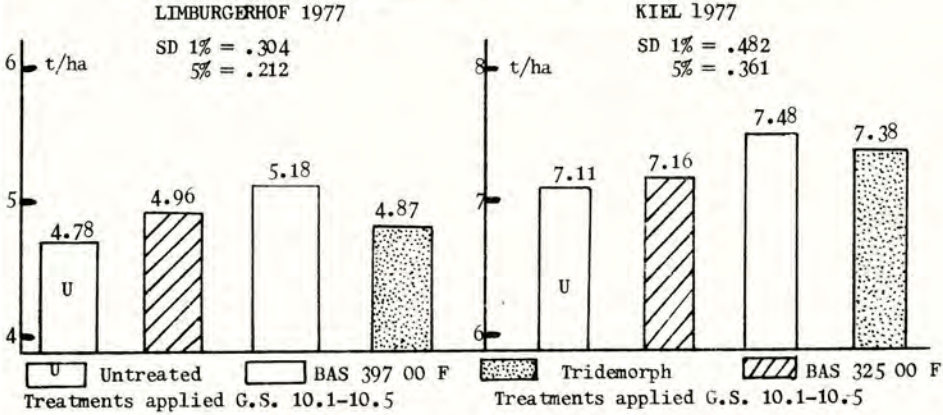
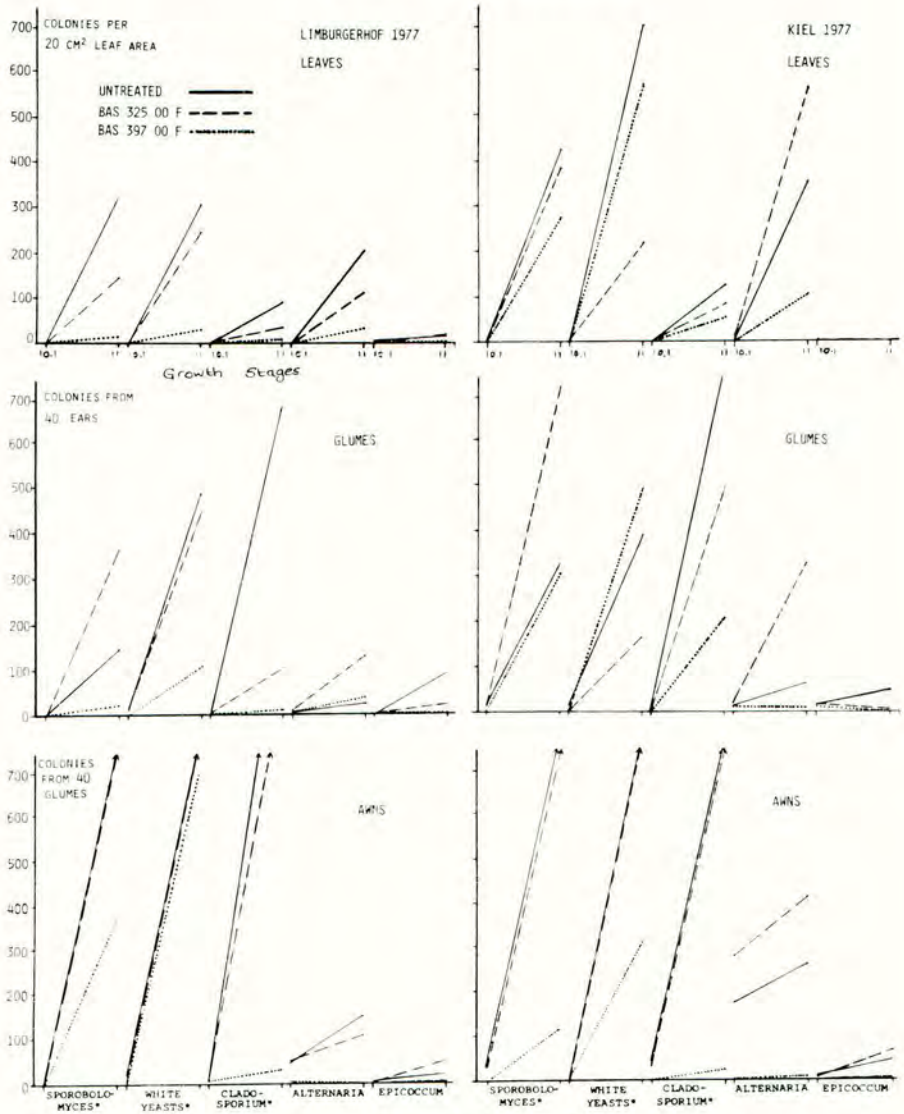


Fig. 2

Changes in microflora of barley leaves, awns and glumes between G.S. 10.1–10.5 and G.S. 11 in response to treatment with BAS 325 00 F and BAS 397 00 F



* Numbers reduced by a factor of 100

DISCUSSION

Both late and early sprays in Limburgerhof trials in 1976 resulted, as observed in previous years, in yields higher than those of the untreated plots. Significant increases following early sprays could be put down to eyespot control, but the foot rot level in the late sprayed plots was as high as in the untreated.

The fact that positive yield effects were obtained in 1977, when the fungicide treatments gave no control of the diseases (foot rots and rusts) observed in the crop, endorses the conclusion that yield increases resulted from other causes.

Yield increases in the absence of disease have been explained for carbendazim generating fungicides by the physiological effects of carbendazim on the plant. Reduced chlorophyll breakdown and delayed senescence have been reported (Weaver, 1972; Tripathi & Schlosser, 1977), which would allow a longer time for photosynthetic activity and consequently more time for grain development. Cytokinin-like effects have been reported for benomyl (Skene, 1972) and a direct link between kinetin sprays on spring barley ears and increased yield has been demonstrated by Aufhammer & Solansky (1976). However, since Frahm (1976) could not biochemically confirm delayed senescence (it must be noted that he also failed to significantly increase yields in field experiments with benomyl on wheat) the question of physiological effects of carbendazim remains unclear. Certainly such effects cannot completely be ruled out for methyl thiophanate, which breaks down to carbendazim in the plant, but these do not explain the even higher yield increases obtained in these experiments with BAS 397 00 F.

Delayed senescence need not, however, be a direct effect of fungicide treatment. Recent studies on leaf surface microflora suggest that phylloplane microorganisms play an active part in the senescence process (Last, 1955; Dickinson, 1967, 1973) and the effects of fungicides on leaf microflora of cereals are well documented (Dickinson, 1973 a & b; Dickinson & Wallace, 1976; Jenkyn & Prew, 1973). In one instance prolonged leaf life could be directly connected with a reduction in the activity of phylloplane saprophytes (Price, 1969).

Several phylloplane fungi have been shown to increase leaf senescence, including Sporobolomyces, Alternaria and Aureobasidium (Skidmore & Dickinson, 1973; Price, 1969). Both Sporobolomyces and Alternaria, as well as Cladosporium were considerably suppressed by the fungicide treatments in our studies. This suppression was most marked on the part of BAS 397 00 F, where delayed senescence was visually apparent and yield increases were greatest.

The effect of fungicides was similar on all organs. Unfortunately the overall microflora population on awns at G.S. 11 was so large that accurate measurements for untreated and BAS 325 00 F were impossible. Nevertheless, the reduction obtained with BAS 397 00 F is obvious, and in view of the importance of the awns for the development of barley grain, such a reduction in senescence causing saprophytes cannot be unimportant.

Since foot rot and leaf disease control could not be identified as factors contributing to the yield increases on the part of BAS 325 00 F and BAS 397 00 F, it appears that the yield responses are in some way connected with the observed effects on the microflora. However, in the case of treatments with tridemorph only minor effects on the microflora were found, confirming the findings of Dickinson (1973 a), but nevertheless increases in yield were obtained in the absence of visible control of foot or foliar diseases. This anomaly cannot be explained and further work is required to investigate the result.

Further work is also required to explain how, in the case of the other treatments, a reduction in the microflora could have such an effect on yield. The

evidence so far suggests that the effect may be indirect, through a delay in senescence. There is a need for a careful study of the effects of individual fungicides on the components of the microflora, combined with investigations on the response of plants to changes in the microflora population. Obviously caution is also required in the interpretation of yield data from experiments with fungicides known to have activity against saprophytes.

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Proceedings 1977 British Crop Protection Conference - Pests and Diseases

THE EFFECT OF DIFENZOQUAT ON MILDEW ON WHEAT AND BARLEY

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Summary Seven field trials are described which were conducted during 1976 and 1977 and were designed to evaluate the effects of the wild oat herbicide difenzoquat on powdery mildew on wheat and barley. Difenzoquat doses of 0.5 to 1.0 kg a.i./ha were applied with different concentrations (0, 0.05 and 0.5% v/v) of wetting agent. Comparisons were made with the fungicide tridemorph in all trials.

Difenzoquat spray treatments containing wetting agent reduced mildew at all doses and progressively greater efficacy was observed at higher doses of difenzoquat and higher concentrations of wetting agent. The more effective difenzoquat treatments were generally equal to tridemorph.

The value, in a mildew protection programme, of difenzoquat applied for wild oat control is discussed, and it is suggested that the effects of difenzoquat on mildew are a valuable alternative or complement to the use of specific fungicides.

INTRODUCTION

The effectiveness of difenzoquat as a herbicide for the control of wild oats (Avena spp.), with excellent selectivity in wheat and barley under conditions in the United Kingdom, have previously been reported (Winfield, 1974; Winfield and Caldicott, 1975). Experiments in other countries showing equal levels of efficacy have been described by Shafer (1974). Difenzoquat is currently used in many countries for control of wild oats in wheat and barley. The recommended dose in the United Kingdom is 1.0 kg a.i./ha of difenzoquat applied with 0.5% v/v wetting agent.

Observations in the field, during the development of difenzoquat as a herbicide for the control of wild oats, suggested that the product reduced the incidence of mildew (Erisyphe graminis) on treated crops. Experiments conducted in France confirmed these observations (Cognet et al, 1977).

The field trials here described were conducted in the United Kingdom during 1976 and 1977 and were designed to evaluate the effects of difenzoquat on cereal mildew in comparisons with the standard tridemorph mildew fungicide. The trials conducted during 1977 were an attempt to measure also the value of difenzoquat treatments in a mildew protection programme when applied for wild oat control.

METHODS AND MATERIALS

Trials were conducted in Eastern and Southern England on commercial crops of winter wheat, winter barley and spring barley. Sites were selected where mildew infections were present, usually at low levels and at times when infestations would be expected to increase naturally. Spring barley crops at trial 5 in 1976,

and at trial 7 in 1977 were sown with seed treated with ethirimol systemic mildew fungicide. The effects of this treatment were breaking down when experimental treatments were applied. Wild oats were not present at any site.

In each trial a randomised block design with four replicates was used. Plot size in 1976 (trials 1 to 5) was either 2.75 m x 16 m or 2.5 m x 15 m. In 1977 (trials 6 and 7) treatments were applied to a 2.5 m strip through plots 3 m x 15 m, thus leaving an untreated strip 1 m wide between treated areas in order that mildew innoculum should be distributed as evenly as possible through the trial area at all times.

All treatments were applied with knapsack spraying equipment at 200 or 225 l/ha using flat fan nozzles at 2.8 bars spray pressure.

Difenzoquat methyl sulphate, formulated as a soluble powder containing 62% active ingredient (difenzoquat), was applied with a non-ionic alkyl phenol ethylene oxide condensate wetting agent containing 90% active ingredient. In trial 1 difenzoquat treatments were in factorial design at three doses (0.25, 0.5 and 1.0 kg a.i./ha) by three concentrations of wetting agent (0, 0.05 and 0.5% v/v). In all other trials difenzoquat was applied at either 0.5, 0.75 or 1.0 kg a.i./ha in spray mixtures containing wetting agent at 0.5% v/v. Tridemorph 75% e.c. was applied as a standard mildew fungicide treatment at 0.53 kg a.i./ha in all trials.

Stages of growth of crops were recorded in decimal coding (Zadoks et al, 1974) at the times of each application and each subsequent assessment of results. In 1976 experimental treatments consisted of single sprays at different times, followed by assessments of results at intervals from 11 days up to 89 days after application. In 1977 experimental treatments compared the effects of single and sequential applications of difenzoquat and tridemorph. In these trials mildew infections were assessed on several occasions up to 58 days after the initial application; and up to 39 days after the second of the two sequential applications.

Mildew infection was measured by estimating the percentage area of mildew pustules on leaves according to the Plant Pathology Laboratory disease assessment key (Anon. 1976). This estimate was made on ten tillers from each plot, taking a leaf from the same position (1st to 4th leaf from the top) on each tiller. Mean data from each plot (x) were transformed to log (x + 1) for statistical analyses.

At harvest, grain yield at three sites in 1976 was assessed in a strip 2.1 m x 13 m cut through each plot with a combine harvester modified for use on experimental plots.

RESULTS

Weather conditions and mildew infection

During 1976 mildew infections developed actively at trial sites during March and April in winter barley and during April in spring barley; infection developed later in winter wheat. May, June and July were generally hot and dry with low atmospheric humidity, and the progress of mildew infections was slow during this period.

In contrast during 1977 mildew was absent on barley until late May or June. Thereafter the disease developed relatively slowly and infections at no time appeared to be sufficiently serious to cause reductions in grain yield.

Trial 1

At the first date of assessment (12 days after treatment) tridemorph gave a significantly greater reduction in mildew infection than the nine difenzoquat treatments (Table 1). At the other three dates of assessment there were no significant differences between treatments.

Mean mildew infection over the four dates of assessment on the factorial analysis of treatments indicated that control increased significantly with difenzoquat dose and wetting agent concentration.

Trial 2 to 5

At trials 2, 3 and 4 (Table 2) the earlier treatments applied between crop G.S. 23 and 31 showed generally small and non-significant effects on mildew infection when assessed between 32 and 89 days after treatment. The later treatments applied between G.S. 32 and 56 at trials 2, 3 and 4 and all the treatments at trial 5 gave significant reductions in mildew cover at the respective dates of assessment, 11 to 43 days after treatment. In general there was little difference between the effects of difenzoquat and tridemorph. However, at the spring barley trials, 4 and 5, tridemorph when applied at G.S. 32 gave a significantly greater reduction in mildew than difenzoquat. Later assessment at trial 5 showed no significant difference. At the winter wheat trial 3, difenzoquat applied at G.S. 37 at 1.0 kg a.i./ha gave a significantly greater reduction in mildew than tridemorph.

At no site did the mildew infection reach high levels on the upper part of the plant and there were no significant increases in yields (Table 3). However, at the two spring barley sites, 4 and 5, the earlier treatments tended to give greater yield increases. There were no consistent differences between difenzoquat doses or between difenzoquat and tridemorph.

Trials 6 and 7

In 1977, at the spring barley sites 6 and 7 (Tables 4 and 5) single applications of difenzoquat and tridemorph resulted in significant reductions in mildew cover at seven of the total eight dates of assessment. Following the first application at both sites there were no significant differences between difenzoquat at 1.0 kg a.i./ha and tridemorph, and only slight differences between difenzoquat doses. Following the second single applications, at trial 6, 34 and 43 days after treatment tridemorph gave a greater reduction in mildew than difenzoquat. There were no significant differences at the other dates of assessment in trial 6 or at any time of assessment in trial 7.

There were no significant differences between the sequential treatments of difenzoquat + tridemorph and tridemorph + tridemorph which both gave the greatest reduction in mildew infection at all dates of assessment after the second application.

DISCUSSION

Results of the seven trials described indicate that difenzoquat consistently effected significant and substantial reductions in mildew infections on both wheat and barley. In general difenzoquat applied at 1.0 kg a.i./ha with 0.5% v/v wetting agent was as effective as the widely used cereal mildew fungicide tridemorph. There were indications that in some cases tridemorph was more effective over the first 10-15 days after treatment and tridemorph was also slightly

Table 1

Effect on mildew infection of difenzoquat at three doses with wetting agent at three concentrations in comparison with tridemorph - 1976

Trial 1. Spring barley cv. Julia

		Mildew % cover			
Days after treatment		12	19	29	35
Crop G.S.		G.S. 45	G.S. 50	G.S. 58	G.S. 58
Leaf assessed		leaf 4	leaf 3	leaf 2	leaf 2
Treatment 27.5.76 at G.S.30					
Difenzoquat (kg a.i./ha)	Wetter concn (% v/v)				
0	0	40.8^{ab}	28.5	13.9	20.0
0.25	0	40.8^{a}	23.5	11.7	8.4
0.25	0.05	21.5^{ab}	23.1	9.7	6.5
0.25	0.5	11.0^{cd}	29.3	9.2	6.5
0.5	0	17.4^{bc}	16.2	8.7	6.2
0.5	0.05	16.4^{abc}	12.2	4.9	4.1
0.5	0.5	9.6^{cd}	6.7	4.1	1.6
1.0	0	22.4^{abc}	16.5	10.3	8.6
1.0	0.05	22.9^{abc}	9.0	4.6	4.2
1.0	0.5	4.8^{d}	7.2	1.8	3.5
Tridemorph					
0.53 (kg a.i./ha)		1.7^{e}	7.1	3.5	1.0
Sig. by 'F' test		0.01	NS	NS	NS
cv (%)		17.1	23.5	35.9	43.8

Mean of four assessment dates (factorial analysis)

Wetter conc (% v/v)	Difenzoquat dose (kg a.i./ha) 0.25	0.5	1.0	Mean	Sig. by 'F' test
0	21.1	12.1	14.5	15.9^{c}	
0.05	15.2	9.4	10.2	11.6^{b}	
0.5	14.0	5.5	4.3	7.9^{a}	
Mean	16.8^{b}	9.0^{a}	9.7^{a}		0.001
Sig. by 'F' test				0.001	

Means are compared using Duncan's New Multiple Range Test. Within columns and rows means with common superscripts are not significantly different at P = 0.05.

TABLE 2

Effect on mildew infection of difenzoquat at two doses in comparison with tridemorph at four times of application - 1976

Trial No.		2			3			4			5		
Crop		Winter barley cv. Maris Otter			Winter wheat cv. Champlein			Spring barley cv. Proctor			Spring barley cv. Golden Promise		
Treatments (kg a.i./ha)		Application date	crop G.S.	Mildew % cover on leaf 2 15.6.76 G.S. 75	Application date	crop G.S.	Mildew % cover on leaf 2 29.6.76 G.S.60	Application date	crop G.S.	Mildew % cover on leaf 2 18.6.76 G.S. 52	Application date	crop G.S.	Mildew % cover on leaf 3 15.6.76 G.S.60	Mildew % cover on leaf 2 25.6.76 G.S. 70
Untreated		-	-	6.0^{a}	-	-	7.9^{ab}	-	-	28.6^{a}	-	-	38.1^{a}	31.7^{a}
Difenzoquat*	0.5	18.3.76	23	5.5^{ab}	22.4.76	30	4.8^{abcd}	7.5.76	23	20.7^{ab}	7.5.76	23	23.2^{bc}	-
Difenzoquat	1.0	"	23	5.6^{ab}	"	30	4.9^{abcd}	"	23	18.0^{abcd}	"	23	19.4^{cd}	-
Tridemorph	0.53	"	23	3.9^{abc}	"	30	4.3^{bcd}	"	23	18.2^{abcd}	"	23	21.6^{bc}	
Difenzoquat	0.5	23.4.76	30	4.1^{abcd}	7.5.76	31	4.9^{abcd}	17.5.76	30	20.8^{ab}	17.5.76	30	13.5^{e}	-
Difenzoquat	1.0	"	30	3.2^{abcde}	"	31	4.0^{bcd}	"	30	13.3^{bcd}	"	30	8.5^{ef}	
Tridemorph	0.53	"	30	3.3^{abcde}	"	31	5.6^{abc}	"	30	11.9^{cd}	"	30	13.2^{de}	-
Difenzoquat	0.5	7.5.76	32	2.0^{bcdef}	17.5.76	37	3.1^{cd}	27.5.76	32	6.8^{e}	25.5.76	32	11.2^{e}	9.5^{b}
Difenzoquat	1.0	"	32	1.0^{ef}	"	37	2.2^{d}	"	32	7.1^{e}	"	32	9.9^{e}	7.3^{b}
Tridemorph	0.53	"	32	1.1^{def}	"	37	6.6^{abc}	"	32	3.9^{f}	"	32	5.8^{f}	6.9^{b}
Difenzoquat	0.5	17.5.76	39	1.0^{def}	9.6.76	56	5.4^{abc}	7.6.76	39	15.5^{bcd}	7.6.76	39	-	7.7^{b}
Difenzoquat	1.0	"	39	0.6^{f}	"	56	3.0^{cd}	"	39	16.7^{bcd}	"	39	-	9.6^{b}
Tridemorph	0.53	"	39	0.6^{f}	"	56	6.1^{abc}	"	39	12.6^{d}	"	39	-	5.7^{b}
Sig. by 'F' test				0.001			0.01			0.001			0.001	0.001
CV (%)				41.3			21.0			10.9			9.9	17.8

Means are compared by Duncan's New Multiple Range Test. Within columns means with common superscripts are not significantly different at P=0.05.

* All difenzoquat treatments included wetting agent at 0.5% v/v.

Table 3

Effect on grain yield of difenzoquat and tridemorph at three sites - 1976

Trial no.	2			4			5		
Crop	Winter barley cv. Maris Otter			**Spring barley** cv. Proctor			Spring barley cv. Golden Promise		
Treatments (kg a.i./ha)	Application date	Crop G.S.	Relative grain yield %	Application date	Crop G.S.	Relative grain yield %	Application date	Crop G.S.	Relative grain yield %
Untreated	-	-	100 (4.81 t/ha)	-	-	100 (2.63 t/ha)	-	-	100 (3.56 t/ha)
Difenzoquat* 0.5	18/3/76	23	106	7/5/76	23	113	7/5/76	23	108
Difenzoquat 1.0	18/3/76	23	100	7/5/76	23	118	7/5/76	23	108
Tridemorph 0.53	18/3/76	23	101	7/5/76	23	113	7/5/76	23	108
Difenzoquat 0.5	23/4/76	30	99	17/5/76	30	113	17/5/76	30	106
Difenzoquat 1.0	23/4/76	30	102	17/5/76	30	117	17/5/76	30	111
Tridemorph 0.53	23/4/76	30	99	17/5/76	30	110	17/5/76	30	111
Difenzoquat 0.5	7/5/76	32	96	27/5/76	32	99	25/5/76	32	109
Difenzoquat 1.0	7/5/76	32	97	27/5/76	32	108	25/5/76	32	107
Tridemorph 0.53	7/5/76	32	93	27/5/76	32	108	25/5/76	32	106
Difenzoquat 0.5	7/5/76	39	104	7/6/76	39	104	7/6/76	39	100
Difensoquat 1.0	7/5/76	39	104	7/6/76	39	105	7/6/76	39	100
Tridemorph 0.53	7/5/76	39	95	7/6/76	39	105	7/6/76	39	104
Sig. by 'F' test			NS			NS			NS
cv %			8.1			7.9			10.2

*All difenzoquat treatments included wetting agent at 0.5% v/v.

Table 4

Effects on mildew infection of difenzoquat and tridemorph applied singly and sequentially - 1977

Trial 6. Spring barley cv. Proctor

Date of treatment	1/6	-		20/6	-	-	-	-
Date of assessment	-	16/6		-	5/7	14/7	21/7	29/7
Days after earlier treatment	0	15		19	34	43	50	58
Days after later treatment	-	-		0	15	24	31	39
Crop G.S.	30	37		39	56	58	60	73
Leaf assessed	-	leaf 5		-	leaf 3	leaf 2	leaf 2	leaf 2
Treatment (kg a.i./ha)			Treatment (kg a.i./ha)					
		Mildew % cover						
Untreated	-	8.7[a]		-	30.2[a]	31.4[a]	37.4[a]	56.4[a]
Difenzoquat*	0.75	1.0[b]		-	10.3[b]	13.4[b]	15.0[ab]	32.8[b]
Difenzoquat	1.0	1.2[b]		-	7.8[b]	15.6[b]	21.9[ab]	31.9[b]
Tridemorph	0.53	0.9[b]		-	9.0[b]	17.3[ab]	18.3[ab]	29.8[b]
Difenzoquat	1.0	0.7[b]	Tridemorph	0.53	0.5[d]	1.2[c]	4.5[c]	7.6[c]
Tridemorph	0.53	1.1[b]	Tridemorph	0.53	0.6[d]	1.7[c]	6.3[bc]	6.6[c]
			Difenzoquat	1.0	2.4[c]	10.6[b]	14.2[bc]	23.6[b]
			Tridemorph	0.53	0.9[d]	2.6[c]	7.1[bc]	24.9[b]
Sig. by 'F' test		0.001		-	0.001	0.001	0.01	0.001
CV (%)		44.1		-	15.8	22.9	28.0	9.5

Means are compared by Duncan's New Multiple Range Test. Within columns means with common superscripts are not significantly different at P = 0.05.

*All difenzoquat treatments included wetting agent at 0.5% v/v.

Table 5

Effects on mildew infection of difenzoquat and tridemorph applied singly and sequentially - 1977

Trial 7. Spring barley cv. Golden Promise

Date of treatment	20/6	-	4/7	-	-
Date of assessment	-	1/7	-	8/7	19/7
Days after earlier treatment	0	11	14	18	29
Days after later treatment	-	-	0	4	15
Crop G.S.	45	58	60	64	73
Leaf assessed	-	leaf 3	-	leaf 3	leaf 2

Treatment (kg a.i./ha)		Mildew % cover	Treatment (kg a.i./ha)			
Untreated		47.8[a]		-	39.1[a]	58.2[a]
Difenzoquat*	0.75	13.2[b]		-	4.7[c]	9.7[bc]
Difenzoquat	1.0	9.8[b]		-	2.5[d]	10.0[c]
Tridemorph	0.53	10.4[b]		-	3.3[cd]	8.4[c]
Difenzoquat	1.0	11.0[b]	Tridemorph	0.53	3.4[cd]	1.6[d]
Tridemorph	0.53	10.0[b]	Tridemorph	0.53	4.5[c]	1.5[d]
			Difenzoquat	1.0	20.9[b]	16.2[bc]
			Tridemorph	0.53	19.2[b]	20.0[b]
Sig. by 'F' test		0.001		-	0.001	0.001
CV (%)		14.7		-	13.5	19.6

Means are compared by Duncan's New Multiple Range Test. Within columns means with common superscripts are not significantly different at P = 0.05.

*All difenzoquat treatments included wetting agent at 0.5% v/v.

more effective at several later times of assessment in spring barley. Conversely there were indications that difenzoquat may be more effective and more persistent in winter wheat.

Due to the somewhat unusual nature and timing of mildew epidemics during both 1976 and 1977, some reserve is necessary when interpreting the effects of experimental treatments observed at intervals of more than 20 to 30 days after application. In 1976 mildew re-infection pressure after treatments was very low due to the hot dry conditions. Also, in 1977, applications were made on light infections and at slightly later stages of growth than would normally be expected. Thus in neither year could the true persistence of difenzoquat treatments, applied at the times recommended for wild oat control, be accurately judged.

It has been shown that the greatest yield response from application of a mildew fungicide can be expected when sprays are applied immediately prior to or during the early stages of mildew epidemics (Jung and Bedford, 1971; Jenkins and Storey, 1975). These conditions often apply between crop growth stages 24 and 30 in winter barley, and between crop growth stages 30 and 31 in spring barley. Thus difenzoquat applied for control of wild oats during crop growth stages 20 to 30 will frequently be applied at an appropriate stage for control of early mildew infections. A subsequent fungicide treatment may be unnecessary and will depend on variety and on mildew re-infection pressure at later stages.

Difenzoquat doses less than 1.0 kg a.i./ha + 0.5% v/v wetting agent, the dose recommended for control of wild oats in the United Kingdom, also caused substantial reductions in mildew, which in many cases were not significantly different from the effects of the higher dose. In view of the weather conditions during these experiments it is clear that further investigations are necessary into the effects of these lower doses before it would be possible to suggest the minimum dose of difenzoquat plus wetting agent for effective and reliable control of mildew.

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Proceedings 1977 British Crop Protection Conference - Pests and Diseases

INSENSITIVITY OF WHEAT BUNT TO HEXACHLOROBENZENE AND QUINTOZENE (PENTACHLORONITROBENZENE) IN GREECE

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Summary Insensitivity to hexachlorobenzene and quintozene was detected by laboratory and field tests in samples of wheat bunt (Tilletia foetida) collected in 1975 and 1976 from different regions of Greece, where hexachlorobenzene had been used for many years as a seed dressing of wheat. These samples were also found to contain strains insensitive to hydantoin and imazalil. The insensitivity levels found were high. Of 31 other fungicide seed treatments tested all but polyoxin AL gave good control of wheat bunt and resulted in increases of grain yield.

INTRODUCTION

Since 1958 hexachlorobenzene has been generally accepted in Greece as one of the most effective seed dressing fungicides for the control of common bunt (Tilletia caries and T. foetida) of wheat. Also from 1958 to 1974 The Seed Production Service of the Ministry of Agriculture has used several seed-dressing products with the active constituent hexachlorobenzene.

Hexachlorobenzene and organically combined mercury products were included as reference treatments in wheat seed-dressing trials, since 1968 in Greece, in which a number of new chemicals were tested each year for their effectiveness against seed and soil-borne bunt and other diseases of wheat (Skorda 1973, 1974). Up to 1972 there was no evidence of insensitivity to hexachlorobenzene of Tilletia spp. in commercial crops. However in 1973-1974 season information from field observations indicated that bunt had became troublesome in certain areas of Greece. Some farmers who had bunted crops that season had apparently dressed their wheat seed according to recommendations with hexachlorobenzene supplied by Seed Centres of the Agriculture Ministry. Samples of this source of hexachlorobenzene formulation were analysed in official laboratories and found to contain the correct amount of active ingredient. In the same year the product was included as a reference treatment in the wheat seed-dressing trials of PBI, Thessaloniki and it was found to be as effective against bunt as in previous years.

As a result of this situation, and noting the work of Kuiper (1965) who reported the failure of hexachlorobenzene to control bunt in Australia, a survey was initiated to determine the presence of bunt insensitivity to hexachlorobenzene in Greece. Samples of the disease were collected from diferent areas of the country and examined in laboratory and field tests.

METHODS AND MATERIALS

Experiments were carried out in 1975/76 and 1976/77 seasons on samples of bunt collected in 1975 and 1976 respectively.

In the experiments bunt balls were collected from naturally infected wheat crops in which seed treatment had been omitted, from infected crops in which seeds were treated with hexachlorobenzene in the principal growing areas and from infected hexachlorobenzene treatments of trials.

In the laboratory tests 11 of the bunt samples from different regions were selected at random and screened for resistance under controlled conditions using soil plates according to the method described by Kiraly (1970). Four replications were used in all tests and known susceptible and resistant strains were used for comparison as standards.

In field trials a mixture of spores apparently insensitive to hexachlorobenzene and collected from different regions, was tested by treating with hexachlorobenzene and other chemicals. In these tests wheat cv. Generoso was inoculated by ball-milling it for a standard time in glass jars with 0.5 percent by weight of bunt teliospores. Inoculated seed was also treated in the same way with the fungicides at rates given in Table 1. Also a treatment was included of seed, non infected, and without any chemical. Most of fungicides were applied dry and adhered very well. The slurry formulations were diluted with the same amount of water to obtain satisfactory coverage and were applied by shaking the chemical and seed together in a large jar. True liquid formulations were applied to a thin layer of seed contained in a shallow tray by a laboratory compressed gas-powered sprayer. The spraying operation was done quickly and the seed shaken immediately to obtain good distribution. After treatment, the seeds were stored for 24 to 48 hours in sealed plastic envelopes before planting.

The trials were of randomized block design with six replicates using plots of 2 X 10 m. Nearly all the crops were drilled in early to late November, which is near normal.

The fungicide formulations used were as follows: Agrosan GN w.p. (1.6% w/w organomercury compounds equivalent to 1% w/w mercury), hexachlorobenzene 12% w.p., quintozene 25% w.p., Terraclor Super X w.p. (20% quintozene and 5% 5-ethoxy-3-trichloromethyl -1,2,4-thiadiazole), chlorothalonil 75% w.p., triadimefon 5% w.p., propineb 65% w.p., Zincoran 80% wp., thiabendazole 26.6% and maneb 57.2% w.p., SN 41703 + SN 43410 w.p., (30% ethy-N (3-dimethylamino-propyl)-thiocarbamate-hydrochloride and 30% 2-isopropylsulfonyl-5-trichlormethyl-1,3,4-thiadiazol), polyoxin AL 10% w.p. (antiobiotic), Kinolat 15 w.p. (copper oxyquinolate 15%), New Davlitine w.p. (mancozeb 52% and 7% Cu in the form of bordeaux mixture), TCMTB 25% w.p. and 15% w.p. mancozeb (S60) 60% w.p. and (M-45) 80% w.p. Vondozeb 79% w.p. (ethylene-1,2-bisdithiocarbamate ions, manganese ions and zinc ions), nuarimol 8% and maneb 32% w.p., nuarimol 97.6% w.p., Vondocarb w.p. (64% mancozeb and 10% carbendazim), Granosan w.p. (15% carbendazim and 60% maneb), Toram w.p. (17.5% methyl thiophanate and 60% thiram), Frumidor w.p. (14% methyl thiophanate and 60% maneb), Mollos Super w.p. (maneb 32% and Cu 9.5% in the form of bordeaux mixture and carbaten 8%), fenfuram 75% w.p., guazatine 30% + fenfuram 10% w.p., guazatine 30% + imazalil 2% w.p., guazatine 30% + fenfuram 10% + imazalil 2%, imazalil 25% w.p., hydantoin 50% w.p., maneb 5317 48% e.c., and maneb 5318 32% e.c. Fungicide afficiency was judged on the increase in yield and percent infection of disease.

RESULTS

Control of common bunt. In laboratory tests of 11 bunted samples nine (82%) with tolerant strains to hexachlorobenzene were found.

Table 1

Effect of seed treatment with fungicides against common bunt in field trials

Treatment	Rate g a.i/ 100 kg seed	Bunted spikes %		Grain yield % of control		Germination %	
		1976	1977	1976	1977	1976	1977
Control non infect.		0.9c	-	121ab	-	96	-
Control infected		20.8a	12.2a	100e	100f	96	96
Agrosan GN	3.2	0.7c	0.8c-e	119a-c	120cd	93	92
Hexachlorobenzene	25.0	11.5b	9.3b	115b-d	102ef	88	96
Quintozene	50.0	-	8.3b	-	108d-f	-	95
Terraclor Super X	150.0	10.0b	7.3b	112b-d	99f	97	95
Polyoxin AL	10.0	13.5b	-	104de	-	94	-
Chlorothalonil	127.5	0.1c	0.7de	113b-d	148a	91	96
Triadimefon	12.5	0.1c	0.0e	124ab	116c-e	70	57
Propineb	162.5	0.1c	0.5de	126ab	125bc	97	98
Zincoram	120.0	1.7c	1.8c	108c-e	117cd	96	97
Thiabendazole/maneb	167.6	0.2c	0.0e	107de	99f	93	97
SN41703+SN43410	180.0	0.1c	1.2cd	113b-d	134b	94	94
Kinolat 15	30.0	1.2c	-	113b-d	-	99	-
New Davlitine	118.0	-	0.5de	-	116c-e	-	97
TCMTB (Tillosan)	37.5	1.1c	-	114b-d	-	93	-
TCMTB (S.davloxan)	22.5	0.8c	-	120ab	-	93	-
TCMTB BST	50.0	0.2c	-	114b-d	-	97	-
Mancozeb (S 60)	90.0	0.0c	-	120ab	-	99	-
Mancozeb (S 60)	120.0	0.0c	-	126a	-	96	-
Control non infect.		-	0.3d	-	119a	97	94
Control infected		20.7a	12.5a	100b	100b	95	96
Imazalil	7.5	8.1c	-	124a	-	96	-
Hydantoin	100.0	14.3b	-	103b	-	96	-
Mancozeb (M-45)	80.0	0.0d	-	124a	-	97	-
Mancozeb (M-45)	120.0	0.0d	-	118a	-	96	-
Vondozeb	197.0	-	0.0d	-	136a	-	95
Nuarimol	14.6	0.0d	0.5d	-	130a	-	96
Nuarimol	19.6	-	0.5d	-	134a	-	96
Nuarimol	24.4	-	0.3d	-	131a	-	94
Nuarimol-maneb	100.0	0.0d	0.0d	-	127a	-	97
Vondocarb	185.0	-	0.2d	-	124a	-	96
Granosan	150.0	0.0d	0.0d	118a	131a	96	93
Toram	178.0	0.1d	-	117a	-	95	-
Frumidor	148.0	-	0.2d	-	121a	-	96
Mollos Super	225.5	0.0d	-	118a	-	96	-
Fenfuram	150.0	0.0d	0.0d	120a	118a	95	93
Guazatine-fenfuram	80.0	-	1.3c	-	120a	-	83
Guazatine-imazalil	64.0	-	2.7b	-	126a	-	96
Guaz.-fenf.-imaz.	84.0	-	0.5d	-	134a	-	95
Maneb 5317	127.0	0.0d	1.8c	126a	123a	99	96
Maneb 5318	96.0	0.0d	0.7d	126a	127a	99	98

Figures suffixed by the same letter (a,b,c,d,e) are not significantly different at the 5% level.

In the field tests bunt infection was higher in the first year of test and lower in the second, because of the high level of drought during the latter season. Table 1 shows that most of the seed treatment chemicals tested significantly reduced the incidence of bunt compared with the control. However hexachlorobenzene quintozene (two formulations), imazalil, hydantoin and polyoxin AL gave very low control of bunt.

Grain yield. In the field experiments in both 1976 and 1977 almost all effective fungicide treatments gave significantly higher yield than the non-treated bunt infected controls and non-effective fungicides. The only exception was thiabendazole/maneb. The increase of yield was not always relative to effectiveness of disease control. There was greater yield benefit with fungicides which control other seed or soil-born diseases in addition to bunt. In other cases lower yield increases occurred because of decreased germination ability after seed dressing, as with triadimefon.

DISCUSSION

Hexachlorobenzene, imazalil and quintozene were very effective against bunt for a period of several years (1958-1972) and gave control equal to that obtained with organomercurial compounds (Skorda, 1973, 1974). After 1972 reports from field usage indicated control of bunt following the use of fungicide seed treatments was less effective.

The tests reported confirm this. The effectiveness of hexachlorobenzene, imazalil and quintozene in field tests was reduced by 50% in 1976 and in 1977 by 30%. Hydantoin was also found to give unsatisfactory control. In 1977 two different proprietary formulations of quintozene were used so that the possibility of a faulty batch can be excluded.

These results strongly suggest that a race or races of T. foetida with a high level of insensitivity towards hexachlorobenzene, quintozene, imazalil and hydantoin has developed in regions of Greece where hexachlorobenzene has been used as wheat seed treatment for a long time. It has been suggested that a specific fungicide may give rise to insensitivity more readily than a non-specific one (Wood, 1960) and hexachlorobenzene is one of the most specific fungicides known. Races of bunt were found insensitive to hexachlorobenzene in Australia (Kuiper, 1965) and the capacity of T. foetida and T. earies to produce new races is well known (Kendric, 1964; Metzger and Kendrick, 1967). The new races tested and reported here were also insensitive to imazalil, while in previous work races sensitive to hexachlorobenzene were also susceptible to imazalil (Skorda, 1974) and hydantoin(Burgaud et al, 1975).

The findings of these trials show also that the inoculum of insensitive races can be increased rapidly, when they are insensitive to a fungicide which is used widely. This discovery necessitates research into the nature of the insensitive race or races and their distribution. Since insensitivity to more than one fungicide was found care will be necessary in recommending alternative fungicidesand in the development of treatment programmes based on the alternation of different fungicide groups from year to year.

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Proceedings 1977 British Crop Protection Conference - Pests and Diseases

FUNGICIDE SELECTIVITY; CHANCE AND NECESSITY

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Summary The introduction of very active systemic fungicides has not been followed by any reduction of world crop losses due to diseases. Some fungicides have a wide spectrum of activity: others control only a few closely-related pathogens. The emergence of insensitive strains of fungi previously sensitive to a fungicide is due to the selective pressure exerted by very effective compounds which are single-site inhibitors on a pathogen population which already included a small proportion of the insensitive strain. Strains insensitive to a particular fungicide may persist in the population for several years after application of that fungicide has ceased. Insensitivity might be overcome by using synergistic compounds, by negative cross-resistance or by incorporating fungicides in carrier vesicles. More effective methods of control may come from fundamental studies of fungal physiology.

INTRODUCTION

Although the newer systemic fungicides are more active and control fungal pathogens better than older, protective compounds the average annual loss to agriculture by plant diseases in the U.S. actually increased from 1942 to 1974 (partly due to demands for improved quality). Fungicides are applied to only 0.5% of the U.S. crop area (May, 1977); plant diseases are controlled mainly by crop rotation and plant breeding. Nevertheless, frequent applications of fungicides are essential for some crops and necessary for others where cultural practices have been ineffectual. Only in glasshouses may the spread of fungal pathogens be curbed by manipulation of the environment (Spencer, 1977). Disappointingly, the introduction of more active fungicides with systemic properties has not reduced world crop losses due to plant diseases.

All fungicides must be selective to kill the fungus without damaging the host plant, and also must have little mammalian toxicity; some fungicides are toxic to other micro-organisms, arthropods or earthworms (Byrde and Richmond, 1976).

Most protective fungicides act by selective accumulation, being taken up rapidly by fungal spores but only slowly and in small amounts by leaves (Somers, 1969) and are not selective in their control of plant pathogens. However, captan and dodine control many fungi but have little effect on powdery mildews, whereas sulphur and dinocap are active only against powdery mildews (Somers, 1963).

Systemic fungicides enter plant cells, therefore show greater selectivity, act at one or few sites and are effective at small concentrations.

Benomyl is effective against many fungi except Phycomycetes, Porosporae and some Basidiomycetes (Bollen and Fuchs, 1970); ethirimol and dimethirimol only against powdery mildews (Bent, 1970); the oxathiins only against Basidiomycetes (Schmeling and Kulka, 1966). Unfortunately the very effectiveness of these extremely active, biochemically specific compounds has resulted in the selection and

spread of strains of fungi insensitive to a previously effective fungicide. The selectivity of fungicides (Siegel, 1975; Byrde and Richmond, 1976) and the emergence (Dekker, 1976, 1977; Fehrman, 1976; Hoffmann and Kiebacher, 1976) and genetics (Tuyl, 1977) of insensitivity have already been discussed. This paper considers how the problems posed by the selectivity of fungicides can be overcome.

SELECTIVITY AMONGST FUNGI

A highly selective fungicide which killed only a particular target fungus and was completely free from undesirable side-effects such as toxicity to man or damage to natural antagonists would meet all the requirements of environmentalists. Unfortunately the cost of developing such fungicides is disproportionately high in relation to profits from sales, and more than one fungicide might be required for a particular crop. Hence both manufacturers and growers prefer broad-spectrum fungicides (Street, 1975).

Fungi are insensitive to a particular fungicide for two fundamental reasons; the active site is either not reached or it is modified. The active site is not reached when the cell membrane is impermeable to the fungicide, or the fungicide is detoxified within the cell or the fungus is unable to activate an inactive compound. The active site may be modified so that the toxicant can no longer bind, or the fungus may have an alternative metabolic pathway which avoids the inhibited step, or finally an insensitive strain may simply be able to synthesize more of the inhibited enzyme (Dekker, 1977).

The varied sensitivity of fungi to streptomycin may be due to differences in membrane permeability. Sensitive Oomycetes take up nine times as much streptomycin as insensitive fungi (Voros, 1965). Insensitive fungi take up large amounts of fungicides when detoxication occurs within the cell. Fenaminosulf is ten times as toxic to Pythium as it is to Rhizoctonia (Hills and Leach, 1962) because Rhizoctonia is able to detoxify the fungicide by an enzyme system which is absent from Pythium (Tolmsoff, 1962). Macroconidia of Fusarium solani can detoxify dodine and release the products into solution (Bartz and Mitchell, 1970).

Insensitivity may also be due to a combination of low permeability and detoxication. Thus insensitive fungi take up less quintozene from solution than sensitive fungi, but they also detoxify the fungicide and excrete the products into the medium (Nakanishi and Oku, 1969a). The situation with ascochitine is more complicated. Sensitive fungi take up more of the antibiotic than insensitive fungi. Highly insensitive fungi take up the same amount as moderately insensitive fungi, but they also have a detoxication mechanism (Nakanishi and Oku, 1969b).

The most serious problem that has resulted from the widespread use of systemic fungicides has been the emergence of races of a pathogen insensitive to the applied fungicide. These races were probably already present in the pathogen population and emerged as a result of the powerful selective pressure exerted by very active compounds which are also single site inhibitors. The biochemical mechanisms responsible for differences in sensitivity between races of a fungus are unlikely to be any different from those responsible for differences between species or genera. Thus strains of Botrytis cinerea, insensitive to quintozene, detoxified the fungicide in the same way as insensitive species of fungi such as Fusarium oxysporum f. niveum (Nakanishi and Oku, 1970). Further examples are given by Dekker (1977).

POSSIBLE METHODS OF OVERCOMING INSENSITIVITY

The chances of emergence of insensitive forms of previously sensitive fungi may be reduced by avoiding the continuous selective pressure resulting from many applica-

tions of fungicides with the same mode of action (Dekker, 1977). Once insensitive strains predominate in a crop, the pathogen can be controlled only by applying a fungicide with a different mode of action. Unfortunately once resistant genes occur in a population they may persist for many years after applications of the toxicant has ceased. Thus four years after it was prohibited to feed pigs with tetracycline to promote growth there was no reduction in the amount of Escherichia coli resistant to tetracycline in the pig population (Smith, 1975). Similarly strains of the rice blast fungus (Pyricularia oryzae) insensitive to kasugamycin have been detected in Japan several years after applications of the antibiotic had ceased (Misato et al., 1977).

It seems unwise to rely on the chemical industry introducing more and more new fungicides which do not show cross-resistance with existing compounds. Ways must therefore be found either of prolonging the life of compounds in present use or of finding alternative methods of control where the emergence of insensitive strains is not possible.

One method of overcoming insensitivity is to take advantage of negatively correlated cross-resistance. Thus strains of the rice blast fungus insensitive to phosphorothiolate fungicides are more sensitive to ethyl 2,4,5-trichlorophenylphosphoramidate than wild-type strains. Synergism also occurred between the two compounds (Uesugi et al., 1974).

Synergism has been used to control insects insensitive to DDT by preventing the organism from detoxifying the insecticide; synergists may also act by increasing the rate of penetration (Margham, 1975). Unfortunately prevention of detoxication of a fungicide does not always make a fungus more sensitive. Most of the captan taken up by fungal spores is detoxified by reaction with soluble thiols. When spores are treated with a non-toxic thiol reagent such as iodoacetic acid, uptake of captan is reduced considerably and hence the toxicity of the fungicide, on a spore-weight basis, is greatly increased. However, the fungistatic activity of captan is unaffected by the treatment, probably because the rate-determining step is the penetration of the cell membrane (Richmond and Somers, 1966).

Where insensitivity is due to membrane impermeability it may be possible to add to the fungicide a compound which increases the permeability of the membrane. Alternatively, fungicides might be incorporated into a non-toxic biodegradable carrier (Gregoriadis, 1977) which could enter a fungal cell to release the toxicant once it has passed through the membrane. This technique has been used to make insensitive tumour cells sensitive to Actinomycin D; the drug was incorporated into lipid vesicles (Poste and Papahadjopoulos, 1976). Whether such methods are applicable to fungi is not known, but the idea seems worth pursuing.

CONCLUSIONS

Although insensitivity to some fungicides or antibiotics may be associated with decreased virulence (Hamilton-Miller, 1974; Fuchs and Viets-Verweij, 1975) it is probable that insensitive strains of previously sensitive fungi will continue to emerge as long as very active, single-site inhibitors are used as fungicides. Insensitive strains have arisen mainly because the newer compounds are so efficient, and few will wish to return to using the older protective fungicides.

The methods suggested here for overcoming insensitivity are mere palliatives. New methods of disease control such as the dichlorocyclopropane fungicides which may act by stimulating the natural disease-resistance mechanisms of the plant (Langcake and Wickins, 1975) are required. More effective biological methods of control may come only from fundamental studies of the physiology and biochemistry of fungal pathogens and the mechanisms of pathogenicity.

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METABOLIC PROFILES OF FUNGI TREATED WITH SYSTEMIC FUNGICIDES - A NEW APPROACH

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Summary Fungal mycelium can be conveniently separated into three complex fractions which, after derivatization, can be separated by gas chromatography on SCOT columns to yield characteristic patterns of peaks (metabolic profiles) representing standing concentrations of the substances of which the mycelium is composed. These characteristic patterns may undergo changes when the fungus is grown in the presence of systemic fungicides or other metabolic inhibitors. The changes may be interpreted to indicate the site of action of the fungicide.

Sommaire Le mycélium fongueux est facilement séparé en trois fractions complexes. Les dérivés de ces fractions peuvent alors être séparés par chromatographie au gaz sur des colonnes SCOT pour obtenir des tracés de pics caractéristiques (profils métaboliques) qui représentent les concentrations actuelles des substances qui composent le mycélium. Ces tracés caractéristiques peuvent subir des changements quand le fongus est élevé en la présence de fongicides systémiques ou d'autres inhibiteurs métaboliques. Ces changements peuvent être pris comme indiquant le point d'action du fongicide.

INTRODUCTION

Changes in the standing concentrations of substances (metabolites and structural components) in fungal mycelia resulting from the action of a systemic fungicide may be interpreted to indicate the mode of action of that fungicide. The information may be initially collected as recognisable patterns of chromatographic peaks, "metabolic profiles", (Horning & Horning, 1971) characteristic of either treated or untreated mycelia. Fungicide-induced changes in metabolic profiles (loss or increase of peaks) may be used to indicate which specific areas of the fungal metabolism are sensitive to particular fungicides. Confirmation of the interpretation may be obtained by biochemical investigations guided by these observations.

Metabolic profiles can be obtained from each of three fractions which, since nothing is discarded, contain between them essentially all the metabolites and structural components of the original mycelium *viz*. (1) a fraction soluble in an organic solvent (2) a water soluble fraction and (3) an acid hydrolysate of the residual fungal mycelium. A principal advantage of this all-embracing approach is that it will immediately reveal changes in metabolism in unexpected as well as in expected areas, so that it is no longer necessary to guess the area for biochemical investigation prior to carrying out the work.

MATERIALS AND METHODS

The chosen fungus is grown at 20° in a liquid minimal-malt medium (Greenaway, 1973), collected by filtration or centrifugation and freeze dried. When the fungus is to be grown in the presence of a fungicide an amount is added which gives a decrease in the rate of growth not more than 50%.

To obtain Fraction 1 the dried mycelium (50 - 100 mg) is extracted in a soxhlet apparatus with 25 ml chloroform/methanol (2 : 1 v.v), the extract is dried and the fats saponified with hot alcoholic KOH and then neutralized with HCl. The residue after chloroform/methanol treatment is re-extracted in a similar way, but using water as the solvent, to give the water-soluble Fraction 2. The residual insoluble constituents of the mycelium are hydrolysed in 2N - HCl at 110°C to yield Fraction 3. Volatile TMS and TMS-MO derivatives are prepared from each fraction essentially as described by Thenot & Horning (1972).

For chromatography a Pye Unicam 104 chromatograph fitted with a flame ionization detector was used. 0.1 - 0.2 µl samples of the volatile derivatives obtained from fractions 1 - 3 are injected without splitting into a 30 m x 0.5 mm. ID surface coated open tubular (SCOT) column coated with silanox and OV1 (German & Horning, 1973), using helium as a carrier gas. We have prepared columns which, when tested with hydrocarbons, have an efficiency of 40,000 plates. A temperature programme of 120° - 300° (3° rise per min) with no initial hold but with a final temperature hold of 15 min was used to accommodate the widely differing boiling points of the derivatives. Under these conditions ergosterol had a retention time of 57.45 ± 0.4 min at a helium flow of 2 ml/min. The peaks are usually sharp and have a half width of about 2-6 sec throughout the run.

In the work reported here we have examined the effects of two systemic fungicides, [α-(2-chlorophenyl)-α-(4-chlorophenyl)-5-pyrimidinemethanol] (EL222) and [S-n-butyl-S'-p-tert-butylbenzyl-N-3-pyrimidylimidodithiocarbamate] (S-1358).

RESULTS

We find, from examination of metabolic profiles of species of Botrytis, Ceratocystis, Cladosporium, Penicillium, Saccharomyces, Cunninghamella and Ustilago, that fractions 1, 2 and 3 yield reproducible metabolic profiles which are characteristic of the particular fungus. These characteristic metabolic profiles undergo changes in the presence of fungicides. Some fungicides affect the pattern of a single fraction, whereas others affect the pattern of more than one.

As an illustration of the application of the method we wish to report the effects of two systemic fungicides (EL 222 and S1358) on the substances present in the Fractions 1 from Ustilago maydis (DC) Corda (IMI 103761). EL 222 is highly toxic ($E.D._{50}$ = 3µM) to U. maydis, whereas S1358 is far less toxic (ED_{50} > 100µM). Fig. 1A (untreated U. maydis) shows that the g.l.c. has separated a large number of components which are however difficult to identify merely on the basis of retention time. This characteristic profile of U. maydis is reliably obtained when the conditions of growth of the fungus, extraction, derivatization and g.l.c. are repeated unchanged. However, when the fungus is grown in the presence of 3µM EL 222 (1B) or 100 µm S1358 (1C) a few peaks are diminished and others markedly increased, although the general metabolic profile still clearly resembles that of the untreated mycelium. There are indications in the literature that both these fungicides may affect sterol metabolism. For example the effects of both triarimol (chemically related to EL 222) on U. maydis (Ragsdale, 1975) and of S1358 on Monilinia fructigena (Kato et al, 1974) and Saccharomyces cerevisiae (Kato & Kawase, 1976) are thought to be primarily due to inhibition of demethylation of intermediates in the pathway of ergosterol synthesis. Since a g.l.c. technique was earlier used to demonstrate that triarimol caused the accumulation of intermediates of ergosterol biosynthesis,

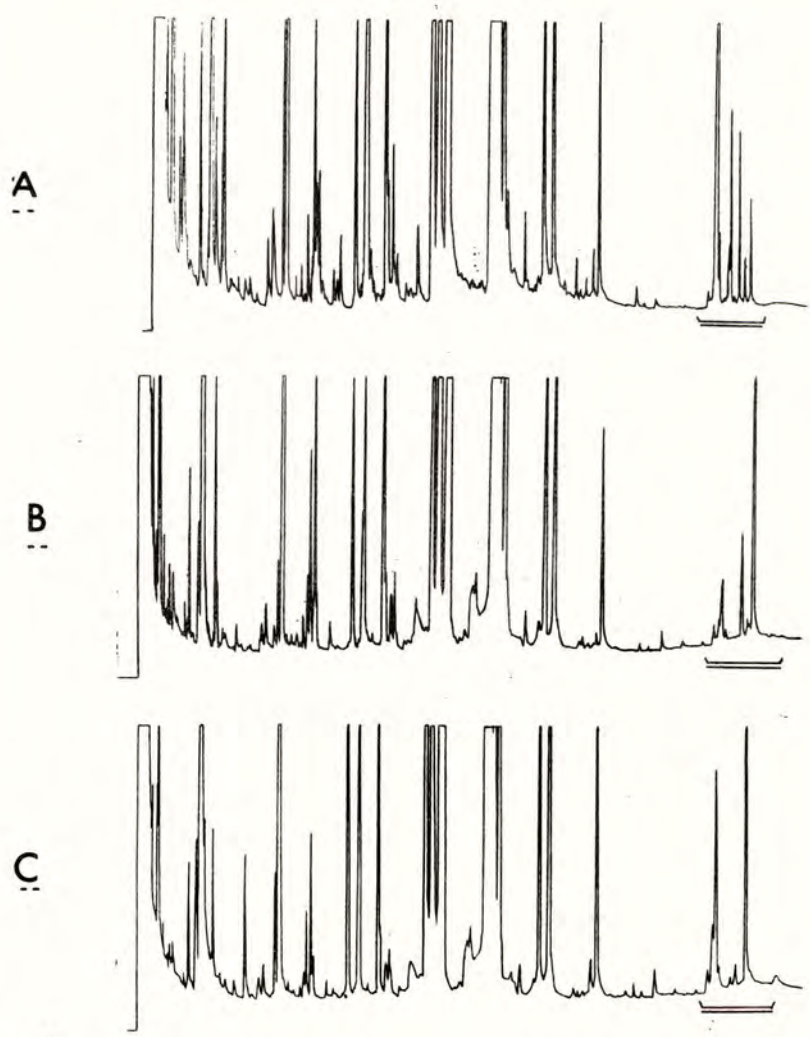

Figure 1

Effect of the systemic fungicides S1358 & EL 222 on metabolic profiles of Ustilago maydis.

Experimental methods as in text. A, No fungicide.

B, Grown in presence of 3μM EL 222. C, Grown in presence of 100 μM S1358.

(Ragsdale & Sisler, 1972) our attention was drawn to the area in our metabolic profiles where ergosterol and related sterols are known to run (the "sterol area"). The sterol areas of A, B, & C are reproduced side by side on a layer scale in Fig. 2. On the basis of published data, (Ragsdale & Sisler, 1972; Sherald & Sisler, 1975) and using retention times of sterol and hydrocarbon markers run through the same columns for orientation, we may tentatively identify several of the peaks in the sterol area (see legend to fig. 2).

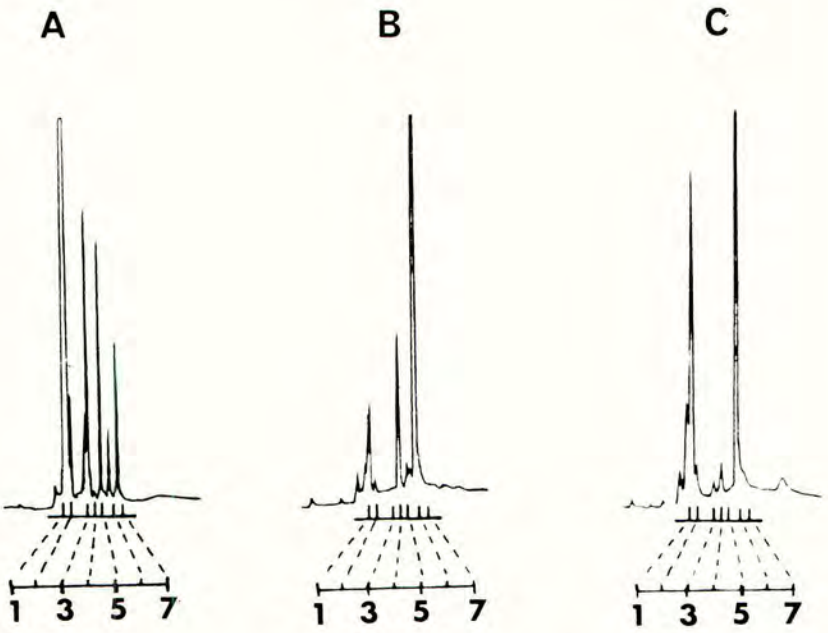

Figure 2

The sterol areas of the metabolic profiles shown in Figure 1. The peaks are tentatively identified as: [1], 24-methylcholesta-5,7, 22-trien-3β-ol (ergosterol); [2], 14α-methyl-24-methylene-5α-cholest-8-en-3β-ol; [3], 24-methylcholesta-5,7-dien-3β-ol; [4], 4α, 14α--dimethyl-24-methylene-5α-cholest-8-en-3β-ol (obtusifoliol); [5], unknown; [6], 24-methylene-4,4,14α-trimethyl-5α-cholest-8-en-3β-ol; [7], 4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol.

Table 1 summarises our data on the relative concentrations of the sterols separated as peaks 1-7, using a Hewlett-Packard Integrator (3380A) to calculate the areas under the curves. The increases and decreases of particular peaks are clearly shown. Making use of the data in Table 1, assuming the tentative identifications of the peaks to be correct, and knowing the pathway of ergosterol biosynthesis in U. maydis (Ragsdale, 1975) the sites of action of S1358 and EL 222 in this fungus can be proposed.

Table 1

Effect of the systemic fungicides S1358 and EL 222 on sterols in Ustilago maydis

Area under Curve (% of total "sterol area")

Peak No.*	Control	S1358	EL222
1	56	30	21
2	7	1	2
3	19	2	0
4	0	3	13
5	9	0	2
6	4	62	62
7	5	0	0

*as referred to in legend to Fig. 2.

The sterol area represented the following percentage of the total substances in Fraction 1; untreated mycelium, 1.2%, with S1358, 1.4% and with EL 222, 1.1%.

Our conclusions are summarised in Fig. 3, namely, that EL 222 is a strong inhibitor of the enzyme responsible for the 4-demethylation of obtusifoliol (accumulations of peaks 4 & 6, diminution of peak 1 and loss of peaks 2, 3 & 5) and that S1358 is a weak inhibitor of the enzyme responsible for the demethylation of 24-methylene-4,4,14α-trimethyl-5α-cholest-8-en-3β-ol (peak 1 retained but peak 6 increased; peaks 2, 3 & 5 diminished; peak 4 small, implying that obtusifoliol is further metabolised almost as fast as it is formed).

Inspection of the metabolic profiles in Fig. 1 also reveals small changes other areas which cannot be interpreted at present.

It is noted that the changes in sterols accompany a reduction in the rate growth of the fungus by 50% in the case of EL 222 although at the concentration S1358 used, where sterol changes were clear, there is no diminution of growth. is may be an indication that the standing concentration of ergosterol had not come critical in the presence of S1358.

The changes observed in fraction 1 are not the only changes which accompany eatment with the fungicides. We also noted changes in the metabolic profile fraction 2, which we are at present unable to interpret, but which at least dicate that the effect of these two fungicides is not confined solely to sterol tabolism.

Preliminary experiments with an experimental ICI systemic fungicide show that

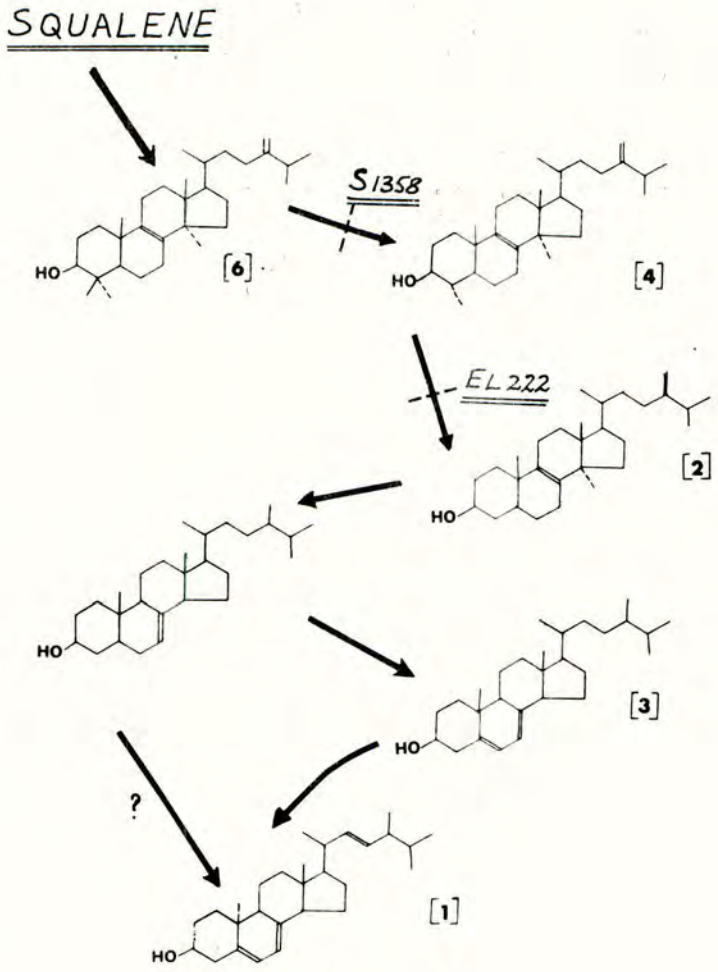

Figure 3.

The possible modes of action of two systemic fungicides on sterol metabolism in Ustilago maydis.

The numbers in square bracket refer to the tentative identifications listed in Figure 2.

it does not noticably alter the metabolic profile of fractions 1 and 3 but produces characteristic changes in a few peaks in fraction 2.

DISCUSSION

Our observations give an illustration of the potential use of metabolic profiles in establishing the mode of action of systemic fungicides and other metabolic inhibitors. However further work in this area is dependent on the positive identification of all those components of the metabolic profile which undergo changes in response to the inhibitors. Such positive identification can only be achieved by the use of the g.l.c. in conjunction with a suitable mass spectrometry system.

Acknowledgements

We wish to thank Eli Lilly & Co., Windlesham, U.K. for a sample of EL222 and the Sumitomo Chemical Co. Ltd., Japan for a sample of S-1358.

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Proceedings 1977 British Crop Protection Conference - Pests and Diseases

MECHANISMS OF ACTION OF FOLIAR SPRAYS OF DAMINOZIDE AND ETHIONINE AGAINST POTATO COMMON SCAB

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Summary Foliar sprays of the growth retardant daminozide or the amino acid ethionine decreased the incidence of potato common scab, caused by soil-borne Streptomyces scabies.

Analogues of daminozide were generally less effective against scab. Daminozide was poorly toxic to S.scabies in plate tests on two media, and did not affect its primary metabolism. Its action on scab is probably indirect.

In plate tests on S.scabies, ethionine was much more toxic than daminozide on Czapek-Dox agar, but its action was antagonised by methionine or metabolic precursors; L-ethionine was more toxic than D-ethionine; and chemical analogues of ethionine were ineffective. Ethionine penetrated potato leaves poorly, but was thereafter found in all parts of the plants; esters of ethionine entered the leaves more readily. Ethionine may act against scab as a direct systemic fungicide.

INTRODUCTION

McIntosh (1975) reported that the incidence of potato common scab, caused by soil-borne Streptomyces scabies, could be decreased by suitably-timed foliar sprays of the growth regulator daminozide ("Alar" or "B-Nine") or the non-protein amino acid DL-ethionine. These effects were found in both glasshouse and field, and have been confirmed by further work (McIntosh, 1976, 1977).

Daminozide and ethionine are chemically unrelated, and are unlikely to act on scab in the same way. They may be, or may be metabolised to, direct-acting systemic fungicides; their action may be within the plant, on the stolon surfaces, or in the rhizosphere. If it is within or on the plant, it may be to prevent infection by S.scabies, or to suppress symptom expression by the plant; if the action is in the rhizosphere, it may be direct fungitoxic action, or it may be to encourage the growth of microflora antagonistic to S.scabies. This paper is an interim report, describing some experiments aimed at clarifying the mechanisms of action, and at finding other chemicals with similar action.

MATERIALS AND GENERAL METHODS

Chemicals Daminozide (N-dimethylaminosuccinamic acid) was technically pure; analogues were made by combining unsym. dimethyl hydrazine, N-aminopiperidine or N-amino morpholine with the anhydrides of succinic, methyl succinic, glutaric, maleic or citraconic acids. Other growth regulators were technically pure or formulated commercial samples. Quintozene, DL-ethionine, its isomers and analogues, and gibberellic acid (GA_3) were of laboratory grade. Esters of DL-ethionine were made by the conventional method.

Plate tests Direct in vitro fungitoxic action was measured against three isolates of S.scabies in "poisoned agar" plate tests on two media: potato dextrose agar (PDA) or Czapek-Dox agar (CDA). After incubation at 25°C for three weeks, fifteen colony diameters were measured for each of five logarithmically-spaced concentrations of each chemical and, from these, EC50's were calculated by standard methods. Similar tests used graded concentrations of ethionine antagonists, added to standard amounts of DL-ethionine; replicated colony diameters were measured as before, and expressed as percentages of the diameters on the corresponding untreated plates. Internal standards for comparison were either quintozene (which is widely used as a soil-treatment for scab control) or DL-ethionine itself.

Glasshouse and growth-room tests The method for measuring the effects of foliar sprays on the incidence of scab on potted plants in the glasshouse has been described (McIntosh, 1975). Experiments on uptake and distribution of ethionine were done on plants (cv. Majestic) in a growth room at 20/15°C.

Analysis Ethionine and methionine in plant tissue were estimated by extraction with ethanol and analysis by GLC.
Other methods are outlined in the appropriate sections below.

RESULTS

Daminozide and Ethionine

Plate tests Table 1 shows weighted mean EC50's from two tests of quintozene, daminozide and DL-ethionine against each combination of the three isolates and two media.

Table 1

EC50s (ppm) of quintozene, daminozide and DL-ethionine against three isolates of S.scabies on two media

	Czapek-Dox agar			Potato dextrose agar		
Isolate	Quintozene	Daminozide	DL-ethionine	Quintozene	Daminozide	DL-ethionine
1	20 ± 5	400 ± 1	4 ± 0.1	14 ± 2	200 ± 8	660 ± 150
2	10 ± 1	1200 ± 70	270 ± 50	14 ± 5	560 ± 50	1000 ± 520
3	∞	560 ± 20	3 ± 0.1	9 ± 1	230 ± 5	100 ± 30

The isolates differed greatly in their susceptibilities, isolate 3 being remarkably resistant to quintozene on CDA. Daminozide was much less toxic than quintozene against the other two isolates on CDA and against all isolates on PDA. DL-ethionine was much less toxic on PDA than on CDA, on which it was more toxic than quintozene against two isolates.

Other experiments on daminozide and ethionine are now described separately.

Daminozide

Comparisons with other growth regulators Of nine chemical analogues of daminozide, only dimethylamino maleamic acid ("CO-11") had comparable effects on scab in glasshouse tests. In four tests with single sprays of freshly-made 1.2% solutions, mean scab indexes were 9.5 (unsprayed), 5.7 (daminozide) and 4.1 ("CO-11"); LSD's were 2.5 ($P=0.05$), 3.3 ($P=0.01$) and 4.2 ($P=0.001$). Yields of tubers were not affected.

Twenty-one unrelated growth regulators were also tested against scab in the glasshouse, mainly as foliar sprays but occasionally (eg. with chlormequat chloride) as soil drenches. Each had its expected effect on plant growth, but none decreased scab incidence.

Two-plant tests These were designed to detect movement of daminozide into the rhizosphere by measuring its effects on plant height in the glasshouse. Two single-stem plants were grown in each pot (McIntosh, 1975), and one of each pair was sprayed with 1.2% daminozide solution, the second plant and the soil being shielded from spray solution. Three weeks after spraying, plant heights were measured.

The figures from the combined results of two such tests were 24cm (sprayed plants), 50cm (unsprayed paired with sprayed plants) and 47cm (unsprayed paired with unsprayed plants); the LSD was 4cm ($P=0.05$). Thus, spraying one plant of a pair did not decrease the height of the other in the same pot, even though the roots were thoroughly interlaced.

Interaction with gibberellic acid Experiments (still incomplete) with simultaneous application of daminozide and gibberellic acid as foliar sprays on glasshouse plants indicate that, while GA_3 (0.005%) nullified the effect of daminozide (0.6%) on stem extension, it did not greatly alter the effect on scab incidence.

Metabolism of S.scabies Some effects of daminozide on the metabolism of S.scabies was measured by feeding ^{14}C-glucose for 2 or 4 h to cultures of isolate 1 on liquid Czapek-Dox medium, without and with added daminozide (0.7 mM). The organism was then killed with liquid nitrogen, extracted and fractionated by conventional methods into the following alcohol-soluble fractions: lipids, organic acids, sugars, soluble nucleotides and amino acids. The amounts of ^{14}C in each fraction were not affected by addition of daminozide to the culture medium.

In other tests on isolate 2, also on liquid Czapek-Dox medium, daminozide immediately decreased oxygen uptake, as measured by an oxygen electrode, at about 5mM and stopped it completely at 11mM.

Ethionine

Plate tests : isomers In tests similar to those described above, the separate ethionine isomers were compared; Table 2 shows mean EC50's from two tests against each of the same three isolates of S.scabies, on CDA only. The L-isomer was clearly much more toxic than the D-isomer, but the differences between the L-isomer and the DL-mixture were slight, possibly because the dose-response lines were very flat. As before, the absolute and relative toxicities of the isomers varied amongst the isolates.

Table 2

EC50s (ppm) of DL-, D- and L-ethionine against three isolates of S.scabies on Czapek-Dox agar

Isolate	Ethionine isomer		
	DL-	D-	L-
1	38 ± 6	200 ± 100	36 ± 2
2	280 ± 40	∞	130 ± 50
3	6 ± 0.6	18 ± 4	5 ± 0.6

Plate tests : analogues In other similar plate tests, the following analogues and homologues of ethionine failed to decrease growth of isolate 3 of S.scabies on CDA at the molar equivalent of 100 ppm of ethionine (S-ethyl-homocysteine): L-cysteine, S-methyl- and S-ethyl-L-cysteine, DL-methionine-S-methylsulphonium chloride ("methyl methionine"), DL-2-hydroxy-4-methylthio butyric acid ("DL-methionine hydroxy analogue"), DL-ethionine sulphone, DL-methionine (i.e. S-methyl-DL-homocysteine), S-allyl-, S-isopropyl-, S-n-butyl-, S-hexyl-, S-phenyl- and S-benzyl-DL-homocysteine. In these tests, DL-ethionine at 100 ppm restricted growth to about 3% of that on untreated CDA.

Plate tests : antagonists In other tests, with the same growth conditions, the effects of three concentrations of DL-ethionine on growth of S.scabies were measured in the presence or absence of graded series of concentrations of DL-methionine, DL-homocysteine (as thiolactone hydrochloride) or DL-homoserine. Each of the three isolates gave essentially the same result; for brevity, each figure in Table 3 represents the combined results from one test on each of the three isolates, on CDA.

Table 3

Growth of S.scabies in presence of DL-ethionine with and without competing amino acids. Colony diameters are expressed as percentages of those on untreated plates (17.2 mm).

DL-ethionine, ppm	Added amino acid	Molar equivalent (ME) of added aminoacid ME/1	ME/4	ME/16	ME/64	nil
500	DL-methionine	89	90	57	25	4
	DL-homocysteine	33	41	16	15	11
	DL-homoserine	15	6	5	-	3
100	DL-methionine	100	78	47	24	9
	DL-homocysteine	68	33	20	20	19
	DL-homoserine	15	8	-	-	5
20	DL-methionine	86	70	46	32	19
	DL-homocysteine	53	42	44	38	36
	DL-homoserine	24	18	-	-	15

DL-methionine was clearly the most effective antagonist, restoring growth to about 90% of normal when present at the same molar concentration as DL-ethionine (ME/1); even the lowest concentration of DL-methionine (ME/64) permitted growth to some extent. DL-homocysteine and DL-homoserine also antagonised the action of DL-ethionine, but were generally less effective than DL-methionine.

Uptake and distribution of DL-ethionine and esters in plants A total of 60mg of DL-ethionine per plant was distributed, as evenly as possible, as discrete 0.1 ml drops of 1% solution on the leaves. At intervals up to 6 weeks after application, the unabsorbed ethionine was washed with water from the leaf surfaces, and the plants were divided into various parts and analysed for ethionine. The results in Table 4 are from one plant, harvested 22 days after application; the figures are typical of those from several experiments.

Table 4

Distribution of DL-ethionine in potato plants 22 days after application to leaves of 60 mg per plant

Tissue	Wt. of ethionine mg.	Fresh wt. of tissue g.	mg/g fresh wt.
Stems	1.48	4.4	0.336
Leaves	7.04	24.0	0.293
Roots	0.01	3.5	0.003
Tubers	0.05	13.0	0.004
Washed from leaf surfaces	25.6		
Total recovered	34.2		

By far the largest amount of ethionine was found on the leaf surface, which it evidently does not penetrate easily. The rest was mainly in the leaves and stems, with only small amounts in the tubers and roots. Only just over half of the applied 60 mg was recovered. Some was presumably lost by breakdown on the leaf surface; evidence from other experiments suggests that some was incorporated into leaf protein.

In an effort to improve penetration, some experiments were done with the n-propyl ester of DL-ethionine which, like other esters, is more phytotoxic than DL-ethionine itself, and must be applied at lower concentrations. Droplets of solutions of DL-ethionine (1.0%) or the ester (0.079% - i.e. 1/16 of the molar equivalent of 1% ethionine) were placed on leaves (four drops per compound leaf). After 24 h, the leaf surfaces were washed with water and the leaves analysed as before. The ester was rapidly hydrolysed to ethionine in leaf tissue. Results of triplicate analyses of leaves of comparable size, expressed as ethionine, were: after application of DL-ethionine, 3.58 mg in surface washings and 0.27 mg in leaves; after application of ester, 0.0 mg in surface washings and 0.29 mg in leaves. The ester penetrated the leaf surface much more efficiently than ethionine. The n-pentyl and n-octyl esters behaved similarly.

In the above experiments, measured amounts of DL-ethionine were applied by placing drops of solution on the leaves. However, under the more practical conditions of glasshouse and field tests on scab control, plants were sprayed to run-off, and retained much more spray solution. Tubers from glasshouse plants, sprayed in this way with 1% solutions of ethionine with the usual precautions against solution reaching the soil, were analysed for both ethionine and naturally occurring methionine. The amounts found (mg/g fresh wt.) were: 26 days after spraying, 0.573 (ethionine) and 0.072 (methionine); and, 38 days after spraying, 0.377 and 0.032. Ethionine was present in much larger amounts than in the drop-placement experiments (Table 4); its level exceeded that of methionine by 7-10 times.

DISCUSSION

Foliar sprays of either daminozide or ethionine certainly decreased the incidence of potato common scab. Experiments aimed at clarifying the mechanisms of action are still incomplete and some were inconclusive. However, certain preliminary conclusions may be drawn.

Daminozide is almost alone, among the growth regulators and close chemical analogues tested, in its ability to decrease scab. The other regulators included the well-known retardants chlormequat chloride, "AMO 1618" and ancymidol, but they all failed to affect scab, possibly because their biochemical mode of action differs from daminozide in some respects (Dicks, 1976). The only chemical to decrease scab to the same extent as daminozide was dimethylamino maleamic acid ("CO-11"), but as it is not very stable in aqueous solution (Dahlgren & Simmerman, 1963), it is less likely than daminozide to be of practical value.

Daminozide was poorly toxic to S.scabies in plate tests (Table 1). It did not affect primary metabolism (measured with ^{14}C-glucose) of S.scabies in culture at the concentration used (0.7 mM); it stopped uptake of oxygen by S.scabies (isolate 2) at 11mM (~1800ppm), but such a high concentration, which corresponds roughly to the EC50 (1200ppm), could not possibly arise in tubers after application of 1% sprays. The effects on respiration and growth may be due to the reported effect of daminozide on membrane permeability (Undurraga & Ryugo, 1970).

The action of daminozide as a retardant is to affect the physiology of the whole plant; it moves to the roots of several plant spp. after foliar application (Moore, 1968; Dicks, 1972), and affects stolon growth in potatoes (Humphries & Dyson, 1967). However, the results of the two-plant tests, along with the finding that daminozide is stable enough in soil for growth of potato plants to be retarded by direct soil application (McIntosh, 1975), suggest that no daminozide passed into the rhizosphere from roots or stolons of sprayed plants. (This contrasts with experiments in which

radio-labelled daminozide was applied to foliage and radioactivity was afterwards found in soil (Undurraga & Ryugo, 1970; Sachs et al, 1975).)

The available evidence suggests that the action of daminozide on scab is indirect, its primary action (possibly independent of its anti-gibberillic acid action) being on the plant rather than the organism.

Ethionine None of the chemical analogues tested had any effect on growth of S.scabies. Ethionine was much more strongly fungitoxic than daminozide to S.scabies and on two isolates more toxic than quintozene. As might be expected from results of fungitoxicity and other tests of ethionine isomers on various organisms, the L-isomer was the most effective, and the action was antagonised by methionine and its precursors (Stekol & Weiss, 1949; Schrank, 1956; Maw, 1961; Moje et al, 1963; Jones & Woltz, 1969). This is consistent with the lower toxicity of ethionine on PDA (containing methionine) than on CDA (sucrose and minerals only).

After foliar application, DL-ethionine was found in all parts of potato plants, including tubers, even though it did not easily penetrate the leaf surface. However esters entered the leaf more readily and might be expected to give more effective scab control. Results of current tests on distribution of esters throughout the plants, and on their effects on scab in glasshouse and field, are not yet available.

After application of sprays to foliage of potted plants in amounts sufficient to decrease scab incidence, ethionine was found in tubers at about 0.5 mg/g fresh wt, or 500 ppm, which corresponds roughly to the EC50's in the plate tests on PDA, and exceeds those on CDA. The amount of free methionine in the same tubers was 7-10 times smaller; if both compounds were equally available to S.scabies, this amount would be enough to decrease the fungitoxic action of ethionine (Table 3).

We consider that ethionine, in contrast to daminozide, probably acts as a systemic fungicide against S.scabies. As the metabolic precursors of methionine (homocysteine and homoserine) antagonised the effect of ethionine on S.scabies, it seems likely that ethionine inhibits the synthesis or subsequent metabolism of methionine in S.scabies.

Many questions on the action of these compounds remain unanswered; work on both is continuing.

Acknowledgements

We thank Miss A.L. Gathergood and Mrs. I.J. Covell for their help; and the following firms for chemical samples: Uniroyal (daminozide); Ciba-Geigy (S-substituted analogues of ethionine); Lilly (ancymidol).

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Proceedings 1977 British Crop Protection Conference - Pests and Diseases

CRITICAL EVALUATION OF STRAWBERRY SPRAY SCHEMES FOR CONTROL OF BOTRYTIS CINEREA RESISTANT TO BENZIMIDAZOL FUNGICIDES

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Summary In order to control resistant Botrytis strains on strawberries alternating and mixture spray schemes are often proposed. The effect of an alternating spray scheme with dichlofluanid and benomyl was examined by regular analysis of the sprayed petals. Fungicides were found on all the petals the day after spraying, but the number of petals with detectable fungicide deposits decreased sharply at the end of the week. Consequently the new flowers were not protected from resistant fungal strains during the week where a benzimidazol fungicide was used in an alternating spray scheme. The effect of a mixture of dichlofluanid and benomyl was studied in vitro by determination of the ED 50 and ED 95. A resistant Botrytis isolate was inhibited to the same degree by dichlofluanid alone as by the mixture. Consequently the addition of a benzimidazol fungicide has no effect on the resistant strains. The results of these laboratory experiments are confirmed in field trials.

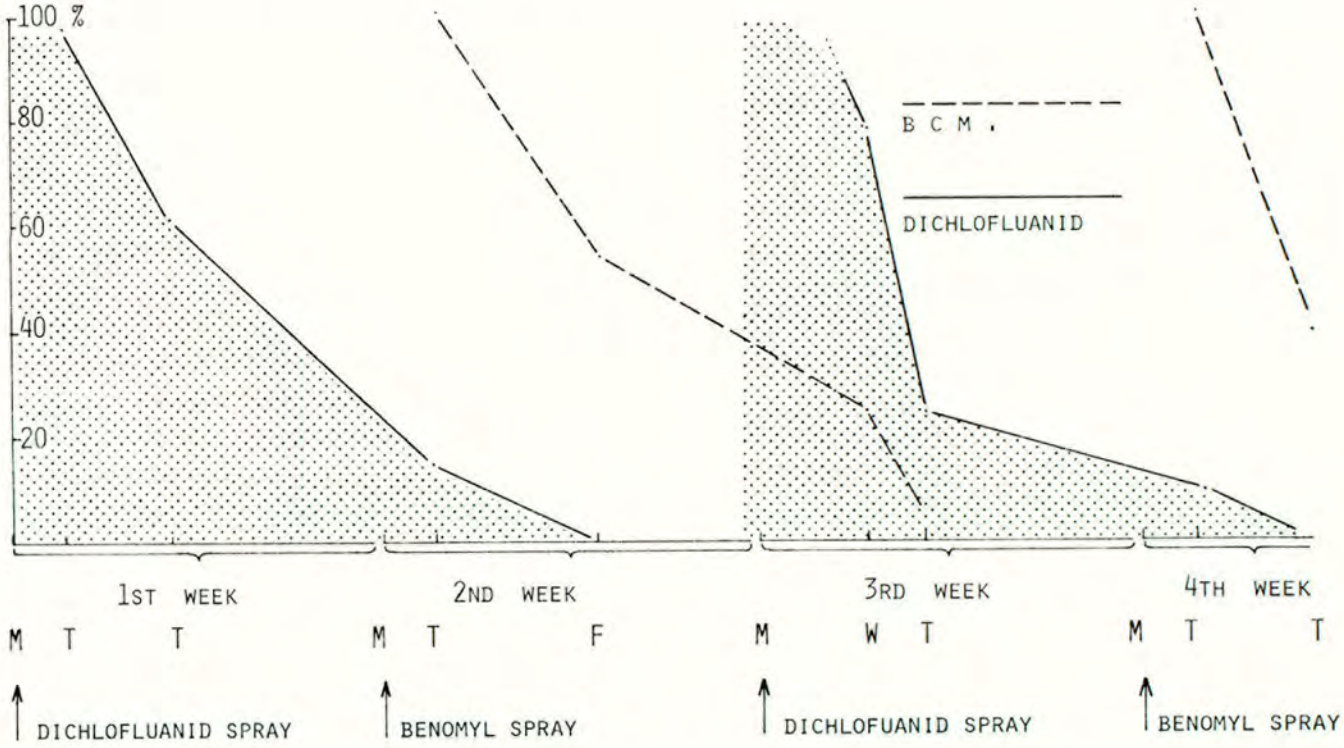
Résumé Evaluation critique des schémas de pulvérisation sur fraises pour la lutte contre le Botrytis cinerea resistant aux fongicides à base de benzimidazole.

Dans la lutte contre les souches résistantes du Botrytis sur fraises on a souvent proposé des pulvérisations alternantes ou aux mélanges. Un schéma de pulvérisation alternant le dichlofluanide et le bénomyl a été étudié par analyse régulière des pétales traitées. Sur toutes les pétales les fongicides ont été retrouvés le lendemain du traitement, mais le nombre de pétales avec des dépots décelables de fongicides diminuait rapidement vers la fin de la semaine. Les jeunes fleurs ne sont donc pas protégés pendant la semaine durant laquelle un fongicide à base de benzimidazole a été utilisé dans un schéma de traitement alternant. L'effet d'un mélange de dichlofluanide et de benomyl a été examiné in vitro par la détermination de l'ED 50 & l'ED 95. Un Botrytis résistant était freiné autant par le dichlofluanide seul que par le mélange. L'addition d'un fongicide benzimidazol à un autre n'a donc pas d'effet sur les souches résistantes de Botrytis. Les résultats de ces expérimentations au laboratoire ont été confirmés par des essais sur fraises en plein champ.

INTRODUCTION

Isolates of Botrytis cinerea tolerant to benzimidazol fungicides have been obtained regularly from strawberry fields sprayed with these fungicides (Jarvis & Hargreaves, 1973, Hartill et al, 1975, Gjaerum, 1975, Abelentsev, 1973). From the numerous reports it is clear that the phenomenon is widespread in several countries. Since applications of benzimidazol fungicides are ineffective to control resistant fungi, adaptations of the spray programmes are required. In the literature two kinds

FIG. 1 PERCENT PETALS (Y) BEARING FUNGICIDES DURING THE FOUR WEEKS OF FLOWERING (X) USING AN ALTERNATING SPRAY SCHEME WITH DICHLOFLUANID OR BENOMYL ON MONDAY



of adaptations are often proposed in order to control resistant fungal strains (e.g. Delp 1976) : 1° alternating a benzimidazol and an other fungicide and 2° using a mixture of both. The effect of these alternative spray programmes was examined by field experiments and discussed at this Conference in 1975 (Jordan & Richmond, 1975). We have now examined both spray schemes by experiments and analyses in the laboratory and in the field.

METHODS AND MATERIALS

Resistance of B. cinerea isolates was tested by growing them on potato dextrose agar without and with 100 ppm carbendazim

Fungicide analyses of petals were carried out by thin layer chromatography (Jamart et al, 1975). Chloroform was selected as a suitable solvent for development of dichlofluanid and BCM on the same chromatogram. The detection limit is about 50 ng for dichlofluanid and 10 ng for BCM. This correspond with 10 and 2 ppm respectively on petal leaflets of about 5 mg. Flowering strawberries were sprayed weekly with Euparen (50% dichlofluanid) or Benlate (50% benomyl) and flowers picked the following days. Each petal leaflet was separately extracted in 1 ml ethyl acetate and spotted. Detection was done by Penicillium expansum.

Sensitivity of a fungal isolate to the fungicides was tested by growing the isolated fungus on potato dextrose agar with a range of different fungicide concentrations. Increased growth inhibition was observed at increasing concentrations and from the inhibition curve (fig. 2) the ED 50 and ED 95 was calculated (Weber,1956).

Spray trials in the field ware carried out in 1977 on a commercial farm. In order to obtain maximum protection we sprayed twice a week (Kamoen & Jamart,1975) during the four weeks of flowering. Euparen and Benlate was used on all plots at half the normal concentration: 1 and 0,4 kg/ha respectively. The fungicides were applied on Tuesday and Friday morning. Artificial infections were always carried out by spraying a spore suspension of a resistant Botrytis strain the evening before each spray. Each treatment consisted of 4 blocks of 20 plants c.v. Red Gauntlet.

RESULTS

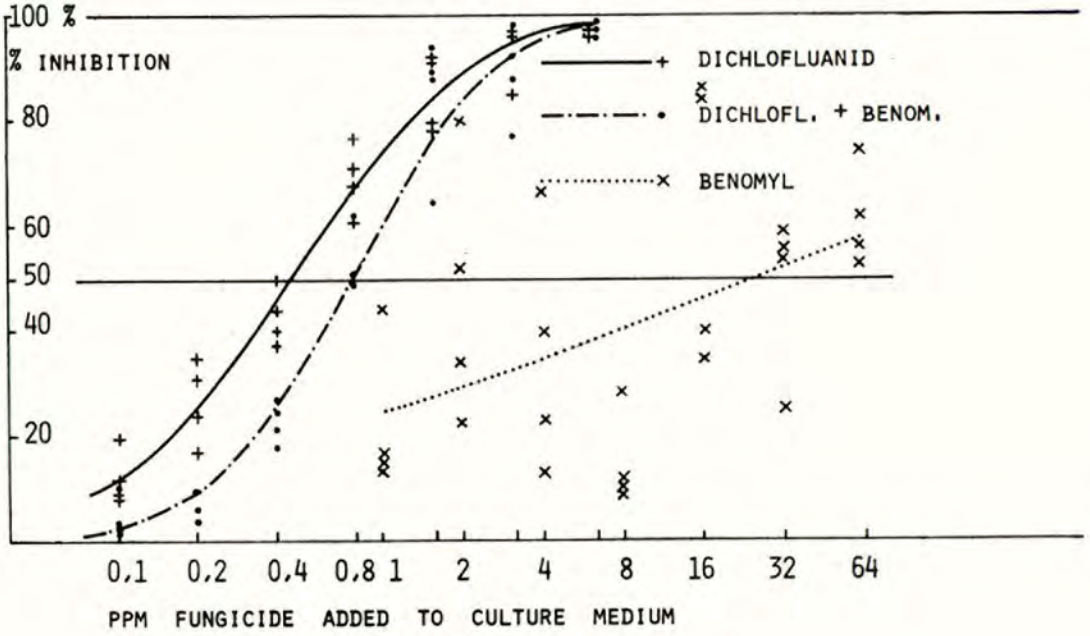
In 1976 and 1977 fifty commercial farms were examined in Belgium for the presence of resistant Botrytis strains. On 45 farms BCM was detected on the calyx of the rotted fruits, and on 32 farms 100% of the isolates were resistant. On 11 farms resistant and non-resistant strains were isolated. Only two farms were found without a resistant strain. This inquiry shows that the resistant strain is rapidly selected and will certainly prevail on farms where benzimidazol fungicides are used.

Examination of alternating spray schemes

In order to test the effectiveness of alternating spray schemes, flowering strawberries were sprayed weekly, the first and the third week Euparen, the second and fourth week Benlate. In order to see how many petals carried fungicide deposits, the petals of open flowers were analysed the following day, three days later etc. From figure 1 (where dichlofluanid and BCM the breakdown product of benomyl are plotted) we can see that the day after spraying the fungicides were detected on all the petals. Three days later about 50% of the petals were without fungicides and at the end of the week petals containing fungicides were rare . This is due to the continuous and fast opening of new flowers from closed green and white buds, whose internal parts were not covered by the preceding treatment. (Kamoen & Jamart,1975).

It is evident that most flowers are protected against Botrytis for a few days following dichlofluanid sprays (fig. 1, dotted zones). There is no protection at all against the resistant strains for more than two weeks when a benzimidazol fun-

FIG 2 INHIBITION OF MYCELIAL GROWTH (%) OF A RESISTANT BOTRYTIS ISOLATE



gicide is used (fig. 1, second and fourth week) due to the absence of dichlofluanid and the resistance of the fungus to benzimidazols.

Examination of a mixed spray scheme

A second spray scheme often proposed is the spraying of a mixture of a benzimidazol and an other fungicide at half the normal concentration. The effect of a mixture of both fungicides was examined by growing a resistant Botrytis isolate on potato dextrose agar with different concentrations of dichlofluanid, benomyl and mixtures of equal concentrations of both. The calculated ED 50 was respectively 0,4 ppm, 25 ppm and 0,8 ppm. The corresponding ED 95 values were 3,5 ppm. ,>100 ppm and 4 ppm (fig. 2). From these results it is clear that the resistant strain is inhibited to roughly the same degree by the dichlofluanid alone as by the mixture.

We can conclude that when resistant strains prevail the use of a mixture spray provides no additional protection in the field in comparison with a non benzimidazol fungicide alone.

Field experiment

Finally a field experiment was carried out in order to confirm the preceding results obtained in laboratory tests.

In 1977, six spray schemes were tested during four weeks of flowering including a control (unsprayed), benomyl (B), different alternating spray schemes (B/D/...), dichlofluanid (D) and a mixture of benomyl and dichlofluanid. The alternating spray schemes were selected so that from treatments II to VI gradually longer periods are covered by dichlofluanid (see table 1).

From table 1 it is clear that the number of infected fruits decreases when the period covered by dichlofluanid sprays increases. No significant differences occured between yields per treatment.

Table 1

Field evaluation of strawberry spray schemes for the control of Botrytis cinerea resistant to benzimidazol fungicides

week:	1st		2nd		3th		4th		1)	yield per treatment	infected fruits		
day:	T	F	T	F	T	F	T	F		kg	number	%	
	Treatments												
I	unsprayed									38,526	487	9,00 a	3)
II	B	B	B	B	B	B	B	B	2)	40,409	324	5,48 b	
III	D	B	B	B	D	B	B	B		39,872	186	3,22 b c	
IV	D	D	B	B	D	D	B	B		42,322	155	2,48 c	
V	D	D	D	B	D	D	D	B		37,909	108	1,87 c d	
VI	D	D	D	D	D	D	D	D		36,339	98	1,84 c d	
VII	mixture									39,963	62	0,97 d	

1) day of the week when the spray was applied T : Tuesday, F: Friday

2) succesive treatments B: benomyl, D: dichlofluanid

3) percentages followed by a character in common are not significantly different at the 5% level (Duncan's multiple range test & arc sin transformation)

DISCUSSION

The results from laboratory and field experiments suggest that if resistant strains prevail, the following conclusions can be drawn for strawberry fruit rot control:
1) with a benzimidazol fungicide alone no control is possible and selection of resistant strains will be stimulated.
2) a mixture gives no better protection than dichlofluanid alone but likely prevents selection by the continuous presence of the dichlofluanid
3) when alternating spray schemes are used the protection will increase according as the period covered by dichlofluanid is increased and the selection will increase inversely proportional to this.

However from our field experiment we found that benomyl is still better than the untreated control. We believe that besides our artificial infection with the resistant strain there was natural infection with sensitive strains. We tested this hypothesis by isolating Botrytis from the growers field next to our experimental plots. The isolates obtained didn't grow on BCM-containing agar and the natural occuring Botrytis seems to be sensitive. Thus where sensitive and resistant strains coexist a mixed spray can still be partial effective.

It has been suggested that redistribution of fungicides from sprayed flowers to unsprayed ones by rain may extend the period of protection. We tested this in glasshouse spraying on plants with closed white buds followed by artificial raining the second and third day. The new opened flowers were analysed on the fourth day and fungicides were found on less than 20% of the flower petals. From this we can conclude that redistribution of fungicides is of no importance for strawberry fruit rot control.

Acknowledgements

We thank for technical assistance Mr. H. Van De Sype, Mr. L. Detavernier and Mr. J. Aerts.

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Proceedings 1977 British Crop Protection Conference - Pests and Diseases

STRATEGIES FOR WHEAT BULB FLY CONTROL IN THE YORKS AND LANCS REGION

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Summary Wheat bulb fly control has relied on the use of relatively inefficient seed treatments on a wide scale, because an accurate forecast of damage could not be made before the crop was sown. A method has now been developed which estimates egg populations by the middle of August and allows for a more sensible choice of control strategies. Alternative methods of wheat bulb fly control have been developed which offer considerable economic advantages over the standard method.

INTRODUCTION

Seed treatments against wheat bulb fly (Delia coarctata) were the first effective form of control to be developed, and they have become the main method used to combat this pest. The phytotoxic effects of many of the treatments tested have been frequently documented, but the standard commercial seed treatments have still given a considerable yield increase over the untreated controls in all published trials. (Bardner, 1958, 1957; Bevan, 1965; Brown & Maskell, 1965; Dixon, 1967; Gough et al, 1961; Griffiths, 1977; Griffiths et al, 1976; Maskell & Gair, 1961; Maskell, 1967, 1970; Mathias & Roberts, 1967, 1969, 1973).

Emergency sprays have found a limited use as supplements to inadequate seed treatment or where seed treatment has been omitted. Their use has also been found to give a considerable yield increase over untreated controls (Brown & Maskell, 1962; Maskell & Davies, 1974; Makepeace, 1969; Griffiths & Scott, 1969).

Granular treatments have been seen to offer a further increase in yield over standard methods but, due to their apparent expense, have been little used in commercial practice (Brown & Maskell, 1965; Catling, 1967; Makepeace, 1965, 1967; Maskell & Gair, 1961; Mathias & Roberts, 1967; Sinclair, 1975; Price & Wright, 1976).

Some early results with protective sprays are reported in this paper; other results of work on the protective use of chlorpyrifos sprays have been reported by Price & Wright (1976).

As part of the continuing trials programme co-ordinated by the Wheat Bulb Fly Study Group of the Closed Conference of Advisory Entomologists, the results of these different control strategies have been compared on a range of sites in Yorkshire and Humberside. The aim of the work has been to identify the most profitable strategies for wheat bulb fly control at different levels of damage. The introduction of a method of wheat bulb fly egg collection in oviposition trays (Oakley & Uncles, 1977) gives a reasonable estimate of the level of egg populations by the middle of August. This should allow for a more rational choice of control strategies than has previously been possible.

PROTECTIVE SPRAYS

Two trials were laid down in the East Riding of Yorkshire (now Humberside) in 1972 to investigate the possible use of a protective spray. It was hoped that if a spray could be applied in February to protect the crop from attack this would allow for an extensive sampling service to be introduced as a basis for the decision on whether or not to treat. Three materials were applied to crops of cultivar Joss Cambier, which had already been treated with chlorfenvinphos seed treatment. In a year when wheat bulb fly larval invasion was delayed an early treatment was applied on February 21 and a late treatment on March 12. The early treatment with pirimiphos-methyl gave a significant yield increase at both sites, but the late treatment had no effect. Omethoate sprays had no significant effect at the first site but gave significant yield increases at the second site at both application dates.

A trial was laid down in 1973 to elucidate the difference in timing requirements of these two materials. The cultivar used was Maris Huntsman and the seed was treated with only a mercurial seed treatment. The results of this trial are shown in Table 1.

TABLE 1 Larval invasion and treatment yields

Date of application	% plants invaded at that date	Treatment yields (tonnes/ha 85% dm) Pirimiphos-methyl at 1.751/ha of a 50% ec	Omethoate at 1.251/ha of a 50% ec
31 Jan	30	7.23	6.36
7 Feb	32	6.77	6.78
14 Feb	65	6.58	7.17
21 Feb	75	6.29	7.01
SE for comparison within treatments		0.125	0.177

In 1974 co-operative trials were carried out nationally on this subject, but wheat bulb fly attack was slow to develop and no significant yield effects were obtained in the trial done in South Humberside. A trial comparing spray materials was also initiated in South Humberside. Here a significant attack developed very early and some interesting results were obtained. A second protective treatment was introduced, this was chlorfenvinphos at 3.5 l/ha of 32% ec. The late treatments were applied on 6 February when 62% of the plants were attacked and 10% of the larvae had developed to the third instar and were ready to begin secondary invasion. These results are shown in Table 2.

TABLE 2 Treatment yields and larval control

Treatment	Date of application	Yield (tonnes/ha at 85% dm)	Nos of larvae per m
pirimiphos-methyl	14 Jan	3.42	23.3
chlorfenvinphos	14 Jan	3.46	14.4
omethoate	6 Feb	3.17	11.5
Untreated		2.19	35.4
SE		0.194	1.8

In 1976 a trial was laid down using a protective spray of chlorpyrifos at 1.7 l/ha of the 48% ec applied on 1 February; this compared well with standard treatments as shown in Table 3.

TABLE 3 Treatment yields and larval control

Treatment	Yield (tonnes/ha at 85% dm)	Live larvae per metre of row
chlorpyrifos spray	7.38	14.3
chlorfenvinphos seed/treatment	6.88	25.4
fonofos granules	7.39	14.3
untreated	6.45	44.9
SE	0.202	1.19

Three successful protective spray treatments have now been identified; as yet though they have not been tested against each other sufficiently to give any indication of their relative merits for this use. The mode of action of the chemicals seems to be different. Chlorfenvinphos was also effective if applied at a higher rate at sowing and seems to be soil acting. Pirimiphos-methyl seems to act mainly by surface adsorption on to the leaf, dead first instar larvae being found in the outer leaf sheaf after treatment. Different soil types, the effect of weather conditions at application and timing requirements may influence the performance of the different treatments.

COMPARISON OF STRATEGIES

In the past four years eight trials have been done from the results of which different treatment strategies can be compared. All strategies have not been represented in all the trials. The details of the trial sites are summarised in Table 4; in all the trials the seed was drilled between 20 and 30 mm deep at 150 kg/ha. Trial D was the spray timing trial where significant damage did not materialise; thus it has been used as a phytotoxicity trial, as has trial H which was located on wheat bulb fly free soil. The potential wheat yield in the field has been taken as the yield of the best treatment in the trial. Table 5 summarises the differences in yield obtained between the best treatment strategy in the trial and each strategy represented. The following treatment strategies have been considered:

1. No treatment applied: untreated controls were included in all trials. The yield losses recorded were remarkably stable around 1.2 tonnes/ha, with one exception in trial B where a very early secondary invasion occurred before the crop had tillered.

2. Emergency spray: a spray application of omethoate was included in two damage trials and one phytotoxicity trial. In both cases the treatment applied at optimum timing has been considered. The cost of treatment has been taken as £10/ha.

3. Protective spray: the trials that included protective sprays have already been mentioned in part. The sprays considered here are chlorfenvinphos in Trials C and F, chlorpyrifos in Trial G and pirimiphos-methyl in Trials A & D.

4a. Chlorfenvinphos seed treatment: this treatment was included in six trials, loadings considered are detailed in Table 6. The check to vigour was similar at both 'undamaged' sites, but a substantial yield loss resulted at Trial D where untreated plots yielded 4.88 tonnes/ha and no yield loss occurred at Trial H where untreated controls yielded 8.39 tonnes/ha. The cost of treatment has been taken £2.50/ha.

4b. Permethrin seed treatment was included in five trials, the loadings are also detailed in Table 6. The cost of treatment has been forecast as £7.50/ha.

TABLE 6 Loadings of seed treatments

Trial	Chlorfenvinphos Loading ppm	Application method	Permethrin Loading ppm	Application method
B	759	commercial	1976	churn dresser
D	759	"	-	-
E	759	'mini-rotostat'	721	'mini-rotostat'
F	1729	"	1098	"
G	834	"	1145	"
H	834	"	1145	"

The treatment applied by churn dresser was done at ADAS Cambridge and the treatments applied by mini-rotostat were done at Rothamsted Experimental Station. The loadings were estimated by Cambridge ADAS in 1975 and 1976 and at Rothamsted Experimental Station in 1977 using standard GLC methods.

5. Fonofos granules applied at drilling This treatment was included in three damage trials and one phytotoxicity trial. The approved rate of 1.4 kg ai/ha is considered in all trials but in trial F 0.11 kg ai/ha was also used. The cost of treatment has been taken as £20/ha.

Taking the current value of wheat as £80/tonne the net cost of the treatment strategy has been calculated at different damage levels. The level has been assessed as the percentage actually damaged of fields placed at risk by their cropping and location. The cost of phytotoxicity plus that of treatment is taken in undamaged fields and the cost of unprevented damage plus treatment in damaged fields. A perfect selection of emergency treatments is assumed in strategy 2, so that treatment is only applied where damage would otherwise result.

This approach follows that proposed by Strickland (1967) and applied to potato cyst nematodes by Jones (1973) and to cereal aphids by George (1975). The resulting costs are shown in Figure 1.

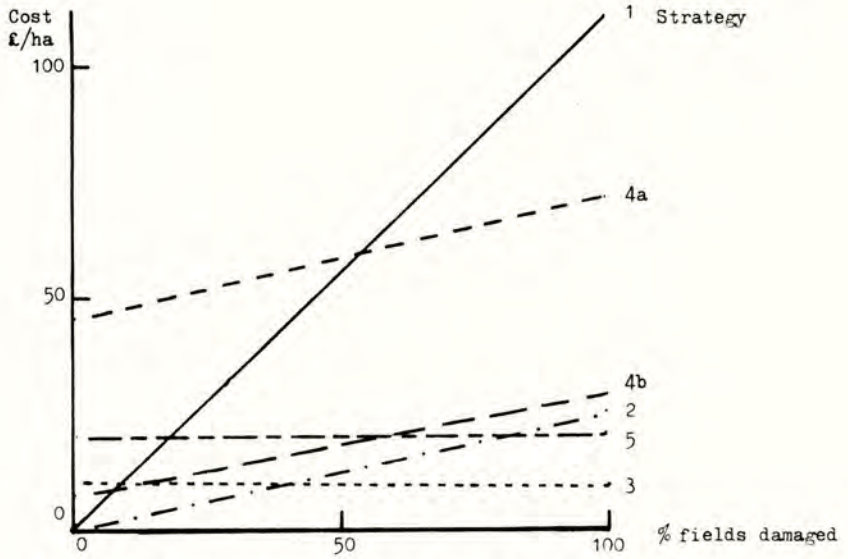

Figure 1. The net cost of wheat bulb fly damage and of control measures at different levels of damage.

DISCUSSION

In any exercise of this nature a number of assumptions have to be made; here the most questionable assumption is that the trials are representative of the overall situation. The yield improvements obtained with chlorfenvinphos seed treatments in these trials are very much of the same order as shown in previous publications (loc cit) and it is suggested that the apparent discrepancy in the effectiveness of this treatment is due to the non-inclusion of more effective treatment strategies in most seed treatment trials. The levels of phytotoxicity and larval control exhibited by this treatment were also within the range shown in previous trials where the seed loading was known. (Griffiths et al, 1975, 1976)

The mean yield loss attributed to the difference between the best strategy and the untreated controls has been taken as an expression of the total damage experienced on the sites. These estimated total losses compare closely with those obtained by Bardner (1968) who used polythene covers to produce infested and non-infested soil on the same site, and also with the losses forecast by the regressions produced by Bardner et al (1970) from individual plots in trials in the Eastern Region of ADAS. This suggests that the best strategies identified in this work are indeed preventing nearly all of the yield loss caused by wheat bulb fly attack.

The emergency spray strategy has not been included in enough trials in this series for any real confidence to be placed in these results alone. Similar yield increases to those obtained in these trials have been obtained in many other trials (loc cit) but the proven timing sensitivity of this strategy makes overall comparison difficult (Griffiths & Scott, 1969, Maskell & Davies, 1974). This timing sensitivity would involve a considerable risk in relying on this strategy for control, and it is probably best kept in reserve in case a planned strategy cannot be applied optimally.

In any future wheat bulb fly control trials it is suggested that several different strategies should be compared to the treatments under test. Further strategic comparisons can then be made and the chance of developing non-optimal strategies excluded.

The original purpose of this study was to establish the most profitable strategies at different damage risk levels. The levels of risk established by the annual surveys in the Yorkshire and Lancashire Region of the ADAS are shown in Figure 2. Whilst damage levels do not always reach these risk levels they have never been exceeded. These levels fluctuate from year to year and between the risk areas recognised in the region. This difference between areas is amplified across the rest of the country where differences between areas within a given year can be greater than overall differences between years. The need to gear the advice given on wheat bulb fly control to local conditions, soil types and experience cannot be over emphasised.

It is unfortunate to note the general rise in wheat bulb fly populations recorded in Figure 2 following the withdrawal of aldrinated fertiliser (Anon, 1964) and aldrin and dieldrin seed treatments (Anon, 1967). It is hoped that a more logical choice of control strategies will reverse this trend; to this end other strategies may be available such as that suggested by Bardner et al (1977), who propose controlling adult flies in emergence sites.

I thank the staff of the Entomology Department at Leeds for practical help, the members of the Wheat Bulb Fly Study Group for constructive criticism and guidance, the staff of Rothamsted Experimental Station and Messrs Maskell and Roberts for assistance with seed treatment, Mr B A Cooper, ADAS Shardlow, for permission to use unpublished results, Analytical Services at Cambridge ADAS and Mr K A Jeffs for GLC analysis, and the many farmers involved for willingly providing trial sites and facilities.

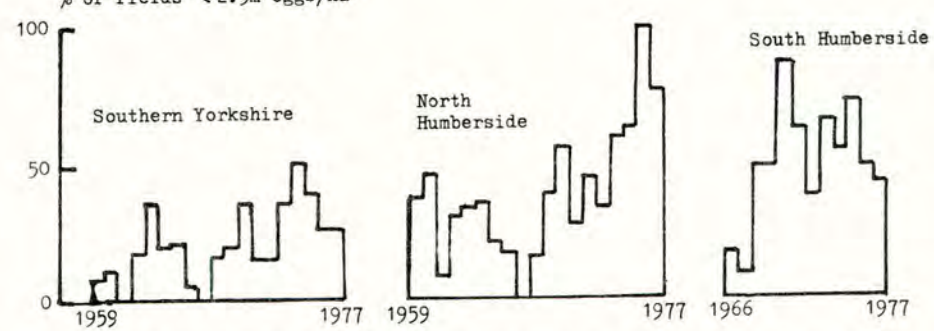

Figure 2. The proportion of fields in damaging categories as shown by annual egg sampling.

TABLE 4

DETAILS OF TRIALS

	YEAR OF HARVEST	LOCATION	EGG COUNT millions/ha	CULTIVAR	SOWING DATE	SOIL TYPE
A	1974	Aldbrough	3.4	Maris Huntsman	11 11 1973	Sandy clay loam
B	1975	Flinton	10.3	Maris Huntsman	17 11 1974	Sandy clay loam
C	1975	Crowle	8.7	Cama	30 10 1974	Humose fine sand
D	1975	Appleby	11.1	Maris Huntsman	5 11 1974	Sandy loam
E	1976	Humbleton	8.8	Maris Huntsman	20 10 1975	Sandy clay loam
F	1976	Womersley	13.6	Maris Freeman	28 10 1975	Sandy clay loam
G	1977	Humbleton	17.3	Maris Huntsman	20 10 1976	Sandy clay loam
H	1977	Brantingham	0	Maris Huntsman	22 10 1976	Silty loam

TABLE 5

Yield difference (tonnes/ha) between strategies and the best strategy in the trial

STRATEGY	TRIAL								MEAN EFFECT	
	A	B	C	D	E	F	G	H	damaged	undamaged
1	- 1.04	- 2.80	- 1.27	0	- 1.18	- 1.20	- 0.94	0	- 1.41	0
2	- 0.12		- 0.29	0					- 0.21	0
3	0		0	0		0	0		0	0
4a		- 1.26		- 1.06	- 0.79	- 0.92	- 0.51	0	- 0.87	- 0.53
4b		0			- 0.07	- 0.46	- 0.56	0	- 0.27	0
5					0	- 0.05	0	0	- 0.02	0

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Proceedings 1977 British Crop Protection Conference - Pests and Diseases

CHEMICAL CONTROL OF THE WHEAT BULB FLY IN SCOTLAND

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Summary Deaths of wild geese in east Scotland in 1971-72 and 1974-75 have been attributed to the ingestion of wheat seed treated with carbophenothion, and to a less extent dieldrin, for control of the wheat bulb fly (Delia coarctata). Likewise in 1971-72, the poisoning of wood pigeons and other birds in Lothian Region was ascribed to the consumption of wheat seed treated with aldrin or dieldrin. Field trials were begun in 1974 to test potential insecticidal alternatives to carbophenothion, aldrin and dieldrin for control of wheat bulb fly larvae. The chemicals chosen were isofenphos, fonofos, pirimiphos-ethyl, triazophos, chlorfenvinphos, permethrin and WL41706 as seed treatments; chlorfenvinphos e.c., chlorfenvinphos and fonofos granules as soil treatments; and chlorfenvinphos, omethoate and pirimiphos-methyl e.c.'s as sprays. The most active (i) seed treatments (ii) soil treatment and (iii) spray against larvae of the wheat bulb fly were respectively (i) isofenphos (0.2% a.i. to wt of seed, 1974-75 trials) and chlorfenvinphos (0.2% a.i. to wt of seed, 1975-76 trials), (ii) fonofos (10% granule at 14.0 kg/ha) and (iii) omethoate (57.5% e.c. at 1.8 l/ha).

INTRODUCTION

The wheat bulb fly (Delia coarctata) is found every year infesting winter wheat crops in Lothian, Central, Fife and Tayside Regions of east Scotland. It is a serious pest in some years, but in most years the numbers of larvae are low enough not to cause serious economic damage. Nevertheless, growers favour taking regular precautions against the occasional serious attack by using chemically treated seed. Wheat seed treatments have been implicated by Hamilton and Stanley (1975) and Hamilton et al (1976) as the cause of death among wild geese in Tayside Region in 1971-72 and 1974-75. The goose deaths were apparently caused by the ingestion of carbophenothion-treated seed. Some goose deaths and deaths of wild pigeons in Lothian Region in 1971-72 were found to be caused by the consumption of wheat seed treated with aldrin or dieldrin (Anon., 1972). Partly as a result of these incidents, aldrin and dieldrin were withdrawn as cereal seed treatments (Anon., 1973), and the distributors of carbophenothion agreed not to sell or promote its use in Scotland. In response to concern regarding the environmental hazards of some insecticidal cereal seed treatments, field trials were initiated in 1974 to test possible chemical alternatives to carbophenothion, aldrin and dieldrin for control of wheat bulb fly. The results of 1974-75 and 1975-76 trials are presented in this paper.

The chemicals tested in the field experiments were applied as seed treatments (1974-75 and 1975-76 trials), granules and sprays (1975-76 trials). Chlorfenvinphos was the only seed treatment to have been tested before 1974 under Scottish conditions (P. Osborne, personal communication) and for which avian toxicological data were available. Bunyan et al (1971) measured the acute oral toxicity of chlorfenvinphos to pheasants, pigeons and quail and found that the chemical was especially toxic to pigeons. This result may be compared favourably with the more general avian toxicology of carbophenothion which was found to be uniformly toxic to geese, pigeons and quail (Jennings et al., 1975).

Table 1

Field Trials - Site Details

	Forfar, Tayside	1974 and 75 Pittenweem, Fife	North Berwick, Lothian	1975 and 76 North Berwick, Lothian
Soil Type	imperfectly drained loam	sandy, clay loam; imperfectly drained	sandy, clay loam; imperfectly drained	sandy, clay loam; imperfectly drained
Previous Crop	narcissi	potatoes	potatoes	potatoes
Egg Count (millions/ha)	2×10^6	12.4×10^6	3.5×10^6	6×10^6
Sowing Date	7.11.74	21.11.74	15.11.74	13.11.75 and 16.12.75
Sowing Rate (kg/ha)	196	196	196	196
Seed Variety	Maris Huntsman	Maris Huntsman	Maris Huntsman	Maris Huntsman
Design	randomised block (4 replicates)	randomised block (4 replicates)	randomised block (4 replicates)	randomised block (3 replicates: seed + soil treatments 2 replicates: sprays)
Plot Size (ha)	0.01	0.01	0.01	0.01
Date of visual score of plant emergence	4.2.75	8.1.75	20.1.75	4.2.76
Sampling Date	3.4.75	15.4.75	4.4.75	23.3.76) seed + 26.4.76) soil treatments 6.4.76 sprays
Harvest Date	28.8.75	1.9.75	1.9.75	28.8.76

METHODS AND MATERIALS

The chemicals tested in the three 1974-75 field experiments were all seed treatments (Table 2).

In the 1975-76 experiments, seed treatments, soil treatments in the form of granules and sprays applied before sowing and foliar sprays applied in the spring were tested (Table 2). The seed treatments were applied using the miniature 'Rotostat' at Rothamsted Experimental Station. All treatments were used on seed sown in mid-November and mid-December, 1975 (Table 1) in an attempt to show whether there was a greater benefit from control of the wheat bulb fly in a later sown crop than in an earlier sown crop. This attempt was unsuccessful because the December-sown wheat failed to establish so that the results discussed in this paper relate only to the November-sown wheat. Egg hatch and the relative proportions of first, second and third instar larvae in the November-sown control plots were monitored weekly from 9 February to 24 May 1976.

Table 2

Field Trials - Chemical Details
(including visual scores of plant emergence in seed treatment plots)

(a) Seed Treatments

Chemical	% a.i. to wt of seed	Visual score of plant emergence 1974-75 (max 40) Tayside	Fife	Lothian	1975-76 (max 30) Lothian
Isofenphos	0.2	28	29	32	-
Fonofos	0.2	34	32	36	-
Pirimiphos-ethyl	0.2	40	40	40	-
Triazophos	0.2	24	36	24	-
Chlorfenvinphos	0.2	30	32	30	21
Permethrin	0.2	-	30	-	27
WL41706	0.2	-	-	-	26
Control (organomercury fungicide only)	-	38	29	40	30

(b) Soil Treatments

1975-76 Lothian

Chemical	Formulation	Rate of application
Chlorfenvinphos	10% granule	16.8 kg/ha
Chlorfenvinphos	24% e.c.	3.5 l/ha
Fonofos	10% granule	14.0 kg/ha

(c) Sprays (1st applied 2 March; 2nd applied 16 March; 3rd applied 1 April, 1976)

Chemical	Formulation	Rate of application
Chlorfenvinphos	24% e.c.	5.6 l/ha
Omethoate	57.5% e.c.	1.1 l/ha
Pirimiphos-methyl	50% e.c.	1.8 l/ha

In both years, all seed was treated with a liquid organomercury fungicide.

The seedlings on seed and soil treatment plots were scored visually for emergence before the wheat bulb fly eggs began to hatch in the spring to index phytotoxic effects of the chemicals. The plots were scored from 0 to 10 depending on the degree of seedling emergence and growth. The treatment which appeared to least affect plant growth was assigned a maximum of 10 for each replicate and all other treatments were indexed relatively (Table 2).

Three thirty-centimetre paired row samples were taken either at random from each plot (1974-75) or at equidistant points along the same diagonal of the plots (1975-76) and counts made of wheat bulb fly larvae (alive and dead) and healthy and damaged shoots. Plots were sampled once in the 1974-75 trials and twice in the 1975-76 trials at approximately one month's interval. The results of the two samples were combined to give a more complete analysis of the effects of the experimental chemicals than could be obtained by a single sample. Plots which were sprayed in the 1975-76 trials were sampled both before and after spraying. Details of trial sites for both years and sowing, sampling and harvest dates are given in Table 1. Chemical formulations and rates of application are given in Table 2.

RESULTS

1974-75 field trials

The most phytotoxic seed treatment at Tayside and Lothian sites was triazophos (Table 2). It was not, however, as phytotoxic as isofenphos at the Fife site. Isofenphos was found to adversely affect seedling emergence at all sites. Chlorfenvinphos and permethrin were also found to affect the emergence of seedlings, but not to the same degree as isofenphos. Fonofos was marginally phytotoxic at all sites. Pirimiphos-ethyl was the least phytotoxic seed treatment. It did not affect seedling emergence at any site.

Isofenphos was the only seed treatment to reduce the percentages of damaged shoots and plants with live larvae relative to controls at all sites (Table 3). Although fonofos and chlorfenvinphos reduced the percentages of plants with live larvae at all sites, the chemicals were found to reduce shoot damage only at the Fife and Lothian sites. Triazophos reduced the percentages of damaged shoots and plants with live larvae only at the Tayside and Fife sites. Pirimiphos-ethyl reduced shoot damage at the Fife and Lothian sites, but the chemical reduced the percentage of plants with live larvae only at the Fife site. Permethrin, which was tested only in Fife, reduced shoot damage, but did not reduce the percentage of plants with live larvae relative to control.

No seed treatment improved the number of healthy shoots in unit length of row compared with controls at any site (Table 3).

Final yields were not improved by the treatments except in the case of isofenphos at the Lothian site (Table 5). Isofenphos was found, however, to reduce yield relative to control at the Tayside site.

1975-76 field trials

All seed and soil treatments were found to reduce significantly the percentages of damaged shoots and plants with live larvae (Table 4). The number of healthy shoots in unit length of row was increased by all treatments except chlorfenvinphos seed treatment which was observed to adversely affect seedling emergence (Tables 2 and 4). Although chlorfenvinphos was phytotoxic, all treatments gave increased yields relative to controls (Table 6).

Table 3

Field Trials 1974-75

Chemical	Site	% damaged shoots	% plants with live larvae	Healthy shoots in a 1.8 m length of row
Isofenphos	Tayside	8.2	1.9	167.5
	Fife	6.4	2.8	267.7
	Lothian	12.7	14.0	252.7
Fonofos	Tayside	12.1	12.3	210.5
	Fife	7.8	6.4	224.2
	Lothian	14.6	14.7	154.0
Pirimiphos-ethyl	Tayside	15.0	22.1	146.8
	Fife	9.6	10.9	280.7
	Lothian	14.1	22.6	262.0
Triazophos	Tayside	11.0	2.4	179.0
	Fife	7.5	6.7	232.0
	Lothian	18.1	24.8	180.3
Chlorfenvinphos	Tayside	14.8	17.8	145.3
	Fife	6.5	0.0	261.7
	Lothian	11.0	14.0	189.3
Permethrin	Fife	9.4	15.5	347.5
Control	Tayside	15.8	24.7	189.7
	Fife	17.3	23.4	283.5
	Lothian	21.0	30.7	226.5
S.E. difference	Tayside	2.2	3.3	27.3
	Fife	2.3	4.0	33.5
	Lothian	2.1	4.6	32.7

The only spray treatment to reduce the percentage of damaged shoots was omethoate. This effect was produced by a single application of the chemical. All omethoate spray treatments reduced the percentage of plants with live larvae. The percentage of plants with live larvae was reduced by chlorfenvinphos and pirimiphos-methyl only after two or three sprays. All sprays of omethoate, the first and second sprays of chlorfenvinphos and the second spray of pirimiphos-methyl increased the number of healthy shoots in unit length of row. All three chemical spray treatments increased yield, independent of the number of spray applications.

DISCUSSION

In the 1974-75 trials, isofenphos was the most active chemical for control of wheat bulb fly larvae; this activity has also been observed by Griffiths *et al.* (1975). The treatment reduced larval invasion of the plants and killed larvae within plants; although it was slightly phytotoxic, yield was not adversely affected. Unfortunately however, the chemical was withdrawn by the manufacturers from further development as a seed treatment for control of wheat bulb fly larvae, and was therefore not used in 1975-76. Although triazophos was markedly phytotoxic, fonofos gave promising results in 1974-75 reducing larval invasion of plants and

Table 4

Field Trials 1975-76 Lothian

Chemical	% damaged shoots	% plants with live larvae	Healthy shoots in a 1.8 m length of row
(a) Seed Treatments			
Chlorfenvinphos	8.3	2.9	88.6
Permethrin	11.0	12.4	128.6
WL41706	18.0	15.8	112.0
(b) Soil Treatments			
Chlorfenvinphos granules	11.2	7.4	112.2
Chlorfenvinphos e.c.	16.2	13.9	111.9
Fonofos granules	10.1	9.5	143.9
Control	30.2	26.1	71.8
S.E. difference	3.5	3.2	17.3
(c) Sprays			
Chlorfenvinphos e.c.			
Spray 1	31.1	39.2	187.0
Spray 2	27.0	25.2	203.5
Spray 3	25.8	25.3	160.5
Omethoate e.c.			
Spray 1	23.7	26.0	224.0
Spray 2	29.3	19.2	205.0
Spray 3	25.0	15.8	234.0
Pirimiphos-methyl e.c.			
Spray 1	28.6	42.4	164.5
Spray 2	25.9	29.7	206.5
Spray 3	29.5	32.5	164.5
Control	33.9	42.3	127.0
S.E. difference	4.3	3.7	24.5

killing larvae within plants, but, like isofenphos, neither was available in 1975-76.

The withdrawal of isofenphos, fonofos and triazophos experimental seed treatments left only three chemicals for testing in 1975-76. Pirimiphos-ethyl seed treatment was not included in the 1975-76 trials because it gave virtually no control of infestation of wheat plants by wheat bulb fly larvae in the previous year's trials. Chlorfenvinphos and permethrin were included in 1975-76 because they gave good control of wheat bulb fly larvae in 1974-75. In both years, chlorfenvinphos, the standard commercial seed treatment, was found to reduce larval invasion and kill larvae within plants. Permethrin reduced larval invasion in both trials, but the chemical killed larvae within plants only in the 1975-76 trials. WL41706, a synthetic pyrethroid like permethrin, was also found to reduce larval invasion and kill larvae within plants in 1975-76.

Soil treatments before sowing and foliar sprays in the spring were investigated in 1975-76 as possible chemical controls which, unlike seed treatments, would not have the same potential for causing deleterious side effects on wildlife. All soil treatments reduced larval invasion and killed larvae within plants. However, it is probable that growers of winter wheat will not generally use the soil treatments because of their high cost compared to the much cheaper and equally active seed treatments. A possible alternative to seed treatment is a spray when wheat bulb fly larvae invade seedlings in spring. Omethoate was the most active spray chemical (see Maskell and Davis, 1974), reducing larval invasion and killing larvae within the plants after only one application. Two applications of chlorfenvinphos and pirimiphos-methyl were found to be necessary to give approximately the same degree of

Tables 5 and 6

Field Trials 1974-76 - Grain Yields at 15% moisture content

1974-75

Chemical	Site	Yield (t/ha)
Isofenphos	Tayside	6.9
	Fife	7.3
	Lothian	7.3
Fonofos	Tayside	7.3
	Fife	8.1
	Lothian	6.8
Pirimiphos-ethyl	Tayside	7.3
	Fife	7.4
	Lothian	7.2
Triazophos	Tayside	6.3
	Fife	7.4
	Lothian	6.8
Chlorfenvinphos	Tayside	7.3
	Fife	7.3
	Lothian	7.0
Permethrin	Fife	7.4
Control	Tayside	7.4
	Fife	7.3
	Lothian	6.8
S.E. difference	Tayside	0.2
	Fife	0.4
	Lothian	0.2

1975-76

Chemical	Yield (t/ha)
(a) Seed Treatments	
Chlorfenvinphos	6.3
Permethrin	6.4
WL41706	6.4
(b) Soil Treatments	
Chlorfenvinphos granules	6.3
Chlorfenvinphos e.c.	6.1
Fonofos granules	6.1
Control	5.4
S.E. difference	0.2
(c) Sprays	
Chlorfenvinphos e.c.	
spray 1	6.1
spray 2	6.5
spray 3	6.1
Omethoate e.c.	
spray 1	6.2
spray 2	6.3
spray 3	6.3
Pirimiphos-methyl e.c.	
spray 1	6.0
spray 2	6.1
spray 3	6.0
Control	5.4
S.E. difference	0.2

control, but neither chemical reduced larval invasion even after three applications. The first spray of omethoate which achieved adequate control of the wheat bulb fly larvae was applied at 50-60% egg hatch when 90% of the larval population was first instar and the remainder second instar. However, a drawback to the application of sprays in February and March is the likelihood of unsuitable soil conditions for mechanical field operations at that time of year.

Acknowledgements

The author wishes to thank Dr. P. Osborne and Dr. D. Graham for helpful comments on the manuscript; Miss A. Walker, Mr. C. Kasperek and Mr. J. Cheyne for technical assistance; and Department of Insecticides and Fungicides, Rothamsted Experimental Station for treating seed.

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Proceedings 1977 British Crop Protection Conference - Pests and Diseases

THE EFFECTS OF FOLIAR FUNGICIDES ON SOME INSECT PESTS OF CEREALS

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Summary The effects of foliar fungicides on Lema melanopa, Sitobion avenae and Metopolophium dirhodum were investigated in laboratory trials. Contact with benomyl did not reduce the hatchability of Lema eggs but the survival of larvae hatching from treated eggs was lower than in the controls. Contact with benomyl did not reduce the survival of Lema adults but increased the mortality of newly hatched larvae. Similarly, the mortality of Lema larvae reared on barley leaves treated with benomyl was greater than in the controls. The contact toxicity of benomyl to adults and nymphs of M.dirhodum was high and the survival of M.dirhodum and S.avenae was reduced when reared on leaves treated with benomyl. Both benomyl and carbendazim reduced the reproductive rate of S.avenae and M.dirhodum but triadimefon did not affect either species.

INTRODUCTION

Since 1969 many foliar fungicides have been introduced into agriculture to control cereal leaf diseases such as mildew (Erysiphe graminis), leaf blotch (Rhynchosporium secalis) and yellow rust (Puccinia striiformis). In particular, compounds such as benomyl, carbendazim and tridemorph are now widely used on both spring and winter barley and winter wheat. For example, in our 62 km^2 West Sussex study area the percentage of cereal fields treated with foliar fungicides has increased from 0% in 1970 to 55% in 1976 (Potts, 1977).

A number of studies (e.g. Catling, 1969; Parr & Binns, 1970; Spadafora & Lindquist, 1972; Nakashima & Croft, 1974) have shown that benomyl has acaricidal properties whilst Stringer & Wright (1973) demonstrated that it would kill earthworms. Benomyl is also toxic to the eggs of the cabbage maggot (Delia brassicae) (Reyes & Stevenson, 1975) and adults of a ladybird (Stethorus punctum) (Colburn & Asquith, 1973). High concentrations of benomyl have also been shown to be toxic to aphids in glasshouses (Binns, 1970; Wilding, 1972).

Apart from these studies there is little information available on the effects of the commonly used foliar fungicides on insects. In view of the scale on which these compounds are now used experiments were carried out in the laboratory to investigate the effects of three systemic fungicides on some insect pests of cereals. The effects of these compounds on other insects will be considered elsewhere.

MATERIALS AND METHODS

Cereal leaf beetles (Lema melanopa) and cereal aphids (Sitobion avenae and Metopolophium dirhodum) were reared on spring barley (cv. Aramir) plants in the laboratory at fluctuating temperatures. Adults and immature stages of the insects used in the tests were obtained from the cultures. The fungicides used in the tests were benomyl (Benlate 50% w.p.), carbendazim (Bavistin 50% w.p.) and triadimefon (Bayleton 25% w.p.) and all amounts of fungicides refer to the active ingredient.

As the eggs of Lema melanopa are uaually cemented to the leaf tissue the contact toxicity of benomyl to them was initially determined by dipping segments of barley leaves, on which eggs had been laid, in suspensions of benomyl ranging from 0.01% to 1.0% a.i. In subsequent tests, however, eggs were successfully removed from the leaf tissue and dipped in suspensions of benomyl. Control eggs were dipped in distilled water. After treatment the eggs were placed on damp filter paper in petri dishes. Numbers of newly hatched larvae were counted every day and the criterion of viability used was their ability to crawl. Each replicate consisted of thirty eggs and each treatment was replicated six times. Larvae which hatched from the eggs were transferred to separate petri dishes and their mortality was assessed after three days. There were four replicates, each of thirty larvae.

A similar procedure was used to determine the contact toxicity of benomyl to the newly hatched larvae and adults of Lema melanopa and to the first instar nymphs and adults of the cereal aphid Metopolophium dirhodum. The insects were dipped in suspensions of benomyl and then placed on filter paper to dry. They were then confined in petri dishes on damp filter paper with segments of untreated barley leaves and mortality was assessed at intervals after treatment. The concentrations of benomyl tested are given in Tables 3 and 5.

The oral toxicity of benomyl to the newly hatched larvae of Lema melanopa and to the first instar nymphs and adults of Sitobion avenae and Metopolophium dirhodum and of carbendazim and triadimefon to adult S.avenae was determined by allowing them to feed on segments of barley leaves removed from plants which had been sprayed to run-off with suspensions of the fungicides. The concentrations of the fungicides used in the tests are given in Tables 4, 6 and 7. As described previously, the insects were kept in petri dishes and mortality was assessed daily. In all tests each replicate consisted of thirty individuals and each treatment was replicated six times.

In further tests the oral toxicity of benomyl, carbendazim and triadimefon to Sitobion avenae and Metopolophium dirhodum was determined by confining adults or first instar nymphs with barley plants. Barley plants were grown from seed in 16 cm plant pots; there were fifteen plants per pot. When the plants were at the four leaf-stage they were sprayed to run-off with 0.15% a.i. suspensions of benomyl or carbendazim or a 0.075% a.i. suspension of triadimefon. Plants sprayed with distilled water acted as controls. Two days later fifty adult or one hundred first instar nymphs were added to each pot and each treatment was replicated four times. Plants and insects were kept in cages in the laboratory and numbers of live aphids were counted either five or fourteen days after treatment.

RESULTS

In the first experiment the hatchability of Lema eggs was generally low (Table 1). Lema females tend to lay their eggs near to areas of leaf damaged by their feeding and a high proportion of the eggs, particularly in the controls, was affected by a fungus which developed on the damaged leaf tissue and then spread to the eggs. Benomyl inhibited the development of this fungus and a greater number of treated than control eggs hatched successfully, although the difference was not significant (Table 1).

Tests carried out with isolated eggs confirmed that benomyl did not affect the hatchability of Lema melanopa eggs, even when high concentrations of the chemical were used (Table 1).

However, the survival of larvae which hatched from eggs treated with 0.5% and

Table 1

Contact toxicity of benomyl to the eggs of Lema melanopa

Treatments	Mean no. (per replicate) eggs hatching	
	Eggs on leaf tissue	Isolated eggs
Control	14.7	24.5
0.01% benomyl	16.7	-
0.1% benomyl	19.5	26.5
0.5% benomyl	-	26.0
1.0% benomyl	18.3	23.0

No significant ($P > 0.05$) differences between treatments

1.0% benomyl was significantly lower than the controls (Table 2). In addition, feeding by those larvae which survived the treatment was much reduced compared with control larvae. There was no significant difference between the survival of larvae hatched from eggs treated with 0.1% benomyl and the controls (Table 2).

Table 2

Mean no. (per replicate) Lema melanopa larvae alive three days after hatching from eggs treated with benomyl

Treatments	Mean no. (per replicate) live larvae
Control	24.0
0.1% benomyl	22.3
0.5% benomyl	17.5 *
1.0% benomyl	8.0 ***

* $P < 0.05$
***$P < 0.001$

Whilst benomyl had no contact toxicity to the adults of Lema melanopa (Table 3) the survival of newly hatched larvae was significantly lower than in the controls (Table 3).

Although the survival of Lema larvae reared on leaves treated with 0.1% benomyl was slightly lower than in the controls, the difference was not significant (Table 4). However, the survival of larvae reared on leaves treated with either 0.5% or 1.0% benomyl was significantly lower (Table 4). As in the previous test control larvae fed more than treated larvae and there was a marked tendency for the latter to leave the leaves and wander round the filter paper in the petri dishes. Similar results were obtained when the first instar larvae of another chrysomelid beetle (Gastrophysa polygoni) were confined with leaves of knotgrass (Polygonum aviculare) which had been treated with benomyl (Vickerman, unpublished).

Table 3

Contact toxicity of benomyl to adults and first instar larvae of *Lema melanopa*

Treatments	Mean no. (per replicate) live insects three days after treatment	
	Adults	Larvae
Control	30.0	23.1
0.1% benomyl	29.5	19.5 *
1.0% benomyl	29.3	4.6 ***

* $P < 0.05$
*** $P < 0.001$

Table 4

Oral toxicity of benomyl to first instar larvae of *Lema melanopa*

Treatments	Mean no. (per replicate) live larvae five days after treatment
Control	26.0
0.1% benomyl	22.4
0.5% benomyl	11.8 **
1.0% benomyl	2.5 ***

** $P < 0.01$
*** $P < 0.001$

The contact toxicity of benomyl to both the first instar nymphs and adults of *Metopolophium dirhodum* was high (Table 5). Within seconds of treatment the aphids became lethargic and paralysis was soon evident.

The mortality of first instar nymphs and adults of both *Sitobion avenae* and *Metopolophium dirhodum* reared on barley leaves treated with 0.15% and 0.30% suspensions of benomyl was also significantly higher than when reared on untreated leaves (Table 6).

Table 5

Contact toxicity of benomyl to first instar nymphs and adults of Metopolophium dirhodum

Treatments	Mean no. (per replicate) live aphids three days after treatment	
	Nymphs	Adults
Control	29.5	30.0
0.15% benomyl	6.0 ***	5.3 ***
0.30% benomyl	1.2 ***	3.0 ***

*** $P < 0.001$

Table 6

Oral toxicity of benomyl to first instar nymphs and adults of Sitobion avenae and Metopolophium dirhodum

Treatments	Mean no. (per replicate) live aphids five days after treatment			
	S.avenae		M.dirhodum	
	Adults	Nymphs	Adults	Nymphs
Control	28.7	28.0	30.0	28.3
0.15% benomyl	16.0 **	17.3 *	18.3 **	15.7 **
0.30% benomyl	6.3 **	10.5 **	4.5 **	8.5 **

* $P < 0.05$
** $P < 0.01$

In further tests the mortality of Sitobion avenae adults reared on barley leaves treated with either 0.15% benomyl or 0.15% carbendazim was significantly greater ($P < 0.01$) than that of adults reared on untreated leaves (Table 7). However, the mortality of aphids reared on leaves treated with 0.075% triadimefon was not significantly different from the controls (Table 7).

There was no significant difference between the numbers of Sitobion avenae nymphs produced on barley plants treated with triadimefon and the numbers produced on control plants (Table 8). However, significantly fewer nymphs were produced on plants treated with either benomyl or carbendazim (Table 8).

Table 7

Toxicity of benomyl, carbendazim and triadimefon to adult Sitobion avenae

Treatments	Mean no. (per replicate) live aphids five days after treatment
Control	29.1
0.15% benomyl	14.3 **
0.15% carbendazim	16.7 **
0.075% triadimefon	27.8

** $P < 0.01$

Table 8

Mean no. (per replicate) live nymphs found on barley plants treated with benomyl, carbendazim and triadimefon five days after the introduction of Sitobion avenae adults

Treatments	Mean no. (per replicate) live nymphs
Control	327.5
0.15% benomyl	48.0 ***
0.15% carbendazim	64.5 ***
0.075% triadimefon	301.0

*** $P < 0.001$

Similarly, when Metopolophium dirhodum nymphs were caged with plants treated with the different fungicides significantly fewer live aphids were found on plants treated with carbendazim or benomyl than on control plants fourteen days after treatment (Table 9). There was no significant difference between the numbers of aphids found on control and triadimefon-treated plants (Table 9).

DISCUSSION

Although benomyl did not affect the hatchability of Lema eggs, the survival of larvae hatching from eggs treated with high concentrations of benomyl was reduced, perhaps indicating an ovicidal effect. Concentrations of benomyl (0.1% and 0.15%) which approximated to those normally used in the field were also toxic to Lema larvae and very toxic to the cereal aphids Sitobion avenae and Metopolophium dirhodum. The data for the two cereal aphid species confirmed the results obtained by Sagenmüller (1977). There was little feeding by Lema larvae and cereal aphids became particularly

Table 9

Mean no. (per replicate) live aphids found on barley plants treated with benomyl, carbendazim and triadimefon fourteen days after the introduction of Metopolophium dirhodum nymphs

Treatments	Mean no. (per replicate) live aphids
Control	619.0
0.15% benomyl	31.5 ***
0.15% carbendazim	73.2 ***
0.075% triadimefon	564.0

*** $\langle P$ 0.001

restless when feeding on treated leaves and it is likely that the chemical also had a repellent effect. Sagenmüller (1977) noted that more cereal aphids settled on untreated winter barley plants than on plants treated with benomyl during the three day period after the application of the chemical. According to Stringer & Wright (1973) earthworms would not feed on leaf material with spray deposits of benomyl; they also suggested that the toxicity of benomyl to earthworms was attributable to the anti-cholinesterase activity of the carbamate moiety of the molecule. The fact that both the carbamate fungicides benomyl and carbendazim but not the triazole fungicide triadimefon were toxic to cereal aphids tended to support this suggestion.

Some preliminary experiments were carried out in 1977 to assess the effect of benomyl on field populations of Sitobion avenae. Whilst the data available so far confirm the results obtained in the laboratory, the effects of this chemical on cereal aphid populations in other situations, other years or in the long term is not predictable. Zimmermann (1976) found that benomyl inhibited the development of cereal aphid pathogens (Entomophthora sp.) in vitro and it is possible that this chemical has similar effects in the field. For example, Nanne & Radcliffe (1971) demonstrated that the application of a number of foliar fungicides to a potato crop resulted in a reduction in the proportion of peach-potato aphids (Myzus persicae) infected by fungal pathogens and consequently higher numbers of aphids in treated than control plots.

Of the other commonly used foliar fungicides tridemorph has been found to increase the reproductive rate of cereal aphids (Sagenmüller, 1977) and also to completely inhibit the development of Entomophthora (Zimmermann, 1976). There is also some evidence (Vickerman, unpublished) that higher populations of cereal aphids are found in cereal fields treated with tridemorph than in untreated fields. Whilst the use of some fungicides may therefore result in a reduction in the numbers of pests such as cereal aphids, others may exacerbate pest problems in the field. It would surely be prudent to investigate the effects of these fungicides on pests and beneficial insects in the field, preferably before rather than after their widespread use on farmland.

Acknowledgements

I would like to thank Mr. D. Hesketh for his help throughout the course of the

work and Bayer U.K. Ltd. for supplying a sample of triadimefon. The work was financed by a research grant from the Agricultural Research Council.

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Proceedings 1977 British Crop Protection Conference - Pests and Diseases

A LABORATORY METHOD FOR TESTING SEED TREATMENTS FOR THE CONTROL OF SLUGS IN CEREALS

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Summary Seed treatment has been investigated as an alternative to molluscidal pellets for controlling the grey field slug (Deroceras reticulatum, formerly Agriolimax reticulatus) in cereals. A simple laboratory test is described for measuring the numbers of slugs killed and seeds protected by different seed treatments.

Preliminary results with a range of chemicals have shown that treating seeds with thiocarboxime or methiocarb at 0.2% a.i./wt of seed decreased feeding and killed more than half the slugs. Seeds treated with 0.2% of ioxynil, bromoxynil or SAN 155 (5-dimethylamino-1,2,3-trithiane-hydrogenoxalate) failed to kill slugs but greatly decreased feeding. Germination tests of seeds treated with the more promising materials are reported.

Résumé On a examiné le traitement des semences comme méthode alternative aux granulés molluscides pour le contrôle de la limace (Deroceras reticulatum, autrefois Agriolimax reticulatus) dans les cultures de céréales. On décrit une méthode simple de laboratoire pour évaluer le nombre des limaces mortellement atteintes ainsi que celui des graines protégées par les différents traitements.

Des résultats préliminaires portant sur une variété de produits chimiques ont montré que le traitement des graines au thiocarboxime ou methiocarb à 0,2% de matière active par poids de graines a réduit la consommation des graines et a entrainé une mortalité de plus de la moitié des limaces. Le traitement des graines avec 0,2% de "ioxynil", "bromoxynil" ou "SAN 155 (5-dimethylamino-1,2,3-trithiane-hydrogen-oxalate)" n'a pas provoqué la mort des limaces mais a considérablement réduit la consommation des graines. On rapporte des tests de germination sur des graines traitées par les produits présentant le plus de promesses.

INTRODUCTION

Slug damage to cereal crops is most severe in winter wheat, particularly on heavy soils. The most important damage is done shortly after drilling because slugs hollow out the seed, showing a preference for the embryo. This results in gaps in the growing crop. Grazing by slugs on older seedlings causes the characteristic shredding of leaves but this seldom reduces yield significantly. Both types of damage are caused mainly by the grey field slug (Deroceras reticulatum, formerly Agriolimax reticulatus) and only occasionally by the garden slug (Arion hortensis). Slug damage has become more serious in recent years with the advent of direct-drilling techniques, since surface trash and drilling slits associated with these cultivations provide favourable conditions for slugs (Edwards, 1975).

Current methods of control rely on baits containing metaldehyde or methiocarb in the form of pellets either broadcast or drilled into the soil with the seed. These formulations are not always reliable because the pellets disintegrate in wet weather. There is a need for a more direct control method in the form of a seed treatment using a chemical which either poisons slugs or repels them. Gould (1962) tested some copper salts and metaldehyde as seed treatments both in the laboratory and the field. He used large doses, 1.0% a.i. to wt of seed, and found that the compounds gave only limited control and caused problems of phytotoxicity and slow flow of seed through the drill. Symonds (1975) reported a laboratory test in which seeds treated with thiocarboxime killed slugs and prevented grain damage. This paper describes a simple laboratory method for screening a wide range of chemicals for their effectiveness as seed treatments before evaluation in the field.

MATERIALS AND METHODS

Seed treatment

Winter wheat seed, cv. Maris Huntsman, was used. Most of the chemicals were obtained as "pure" or technical materials of >90% purity. These were formulated with talc to give 20% dusts. Exceptions were bendiocarb (80% dust), tazimcarb (80% dust) thiabendazole (60% dust), methiocarb (50% dust) and benomyl (50% dust), which were all diluted with talc to 20% dusts. "San 281" was available only as a 25% e.c. and this was mixed with talc to give a 5% dust. Carbendazim and permethrin were used as the 10% and 25% commercial seed treatments. The dusts were stuck to the seeds with 3% methyl cellulose. Chlorfenvinphos was applied as a 32% Birlane liquid seed dressing. All materials were tested at 0.2% a.i./wt of seed.

Neem treatments were prepared by sticking 5g of crushed seeds of neem (*Azadirachta indica*) to 100g wheat seed with methyl cellulose. Chemicals not identified by common names are listed below:-

PP 199 : 2'-chloro-2,4-dinitro-5',6-di-(trifluoromethyl)-diphenylamine

SAN 155 : 5-dimethylamino-1,2,3-trithiane-hydrogenoxalate

SAN 281 : N,N-dimethyl-2-[(1,1-dimethylethyl)dithio]-1-[[(1,1-dimethylethyl)-dithio]methyl]-ethanamine.

Test procedure

Thirty-one materials were tested against the grey field slug and ten of these were also tested against the garden slug. Slugs collected from the field, starved for three days, were confined individually in 75 x 20 mm glass tubes, each tube containing a single treated seed on damp cotton wool. The tubes were closed with perforated plastic lids and placed in a controlled environment of 12h light at 15°C followed by 12h darkness at 3°C for up to ten days. There were ten tubes (replicates) for each treatment. Groups of treatments tested at the same time (called an experiment) always included thiocarboxime as a standard and untreated seed as a control. Six experiments were done on the grey field slug and two on the garden slug. Numbers of live and dead slugs and numbers of seeds hollowed were counted periodically.

Germination tests

The effects of the treatments on germination of cereal seeds were tested in two ways.

Test A Seeds were placed between the inside wall of a glass crystallising dish and a piece of filter paper, held in place by filling the dish with moistened silver sand. Each treatment consisted of four dishes of twelve seeds. Lengths of germinated shoots were measured at eight days.

Test B Seeds were sown in pots of John Innes Compost. Each treatment consisted of five pots of twenty seeds. Emerged shoots were counted at eight days and fourteen days.

RESULTS

Variability between different experiments was assessed by comparing the results with untreated (control) seeds and thiocarboxime-treated seeds since these were included in every experiment. Although not all slugs survived in the presence of untreated seed and not all untreated seeds were damaged, the results were always markedly different from those with thiocarboxime-treated seed (Table 1).

Table 1

Results from controls (C) and thiocarboxime treatments (T) in six experiments

Expt.No.	Live slugs				Nos. damaged seeds			
	Day 3		Day 9/10		Day 3		Day 9/10	
	C	T	C	T	C	T	C	T
1	10	5	8	5	5	1	10	4
2	9	5	9	3	4	0	9	1
3	10	5	10	5	7	1	10	2
4	10	5	10	4	8	2	10	5
5	10	6	10	2	6	0	10	2
6	10	2	9	2	10	1	10	2
Mean	9.8	4.7	9.3	3.5	6.7	0.8	9.8	2.7

Most other treatments were tested only once (10 slugs/treatment) because slugs were scarce in 1976 when most of the work was done. Although statistical analysis of the results is not possible, the inclusion of untreated and thiocarboxime-treated seed in each test provided an internal check of its reliability.

To compare all the treatments tested in different experiments (Tables 2 and 3), the total numbers of live slugs and damaged seeds have been expressed as a percentage of their corresponding control.

In tests with the grey field slug (Table 2) only thiocarboxime gave less than half as many live slugs as the controls at the end of the test. No other treatment was as effective, but methiocarb, γ HCH, permethrin, copper oxychloride, copper sulphate and benomyl killed some slugs. Several compounds prevented damage to seeds. On the 3rd day of the test, thiocarboxime, methiocarb, chlorfenvinphos, the three copper salts, SAN 155, capsaicin, ioxynil and bromoxynil all gave less than half as many damaged seeds as the controls. However this effect persisted to the end of the test only with thiocarboxime, methiocarb, ioxynil and bromoxynil.

No treatment killed many garden slugs (Table 3). On the third day seed damage was less than 50% of that in the controls in seeds treated with copper sulphate, sodium pentachlorphenolate, SAN 155 and ioxynil. This level of protection did not last to the end of the test with any treatment.

Germination Tests

All treatments tested had an adverse effect on seedlings grown in the absence of compost. However, when seeds were sown in John Innes Compost, there was no serious effect on germination except with sodium pentachlorophenolate (Table 4).

TABLE 2

Effectiveness of chemical seed treatment against the grey field slug

Treatment	Numbers (as % of control) of Live slugs Day 3	Live slugs Day 9/10	Damaged Seeds Day 3	Damaged Seeds Day 9/10
Thiocarboxime*	48	38	12	27
Bendiocarb	111	100	150	111
Methiocarb	89	56	25	44
Dioxacarb	100	67	175	89
Tazimcarb	90	90	86	90
ɣ HCH	78	67	50	89
Permethrin	100	78	125	89
Chlorfenvinphos	111	100	25	89
Phorate	100	100	129	100
Fonofos	100	100	143	100
Copper oxychloride	100	60	13	90
Copper chloride	100	100	0	90
Copper sulphate	100	70	0	60
Benomyl	100	80	67	60
Carbendazim	100	111	100	100
Thiabendazole	100	111	100	100
SAN 155+	100	93	5	53
SAN 281	100	100	50	80
"Neem"	100	111	100	100
Capsaicin	90	100	30	90
PP 199	100	90	100	90
Metaldehyde	100	100	80	90
Na pentachlorophenolate	100	90	67	70
2,6,dichlorophenol	100	90	117	90
2,4,dibromophenol	100	100	117	80
3,5,dibromo-o-cresol	100	100	100	90
2-chloro-5methylphenol	100	100	90	100
4-chloro-2methylphenol	100	89	90	100
4-chloro-3methylphenol	100	111	90	100
Ioxynil	100	100	0	0
Bromoxynil	90	90	0	10

* Mean of 6 experiments

\+ Mean of 3 experiments

Table 3

Effectiveness of chemical seed treatments against the garden slug

Treatment	Numbers (as % of control) of Live slugs Day 3	Live slugs Day 9/10	Damaged seeds Day 3	Damaged seeds Day 9/10
Thiocarboxime[x]	100	85	66	83
Methiocarb[x]	95	85	55	83
Dioxacarb	100	80	57	100
ɣ HCH	100	90	71	100
Chlorfenvinphos	100	80	57	89
Copper sulphate	100	70	0	67
Na Pentachlorophenolate	100	90	14	56
Capsaicin	100	90	57	100
SAN 155[x]	100	100	0	80
Ioxynil[x]	100	95	13	69

[x] mean of 2 experiments.

Table 4

Germination Tests

Treatment	Test A Mean length of shoot (mm) at 8 days	Test B % Germination at 8 days	Test B % Germination at 14 days
Thiocarboxime	9	14	75
Methiocarb	19	54	86
Copper oxychloride	18	26	77
Copper chloride	14	38	75
Copper sulphate	14	39	72
SAN 155	9	42	65
SAN 281	9	4	63
Na Pentachlorophenolate	0	0	0
Control	26	56	78
* Ioxynil	3	33	49
* Bromoxynil	1	53	61
* Control	21	55	63

* Tested on a separate occasion to the other treatments

DISCUSSION

All ten chemicals tested against both the grey field slug and the garden slug were much less toxic to the latter species and less effective in preventing it from damaging the seed.

Of the materials tested on the grey field slug, thiocarboxime killed most slugs and controlled seed hollowing well. However its great toxicity to humans and wild life makes it hazardous for use as a seed treatment.

The molluscicidal properties of ioxynil and bromoxynil were first reported by Wain (1963) who described their effect on two water snails, hosts of Schistosoma mansoni. Stephenson (1967) reported that ioxynil acts as a contact and stomach poison to slugs and is also strongly repellent. In the tests reported here, ioxynil and bromoxynil were the only treatments to protect seeds completely from damage by the grey field slug. The repellent effect presumably prevented the slugs from acquiring a lethal dose. Both compounds have been developed as contact herbicides for the control of broad-leaved weeds in cereals and the germination tests suggest that there might be problems of phytotoxicity in soils with a low organic content. Other analogues of ioxynil and lower rates would be worth testing in the hope of eliminating the phytotoxic effect whilst retaining repellency to slugs. Sodium pentachlorophenolate prevented some slug damage but was also extremely phytotoxic. The other phenolic compounds tested had little or no effect on slug feeding or mortality.

The three copper salts were initially effective in preventing seed damage. This supports Gould's (1962) suggestion that some copper seed dressings are mild repellents but are unlikely to kill slugs.

SAN 155 and SAN 281 were examined because they belong to a novel class of pesticides related to nereistoxin, a naturally occurring substance found in marine annelid worms (Lumbrinereis spp.). The initial protection by SAN 155 was very effective and the damage recorded on the tenth day of the test was only slight. Performance might be even better in the field where slugs would have a choice of food.

Several chemicals gave good initial protection to the seeds, but the effect did not last. Possible reasons are chemical breakdown, leaching of chemical from the seed, or habituation to the chemical by the slug. The last factor may be less important under field conditions because the slugs would not be confined close to the seeds and would have a choice of food. Thus chemicals which gave protection for three days in these tests may be more effective in the field, and would be worth investigating further. In addition, more chemicals need to be screened to find a seed treatment with molluscicidal properties similar to thiocarboxime.

Acknowledgements

We thank Mr. M.H. Sherwood for technical assistance.

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Proceedings 1977 British Crop Protection Conference - Pests and Diseases

CROP PROTECTION BY CULTIVAR DIVERSIFICATION

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Summary Growing large acreages of single cereal cultivars increases the vulnerability of the crop to changes in pathogenicity. The development of increased virulence for a widely grown cultivar can render a large proportion of the national acreage liable to disease and subsequent yield loss. This risk can be lessened by farmers growing at least three different cultivars, each containing a different resistance factor. Schemes for cultivar diversification for crop protection against wheat yellow rust (Puccinia striiformis) and barley mildew (Erysiphe graminis f.sp. hordei) are described.

INTRODUCTION

The United Kingdom acreage of winter wheat and spring barley is dominated by a few, very widely grown cultivars (Table 1). In winter wheat, four cultivars accounted for 74% of total seed sales in 1975 - 76, with Maris Huntsman alone accounting for 34%. In spring barley, the situation is only slightly less extreme with five cultivars accounting for 61% of total seed sales during the same period.

A disadvantage of this type of crop production system is its vulnerability to increases in pathogenicity. Increased virulence for a widely grown cultivar, such as that detected in 1972 in Puccinia striiformis for the hitherto resistant winter wheat cultivar Joss Cambier (Priestley & Doling, 1974), can render a large proportion of the national acreage liable to a severe epidemic and subsequent yield loss. The magnitude of this risk can be judged by the rapidity with which farmers changed from Joss Cambier to other cultivars; seed sales of this cultivar dropped from 27% of total seed sales in 1971 - 72 to only 3% in 1972 - 73. Examples of the hazards of increased pathogenicity to other crops dominated by small numbers of cultivars have been given by Day (1973).

A practical way of reducing the vulnerability of the cereal crop is to grow at least three cultivars on each farm. If the cultivars are deliberately selected so that each contains a different specific resistance factor, a degree of mutual protection is generated for each cultivar. This is additional to the direct effect of the genetic resistance factors present in each cultivar. It arises because the pathogen is unlikely to acquire increased virulence for all the different specific resistances simultaneously. This has the effect of inhibiting the spread of disease between cultivars and thus affording a degree of additional crop protection.

In order to assist farmers in the selection of cultivars to grow together, two schemes have been developed under the auspices of the Physiologic Race Survey of Cereal Pathogens. These are for the protection of winter wheat against Puccinia striiformis, and winter and spring barley against Erysiphe graminis f.sp. hordei.

Table 1

Seed sales of winter wheat and spring barley by cultivar in the United Kingdom 1975 - 76, showing extent to which small numbers of cultivars dominate the market (after M.A.F.F., 1976)

cultivar	sales (tonnes)	% total sales
Winter wheat		
Maris Huntsman	55630	34.2
Bouquet	27703	17.0
Atou	19764	12.1
Flinor	16728	10.3
total 4 cvs	119825	73.6
total other 19 cvs	42882	26.4
total all cvs	162707	100.0
Spring barley		
Golden Promise	34251	16.7
Mazurka	29528	14.4
Maris Mink	24997	12.2
Midas	18807	9.2
Hassan	17516	8.5
total 5 cvs	125099	61.1
total other 27 cvs	79771	38.9
total all cvs	204870	100.0

CULTIVAR DIVERSIFICATION FOR PROTECTION AGAINST WHEAT YELLOW RUST

Determination of specific resistances in cultivars

Zadoks (1961) postulated that specific resistances against P. striiformis were of two types; 'overall' resistances effective at both seedling and adult plant growth stages, and 'mature (= adult) plant' resistances effective only at the adult plant growth stage.

Tests are carried out both on seedlings and adult plants to determine the specific resistances in cultivars. Each cultivar is inoculated with a series of isolates of known specific virulence, and the responses are compared with those of control cultivars of known specific resistance.

In the seedling tests, uredospores are inoculated onto the first leaf of each cultivar. Pots containing the seedlings are sealed in Polythene bags containing a small amount of water and then placed in a refrigerator at 7°C for 48 h. Rapid lowering of the temperature induces dew formation within the bags and this promotes spore germination and leaf penetration. The seedlings are then transferred to a controlled environment cabinet (18°C / 16 h light and 11°C / 8 h dark) for 14 - 18 days after which they are assessed for reaction type (types given in Doling, 1967). A low reaction type indicates cultivar resistance.

Adult plant tests are carried out by creating epidemics in replicated cultivar tussocks grown in Polythene tunnels (Priestley & Doodson, 1976). Each tussock is inoculated with a uredospore : talc mixture at growth stage 20 (for explanation of growth stage codes, see Zadoks et al, 1975). The Polythene provides a humid

Table 2

Winter wheat cultivars grouped according to their phenotypic resistance factors to Puccinia striiformis (yellow rust)

1	2	3	4
Atou	Grenade	Kinsman	Mega
Bouquet	Maris Huntsman	Maris Freeman	
Chalk	Maris Nimrod	Maris Ranger	
Flanders	Sportsman		
Flinor			
Gamin			
Kador	5	6	7
Maris Fundin			
Maris Widgeon	Cappelle-Desprez	Hobbit	Clement
Val	Champlein	Score	
West Desprez	Hawk		

atmosphere which encourages the rapid development of a polycyclic epidemic and also minimises cross contamination between isolates. The tussocks are assessed for Percent Attack (= percentage leaf area infected) at growth stages 37, 45, 58 and 70 using a modified version of the basic International Scale described by Doling (1967). A relatively low Percent Attack value indicates cultivar resistance.

The most important specific resistances identified to date have been allocated sequential numbers (Priestley & Byford, 1977); R 1 - R 10 are 'overall' resistances, R 11 - R 14 are 'adult plant' resistances. The relationship between these phenotypic resistance factors and previously published resistances 'genes' is being investigated.

Many of the currently important winter wheat cultivars have combinations of specific resistances. In some cultivars, combinations involving both types of resistances occur, eg Hobbit probably contains R 2, R 3, R 4 and R 14.

Groups of cultivars

Table 2 shows a selection of the currently important winter wheat cultivars grouped according to their resistance factors. The cultivars in group 1 contain what are probably a number of different 'adult plant' resistances which are, at present, effective against all known United Kingdom isolates of P. striiformis. Those cultivars in group 2 contain R 2 and R 3, those in group 3 contain R 6, that in group 4 contains R 12, those in group 5 contain resistances which are being further investigated, those in group 6 contain R 14 and that in group 7 contains R 9.

Selection of cultivars to grow together

1) Cultivars in group 1 have the highest level of resistance and can be grown together or with cultivars from other groups.
2) If the more susceptible cultivars in groups 2 - 7 are grown, do not grow together cultivars from within groups 2, 3, 5 or 6, but select cultivars from each of the different groups.

Table 3

Winter and spring barley cultivars grouped according to their phenotypic resistance factors to Erysiphe graminis f.sp. hordei (mildew)

0	1	2	3
Clermont	Igri	Armelle	Midas
Freegold	Malta	Berac	
Golden Promise	Senta	Imber	
Hoppel	Sonja	Julia	
Maris Otter	*Astrix	Katy	
Mirra	*Athene	Mosane	
		Zephyr	
4	**5**	**6**	**7**
Lami	Hassan	Ark Royal	Tyra
Lofa Abed	Maris Trojan	Tern	
Mala Abed	*Aramir	Wing	
Vada	*Athos	*Mazurka	
Varunda	*Maris Mink		
*Abacus	*Porthos		
*Georgie	*Uta		
*Luke			
*Sundance			
*Universe			

* do not grow with group 2 cultivars (see text)

CULTIVAR DIVERSIFICATION FOR PROTECTION AGAINST BARLEY MILDEW

Determination of specific resistances in cultivars

Specific resistances are determined by inoculating each cultivar with a series of isolates of E. graminis f.sp. hordei of known specific virulence and comparing the responses with those of control cultivars of known specific resistance.

All tests are carried out using first seedling leaves. Conidia are inoculated onto detached leaf segments maintained on dilute water agar containing benzimidazol a senescence inhibitor, in polystyrene boxes (Wolfe, 1969). The conidia are introduced into a small tower and allowed to settle onto the detached leaves arranged in the base (Wolfe & Schwarzbach, 1975). After inoculation, the leaf segments are maintained at 10 - 12°C for 10 - 14 days in controlled environment cabinets. The number of mildew colonies present on each leaf is then counted. A relatively low value indicates cultivar resistance.

The most important specific resistances have been allocated sequential numbers (R 1 - R 8), and the relationship between these phenotypic resistance factors and previously described resistance 'genes' has been reported (Wolfe & Wright, 1977).

Groups of cultivars

Table 3 shows a selection of the currently important winter and spring barley cultivars grouped according to their resistance factors. Those cultivars in group do not contain any relevant resistance factors. Those cultivars in group 1 contai R 1 or R 1 + 2, those in group 2 contain R 2, that in group 3 contains R 3, those

in group 4 contain R 4 or R 4 + 2, those in group 5 contain R 5 or R 5 + 2, those in group 6 contain R 6 or R 6 + 2, and that in group 7 contains R 7.

Selection of cultivars to grow together

1) Cultivars within each group should not be grown together.
2) The most useful combinations are between cultivars in groups 3, 4, 5 and 6.
3) Cultivars marked with an asterisk should not be grown with group 2 cultivars.

DISCUSSION

The principle of cultivar diversification for crop protection is not a new one. Indeed, advice on this subject for protection against wheat yellow rust has been given for some years (eg N.I.A.B., 1971). What is now proposed is an enlargement of the yellow rust scheme to incorporate more cultivars, and an extension of the diversification principle to include protection against barley mildew.

Experience with yellow rust has shown that the aim of diversification against this disease is to limit the potential threat of new virulences by ensuring that the winter wheat acreage is not dominated by a few, vulnerable cultivars. The size of this threat is illustrated by the findings of Walker & Roberts (1974) who estimated that 56% of the acreage of Joss Cambier in the epidemic year of 1972 was rated by farmers as being severely infected with yellow rust. The estimated mean yield loss of the crops rated as severely infected compared with those without severe rust was 0.7 t/ha. Examination of data from cultivar trials during the period 1957 - 76 has shown that epidemic years of yellow rust, such as 1972, occur on average about every three or four years (Priestley, 1978).

The situation in barley mildew is rather different in that disease surveys have shown that it is a severe problem in most years (King, 1973; Cock, 1975). The immediate aim of the diversification scheme for mildew is to use the presently available resistances more efficiently in an effort to reduce yield loss. The mildew resistance R 2 is present, either alone or in combination with other resistances, in a large number of cultivars. Growing such cultivars has, in the past, created a strong selection pressure for the corresponding virulence factor V 2. The result of this is that the frequency of V 2 is very high in the present mildew population. In practice, this means that diversification between cultivars containing R 2 alone (group 2 in Table 3) and those containing R 2 combined with other resistances (marked with an asterisk in Table 3) will have little beneficial effect in protecting the crop from mildew attack. On the other hand, it appears that there is a restriction to the rapid production of high frequencies of combinations of virulence factors V 3, V 4, V 5 and V 6, as these are rare in the present mildew population. Diversification between cultivars in groups 3, 4, 5 and 6 (Table 3) is therefore likely to be beneficial, at least in the short term.

The two diversification schemes will be revised annually by the Physiologic Race Survey Committee to take account of new cultivars. It is envisaged that these revised versions will be published in various forms shortly afterwards in the N.I.A.B. Farmers leaflet No 8 'Recommended varieties of cereals', the Scottish Agricultural Colleges leaflet 'Recommended varieties of cereals', and the A.D.A.S. booklet 'The use of fungicides for the control of cereal diseases'.

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