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OBSERVATIONS ON THE EFFECTS OF SOME SYSTEMIC CHEMICALS APPLIED TO CEREALS IN TRIALS AT THE N.I.A.B.

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Summary The N.I.A.B. is concerned with the testing of varieties of cereals for performance in yield and in quality. None of the varieties at present available are completely resistant to all of the major cereal diseases. Most of the varieties in the Recommended List (Farmers' Leaflet No. 8, 1969), although satisfactory in many respects, are susceptible to varying degrees to one or more diseases. The effective use of systemic chemicals could thus improve the yields of such varieties.

The N.I.A.B. has been examining the performance of varieties in the presence of systemic chemicals because varieties may react differently when treated and because the use of such chemicals permits assessments of infection to be related to losses in yield. These problems are considered in relation to barley yellow dwarf virus, mildew and eyespot.

INTRODUCTION

In a series of trials from 1967 to 1969 chemicals have been applied to cereals to investigate differences in varietal response to treatment and to study the effects of various aspects of infection upon yield. The three chemicals discussed in this paper are considered representative of those in use or under active development for the control of the aphid vectors of barley yellow dwarf virus, of cereal mildew and of eyespot.

Demeton-s-methyl is used at the N.I.A.B. to determine yield losses due to natural infections of BYDV in large plots of both winter and spring sown cereals. The performance of spring barley varieties in the absence of mildew (Erysiphe graminis)has been studied using ethirimol, which has also proved useful in studying the effect of time of infection upon yield. Similarly, the investigation into performance of varieties of wheat in trials where infection with eyespot (Cercosporella herpotrichoides is controlled has been facilitated by the use of benomyl.

The possible widespread use in agricultural practice of such chemicals raises problems in assessment of varietal performance. Tests of the type described below will, it is hoped, provide additional data on any differences in varietal reaction and of the economic importance of various cereal diseases.

1. METHODS AND MATERIALS - BYDV

For control of BYDV demeton-s-methyl (58% a.i. w/v) was applied to cereals from emergence to harvest at ten day intervals by means of a 2.5 gal knapsack sprayer at a concentration of 60 fl oz/20 gal to run-off. Sprayed and unsprayed replicates (35yd x 2 yd) were laid out alternately and each plot was separated from its neighbour by a barrier replicate of the same variety. The trial was subject to natural infection and yield was assessed as weight of grain/plot at 14% moisture content.

RESULTS - BYDV

Yield trials using demeton-s-methyl to control infestation by aphids, primarily Sitobion avenae and Rhopalosiphum padi, which carry BYDV all showed increases in yield.

Table 1

Insecticide - effects of aphids and BYDV on cereals - percentage increases in yield

Demeton-s-methyl sprayed at 10 day intervals from emergence to harvest.

Crop	Year: No. of replicates:	1967 1 ⁺+	1968 5	1969 5
Spring barley		6.4*	-	6.8*
Spring wheat		6.0*	-	-
Winter oats		-	8.3*	9.4***
Winter wheat		-	3.4	7.3***

⁺+ 1967 - Spring barley contained single plots of ten varieties and spring wheat single plots of eight varieties. Trials in 1968 and 1969 were all of one variety (replicated) per crop.

* ** *** - Significantly different from untreated plots at 5.0%, 1.0% and 0.1% respectively.

Yield data were variable for several reasons, yield increases in 1968 being particularly low despite abnormally large aphid populations mainly because of poor harvest conditions. The 1967 trials were not of replicated plots of one variety as in 1968 and 1969, but consisted of single plots of different varieties, the yields of which indicated possible differences in varietal response in treatment.

Table 2

Insecticide - effects of aphids and BYDV on cereals - examples of yield effects in different varieties in 1967

Demeton-s-methyl sprayed at 10 day intervals from emergence.

Crop	Variety	% increase in yield in sprayed plot
Spring wheat	Kloka	- 0.7
	Rothwell Sprite	- 4.4
	Arin	10.7
	Troll	17.7
Spring barley	Maris Concord	- 5.7
	Proctor	0.2
	Mosane	3.1
	Deba Abed	6.3
	Zephyr	16.3

The spraying interval of 10 days was thought to be possibly insufficient for maximum control. Leaves of winter wheat sprayed at different growth stages with demeton-s-methyl were therefore presented to the primary vector of BYDV - the bird cherry aphid (Rhopalosium padi) - every day following spraying until the aphids began to feed on the leaves. A linear decline in the duration of effective protection from 16 days to 9 days was recorded between growth stages 2 and 10.5.5. Maximum protection was therefore assumed to occur under a 10 day regime until approximately the beginning of flowering, i.e. growth stage 10.5.

DISCUSSION - BYDV

Initial spraying trials using demeton-s-methyl indicated apparent differences between varieties in their reaction to treatment. Routine infection tests at the N.I.A.B. have since shown that these differences are primarily due to inherent variations of varieties in their response to BYDV. The essential differences are between wheat, which is slightly more tolerant than oats and barley, and spring cereals which are more tolerant than winter cereals. Some of these differences can be seen in the data from spray trials (Table 1).

Although the potential yield loss in some varieties of winter barley and oats exceeds 80% (Saunders and Doodson, 1969) the average yield loss in most years at Cambridge has not exceeded 10%. By late July the majority of cereal crops (James, 1969) and grass leys (Doodson, 1966) are infected with BYDV most of which would appear to be derived from aphid infestations occurring relatively late in the growing season. Stage of growth at the time of infection is particularly important. The difference between potential and actual yield losses is therefore directly related to the dynamics of the viruliferous aphid population. At present it is questionable whether or not the naturally occurring losses due to BYDV can be reduced by the economic control of aphid populations. Further investigations into this problem are planned for the 1969/1970 season.

2. METHODS AND MATERIALS - MILDEW

Seed in mildew trials was dressed with ethirimol (80% a.i., 5-n butyl-2 ethylamine-4 hydroxy-6 methyl pyrimidine) at a rate of 2 lb/acre as a powder formulation. Mildew trials were located at all fourteen regional trials centres of the N.I.A.B. (Guide to varieties under trial, observation and multiplication, 1969). Each trial was composed of one 35 yd x 2 yd plot of each of ten varieties of spring barley. Duplicate plots of each variety were sown at each centre using undressed seed, although cultural conditions were not necessarily identical to those using treated material. A single trial of replicated plots of the same size of five varieties of spring barley was also sown at Cambridge in 1969. This contained both treated and untreated seed. The percentage level of mildew infection was assessed in all trials by the method of Large and Doling (1962). Yields in all trials were assessed as weight of grain/plot at 14% moisture content.

Effect of time of infection by mildew was investigated in glasshouse experiments. Seed of the spring barley variety Proctor was sown in 9 inch pots filled with John Innes compost. Seed of one replicate of sixteen pots was dressed with ethirimol at 2 lb/acre equivalent and served as an uninfected control. After a vernalisation period of six weeks, plants were thinned to six plants per pot and placed in the glasshouse utilising supplementary fluorescent lighting to obtain a 16 hour day routine. Subsidiary treatments were applied to control plants every fortnight by spraying with a 1000 p.p.m. solution of ethirimol to run-off which ensured almost complete control of mildew. Other replicates, each of sixteen pots, were infected with mildew at successively later growth stages; i.e. growth stage 2-3, 6 and 10 (Large, 1954), by dusting the plants with conidia of mildew. At harvest yield was assessed as weight of grain/plant.

RESULTS - MILDEW

The replicated trials at 12 of the regional trial centres produced significant alterations in the relative yields of all ten spring barley varieties. The results in Table 3 are incomplete and have yet to be statistically analysed.

Table 3

Fungicide - effects of ethirimol on the relative yields of spring barley varieties

Varieties	Relative yields (Sultan as 100%)		Mean % mildew in replicates	
	Treated	Untreated	Treated	Untreated
Sultan	100	100	0	0.9
Julia	102	89	0	4.2
Zephyr	104	93	0.01	9.5
Impala	99	88	0.01	7.3
Inis	104	90	0	5.6
Vada	97	85	0	1.9
Deba Abed	97	87	0	2.5
Mosane	100	89	0	7.7
Maris Badger	97	82	0.1	12.2
Proctor	99	88	0.01	8.6

Minor differences between cultural practices on treated and untreated plots made direct comparison of yields difficult. Differences in relative yields were not clearly associated with levels of mildew infection. Varietal response was sometimes much greater than expected, especially in Julia and Inis. The replicated trial at Cambridge in 1969 confirmed this conclusion and indicated that response was often greater than that estimated by the level of mildew using the method of Large and Doling (1962).

Table 4

Fungicide - effect of ethirimol upon the yields of five varieties of spring barley

Varieties	Mean % increase in yield in treated plots		Mean % mildew in trials	
	Recorded	Estimated	Treated	Untreated
Sultan	-3.9	5.5	2.5	5.0*
Deba Abed	6.8	7.0	2.3	8.3*
Julia	15.7***	7.0	1.3	7.5**
Impala	16.3***	11.0	3.4	17.5***
Proctor	18.9***	9.0	3.5	12.5***
Mean	10.8***	8.0	2.6	10.2***

* ** *** Significantly different from untreated plots at 5.0%, 1.0% and 0.1% respectively.

Ethirimol has also proved useful in studying the effects of time of infection with mildew upon yield. Reductions in grain weight/plant in glasshouse trials were clearly associated with time of infection (Table 5).

Table 5

Fungicide - effect of time of infection upon yield of Proctor spring barley in glasshouse trials

Control seed dressed with ethirimol at 2 lb/acre equivalent.

Growth stage when inoculated	% loss of yield	% mildew at G.S. 10.5.4.
2.5	98.3**	97.5
6	76.2**	95.5
10	51.0**	79.3
Control	0	2.4

** Significantly different from control plants at 1.0%

DISCUSSION - MILDEW

Trials at the N.I.A.B. have indicated that the control of mildew by the application of ethirimol may alter the performance of varieties and may also indicate the importance of early infections in reducing yields. The relative yields of spring barleys were not greatly altered in the absence of mildew except for the highest yielding variety (Sultan) which dropped to equal fourth place. Direct comparisons between untreated and treated plots of the same variety were made in only one trial at Cambridge but in both this trial and in the trials at regional centres there appeared to be apparent differences between the response of individual varieties to ethirimol. Julia in particular produced yield increases twice as great as expected from the levels of mildew. In the yield trial only Deba Abed produced additional grain corresponding to the expected increase. It is possible, however, that part of the increase in yield in both these and some other winter-sown trials may be associated with the stage of growth at the time of infection. Glasshouse trials using ethirimol to prevent mildew from attacking uninfected control plants indicated that reduction in yield were significantly higher with increasing earliness of infection.

3. METHODS AND MATERIALS - EYESPOT

Varieties of wheat in eyespot trials were sown in four rows/1 yd x 1 yd plots, each plot being separated by a single yard row of oats. All plots were inoculated by inserting straws into the soil at 6 inch intervals along each row. Each plot was divided in half across the rows by a polythene sheet. One half of each plot of each variety was sprayed using a 2.5 gal knapsack sprayer with a 2.27 a.i./gal solution of benomyl (methyl-1-butylcarbamoyl-2-benzimidazolecarbamate) to run-off at growth stage 9. Eyespot infection was assessed before harvest by recording number of whiteheads, degree of lodging and mean severity of lesions, and yield of grain/plot at harvest.

In glasshouse trials 80 to 100 seedlings/variety sown in 5 inch pots filled with John Innes compost were inoculated (Macer, 1966) by inserting infected straws over shoots just as they emerged. Seedlings were sprayed with benomyl (2.27 a.i./gal) when the second leaf began to emerge. Unsprayed seedlings infected with eyespot in the same manner served as controls. Eyespot infection was assessed as mean severity of lesions at the base of the seedlings after nine weeks of growth by measuring the depth of penetration of the fungus through successive leaf sheathes.

RESULTS - EYESPOT

The wide spectrum fungicide - benomyl- proved very effective in controlling eyespot. Eyespot on artificially infected seedlings was completely controlled in glasshouse tests by the application of a spray of benomyl just as the second leaf emerged. In the field trials lodging and whiteheads did not occur in treated plots despite maximum levels of 40% and 35% respectively in control plots. The incidence of severe lesions was rarely more than 5% of those in unsprayed plots. There were significant increases in yields of susceptible varieties as well as indications of differences in varietal response.

Table 6

Fungicide - effects of benomyl in controlling eyespot expressed as % increases in yield

Reaction type of variety	Number of varieties	Mean % increase in yield	Example varieties	% increase in yield
Resistant	18	8.6	Cappelle-Desprez	-4.6
			Joss Cambier	9.5
Moderately resistant	10	15.1*	Elite Lepeuple	-3.8
			Pv 1	25.2
			Pv 2	38.8
Susceptible	21	42.5**	Champlein	44.3
			Hybrid 46	69.5

* ** Significantly different from unsprayed plots at 5.0% and 1.0% respectively.

DISCUSSION - EYESPOT

The effect of benomyl on eyespot infections in wheat was very marked. In all treated plots there were no whiteheads, no lodging and very few severe lesions at the bases of the stems. Increases in yield occurred even amongst some resistant varieties and were far greater than were anticipated in some susceptible varieties, e.g. Hybrid 46. This phenomenon is not, however, surprising in view of the wide spectrum of fungicidal activity for benomyl

The use of systemic chemicals such as those mentioned in this paper in conjunction with resistant varieties could appreciably increase the national yields of cereal crops by controlling cereal pathogens.

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PATHOLOGICAL AND PHYSIOLOGICAL ASPECTS OF CEREAL MILDEW CONTROL USING ETHIRIMOL

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Summary Experiments were made on the effects of mildew (*Erysiphe graminis* f. spp.) and mildew control on the yield and yield components of the major cereals using ethirimol and resistant varieties. Greatest control of mildew with ethirimol was obtained with spring-sown wheat and barley: control was less effective on autumn-sown crops and with oat mildew. Grain yield was increased as a result of mildew control, the amount being dependent on environmental conditions and variety. In one experiment, mildew reduced yield partly through a reduction in tiller number and, presumably, partly as a consequence of a large reduction in root dry weight. Ethirimol applied to resistant varieties reduced yield/tiller and also root dry weight, which indicates that in certain conditions the real effect of mildew on susceptible varieties may be greater than that indicated by the use of ethirimol for control purposes.

INTRODUCTION

In 1968 a small quantity of ethirimol (5-butyl-2-ethylamino-4-hydroxy-6-methylpyrimidine) was obtained from I.C.I. Ltd., for trials on the control of cereal powdery mildew (*Erysiphe graminis* f. spp.) (Bebbington *et al.*, 1968). In a field experiment in that year a yield increase of approximately 25% was obtained with the barley varieties Maris Concord and Zephyr by controlling a severe mildew infection with ethirimol applied as a seed dressing at 1.5 lb a.i./ac (Johnson *et al.*, 1969). The interest generated by this and by the results of I.C.I. trials led to a range of experiments in 1969. The objectives of these experiments were:-

i. to repeat the findings of 1968 and analyse the effects on growth components and components of yield of the major cereals.

ii. to assess the comparative response of wheat, barley and oats to mildew control by ethirimol.

iii. to determine a varietal response if any, to mildew control.

iv. to compare mildew control in autumn- and spring-sown cereal crops.

v. to determine the effectiveness of mildew control in different environmental conditions.

vi. to determine any cytological effects of ethirimol on barley.

To obtain these objectives, seven experiments were carried out in 1969 with the collaboration of six other workers at the Plant Breeding Institute. The size of the overall project meant that the analyses of growth and yield components were not complete before conference publication. This paper is therefore a preliminary report on some of the main findings; various parts of the project will be published separately after further analysis.

METHOD AND MATERIALS

The seven experiments may be summarised briefly as follows:-

1. Oat mildew: glasshouse experiment (with G. Jenkins). Selected seeds of a resistant oat variety, Mostyn, and of a susceptible variety, Condor, were sown in sand-filled pots in the glasshouse. Half of the material was seed treated with ethirimol at a dose equivalent to 0.75 lb a.i./ac and subsequently with suspensions of ethirimol at the same level of application; the remainder were untreated. Plant samples were taken at intervals for growth analysis. The experiment was repeated partially, using soil compost to replace the sand medium. The pots at all stages were watered from above, with the periodic addition of culture solution (modified Hoaglands) to the sand-filled pots. Mildew was inoculated from susceptible spreader pots at the first leaf stage; after the initial inoculation the epidemic became self-perpetuating at a severe level.

2. Ethirimol/Oxycarboxin trial (with R. Johnson). Plots (8 ft x 4 ft) of the winter wheat varieties Viking (mildew and yellow rust susceptible) Minister (mildew susceptible, yellow rust resistant), TP229 (mildew resistant, yellow rust susceptible) and TP114 (resistant to mildew and yellow rust) were sown in the autumn, 1968. The varieties were subjected to four treatments, namely, ethirimol seed dressing for mildew control (0.75 lb a.i./ac) plus oxycarboxin spray (3 lb a.i./ac) immediately prior to ear emergence for yellow rust control, ethirimol alone, oxycarboxin alone, and no chemical treatment. The trial was laid out in randomised blocks with four replicates. The mildew epidemic was allowed to develop naturally; yellow rust spores were inoculated artificially in April 1969 on all plots.

3. Sowing date trial Four small plots (4 ft x 2 ft) of each of the barley varieties Proctor, Maris Otter and Maris Puma and of the wheat varieties Minister and Viking were sown in the field at intervals of six weeks from November through to April. Half of the plots at each date were sown with seed dressed with ethirimol at 3 lb a.i./ac; the remainder were untreated.

4. Irrigation/Nitrogen/Ethirimol trial (with R. N. H. Whitehouse, G. C. M. Sage). Fifteen varieties of spring cereals, (ten barley varieties and five of wheat) were subjected in the field to all combinations of the treatments droughted vs. irrigated and low vs. high nitrogen fertiliser application. The fifteen varieties x four treatments were sown in two replicates using plots 12 ft x 5 ft. Each plot was split for ethirimol seed dressing treatment vs. none at the rate of 0.75 lb a.i./ac. The mildew epidemic was allowed to develop naturally. The droughting effect was obtained by inserting plastic guttering between the plant rows at the base of the plants; most of the falling rain was thus drained away from the area of the root system. Overhead irrigation of the remaining plots reduced the water stress to a low level. Nitrogen fertiliser was applied at either 60 units/ac (low) or 120 units/ac (high), per plot.

5. Glasshouse barley trial (with R. N. H. Whitehouse). Irrigation with dilute culture solution was used for the cultivation of 17 barley varieties grown in grit-filled pots in the glasshouse. Half of the seeds sown were dressed with ethirimol at the 0.75 lb a.i./ac rate: subsequently the seedlings developing from the treated seed were further treated with a suspension of ethirimol at the same rate at the second leaf stage, applied to the grit. A plot consisted of eight plants, two per pot: the trial thus consisted of 272 plants, which was replicated five times. The varieties were selected to cover a range of morphological types and mildew reactions; four of the varieties were also used in trial No. 4 above. Primary mildew inoculation was carried out by brushing spores onto the first seedling leaf of all plants.

6. Cytological trials (with M. D. Bennett) Seeds of the barley varieties Julia and Sultan were germinated on Petri dishes and then transplanted to thimbles suspended over plastic bowls. The bowls contained aerated dilute culture solution or distilled water, together with ethirimol at various levels, i.e. 0, 0.16, 1.6 or 16 ppm a.i.

7. **Farm trial** (with E. T. Whitmore). A 20 acre field approximately four miles east of the Institute was used for a field scale trial. The field was divided, one half being sown with Proctor spring barley, seed dressed at 0.75 lb a.i./ac, the other half being sown with untreated seed. Both seed lots received the normal organo-mercurial seed-dressing. The two ten acre plots were harvested by combine in the normal way and total yields measured.

RESULTS

1. Ethirimol and mildew control

The greatest degree of mildew control by ethirimol was obtained with spring barley. For example in trial no. 5 (glasshouse barley trial) there was no development of mildew of the treated plots. This contrasted with trial no.1 (oat mildew: glasshouse experiment) where only a low degree of control was obtained despite repeated applications of ethirimol suspension to the sand-medium. Mean mildew scores for the oat experiment and the degree of control expressed as a percentage of mean scores from treated vs. untreated plants are given in Table 1.

Table 1

Degree of control of oat mildew obtained by application of ethirimol

No. of weeks after sowing*	Condor Trt.	Condor Untrt.	Mostyn Trt.	Mostyn Untrt.	Trt. as % of Untrt.
5	0.6[I]	0.9	0.0	0.1	60
6	2.1	2.9	0.2	0.2	73
7	2.6	4.2	0.2	0.4	61
9	1.8	2.9	0.3	0.6	60
10	5.1	6.2	0.3	0.2	84
11	4.7	6.2	0.3	0.5	75
12	4.2	5.3	0.3	0.7	75
14	6.2	5.7	2.6	3.4	97
Means	3.4	4.3	0.5	0.8	73

* Ethirimol applied at 0.75 lb a.i./ac equivalent at sowing date, 5 weeks, 6½ weeks and 10 weeks after sowing.

[I] Scores given are means from 0-9 scale (0 = no mildew) based on whole plant score.

The low level of mildew control may have been due to rapid leaching of the active compound from the sand medium. The experiment was therefore partially repeated using soil compost. The degree of control thus obtained was similar to that shown in Table 1. Complete control was obtained with one pot of Condor seedlings however when a large quantity of the ethirimol suspension was added to the pot.

Two field experiments were designed to give information on the duration of the ethirimol effect. In the first, trial no. 2 (Ethirimol/oxycarboxin trial) there was no observable effect on the mildew epidemic, with ethirimol applied at normal field rate to seed sown in October. The epidemic on the two mildew susceptible varieties Viking and Minister did not develop until late in the season and then only to a relatively low level. There was also no effect of ethirimol on the components of growth and yield which were measured, nor was there any interaction with the use of oxycarboxin, either on yellow rust development or on components of growth and yield.

In trial no. 3 however (sowing date trial), in which ethirimol was applied at 4x normal field rate, i.e. 3 lb a.i./ac, control of mildew on autumn-sown Viking and Minister and also on winter barley varieties was obtained. Data from the earliest sown (6/11/68) and latest-sown (30/4/69) plots from two scoring dates are given in Table 2.

Table 2

Mildew scores from early-sown (E) and late-sown (L) plots of wheat and barley treated and untreated with ethirimol at 3 lb a.i./ac

Variety	± ethirimol	No. of weeks from sowing date E : 28	L : 4	E : 32	L : 8
Minister	+	4.5*	0.0	0.5	0.5
	-	6.5	1.5	0.8	3.5
Viking	+	1.5	0.0	0.3	0.0
	-	2.5	2.5	0.3	0.5
Proctor	+	1.0	0.0	0.0	2.5
	-	5.5	4.5	1.5	4.5
Pioneer	+	1.0	0.0	0.0	2.5
	-	3.5	4.0	0.5	3.0
Maris Otter	+	1.0	0.0	0.0	3.5
	-	4.0	3.5	0.5	3.5
Maris Puma	+	1.0	0.0	0.0	3.5
	-	5.5	2.0	0.3	4.5
Treated scores as percentage of untreated	wheat	67	0	73	13
	barley	22	0	0	78

* means of score on 0-9 scale (0 = no mildew)

In the early-sown material control of mildew on wheat was less effective than on barley at both scoring dates; the overall decline in the level of infection was a reflection of increasing plant maturity. In the later-sown material, mildew control on both wheat and barley seedlings at four weeks was complete. Later, at eight weeks, although control on the wheat was still effective, mildew developed to a relatively high level on the ethirimol-treated barley plots. This was at least partly due to the general level of the barley mildew epidemic in the area which was much higher than that of the wheat mildew epidemic. It may have also been partly due to the rapid growth of the barley plants at that stage causing the roots to leave the soil area containing the ethirimol.

2. Ethirimol, mildew control and yield

In trial no. 4 (irrigation/nitrogen/ethirimol trial) using spring-sown wheat and barley varieties, control of mildew by ethirimol was effective on both species.

In general, more mildew developed on the treated plots of the highly susceptible varieties than on those of less susceptible varieties. This effect was probably a reflection of the higher inoculum potential in plots of susceptible varieties since the plots were split for fungicide treatment. There was some evidence however, of a differential response: mildew attacks on the varieties Proctor and Milns Golden Promise being less well controlled than those on Deba Abed and Impala (see Table 3). The spring wheats as a group behaved similarly to the barley varieties at the first scoring date, but at the second occasion the level of mildew control had declined further than on similarly susceptible barley varieties.

Table 3 also includes the grain dry weight yields which show a trend from increased yield with ethirimol treatment on susceptible varieties (group i) to decreased yield with ethirimol treatment on resistant varieties (group iii). The varieties were selected for division into three groups, i. mildew susceptible; ii. intermediate; iii. resistant; the grouped means and significance levels are given in Table 3. Thus mildew control on susceptible varieties gave a yield increase of approximately 5%, whereas ethirimol applied to resistant varieties gave a yield decrease of 7%. If this deleterious effect of ethirimol occurred additively on the susceptible varieties, the real reduction in yields on those varieties caused by mildew would be 7 + 5 = 12%. This reduction occurred despite a mildew attack on the treated controls which was at a higher level than that on the untreated intermediate group of varieties.

Table 3

Mildew scores at two dates and grain yields from wheat and barley varieties in a field irrigation/nitrogen/ethirimol trial, treated (+) or untreated (-) with ethirimol at 0.75 lb a.i./ac

Wheat, W, or barley, B.	Variety	Mildew at 3/6/69 -	+	Mildew at 19/6/69 -	+	Grain yield g/plot -	+
B	Milns G. Prom.	6.9	3.9	7.5	4.6	473	517
B	Proctor	6.5	3.8	5.5	3.4	424	427
i. B	Deba Abed	6.1	1.5	5.5	1.8	472	479
W	TB435	5.6	1.8	6.0	4.6	402	411
B	Impala	4.6	1.4	6.2	2.6	389	446
B	Maris Druid	4.6	2.1	5.1	2.0	409	412
B	Midas	3.0	1.1	3.7	1.9	515	550
ii. B	V.34	1.8	0.7	1.8	0.8	552	534
W	TB306	1.6	0.6	2.9	1.7	380	332
B	Sultan	1.0	0.6	2.5	0.7	477	483
B	Canon	1.0	0.7	1.3	0.7	500	463
B	HB551	0.7	0.6	0.8	0.6	532	501
iii. W	Kolibri	0.6	0.6	0.7	0.6	422	394
W	TW161/1	0.6	0.6	0.6	0.6	408	400
W	TW161/2	0.6	0.6	0.7	0.6	460	401
Grouped means	i. susceptible	5.7	2.4***	6.0	3.1***	428	449*
	ii. intermediate	1.8	0.8***	2.7	1.8***	481	475 n.s.
	iii. resistant	0.7	0.6 n.s.	0.8	0.6 n.s.	464	432**

n.s. = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Data analysed so far indicate that both ethirimol and mildew caused an increase in lodging. In the case of ethirimol this was probably partly due to an increase in straw length; mildew on the other hand decreased weight of straw per unit of tiller length which was probably responsible in part for the increase in lodging caused by the disease.

Trial no. 5 (glasshouse barley trial) produced an overall result similar to that described above for trial no. 4, but the analysis of factors leading to yield loss has been further developed (Table 4).

Table 4

Mildew scores, grain yield, root dry wt. and tiller no. of groups of mildew susceptible (i) and resistant (ii) barley varieties treated (+) or untreated (-) with ethirimol at 1.5 lb a.i./ac in the glasshouse

	Variety	Mildew score	Grain yield g/plot		Root dry wt. g/plot		Tiller no/plant	
			-	+	-	+	-	+
	Proctor	3.5	26	27	2.6	2.8	3.8	3.7
	Milns G. Prom.	3.3	19	23	1.8	1.6	3.6	4.0
i.	Zephyr	2.8	23	28	2.1	2.4	2.8	3.8
	Impala	2.5	25	30	1.9	2.5	3.3	4.1
	HB464	2.1	23	23	3.3	3.7	3.0	3.3
	Maris Canon	0.8	38	38	3.6	2.3	4.7	4.8
	HB465	0.5	17	23	2.3	2.4	2.8	3.5
ii.	Israeli 46	0.3	31	26	2.5	1.6	5.3	5.7
	Monte Cristo	0.3	27	20	9.0	6.7	4.0	3.7
	Akka	0.1	20	19	2.3	1.3	2.9	2.8
Grouped means	i. susc.	2.8	23	26*	2.4	2.6***	3.3	3.8*
	ii. res.	0.4	27	25 n.s.	3.9	2.9***	3.9	4.1 n.s.

n.s. = not significant, * = $P < 0.05$, *** = $P < 0.001$

Loss in grain yield through the action of mildew or ethirimol was not so marked as in the field experiment although the trend was in the same direction. The loss due to mildew was caused by a decrease in tiller number per plant and a reduction in root weight. Reduction in root weight by mildew is based on the assumption that the loss in root weight observed with resistant plants treated with ethirimol also occurred on the mildew susceptible varieties but that the effect was masked by a reduction of similar size caused by the disease. The effect of mildew in reducing tiller number and root weight, leading to yield loss, was previously found by Last (1962); the effect on tillering was also noted by Dr. D. H. Brooks (pers. comm.).

The cytological trials, trial no. 6, confirmed that both mildew and ethirimol could have deleterious effects on plant growth under certain conditions. In mildew-free ethirimol-treated material chiasma frequency in pollen mother cells was decreased significantly by 4% in plants subjected to 16 ppm ethirimol a.i. in culture solution: this was confirmed in tillers taken from Maris Otter in trial no. 3 which had received 3 lb a.i./ac ethirimol as a seed dressing. In addition, chromosome volume in root tips of plants grown in culture solution with 16 ppm ethirimol was reduced by one-third (Dr. M. D. Bennett, pers. comm.).

In the absence of mildew, it was found that ethirimol reduced grain yield, tiller number and root growth of the barley varieties Julia and Sultan: these effects increased with higher concentration of ethirimol in the culture solution from 0.16 to 16 ppm. In the presence of mildew, the effects were similar except that increasing the ethirimol concentration from 0 to 0.2 ppm, which gave complete mildew control, produced a significant increase in shoot and root development. The improvement in growth caused by eliminating mildew presumably masked the lesser deleterious effect of ethirimol on plant growth.

Analyses of nitrogen content and total sugar content of leaves and roots were also made. Little change in nitrogen percentage occurred except for an increase in root nitrogen percentage in the treatment with 16 ppm ethirimol. The leaf sugar analysis showed a large decrease in total leaf sugar content caused by mildew infection.

DISCUSSION

The ability of ethirimol used as a seed-dressing to control cereal mildew attacks for a long period, was confirmed. Control was most effective on spring-sown wheat and barley, less effective on autumn-sown crops and probably least effective on oats. In the field trial, trial no. 7, with spring-sown Proctor, control was completely effective in the early stages of growth. Eventually, however, fungus colonies appeared and measurements showed that these were fewer in number and smaller in size than on untreated plants, which indicated that ethirimol reduced both fungus establishment and growth.

In general, control of the mildew infection by ethirimol leads to increased yield. Evidence for this comes from many I.C.I. field trials (Dr. D. H. Brooks, pers. comm.) from the 1968 Institute trial and from the trials described above including the field trial in which the yield of Proctor was 35 cwt/ac in ten acres grown from treated seed, compared with 32 cwt/ac in ten acres grown from untreated seed, in which a moderate mildew epidemic developed. However, the range of experiments described above indicated overall that ethirimol can have a deleterious effect on plant growth and yield. The size of the effect depended on environmental conditions and variety and was less than that caused by mildew.

The uniformity of the effects caused in the trials described above may be seen in the following comparison. Four of the varieties in the glasshouse trial were also used in the field trial, namely Proctor, Milns Golden Promis, Impala (all mildew susceptible) and Maris Canon (mildew resistant). The percentage change in grain yield caused by ethirimol treatment on these varieties in the two experiments is shown in Table 5.

There was a close similarity of results obtained in the two different environments, Impala being greatly affected by ethirimol treatment, whereas Proctor was not. This difference may be due either to Proctor being more tolerant than Impala to mildew attack or that Proctor is more sensitive to treatment with ethirimol than Impala.

In comparing Proctor and Impala however, ethirimol treatment had little effect on the tiller number and root weight of Proctor whilst causing a considerable increase of these features in Impala. This difference suggests that Impala was very sensitive to mildew attack: it is not possible to determine in Proctor, however, the degree of sensitivity to ethirimol treatment or of tolerance to mildew.

Although the deleterious effect of ethirimol probably restricted the yield increase resulting from mildew control in the Institute experiments, it is likely that the differential between gain through mildew control and loss through ethirimol application would be greater in field crop cultivation than in research station trials. The reason for this is that in the experimental work, because of the small size of plots used and the limited geographical separation of control and treated plots, the treated plots were continuously subjected to a high level of inoculum which frequently led to the development of late, but considerable, epidemics in treated material. The effect of mildew control would threfore tend to be less than that observed in the field.

Table 5

Comparison of % yield change of four barley varieties following treatment with ethirimol in a field and a glasshouse experiment

Variety	Mildew suscept. (S) or resist. (R)	Irrig./nitr./ ethirimol trial	Glasshouse barley trial
Proctor	S	101	103
Milns G. Prom.	S	109	119
Impala	S	114	123
Maris Canon	R	93	104

Thus, although the general use of ethirimol to control mildew in the field should undoubtedly lead to yield increases compared with the present levels, the degree of increase obtained may be an underestimate of the severe damage caused by mildew: this point requires more detailed examination. It is also necessary to investigate in a similar way, other chemicals being introduced for mildew control, to determine whether they have any effect on plant growth in the absence of mildew.

Further lines of investigation which would be of interest are to use mildew fungicides to determine more precisely the tolerance or sensitivity of different varieties to mildew attack, and, the corollary of this, to determine whether different physiologic races of the fungus can cause differences in yield loss.

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RESPONSE OF BARLEY VARIETIES TO THE CONTROL OF POWDERY MILDEW WITH CYCLOMORPH AND TRIDEMORPH

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Summary The recent expansion of the area under barley cultivation particularly in the north of the German Federal Republic, has led to an increase in the incidence of powdery mildew (*Erysiphe graminis*). More than 100 trials were evaluated during the years 1965 to 1969, in which cyclomorph and tridemorph were applied to control the disease, in both susceptible and resistant varieties of spring barley. Two variety groups with different responses were distinguished. Some susceptible varieties reacted with up to 12% higher yields, while others, under similar infection pressure and control measures, showed only up to 8%. In spite of lower mildew infection in some resistant varieties, unexpectedly higher yields were observed, and the probable causes are discussed.

INTRODUCTION

The cultivation of barley in the Federal Republic of Germany has greatly increased during the past ten years. Table 1 shows that this trend is particularly apparent in Northern Germany where the area under cultivation has been doubled in nine years, thereby embracing 18.2% of the arable land. In the remainder of the Federal Republic the increase was 12%, and spring barley represents close to 16% of the total area of arable land. In the same period winter barley showed an increase of 4.3%. In Northern Germany spring barley now represents about 40% of the barley acreage, compared with 90% in the remainder of the Federal Republic.

Table 1

Area under barley in the Federal Republic

	Area under barley in 1000 ha		Proportion of spring barley (%)	
	1959	1968	1959	1968
Northern Germany *	304.5	604.3	38.6	42.6
Rest of the Federal Republic **	646.6	725.9	91.7	87.4

* Schleswig-Holstein, Niedersachsen, Nordehein-Westfalen, Bremen, Hamburg
** Hessen, Rheinland-Pfalz, Saarland, Baden-Württemberg, Bayern.

The overall increase in barley acreage, and preponderance of winter barley in Northern Germany, provides a large source of overwintering infection. Even under climatic conditions adverse to fungal survival, the supply of conidiospores appears to be large enough to initiate a heavy infection of mildew in spring sown barley, in three or four out of five years.

The increase in the barley area of Northern Germany also increases the possibility of occurrence of new biological races (biotypes) of mildew. In this way, infection could occur in varieties previously considered resistant. Thus, when breeding for resistance, there is a constant confrontation of new problems, since selection can only be made against already known biotypes. Of the seven spring barley varieties which comprise more than 75% of the barley grown for seed production in the Federal Republic, none can at present be regarded as fully resistant. In 1966 four of them were still classified as resistant in the official guide. It is for this reason that breeders (and growers) have for some years been interested in a successful chemical method of controlling mildew on spring barley. For them, the help offered by developments in modern chemistry is a logical supplement to their efforts of breeding for resistance.

METHOD AND MATERIALS

Field trials for mildew control in spring barley were commenced in 1961. Two active substances were employed from the new class of morpholine-derivatives, which showed excellent preventive and curative actions against powdery mildews (Pommer et al 1967; Kradel et al 1967, 1968). The compounds are cyclododecyl-2,6-dimethyl-morpholin-acetate (proposed common name cyclomorph) and N-tridecyl-2,6-dimethyl-morpholin (proposed common name tridemorph) (Koenig et al 1965; Kradel et al 1969a). An emulsifiable formulation is on the market containing 750 g/l tridemorph. Both compounds are translocated in cereals and thus can be described as systemic fungicides. They remain active for a period of three to four weeks.

During the years 1965 to 1969 more than 100 replicated field and grower trials were evaluated. In addition, many variety trials, with at times more than 20 spring barley varieties, were carried out. The active substances were always applied only once in the period between the end of tillering and the beginning of shooting. The rates of application for a comparable control of mildew were:

Cyclomorph 1.0 to 1.5 kg a.i./ha
Tridemorph 0.4 to 0.6 kg a.i./ha

RESULTS

In all, more than 40 barley varieties were examined in these trials. However, because of the preliminary nature of the trials the data given only reveal trends which could form the basis for further testing. In this report we shall deal with groups of varieties with a similar behaviour, and not with results from individual varieties.

Two groups can be distinguished amongst the varieties that were more severely infected (Table 2). Over a period of five years in group A, mildew control resulted in an average yield increase of 480 kg/ha, or 12%. The increase in yield of the varieties in group B was only 310 kg/ha, or 8%. No great difference existed between the groups A and B, either in the intensity of infection (vide untreated) or in the effect of the mildew control treatment.

Table 2

Effect of mildew control on yield of susceptible varieties 1965 to 1969

	Infection of mildew (Scale of 1 to 9)*	Yield kg/ha	Yield relative
Group A			
Untreated	6.2	3860	100
Treated	3.2	4340	112
Number of trials	85	91	
Group B			
Untreated	5.8	3950	100
Treated	3.1	4260	108
Number of trials	104	107	

* 1 = No infection 9 = Total infection

In the resistant varieties certain differences are also to be seen (Table 3).

Table 3

Effect of mildew control on yield of fully resistant varieties 1967 to 1969

	Infection of mildew (Scale of 1 to 9)*	Yield kg/ha	Yield relative
Group A			
Untreated	2.5	4070	100
Treated	1.8	4440	109
Number of trials	40	46	
Group B			
Untreated	2.0	4390	100
Treated	1.5	4490	102
Number of trials	11	12	

* 1 = No infection 9 = Total infection

As was expected, the mildew infection was low in all the untreated plots of the resistant varieties. Treatment against mildew on these varieties thus only leads to an insignificant decrease in infection. Nevertheless, in 46 trials during 1967 to 1969, an average increase in yield of 370 kg/ha, i.e. 9%, was achieved on the resistant spring barley varieties grouped under A. On the other hand, the varieties of group B showed the typical behaviour for resistant spring barley, i.e. no higher yields after chemical treatment against mildew.

DISCUSSION

The results as presented above should be interpreted with some reserve. As explained at the beginning, the figures are from control trials and tests, which were not planned originally for the purpose of serving as a variety test in conjunction with chemical control of mildew. Nevertheless, the observed differences are worthy of comment. Some of the mildew susceptible varieties react to chemical treatment with an average higher yield of 12%, while others under comparable infection pressure only reach 8%. This points to possible differences in (a) the genetically fixed yield level, (b) in the physiological reaction to a mildew infection, and (c) to specific varietal reactions to the active ingredients. Particularly remarkable are the observed higher yields of some of the fully resistant varieties. This may be associated with the defensive response of the plant to infection, and the following tentative hypothesis is put forward.

Ring-shaped leaf necroses are often found near primary infections on resistant varieties, and point to a defensive reaction of the barley (Honecker, 1934). Similar defensive necroses are known from potato varieties that are resistant to potato wart (Synchytrium endobioticum), potato blight (Phytophthora infestans) or potato eelworm (Heterodera rostochiensis). This active defence reaction of the plant requires energy, which in turn is no longer available for yield increases. If these primary infections are stopped through chemical treatment, then the defensive reactions are not mobilised and the whole assimilation output of the plant might be utilised for grain production.

There may also be undetermined effects of these chemicals on fungi that parasitise the leaves, glumes and awns on both susceptible and resistant varieties. Some work on this problem has already been started.

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SPRAY TIMING AND THE CONTROL OF MILDEW IN SPRING BARLEY

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Summary In 1968 at Ollerton, Nottinghamshire, the control of mildew obtained by spraying plots of Zephyr barley with drazoxolon on 7th May, 21st May, 5th June, 18th June, 2nd July and 16th July was compared, with the control obtained by spraying plots once only at each of the above dates, and with unsprayed plots. In 1969 the experimental details were similar except that the plots were of a size suitable for combine harvesting. Yields in 1968 were obtained by a sampling method. A significant reduction in the level of mildew on leaf 2 at G.S. 10.5.4. was obtained by a single spray applied on 5th June. There was some indication of an increased quantity of better quality grain but the differences were not significant. In 1969 the epidemic developed later at the two trial sites but again a significant reduction in the level of mildew was obtained by the comprehensive programme and by single sprays applied on 18th and 30th June at Gleadthorpe E.H.F. and on 4th, 18th and 30th June at Osgathorpe, Leicestershire.

An examination of the weather data in relation to the incidence of mildew suggested that in the period of these trials epidemic development of the disease occurred when the mean of the maximum temperature for seven days exceeded 20°C. A single spray after such a period in 1968 and 1969 gave an effective reduction in the incidence of mildew.

INTRODUCTION

On the sandstone soils of the East Midlands mildew in spring barley is always important and in some seasons is sufficiently severe to limit the economic profitability of that crop. It was considered necessary to examine the feasibility of preventing epidemic development of the disease by applying a single fungicidal spray, coincident if possible with the routine herbicide spray.

METHOD AND MATERIALS

1968 Trials were carried out on two commercial farms in the Ollerton area of Nottinghamshire. The results from one site only are reported here because harvest difficulties prevented completion of the trial at the other.

Drazoxolon (4-(chlorophenylhydrazono)-3-methyl-5-soxazolone) was applied with a knapsack sprayer to plots of Zephyr barley at a rate of two pints (1 lb a.i.) per acre (1.12 kg/ha). The crop was sown by the farmer on 18th April at a rate of 8 stone per acre (125 kg/ha) and all plots were delimited by applying paraquat with a dribble bar to eliminate the appropriate discards. The plots, 15 yd x 3 yd (13.72 m x 2.74 m) were each replicated once in four randomised blocks with a one yd discard between plots and blocks. Six of the eight plots in a block were sprayed once only on the following dates, 7th May, 21st May, 5th June, 18th June, 2nd July and 16th July. A further plot was sprayed six times on each of those dates and the remaining plot was not sprayed. Disease assessment on the top two leaves of each of ten tillers per plot was made with the area diagram key (Large, 1962) at fortnightly intervals. (Leaf 1 is always the topmost leaf.)

At G.S. 10.5 the number of fertile tillers in three 1 ft lengths of drill per plot was recorded. At harvest plant samples from eight 1 yd lengths of row per plot were cut, tied by hand, and later threshed by hand. Yield data was obtained from these grain samples.

1969 These trials with minor modifications were repeated at Gleadthorpe E.H.F. and Osgathorpe, Leicestershire. The spray schedules were similar and the fungicide was drazoxolon.

1968 Incidence of Mildew (Table 1)

Mildew was below 1% on all plots until the first week of June (G.S. 9-10) but in the following three weeks it developed rapidly to reach about 45% on leaf 2 of the unsprayed plots by mid-July (G.S. 11.2).

An excellent control of the disease was obtained by the complete spray programme, mildew being only 2% on 9th July (G.S. 11.2). Of the single spray treatments, those applied on 21st May, 5th June, or 18th June, in the early stages of the epidemic, gave a more effective control of the disease than those applied on 2nd July, or 16th July, by which time the disease was well established.

The single spray applied very early (7th May) appears also to have been largely ineffective. The single spray applied on 5th June immediately before epidemic development of the disease began gave a control of mildew at G.S. 10.5.4 which was almost as good as that obtained with the complete spray programme.

Table 1

Mean Percentage Mildew on Leaf 2 of Zephyr Barley 1968

Treatment		Date of Assessment 11 June (G.S.9-10)	25 June (G.S.10.5.4)	9 July (G.S.11.2)
1	Complete schedule	0.38	1.1	2.0
2	7 May only	0.54	7.5	32.8
3	21 May "	0.55	11.7	23.5
4	5 June "	0.94	4.4	18.1
5	18 June "	0.60	9.8	20.8
6	2 July "	1.15	15.9	33.7
7	16 July "	0.97	17.9	31.0
8	Unsprayed	0.76	11.8	43.7
s.e. per plot		-	2.84 (28.4%)	-

1968 Yield (Table 2)

The various components of yield were analysed, but statistically significant differences were obtained only with the 1,000 grain weight, and weight of grain retained by a 2.8 mesh sieve. The high coefficient of variation with this latter component makes the result obtained with this sampling technique questionable in this experiment.

Fig. 1

Barley mildew progress curves

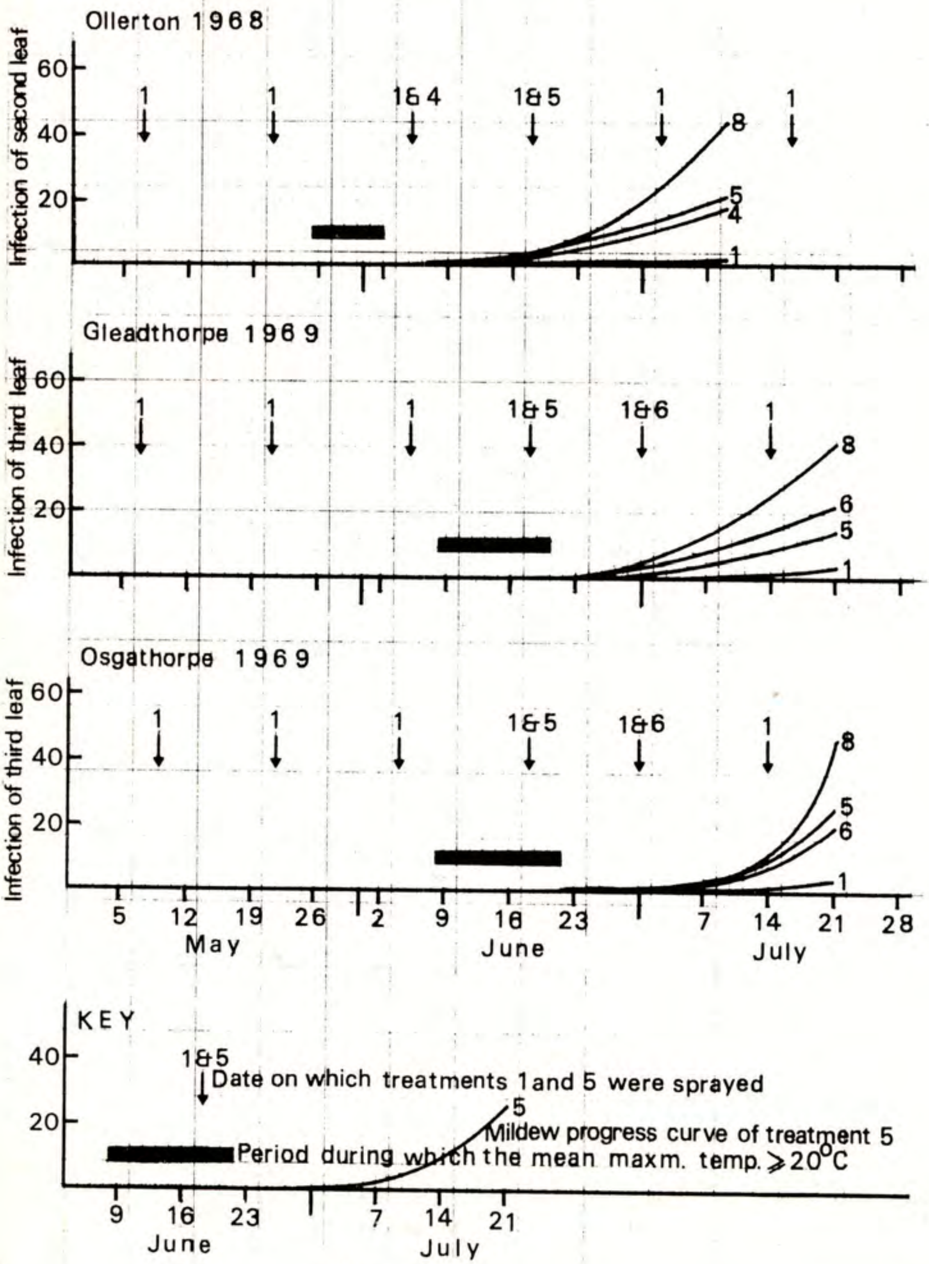

Table 2

Components of Yield 1968

Treatment		Total Yield cwt/acre (kg/ha)	Weight of Grain per 100 heads (g)	1000 Grain Weight (g)	Weight of Grain Over 2.8 Mesh Sieve (g)
1	Complete schedule	31.8 (3992.8)	77.4	36.0	12.19
2	7 May only	29.0 (3641.0)	71.4	33.6	4.77
3	21 May "	29.4 (3691.4)	67.3	33.2	4.13
4	5 June "	28.5 (3578.0)	71.6	34.4	5.65
5	18 June "	28.0 (3515.0)	67.8	34.0	3.55
6	2 July "	28.4 (3565.4)	66.3	32.3	2.71
7	16 July "	28.1 (3527.6)	69.6	30.5	2.97
8	Unsprayed	28.1 (3527.6)	69.1	33.9	3.48
s.e. per plot		2.52(8.74%)	7.90(11.2%)	1.83(5.5%)	2.4(48.9%)

1969 At both sites mildew was first seen on the 9th June. The relative levels of disease showed an appreciable increase between 9th and 27th June at Gleadthorpe, and between 25th June and 9th July at Osgathorpe. On leaf 3 of the unsprayed plots on 11th July the mildew at Gleadthorpe was 41% and at Osgathorpe 4% compared with 4% and 0.5% respectively where a complete spray programme had been applied.

The most effective single sprays were those applied on 18th or 30th June at Gleadthorpe, and on 4th, 18th or 30th June at Osgathorpe.

In Figure 1 is shown the progress of mildew on plants which were either not sprayed, or sprayed six times at 14 day intervals, or sprayed once only - on 4th and 18th June, 1968; 18th and 30th June at Gleadthorpe and Osgathorpe 1969.

DISCUSSION

Epidemic development of *Erysiphe graminis* on spring-sown barley in 1968 and 1969 did not occur until late June. Increases in yield of about 10% were obtained in those seasons by applying six sprays of drazoxolon at fortnightly intervals. The increase in yield obtained by Last (1955), in a season in which epidemic development of mildew occurred in mid-May, was 22% from a mildew-susceptible variety.

The increase in grain quality and quantity, resulting from a single spray of drazoxolon applied at a specific point in time, may not adequately reflect the economic return which is possible when mildew becomes epidemic at a date earlier than in these trials. If, however, a single spray is to be applied it is necessary to predict when it should take place. The work of Benada (1962), Last (1963), Manners and Hossain (1963) indicates that the optimum temperature for sporulation of *E. graminis* is 20 to 25°C. The daily temperatures during the present trials were obtained from the Meterological Station appropriate to the experimental site (Gleadthorpe E.H.F. 1968 and 1969; Sutton Bonington 1969), and examination of this data suggested that the epidemic development of mildew, shown as progress curves in Figure 1 occurred in 1968 and 1969 after a period when the mean of the maximum temperature for seven consecutive days was at, or exceeded, 20°C.

Data was also obtained from other spring-sown barley trials, commercial crops in the East Midland Region in 1968 and 1969, and from the work of Last (1955, 1966), Large and Doling (1962). This data supports the view that, although other factors may modify the effect of mildew on yield, the disease is unlikely to be severe and become epidemic on spring-sown barley until the temperature parameter mentioned above has been operative. This will have obvious implications in advising farmers on the economic practicability of spraying spring barley crops to control mildew.

Single sprays of drazoxolon to control mildew in spring-sown barley crops, if applied with a herbicide spray, or before 26th May, 1968, and 9th June 1969, would have been ineffective in controlling the disease. The programme in which the fungicide was applied six times at fortnightly intervals gave a very effective control of mildew.

Single sprays of drazoxolon applied on, or soon after, the mean of the maximum temperature for seven consecutive days exceeded 20°C gave an effective reduction in the incidence of mildew.

Further work is needed to establish whether a single spray will in all seasons, with varying times of epidemic development of the disease, yield an economic return. The experiments described here suggest a method for timing the application of a single spray.

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WINTER BARLEY AS A SOURCE OF MILDEW FOR SPRING BARLEY CROPS

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Summary Work done over two seasons in Cambridgeshire has shown the importance of winter barley as a source of early mildew infection for neighbouring spring sown crops, but no direct relationship has been found between nearness to winter barley and the final level of the disease on the spring crops. No conclusive evidence was obtained to suggest that early differences in mildew levels were reflected in final yield or quality of grain. Observations made in 1969 indicated that, in this year at least, volunteer plants in undersown leys were unimportant as mildew sources for spring barley crops in Cambridgeshire.

INTRODUCTION

The Danish decision to ban the growing of winter barley (Stapel & Hermansen, 1968) has led to increasing interest in this country in the possible dangers of growing this "green bridge" which may carry through the winter the leaf diseases which James (1969) has estimated have recently cost us over 20% of our national barley yield. Since 1967 various N.A.A.S. departments have been endeavouring to assess the importance of winter barley in the epidemiology of leaf diseases, in particular of mildew (*Erysiphe graminis*) which is by far the most important foliar disease of barley. This paper describes work on this problem carried out during 1968 and 1969 in Cambridgeshire.

METHODS

Observations were made in three areas of the county:-

1. the Fens around Wicken - almost all the barleys in this area are spring sown but one or two winter crops are always grown on the thin soils of the low ridge of Corallian limestone which occupies an area of about 530 acres in the west of Wicken parish,

2. an area in east Cambs. where quite a number of winter barley crops are grown on the Rendzina soils of the chalk ridge,

3. an area of about 16 square miles on the boulder clay plateau of west Cambs. Apart from a single crop near its western edge this area had been kept free of winter barley by an agreement reached with the farmers concerned.

Observations were made at Wicken in both 1968 and 1969. The other two areas were studied in 1969 only.

The approach adopted was to trace any disease gradients which might exist in spring barley fields adjacent to autumn-sown crops, and to compare the disease levels in these fields with those in fields more distant from the overwintering crops. In 1969 observations were also made in spring barley crops adjacent to undersown leys.

Assessments of disease severity were obtained by scoring the percentage cover of mildew on the leaves using the standard area diagrams of a key produced by the N.A.A.S. and the Plant Pathology Laboratory, Harpenden. Assessments were normally made on the lowest green leaves. In the text of this paper leaves are numbered from the top, leaf 1 being the uppermost expanded leaf. Where growth stages are quoted the Feekes Scale (Large, 1954) is used.

A disease gradient could generally be detected quite readily by assessing leaves taken at intervals along a line running directly away from the suspected source of inoculum. More accurate information on disease levels was obtained by assessing a number of leaves along each of a series of traverses running parallel to the edge of the suspected source crop and at the required distances from it; it was from such assessments that the figures quoted in the tables were normally obtained. To obtain a figure for the average level of infection present in a field a number of leaves were assessed along a single traverse running either diagonally across the field or at right angles to any nearby disease source.

In one spring barley crop in each year an attempt was made to correlate yield with early differences in mildew severity. At harvest time a number of one yard lengths of drill were cut by hand from traverses running parallel to the edge of a winter barley crop, the samples were later threshed and yield of grain recorded. In 1969 grain quality was assessed by separating the grain into four fractions over a set of standard sieves. Also in 1969 ear and straw measurements were made on plants from each of twelve one foot lengths of drill taken from each traverse.

RESULTS

<u>Note</u>:- The maps (Figures 1 to 4) do not cover all the fields studied but they show the positions of those crops referred to most frequently in the text.

<u>1a. The Fenland area 1968</u> (Figure 1)

On the Corallian ridge were three February-sown barley crops of variety Maris Badger (Fields A, B & C in Fig.1) drilled to within a few inches of October-sown crops of the winter variety Maris Otter. All the other barley crops in the area were on soils which were deeper and richer than those on the ridge.

In early April, mildew, leaf blotch (<u>Rhynchosporium secalis</u>) and brown rust (<u>Puccinia hordei</u>) were all found at low levels in the winter barley crops. By the end of the month mildew could also be found on those parts of crops A, B and C nearest to the winter barley. From a consideration of our field observations and of meteorological data it was possible to deduce that first infections in fields B and C had taken place on 14th-15th April when winds were in the north east, first infections in field A occurred during a period of south westerly winds a few days later.

Table 1 summarises the results of observations made during the season on Field C.

The mildew gradient seen in May in field C, and similar gradients found in fields A and B, suggest very strongly that at this time of the year the winter barley crops were by far the most important, if not the only, sources of infection for the adjacent spring barley. Gradients could also be found in fields D and E suggesting that the first infected spring sown crops had passed on the mildew to other crops lying near them. During June the gradients in all these fields gradually disappeared. By mid June the highest level of mildew recorded on field C was no longer in the drill adjacent to the winter barley but some yards out into the spring crop, a similar phenomenon was noticed in field B. Field E was late sown and became very heavily mildewed, back infection from this crop may have hastened the levelling out of the gradient in field C.

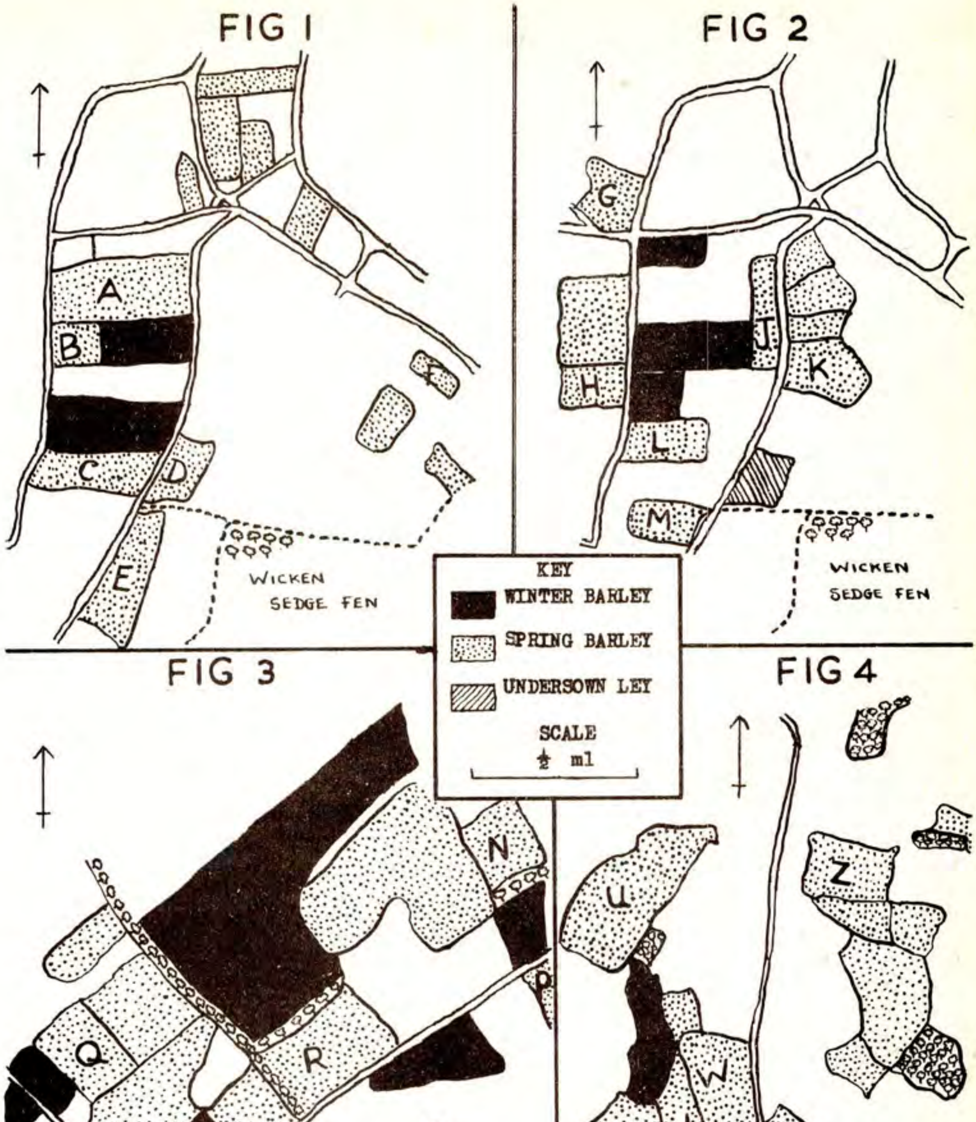
FIG 1
A
B
C
D
E
WICKEN
SEDGE FEN
FIG 2
G
H
J
K
L
M
WICKEN
SEDGE FEN
KEY
WINTER BARLEY
SPRING BARLEY
UNDERSOWN LEY
SCALE
½ ml
FIG 3
N
P
Q
R
S
T
FIG 4
U
V
W
X
Y
Z

Table 1

Field C - Disease assessments and yield measurements

Date	Growth stage	Leaf assessed	0	10	20	40	50	60	80	100	120	150	180	200	300
			Yards from winter barley												
Mildew (% leaf cover)															
1.5.68		4	8.5		2.4	0.8		0.4	0.3				0.0		
16.5.68		4	47		20	13					5		2		
18.6.68	10.4	3	18		24	13		13	16	10		9			7
4.7.68	11	3	40		44					39				36	38
Brown rust (% tiller infected)															
11.6.68	10.1		25		14				0.5						
18.6.68	10.4		80		70	50		40	10	20		50			20
4.7.68	11		100		100					94				83	86
Yield (g/5 yd drill)			283	303	294		268					271			307

In addition to the mildew gradients, gradients could also be found which suggested that the winter barleys were the main sources of brown rust for adjacent spring sown crops. Leaf blotch, though quite abundant in the Maris Otter, never built up in the adjacent Maris Badger crops though occasional lesions were seen on the drills closest to the winter barley.

As will be seen from Table 1 there was no indication that the disease gradients seen early in the season were in any way reflected in the final yield of grain.

In late May, mildew levels in spring barley crops lying between 600 yd and 2000 yd from the winter barley fields were generally comparable with, or rather lower than, the lowest levels recorded at that time on the crops close to the winter barley (1-2% on the lower leaves). It was impossible to tell where the mildew on these more distant fields had come from; spores may have blown this far from either the winter or the early infected spring crops, but other sources of inoculum might also have been involved. Field F, for example, which showed a higher than average level of infection, was later found to have been sited quite close to an undersown ley. By mid June mildew levels on the outlying fields were generally higher than those in the crops contiguous with the winter barley, this may be accounted for by the fact that plant growth was more lush, and disease build up therefore more rapid on the richer soils of the outlying fields than on the thin soil of the Corallian ridge.

<u>1b. The Fenland area 1969</u> (Figure 2)

When the site was first visited in late April no mildew could be found in the northern winter barley field (which followed a crop of the mildew resistant cultivar Sultan) and, though the disease was present on the other two fields (which followed crops of cv. Maris Badger), levels in the southern field were very low indeed. Mildew eventually appeared in all three fields but levels remained low throughout the season.

Table 2 illustrates the results of early season mildew assessments on crops of Maris Badger adjacent to the winter barley fields.

Table 2

Mildew levels in the Fenland fields - 1969 (% mildew on leaf 3)

Field	Date of assessment	Distance from edge of crop nearest the winter barley: 0	5	10	15	20	25	30	40	50	65	75	100	150
L	30.5.69	0.7		0.3		1.3			0.2				0.1	
	6.6.69	1.5				0.6				0.3			0.3	
H	30.5.69	9.0			16.9				5.4		5.9		2.0	
	9.6.69	15.5		15.5			12.1			9.4		6.1	5.5	
J	30.5.69	4.4		1.9			0.8							
	10.6.69	4.6	3.2	2.8				0.8						
K	10.6.69	4.8		2.9			1.2						1.2	1.3

The mildew gradients indicated in the table suggest that early in the season, despite the very low levels of mildew in them, the winter barleys were again the most important sources of inoculum for spring sown crops lying close to them. It is interesting to compare the mildew levels found in field L (an exposed field on the Corallian ridge) with those found in field H (a low lying field surrounded by high hedges). On both these fields the highest mildew levels recorded in May were not in the drills nearest the winter barley but a few yards out into the crop - a similar phenomenon was noticed at this time in field G. On 10th June five spring barley crops situated over 900 yd from the winter barley were examined, mildew was present in all of them but in no case did the level on the lower leaves exceed 1%.

The undersown ley shown in Fig. 2 contained many self sown plants of cv. Maris Badger but careful examination in the spring failed to reveal any overwintering mildew on these plants, neither was it possible to detect any disease gradient in Field M. It appeared therefore that the ley had not acted as a source of mildew inoculum.

2. <u>The east Cambs. area</u> (Figure 3)

Near the bottom of the chalk slope at an elevation of 100-150 ft above sea level lay a number of spring barley crops closely associated with fields of winter barley. Five of these crops, all of them cv. Proctor drilled in March, were studied in some detail. Comparison of disease levels in these crops with those in crops more distant from winter barley was made difficult by the fact that most of the other spring barleys in the area were on higher ground, in more exposed positions, and often on thinner soils, than were those near the autumn sown crops. Differences of variety and sowing date also complicated the picture. Valid comparisons could, however, be made on field T and on another field (designated field TD) which lay to the south east of the area shown on the map some 700 yd from the nearest winter barley; both these fields were similar in elevation, variety and sowing date to the crops closer to the winter barley. Results of disease assessments made in these various fields are shown in Table 3.

Mildew gradients were observable in May on all the spring sown fields close to the winter barley. Field Q was exceptional in that there the peak of disease severity was not in the drill adjacent to the winter barley but some yards out into the crop (see Table 4). By 9th June this peak appeared to have moved yet further into the crop and a second peak towards the far side of the field suggested that disease spread was also occurring from the heavily infected, late sown crop beyond.

Table 3

Disease assessment in east Cambs. fields (% mildew on lower leaves)

Date	Leaf	Field Q	Field N	Field P	Field R	Field S	Field T	Field TD
20.5.69	4	25* to 2+	50* to 5+	43* to 1+	-	-	<1	<1
9.6.69	4	23	43	43	50	37	11	8
15.7.69	2	-	36	-	-	-	-	31

* near to winter barley + distant from winter barley

During late June the original gradients disappeared as may be seen from the following assessments done on Field P on 9th July:-

Distance from north west edge of field:-	1 yd	50 yd	100 yd	200 yd	400 yd
% mildew on leaf 2 :-	8	28	30	32	31

Field P was divided from the winter barley by an embankment some 12 ft high which carried a main road across a shallow valley. The apparent reversal of the mildew gradient at the north west end of the field seems to indicate that the embankment was influencing the disease pattern there. As the disease gradients levelled out on fields close to the winter barley, so did the overall differences in mildew level between these fields and comparable fields more distant from the autumn sown crops. In spring crops on the higher land in the area, however, mildew generally remained at rather a lower level.

Pre-harvest samples were taken from field Q on 13th August. Table 4 shows the results obtained from this sampling in relation to the differences in mildew level seen in late May.

It may be that the figures for samples taken from the traverse nearest to the winter barley should be discounted as representing no more than a headland effect, but even if this is done, no convincingly consistent pattern emerges to suggest that either yield or quality of grain was simply related to distance from winter barley (and hence to early mildew gradient). There are, however, suggestions of gradients in ear length and, more particularly, straw length. The possibility of soil fertility gradients within the field cannot, of course, be ruled out, and obviously many more fields would need to be examined before any firm conclusions could be drawn from such observations.

On the higher land east of the area shown in Fig. 3 were four undersown leys. Examination of these in April failed to reveal the presence of any volunteer barley plants in them though it was, of course, impossible to be certain that plants had not been overlooked. Nine crops of cv. Proctor adjacent to, or very close to, the leys were investigated. No mildew gradient was detected in any of these fields during May and June though, inexplicably, a slight gradient was picked up in one of them in August.

Table 4

Crop performance in relation to early disease gradient

	Yards from winter barley										
	0	1	5	10	20	40	50	80	100	150	300
% mildew on leaf 4 20.5.69		15		25			8		2		
Length of straw (cm)	72.6		66.8	68.8	69.3	69.6		72.1		72.0	76.8
Length of ear (cm)	4.7		4.2	4.5	4.8	4.9		4.9		4.9	5.2
Yield of grain(g/4yd drill)	249		317	245	228	243		244		272	261
% grain retained by 2.8 mm sieve	33		23	19	22	23		24		21	19
% grain retained by 2.5 mm sieve	58		65	67	64	67		66		66	66
% grain retained by 2.2 mm sieve	6		9	11	11	8		9		10	12

3. The west Cambs. area (Figure 4)

While observations were made on a number of fields in the area, effort was concentrated on four crops of cv. Zephyr (fields V, W, X & Y) which, being all on the same farm, could be compared in the knowledge that all would have received similar treatment so far as general husbandry was concerned. Two of these fields were adjacent to the one autumn sown crop which lay on the edge of the otherwise winter barley free area.

Very little mildew appeared to overwinter in the autumn-sown crop and in early May only occasional pustules could be found in the field. No overwintering mildew was found in two undersown leys, which lay in the north of the area (not shown on Fig. 4), even though these fields contained very many self sown barley plants.

By 22nd May the Zephyr crops were well tillered. Occasional mildew pustules (more abundant nearer to the winter barley) could be found in fields V and W. A scattering of pustules was also present in field X but no disease at all was seen in field Y. At this time occasional pustules were also found on a Zephyr crop adjacent to one of the undersown leys but no gradient was detected to suggest that the ley was the source of the disease.

Mildew was first noticed in field Y in early June, a 1% level of infection being present on the lower leaves. In field V at this time average levels of mildew on leaf 3 varied from 2.3% near the winter barley to 0.7% at the other end of the field. By 12th June mildew had appeared in the late sown field U which was separated from the autumn sown crop by a narrow strip of meadowland. There was no suggestion that the winter barley had been the source of mildew for this field. In mid-July the following results were obtained from the Zephyr crops:-

Field:-	V	W	X	Y
% mildew on leaf 3:-	23	20	20	24

By this time there were no obvious differences in mildew level between the fields though there was an indication of a gradient persisting on field W in which levels varied from 24% near to the winter barley to 15% at the other end of the field.

Final levels of mildew were generally quite low throughout the west Cambs. area. Assessments done on 1st August showed up to 10% on the second leaf in field W and up to 15% in field X. Levels in the Zephyr crops near the ley were lower (up to 5% on leaf 3) and comparable with the general level seen on crops of cv. Deba Abed (field Z and others not on the map) near the middle of the winter barley free area. It was of interest, however, that the highest mildew levels recorded anywhere in the area in early August were found in part of one of the Deba crops which had received extra nitrogen - once again final level of mildew in the crop was more dependent on conditions in the field than on proximity to winter barley.

DISCUSSION

Hermansen (1968) demonstrated that the mildew seen in spring barley crops in Denmark was for the most part exophytotic in origin, but that enphytotic disease development could occur in the vicinity of autumn sown crops. Since much more winter barley is grown by the British than was grown by the Danes it is likely that this crop plays a more important part in the epidemiology of foliar diseases in this country than it did in Denmark. The frequency with which winter barley is grown in England does, however, make it more difficult to assess its importance in this respect since it is difficult to find an area of the country in which the development of epiphytotics in the absence of the autumn sown crop can be studied.

In our investigation the disease gradients found at all three sites may be taken as evidence that early in the season the winter barley crops were by far the most important, if not the only, sources of mildew for spring sown crops adjacent to them; it was of interest that gradients could be found in the spring crops even when mildew levels in the adjacent winter barley were very low. It was more difficult to determine to what extent the winter crops acted as sources of inoculum for spring sown fields more distant from them. In such distant fields low levels of mildew could generally be found quite early in the season (late May in 1968, early June in 1969) and while it is possible that this mildew had come from winter barley crops a few miles away, or from early infected spring crops closer to the winter barley, other sources of infection could also have been involved. Hermansen postulated that the low levels of mildew found in Danish spring barley crops in late May were the result of spores blowing in from sources of inoculum in Germany. It is possible that some of the early infections seen in this country are also of exophytotic origin as spore clouds could conceivably blow over from the Continent.

Other potential sources of mildew inoculum are volunteer barley plants, particularly those in undersown leys. Hermansen found such volunteers to be of negligible importance in Denmark, mainly because so few of them survived the winter, but in our milder climate they obviously cannot so easily be discounted. In 1968 a high level of early infection in one field of spring barley was tentatively attributed to proximity to an undersown ley, but no evidence was found to suggest that leys were acting as mildew sources in 1969.

As the season progressed mildew gradients in spring crops near to winter barley fields became much less pronounced. In early June mildew levels in spring crops were often higher some yards away from the winter barley than they were in the drills nearer to it (similar anomolous disease patterns were occasionally seen much earlier in the season), the cause of this phenomenon is not known.

Last (1966) has shown that a temperature of 20° to 25°C is optimal for mildew development. Rosser (1969) suggested that under field conditions most rapid development of the disease might be expected when the mean seven day maximum temperature exceeds 20°C, and Ann (personal communication) found that the disease gradient in a spring barley adjacent to a winter crop levelled out soon after this critical temperature had been reached. In Cambridgeshire in 1969 Rosser's temperature criterion was met throughout most of late June and July; during this period not only did the original disease gradients level out, but the differences in mildew level between spring sown crops close to, and those distant from, winter barley were greatly reduced. The fairly rapid build up of disease occurring at this time appeared to overshadow the effects of proximity to winter barley, so that the final level of mildew was determined less by the amount of inoculum present in a field in early spring than by the environmental conditions prevailing in that field later in the season. It remains to be determined whether the rapid build up of mildew in mid summer is due solely to the multiplication of the disease already in the crops, or whether sources of inoculum outside the area, or even outside the country, might also be playing a part.

Thus, while it is possible that a winter barley crop may be, at least in early spring, an important (or even the main) source of mildew for the spring barley crops for some considerable distance around, the ultimate level of disease in those crops will not necessarily depend on their proximity to the winter barley. Nearness of spring to winter crops could, however, be important in that the original differences in mildew levels could be reflected in differences in crop performance. Such few observations as we have made on this subject have failed to demonstrate conclusively that grain yield and quality vary in relation to early mildew levels, though such variations in crop performance as were observed suggest that this topic would repay further investigation.

A great deal of work obviously needs to be done before the importance of winter barley as a disease hazard to spring sown crops in Britain can finally be assessed.

Acknowledgements

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THE APPEARANCE OF LEAF STRIPE (CEPHALOSPORIUM GRAMINEUM) IN CEREALS GROWN UNDER CONDITIONS OF MINIMUM CULTIVATION

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Summary In a survey carried out in 1968, leaf stripe (*Cephalosporium gramineum*) was found in traces in 30% of minimal cultivated crops examined, and in some cases was more severe than on conventionally grown crops alongside. Field and laboratory studies showed that survival of the fungus was longer on stubble lying on the surface than on stubble buried by cultivation. Several naturally infected weed grasses were found during the survey. Measures to control grass weeds and ensure the removal of surface straw by burning or cultivation should help keep the disease in check.

INTRODUCTION

Leaf stripe, which occurs in all cultivated cereals and in a number of wild and cultivated grass species, was first recorded in Japan by Nisikado et al. (1934). They identified the causal agent as a fungus belonging to the form genus *Cephalosporium* and described it as a new species *Cephalosporium gramineum* Nisikado and Ikata. The fungus appeared to be closely related to other species of *Cephalosporium* which are responsible for vascular wilt diseases in a number of annual crops and in some tree species. They observed the growth of the fungus within the vascular tissue of the host and attributed the stripe symptoms to the production of a systemically transported toxin by the fungus.

Infected plants typically have narrow, well defined yellow-brown stripes extending the length of the leaf sheath and blade. On more severely infected plants the stripes may coalesce and the whole leaf may become chlorotic. Symptoms are readily observed in the lower leaves of infected wheat plants at the early tillering stage, but can be recognised earlier if the plants are not etiolated as a result of poor growing conditions during spring. In spring barley, symptoms are visible at the 4 leaf stage.

Successive leaves are invaded as the plant matures and at pollination very little functional leaf tissue remains; even the vascular bundles of the glumes are infected, the glumes become chlorotic and the ear appears to ripen prematurely. Grain is shrivelled at harvesting, partly due to lack of moisture following blocking of the vascular system, but also due to the lack of synthesised products following the reduction in functional tissue.

The disease has been extensively studied by Bruehl and co-workers (1956) in America where in localised areas it is important in cereals, particularly in irrigated wheat. In this country it was first recorded by Gray and Noble (1960) and more recently has been investigated by Slope and Bardner (1965). The disease was first observed at Jealott's Hill Research Station in 1966 in a direct-drilled, continuous wheat trial. Following its appearance there, a survey was carried out in a large

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number of direct-drilled and minimal cultivated crops. This paper outlines results of this survey, and some investigations of the biology of the disease, particularly in relation to factors which could affect its distribution in different cultural conditions.

Experimental observations

The first observation of leaf stripe at Jealott's Hill was in June 1966 in a long term direct-drilling trial - experiment CP1 (Brooks, 1967). The site of the experiment was in an area of heavy clay soil liable to waterlogging; these are the conditions described by Bruehl (1956) as being particularly suitable for attacks of leaf stripe. In experiment CP1 there was appreciably more leaf stripe in the plots which had been direct-drilled than in the plots which had been ploughed. More detailed disease assessments were carried out in 1967 and 1968. Results of assessments made at growth stage 10·5 are summarised in Table 1.

Table 1.

Incidence of leaf stripe (Cephalosporium) in Cappelle wheat either direct-drilled or drilled into a conventionally ploughed soil.

Treatments	Units of nitrogen top dressing/acre	Mean percentage infected tillers from 10x1 yd sampling units 1966/67 season	1967/68 season
Conventionally ploughed soil	0	0·2	0·2
	40	0·5	0·1
	80	0·6	0·5
	120	0·6	0·4
Direct-drilled	0	7·0	5·2
	40	7·4	8·4
	80	8·4	7·4
	120	7·9	7·4

These results clearly show a significantly ($P < 0{\cdot}01$) higher incidence of disease in the direct-drilled treatments, but no influence of nitrogen top-dressings on disease development. This last finding was confirmed in several other experiments where various amounts of nitrogen top-dressing and combinations with other nutrients has no significant effect on disease incidence. The disease was however, sometimes more difficult to see on low nitrogen treatments where plants were slightly chlorotic, than on high nitrogen treatments where the stripes contrasted vividly with the dark green leaves.

Since there was an obvious association in this trial between the technique of direct-drilling and occurrence of leaf stripe, a survey was carried out during 1968 on 60 sites in various parts of England where direct-drilling or minimal cultivation following paraquat application was carried out for trial purposes or commercial practice. At all sites conventionally drilled areas were available for comparison. Results are summarised in Table 2.

Of the 60 sites examined, positive identifications were made at 18 sites. Of these, 14 sites provided no more than 1 or 2 infected plants in an area of 1 to 2 acres, and in 2 others showing a higher disease incidence, assessments indicated less than 1% infected tillers. The remaining 2 sites, where an incidence of 3 to 4% infection was recorded, were, in fact, long term experiments where wheat was direct drilled into a wheat stubble without ploughing for 3 consecutive years.

Table 2.

Summary of survey of leaf stripe in cereal crops grown under conditions of minimum tillage

	Winter wheat	Spring wheat	Winter barley	Spring barley	Oats
Number of sites examined	21	3	3	32	1
Number of sites where infected plants were observed	14	1	0	3	0

Because of the very low incidence of leaf stripe in the trials it was not possible to obtain figures demonstrating differences between cultural treatments. Nevertheless it was obvious that in direct-drilled trials there was a distinct tendency for more disease to occur in the direct-drilled plots than in the ploughed plots. Even in the direct-drilled plots however, the disease did not reach serious proportions. Where other forms of minimal cultivation were practiced (e.g. chisel ploughing) disease levels were lower and more variable. There was little correlation between disease incidence and soil type, but most infected sites appeared to share the common factor of a moderate or heavy invasion of weed grasses, particularly couch (Agropyron repens), meadow grasses (Poa spp.) and wild oats (Avena fatua). Isolations of the disease were made from several grass species. As a result, it was tentatively concluded (Howell and Burgess, 1969) that leaf stripe might be an endemic disease of grasses, only appearing in cereals to any extent where conditions are favourable for infection and survival of the pathogen.

The incidence of grass weeds could explain some of the cases of build up in direct-drilled plots, but not all. It seemed that a more likely explanation could be that inoculum build up occurred because the fungus survives longer on straw lying on the surface following direct-drilling than on straw buried by ploughing.

Experiments were therefore set up to investigate survival of C. gramineum in naturally infected wheat straw. The fungus is known to survive and propagate within infected straws in the form of mycelium, micro-conidia and characteristic sporodochia.

In the first experiment naturally infected wheat straw was cut into 2 in lengths. Some were dipped for one minute into a paraquat solution equivalent to 24 oz ion/acre. All of the straws were then wrapped individually in nylon and either buried in field soil at a depth of 4 in or placed on the surface. The plots were subsequently drilled with Cappelle wheat.

Samples of straw were recovered at intervals of 3 months and after thorough washing in tap water the straws were plated out on acidified cornmeal agar and incubated at 10°C. One week later the plates were examined for mycelial growth (Fig.1) and after a further 3 weeks for production of the sporodochial stage (Hymenula cerealis).

The results indicated that

(i) the fungus survived longer on straw lying on the surface than on buried straw

(ii) that treatment with paraquat lengthened survival on straw lying on the surface, but not on straw incorporated in the soil

(iii) that the fungus declined more rapidly in straw incorporated in direct-drilled soil than on straw incorporated in ploughed soil. This could be due to greater microbial activity in the direct-drilled soil than in ploughed soil (Brooks, 1967). This was supported by an observation that more saprophytes grew out on to agar plates from straw taken from direct-drilled soil.

Fig. 1. Survival of C. gramineum in straws in the field

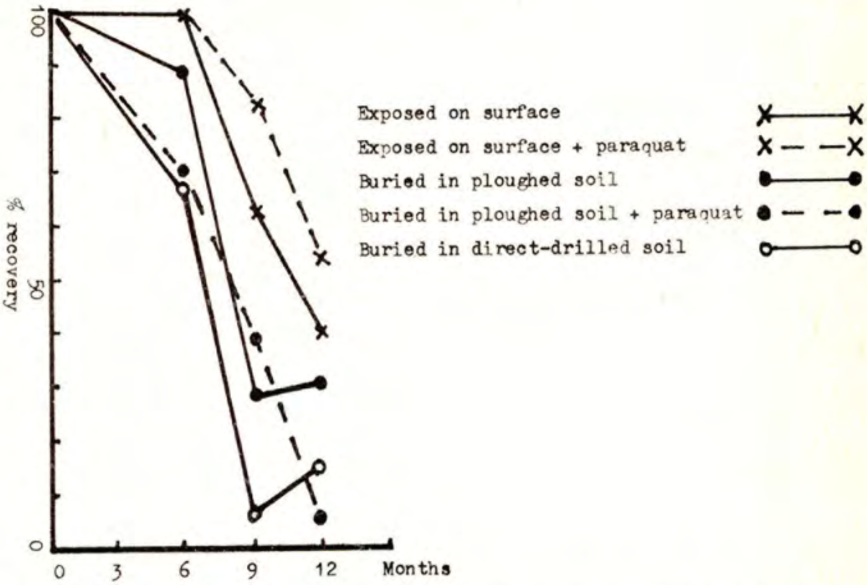
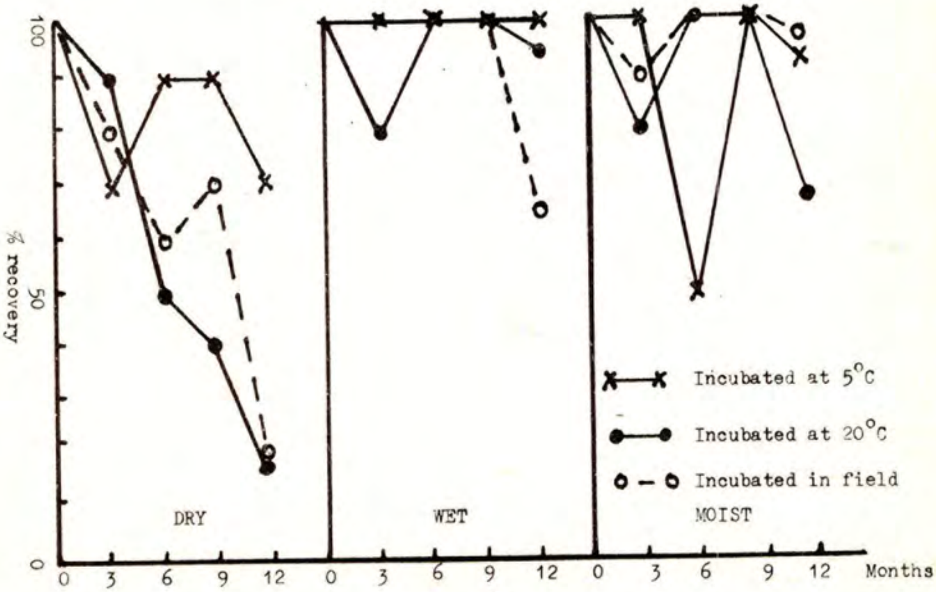


Fig. 2. Survival of C. gramineum in straws held under controlled conditions



Sporodochium production was more variable, but the results suggested that in buried straws the potential to produce sporodochia declines steadily, but on surface straws the potential depends on weather conditions. Straws which had apparently lost the potential to produce sporodochia on agar plates, regained the ability to do so after a period of high rainfall.

A second experiment investigated the effect of temperature and moisture on survival.

Lengths of naturally infected wheat straw were placed in glass phials kept in (i) a dry atmosphere (ii) a saturated atmosphere (iii) submerged in water. The phials were stored at 5°C and 20°C, and in the field. Again samples were taken at intervals of 3 months to determine survival by plating on agar. Results are shown in Fig. 2.

This experiment showed that in the absence of saprophytic competition, survival was greater at 5°C than at 20°C and greater on moist straw than in dry straw. Moist, cool conditions favoured survival in straw, at least in the absence of competition from soil saprophytes. This supports the field observations of Bruehl (1956).

From these experiments it can be argued that paraquat-sprayed stubble lying on the surface of direct-drilled plots allows greater survival than straw incorporated by ploughing. Thus survival could have been enhanced particularly in the cool, damp soil described in experiment CP1. It is interesting that in another direct-drilled experiment near to CP1, but where the stubble was burnt completely prior to drilling, very little leaf stripe occurred and there was no difference between ploughed and direct-drilled plots.

The relatively long survival as mycelium, and ability to sporulate profusely both in and on the soil, show that the fungus is potentially infective for long periods. Because the disease is rare in practice, one must assume that other factors are limiting.

DISCUSSION

From the observations described it is clear that the conditions prevailing in cereal stubble on heavy, clay soils prone to water-logging during winter, and infested with grass weeds, are best suited for survival of *Cephalosporium gramineum* and for a build up of fungus inoculum. It has also been shown that the fungus survives for longer on straw lying on the soil surface than on straw incorporated into the soil by ploughing. It is not surprising, therefore, that where direct-drilling experiments such as CP1 have been sited on heavy, poorly drained soils there has been a slightly greater build up of the disease on the direct-drilled plots than on the ploughed plots. Since direct-drilling is not advocated on such soils, these conditions would not normally arise in commercial practice.

It seems reasonable to assume, therefore, that leaf stripe is not likely to constitute a problem in cereals except under special conditions such as the repeated use of direct-drilling on unsuitable soils where a build up of the fungus is allowed to proceed unchecked. Even on these potentially dangerous sites, the control of grass weeds, and attention to stubble hygiene and burning should keep the disease in check.

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EFFECTS OF SOIL FUMIGATION ON CEREAL FOOT AND ROOT PATHOGENS

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Summary On soil where cereals had been grown frequently a formalin drench applied a few weeks before sowing spring wheat greatly decreased root infection by Ophiobolus graminis and significantly increased yield. Unless formalin was re-applied for the subsequent crop, take-all and cereal cyst-nematode infection increased greatly and yield decreased. On soil recently ploughed from grass, a formalin drench neither increased the yield of spring wheat nor prevented take-all increasing in subsequent crops.

Formalin applied during September for winter wheat decreased take-all in the spring where applied after ploughing, but not where applied to stubble: by harvest it had little effect on take-all incidence or yield.

Formalin had little effect on the incidence of brown foot rot (Fusarium spp), or brown root rot (Pythium spp) but often decreased eyespot (Cercosporella herpotrichoides).

INTRODUCTION

Promising crops of cereals growing on light shallow soils often stop growing during dry weather in June, wither, and yield little grain (Cook 1963). This trouble is worst in crops given abundant nitrogen fertilizer, and is associated with brown foot rot caused by species of Fusarium (Slope 1964). To indicate the relative importance of Fusarium as a primary pathogen and of water stress and nitrogen fertilizer as predisposing factors, an experiment on spring wheat was started at Woburn in 1964. The experiment failed in its initial purpose because the spring wheat was severely attacked by take-all and cereal cyst-nematode, but striking increases in plant growth and yield were obtained after partially sterilizing the soil with a formalin drench. For example plots irrigated when necessary and given 1.2 cwt nitrogen per acre yielded only 18 cwt, whereas those treated with formalin yielded 37 cwt grain per acre (Widdowson and Penny, 1965). These increases are attributed mainly to the control of take-all (Slope 1966) and cereal cyst-nematode (Williams 1966); Fusarium infection did not appear, probably because the month of June was wet. These striking results led to experiments on heavier soil at Rothamsted to study: (1) the control of take-all and other soil-borne diseases on land frequently cropped with cereals; (2) the rate of reinfestation after soil fumigation; (3) the effect on yield and on the increase of take-all after fumigating clean land recently ploughed from grass.

METHODS AND MATERIALS

In 1965 two experiments with spring wheat, comparing four nitrogen dressings (0, 0.5, 1.0 and 1.5 cwt N/ac as calcium nitrate), with and without formalin (266 gal of 38% formaldehyde in 4,000 gal water/acre), were started on two adjacent fields at Rothamsted. Little Knott, where the eight treatments were replicated four times, had grown cereal crops in 19 of the last 21 years. Pastures, where the treatments were replicated twice only, had been in grass for ten years before ploughing for spring wheat in 1964. In February 1965 basal P K fertilizer was broadcast on the 7 x 20 ft (0.0032 acre) plots, and formalin applied by watering can. Half the nitrogen was applied in March just before drilling Opal wheat and the remainder applied in May.

In February 1966 Little Knott was treated as 2 blocks of 16 plots and Pastures as 2 blocks of 16 half-plots to compare factorially formalin and no formalin in 1966, with formalin, and no formalin in 1965. Nitrogen was applied to whole plots as before, and Kloka wheat drilled in March.

For the 1967 crop formalin and no formalin were again compared factorially with formalin and no formalin in the previous year. A 4×2^3 confounded design was used in 2 blocks of 16 plots on Little Knott and 2 blocks of 8 split plots on Pastures. The formalin was applied in September 1966, either to stubble or shortly after ploughing and Cappelle winter wheat was drilled during October. Nitrogen fertilizer was applied as before.

For the 1968 crop there were no more replicates for testing formalin and no formalin factorially with previous applications. The design used was a $\frac{1}{2}$ replicate of 4×2^4 in 4 blocks of 8 plots on Little Knott and in 2 blocks of 8 split plots on Pastures. On Little Knott several interesting treatment sequences had to be sacrificed; for example no plot received formalin every year or had not received formalin in any year. (Table 1). The formalin was applied in September 1967 after ploughing and Cappelle wheat was drilled in October. Nitrogen was applied to the same plots as before in March and May.

Each plot was sampled three times between April and July; each sample contained about 50 plants lifted from four separate 6 in lengths of drill. Growth and vigour were estimated by recording the sample height from soil level to uppermost leaf tip and the number of tillers per plant. After washing, each plant was scored for the presence or absence of take-all, eyespot (Cercoporella herpotrichoides), sharp eyespot (Rhizoctonia solani), brown foot rot (Fusarium spp), brown foot rot (Pythium spp), and abnormal proliferating roots (Heterodera avenae). Severe take-all (more than 50% of the root system affected) was also recorded.

RESULTS

Little Knott experiment 1965-68. Table 1 shows that formalin had large effects in 1965. It greatly decreased take-all incidence in spring and a large difference persisted until harvest. In June only 20% of plants in treated plots had roots deformed by eelworm compared with 44% in untreated plots, and the improved growth after formalin resulted in 28% more grain. Formalin decreased eyespot in July from 31 to 12% straws infected, but it increased Pythium root rot from 3 to 14% and Fusarium foot rot from 2 to 5%.

In 1966 the decrease in take-all and the increase in plant growth after a fresh application of formalin were even greater than in 1965. However, where formalin had been applied in 1965 but not again in 1966, take-all was more prevalent and severe than in untreated plots. Damage by eelworm was less than in 1965, but where formalin had been applied in 1965 27% of plants were damaged by eelworm compared with 19% in untreated soil and fewer than 5% in soil treated with formalin in 1966. These differences in pest and disease incidence significantly increased grain yields where formalin was applied in 1966 and decreased yields where it was applied in 1965 only. (Table 2). There was very little eyespot, sharp eyespot, brown foot rot or brown root rot on straws in July, but in June there were eyespot lesions on 14% straws in untreated and on 4% in formalin treated plots. There was no effect of previous formalin on eyespot.

In contrast to its effect on take-all in spring wheat, formalin applied for the 1967 crop of winter wheat only slightly decreased take-all in April or July and yields were unaffected. However, the adverse residual effect of previous formalin was large, in both April and July, and decreased grain yield greatly. A comparison of the two times of applying formalin showed that, where applied to the stubble before ploughing, it had no effect on disease incidence, which averaged 23 and 69% plants infected in April and June respectively compared with only 8% and 38% respectively where formalin was applied after ploughing, as in previous years. By July these differences in take-all had almost disappeared (Table 1).

Table 1

Percentage number of plants with take-all in July. Little Knott 1965-8

1965 Spring Wheat	Take-all Total	Take-all Severe	1966 Spring Wheat	Take-all Total	Take-all Severe	1967 Winter Wheat	Take-all Total	Take-all Severe	1968 Winter Wheat	Take-all Total	Take-all Severe
0	93	54	0	71	34	0	78	7	F	91	52
						F	67	25	0	96	42
			F	9	5	0	99	67	0	32	12
						F	100	37	F	38	1
F	33	9	0	92	68	0	62	1	0	34	4
						F	59	14	F	72	24
			F	28	13	0	100	66	F	65	20
						F	85	35	0	56	33
Means											
0	93	54		82	51		85	35		54	23
F	33	9		18	9		77	28		66	24

0 = No formalin
F = Formalin

Table 2

Yields in cwt/ac at 15% moisture content. Little Knott 1965-8

1965 Spring Wheat	Grain	Straw	1966 Spring Wheat	Grain	Straw	1967 Winter Wheat	Grain	Straw	1968 Winter Wheat	Grain	Straw
0	24.5	41.8	0	29.4	36.7	0	31.0	37.3	F	28.4	35.0
						F	28.8	42.9	0	26.9	26.7
			F	37.0	52.9	0	15.5	28.8	0	33.5	38.3
						F	18.9	33.8	F	35.4	36.9
F	31.7	49.7	0	24.0	31.6	0	30.6	40.0	0	33.2	35.6
						F	29.7	45.7	F	30.5	33.0
			F	35.9	50.1	0	15.4	29.8	F	33.8	37.5
						F	21.1	38.0	0	31.0	31.0
Means											
0	24.5	41.8		26.7	34.1		23.1	34.0		31.1	32.9
F	31.7	49.7		36.5	51.5		24.6	40.1		32.4	35.6
S.E.	± 0.61	0.77		± 0.64	0.83		± 1.10	± 0.94		± 1.15	1.55

There was much less eelworm damage on winter wheat than on the spring wheat in previous years and it probably had no measurable effect on yield. Plants with eyespot lesions in April were decreased from 29% to 17% by formalin applied before or after ploughing, but this difference had almost disappeared by July, when untreated and treated plots had respectively 37% and 34% straws infected. Pythium root rot was found mainly in April, and Fusarium foot rot in July, but formalin did not affect their incidence.

Formalin applied after ploughing for the 1968 winter wheat crop decreased take-all in spring but by harvest there was rather more disease in the fumigated than unfumigated plots, and yield was unaffected. Formalin applied for the previous crop was again slightly detrimental. The residual effect of formalin seemed to be related to the degree take-all was controlled in the previous crop. Where formalin controlled take-all well and increased crop growth, as in 1965 and 1966, the adverse residual effects in the following year were large. Where take-all and growth were little affected, as in 1967, the residual effects were also small.

<u>The Pastures experiment 1965-68.</u> **Formalin** applied in 1965 for the second spring wheat crop after 10 years of grass had no effect on growth or yield (Table 4), and no residual effect on the increase of take-all in three subsequent wheat crops (Table 3).

Table 3

<u>Percentage number of plants with take-all in July. Pastures 1965-8</u>

1965 Spring Wheat	Total	Severe	1966 Spring Wheat	Total	Severe	1967 Winter Wheat	Total	Severe	1968 Winter Wheat	Total	Severe
0	2	0	0	56	30	0	85	33	0	44	18
									F	77	50
						F	82	34	0	54	22
									F	63	34
			F	22	13	0	95	56	0	58	36
									F	29	10
						F	62	34	0	50	18
									F	22	10
F	2	0	0	51	35	0	68	26	0	27	7
									F	31	14
						F	61	25	0	62	28
									F	41	16
			F	22	14	0	96	52	0	16	8
									F	42	23
						F	86	40	0	35	16
									F	69	34
Means											
0	2	0		54	32		86	42		44	19
F	2	0		22	14		72	33		47	24

No cereal root eelworm damage, and only traces of eyespot (2%), Fusarium foot rot (3%) and Pythium root rot (5%) were found. By 1966 take-all had become prevalent in the third spring wheat crop. Formalin greatly decreased the percentage of plants affected, and increased the mean height from 37 to 41 inches. Straw yield was

Table 4

Yields in cwt/ac at 15% moisture content. Pastures 1965-8

	1965 Spring Wheat			1966 Spring Wheat			1967 Winter Wheat			1968 Winter Wheat	
	Grain	Straw		Grain	Straw		Grain	Straw		Grain	Straw
0	27.2	55.1	0	35.7	50.8	0	29.5	56.6	0	27.0	42.2
									F	19.4	33.5
						F	32.2	71.8	0	28.2	36.4
									F	23.6	40.5
			F	36.8	61.6	0	25.7	52.7	0	26.8	43.6
									F	25.1	34.2
						F	34.2	69.6	0	24.8	28.8
									F	21.9	37.6
F	27.8	58.3	0	35.7	50.8	0	32.4	64.8	0	28.0	35.3
									F	23.0	44.5
						F	32.2	71.4	0	28.4	36.8
									F	21.0	31.4
			F	37.6	63.4	0	28.5	57.2	0	23.4	36.8
									F	24.4	44.0
						F	24.8	56.4	0	30.0	44.8
									F	22.0	36.2
Means											
0	27.2	55.1		35.7	50.8		29.1	57.8		27.1	38.1
F	27.8	58.3		37.2	62.5		30.9	67.2		22.5	37.7
S.E.	± 0.83	1.02		± 0.86	0.62		± 0.87	1.28		± 0.62	0.86

increased by 24%, but the crop lodged severely and grain yield was increased only where no nitrogen was applied. Lodging was associated with excess of nitrogen as only 14% of the straw had eyespot lesions in untreated and 2% in formalin treated plots.

As on Little Knott, the residual effects of formalin significantly increased take-all and decreased yield in the 1967 winter crop. Formalin applied for the 1967 crop decreased take-all where applied after ploughing but not where applied to the stubble before ploughing. By contrast, eyespot, found on 38% straws in untreated plots, was decreased more by applying formalin to stubble (15%) than after ploughing (24%). Formalin consistently increased straw at all rates of nitrogen but grain yields were limited by lodging and formalin increased them greatly where no nitrogen was given and decreased them where most nitrogen was applied.

Formalin applied for the 1968 crop had little effect on take-all or eyespot but greatly decreased grain yield, presumably because it increased lodging in a wet summer. Formalin applied the previous season again increased take-all but the shorter crop lodged the least and yielded the most grain.

DISCUSSION

These experiments showed that take-all can be greatly checked and yields increased on infested sites by treating soil with formalin. They also showed that formalin applied to an uninfested site did not prevent take-all increasing in following crops. The reasons for the unexpected ineffectiveness of formalin for winter wheat are being investigated.

The adverse residual effect of formalin seemed to be related to the degree of control of take-all it achieved in the previous wheat crop. It is easy to suggest that soil fumigation destroys many of the antagonists that may hold a pathogen in check, but this is difficult to prove. Other mechanisms are involved, Widdowson and Penny (1970) showed that the formalin applied to Little Knott in 1965 and 1966 increased plant growth so much that the nitrogen uptake in grain and straw was increased by 25% at each rate of nitrogen. This was approximately equivalent to the removal of an extra 1.0 cwt N per acre, and possibly decreased the growth of the following wheat crop. The residual effects of formalin on yield were greatest without nitrogen fertilizer but were not eliminated by the largest amount given. Even formalin newly applied did not eliminate them, so that yields were always larger where formalin was given for the first time than where it had been applied more than once. So it seems that the residual effect of formalin depends both on nutritional and microbiological factors.

Ebbels (1969) showed that dazomet had a similar residual effect to formalin, that more dazomet improved the control of take-all in the year it was applied and increased the severity of the residual effect in the following crop. Another soil fumigant D-D mixture had no effect on take-all in the year applied and no adverse residual effect, although it kills many soil microbes.

Treating soil with formalin, were it economically feasible, is not a panacea for controlling diseases where cereals are frequently grown. Apart from undesirable residual effects on take-all and yield neither formalin, dazomet nor D-D were efficient in controlling other diseases such as eyespot, Fusarium foot rot and Pythium root rot (Ebbels 1969). Grain yields after using formalin were never as great as those expected from crops where diseases are controlled by a suitable rotation of crops.

Acknowledgments

These experiments are part of a series, the yields of which have been discussed in detail by F.V. Widdowson and A. Penny (1970). Their contribution, and that of D.B. Slope is gratefully acknowledged.

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TREATMENT OF CEREAL SEED BY THE COMBINATION OF CARBOXIN WITH COPPER OXYQUINOLATE

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Summary This paper summarises the results obtained in France after three years of study with 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide (proposed common name: carboxin) which was used both alone and mixed with copper oxyquinolate in order to control the seed borne diseases of cereals. The main part of the work concerns wheat, barley and oat seed. The appendix deals with the potential uses of this combination and also of the combinations of carboxin with thiram and with maneb in the seed protection of sugar beet, maize, sorghum and market garden crops.

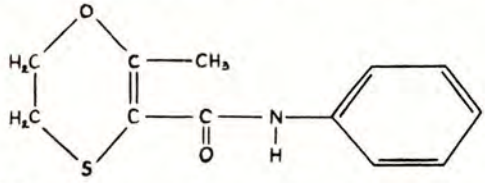
INTRODUCTION

Von Schmeling and Kulka (1966) recently demonstrated the remarkable properties of a new chemical family, the oxathiins. One of these substances, carboxin, proves to be remarkably effective against the Ustilago spp. including loose smut of wheat (Ustilago tritici) and loose smut of barley (Ustilago nuda). It is a well-known fact that these two diseases have always been thought to be resistant to all chemical treatments, and so the only remaining preventive method consisted of subjecting the contaminated seed to hot water treatment. The discovery of carboxin offers growers an efficient method of treatment against loose smut of wheat and barley, consisting simply of dressing the seed. It therefore constitutes an important step forward in the fight against plant diseases. In addition, this discovery will have even greater consequences, in opening up new and fruitful investigations into genetics. Geneticists and plant breeders will now be able to orientate their work towards obtaining new cereal varieties with high quality and yields, without having to concerne themselves with susceptibility to the smuts.

The outstanding character of carboxin lies in the fact that it is a systemic fungicide. This means that it is capable of penetrating the grain and then spreading into the embryo tissues, thereby effecting a real internal disinfection. It is because of this systemic property that carboxin is able to destroy the mycelium contained in the embryo of smut infected seed. Under natural conditions, this entry occurres as soon as the grain begins to swell on impregnation with soil moisture. Laboratory tests show that carboxin is exceptionally active on smuts. The growth of Ustilago zeae on agar, for instance, is entirely prohibited when carboxin is incorporated at 1 ppm. Field results are equally spectacular. (Richard and Cognet, 1967, 1968 a, 1968 b, 1968 c, Ventura et al, 1968)

METHOD AND MATERIALS

Physical and chemical properties of carboxin
Chemical name: 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide
Structure:



Molecular weight: 235
Physical appearance: white crystalline solid with a double structure
Solubility in water at 25°C: 170 ppm

Toxicological properties The toxicity of carboxin is extremely low. The "Food and Drug Research" laboratories (1966) give the following figures:

Acute toxicity for albino rats: L.D.50: 3,200 mg/kg

Chronic toxicity for albino rats: a daily ingestion of 200 ppm for 90 days permitted no particular macroscopic or microscopic observations. In addition, after twelve months of feeding at 100, 200 and 600 ppm there was no significant increase in mortality of animals treated.

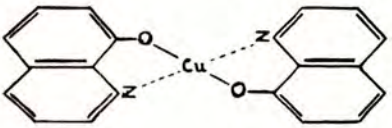
Acute dermal toxicity for adult rabbit: L.D.50: 8,000 mg/kg

Toxicity for fish: approximately 1,000 times less than with DDT for blue-gill sunfish (Weir, 1966), between 10 and 100 times lower than for DDT on rainbow trout (Weir, 1967)

Toxicity for wild life (Weir, 1966): bob-white quail, aged three days, were fed daily for five days approximately 3 g of food containing up to 5,620 ppm of carboxin: the L.D.50 is above this figure (note that the L.D.50 of DDT is between 790 and 1,060 ppm).

Other studies carried out in France indicate that the L.D.50 for red and grey partridge is higher than 2,000 mg/kg (Giban, 1968)

Physical and chemical properties of copper oxyquinolate
Chemical name: hydroxy-8 copper oxyquinolate
Structure:



Molecular weight: 351.6
Physical appearance: olive-green powder
Practically insoluble in water (0.8 ppm at 25°C)

This fungicide has been known for some time and was introduced for seed disinfection for the first time in France in 1959. Copper oxyquinolate is active on most externally carried fungi, has very low toxicity and holds several advantages with regard to formulation, in particular when used at a low rate (30 g per 100 kg seed), which enables it to be combined with other insecticidal or repellent products. It is now very widely used as a cereal seed powder or slurry treatment, not only in France but also in North Africa.

Toxicological properties (Gerard-Mazilier, 1953) Acute oral toxicity for rats: L.D.50 higher than 10,000 mg/kg

Chronic toxicity for rabbits: ingestions of 1,000 mg/kg repeated three times a week for two months did not show at autopsy any apparent sign of toxicity, nor any histopathological tissue change, especially of the renal parenchyma.

Subcutaneous toxicity for mice: subcutaneous injections of copper oxyquinolate in an oil suspension made for three days at 500 mg/kg showed no signs of toxicity.

Transcutaneous toxicity for man: the product, in either water or oil solutions, was rubbed on the skin and caused no irritation or sensitization of the skin. Furthermore, the formation in situ of copper oxyquinolate (by soaking the skin first in a solution of oxyquinoline sulphate and then in a solution of copper sulphate) also did not cause the slightest irritation or sensitization.

Toxicity for wild life (Grolleau, 1965)

Acute oral L.D.50 for bantam: 655 mg/kg

Acute oral L.D.50 for red partridge: 157 mg/kg

Experimental methods and design A randomized block design with six-fold replication was used in the field trials. Plots were hand sown. The cold chamber tests were conducted according to the method described by Kietreiber (1961) and Champion (1969) on a substrate of blotting paper at a temperature of 10°C. Finally, the Ulster method (Muskett and Malone, 1941) was employed for the other laboratory tests. The seeds were treated by dry dressing. Throughout this paper, doses are expressed in grams active ingredient per 100 kg seed.

RESULTS

1. Carboxin used alone The activity of carboxin on the various loose and covered cereal smuts is expressed in Table 1 as a percentage efficiency in relation to untreated according to the dose applied per 100kg of seed. These figures summarise the results of 25 trials on different varieties which, in general, carried high contamination levels. The activity of carboxin with regard to seed pathogens other than the smuts e.g. *Helminthosporium*, *Fusarium* and *Septoria*, is not adequate. Therefore in order that growers may be able to use just one product for the efficient control of all seed-borne diseases, it was desirable to combine carboxin with a fungicide which would complete its range of activity.

2. The combination of carboxin with copper oxyquinolate There are four main reasons for the choice of copper oxyquinolate. The use of this substance for the protection of wheat, barley and oat seed is already extremely widespread in France. It is necessary to use a fungicide requiring low content in formulated treatments, which frequently contain, apart from carboxin, one or more insecticides and a bird repellent. It also has a very low toxicity, which means that this valuable advantage of carboxin is retained. Finally, and above all, a synergism was discovered between carboxin and copper oxyquinolate which has been demonstrated particularly on *Fusarium*, *Septoria* and *Helminthosporium*.

Laboratory and cold chamber tests: demonstration of the synergism of the combination This discovery of synergism between carboxin and copper oxyquinolate was made as a result of some tests carried out to determine if any antagonistic effect existed between the two substances. It soon became apparent that the activity of the mixture was higher than the sum of the efficiencies of the two fungicides taken separately, as is shown in Table 2 and Figs. 1 and 2.

Table 1

<u>Percent efficiency of carboxin on loose and covered smuts in relation to untreated</u>

Cereal	Disease	Number of trials	Varieties	Average contamination level %	Dose of carboxin per 100 kg seed		
					60 g	75 g	100 g
w. barley	loose smut	8	Arès, Hatif de Grignon Y (not yet named)	14.16	94.03	96.89	99.05
sp. barley	loose smut	9	Ceres, X (not yet named)	7.81	90.93	94.17	97.05
sp. barley	covered smut	2	Rika (INRA-FRANCE)	27.13	100	100	100
			Carlsberg(INRA-MAROCCO)	2.51	100	100	100
w.wheat	loose smut	5	Cappelle	7.92	91.20	90.11	95.59
oats	loose smut	1	Noire de Moyencourt (INRA-FRANCE)	53.9 (artificial contamination)	100	100	100

Table 2

Synergism on Fusarium nivale: Ulster method

Carboxin g/100 kg	Copper oxy-quinolate g/100 kg	% efficiency in relation to untreated
56.2	0	66.6
0	10*	20
56.2	10	93.3

Calculation of the synergistic effect:

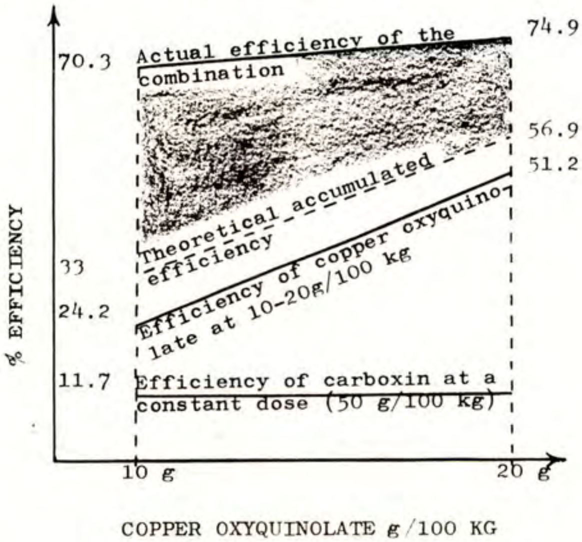
Activity of copper oxyquinolate	20.00%
Activity of carboxin: 66.6% of (100-20)	53.28%
total :	73.28%
Activity of the mixture	93.3 %

Synergism coefficient $\frac{93.3}{73.28} = 1.27\%$

* The recognised dose for copper oxyquinolate is 30 g/100 kg. In research on synergistic effects, it is usual to experiment on products at variable doses well below the normal rates of use.

Fig. 1

Synergism on Septoria nodorum cold chamber tests

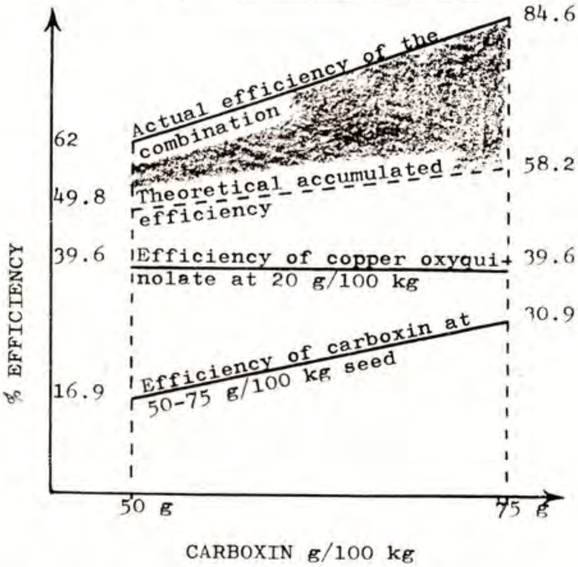


The shaded zone in Fig. 1 represents the synergistic action resulting from the combination of carboxin with copper oxyquinolate (both used at doses well below the normal rates of use). In this example, the synergism coefficient for a mixture containing 50 g of carboxin and 10 g of copper oxyquinolate is in the region of 2, as is proved by the following calculation:

activity of carboxin	11.7%
activity of copper oxyquinolate	
24.2% (100-11.7)	21.3%
total	33.0%
activity of the mixture	70.3%
synergism coefficient $\frac{70.3}{33}$ =	2.1

Fig. 2

Synergism on Helminthosporium gramineum: Ulster method



Again, in Fig. 2 the shaded area measures the synergistic effect observed against this pathogen.

Field trials During the years 1967, 1968 and 1969 a large number of trials were carried out in France and in North Africa, both by official or professional organisations and by the research department of La Quinoleine. These trials were made on loose and covered smuts of winter and spring barley, loose smut of wheat, loose smut of oats, bunt of wheat and leaf stripe of barley.

The results obtained, which are summarised in Fig. 3, led the French Ministry of Agriculture to grant commercial clearance in 1968 for the formulation combining the following quantities of fungicides per 100 kg of barley, wheat or oat seed:

carboxin : 100 g
copper oxyquinolate : 30 g

Efficiency Fig.3 compares the activity against eight cereal seed pathogens of the carboxin and copper oxyquinolate combination with that of the organomercurial used for reference, each product was used at its recommended dose per 100 kg. The tests directed against Septoria and Fusarium were carried out in a cold chamber, the remainder in the field. Under these conditions, the combination of carboxin with copper oxyquinolate reveals an efficiency which is at least equal to that of the reference product which is not active against either Ustilago nuda or Ustilago tritici

Crop safety Phytotoxicity tests on wheat (varieties: Cappelle, Champlein, Moisson, Prestige, Rex, Splendeur) and on barley (varieties: Ares, Aurore, Carlsberg, Ceres, Delisa, Hatif de Grignon, Ingrid, Gregor, Mamie, Piroline, Rika, Trait-d'Union, Zephyr) showed that the treatment of cereal seeds with the combination of carboxin and copper oxyquinolate has no influence, either positive or negative, on their development (Muller, 1968). No particular action was seen on the date of emergence, the percentage of germination, the number of tillers per plant, the date of ear emergence, the date of flowering, or the height of the stems. Likewise, a precise measurement of the fertility of the ears and the weight of 1,000 grains showed no significant effect, as seen in Table 3. For this study 50 ears per plot were harvested and threshed separately. No significant difference is revealed from the statistical analysis of the results of this series of tests.

Table 3

Crop safety

Cereal	Number of grains per ear		Weight of 1,000 grains (in g)	
	Carboxin + Cu oxyquinolate	Untreated	Carboxin + Cu oxyquinolate	Untreated
wheat	39.1	37.1	47.4	47.3
barley	41.3	40.6	44.9	44.2

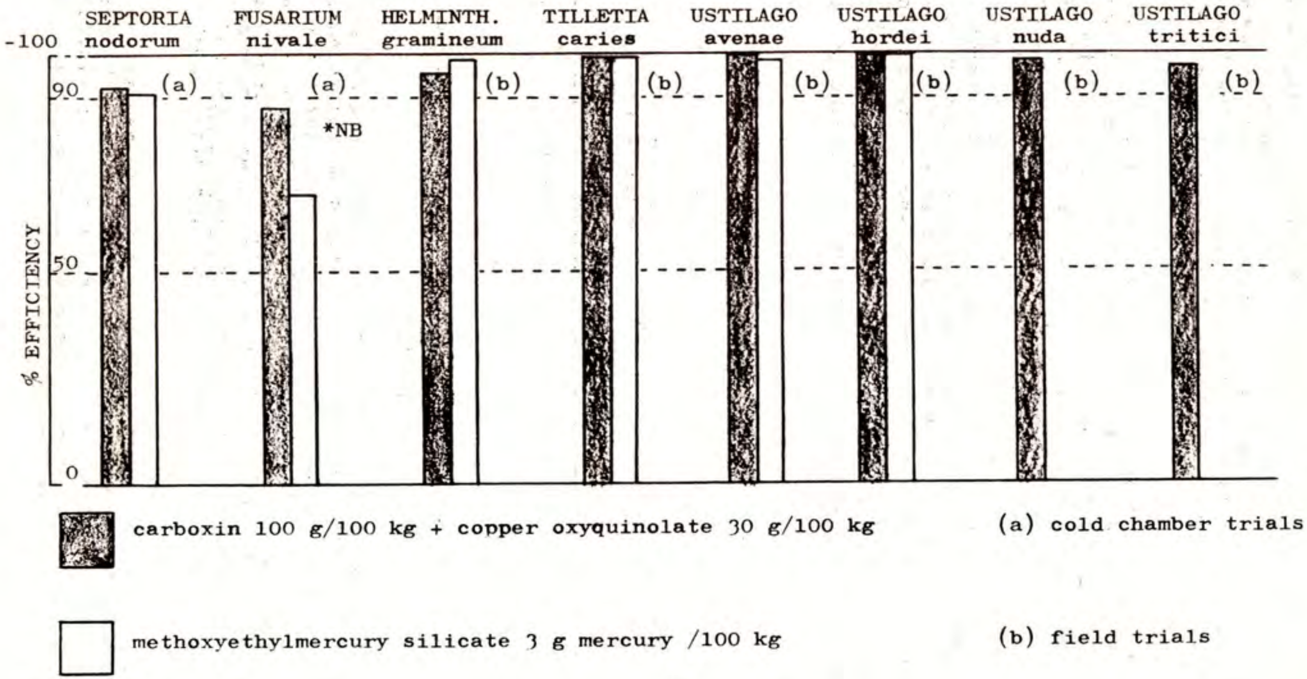
DISCUSSION

The combination of carboxin with copper oxyquinolate proves to be the only product in the world currently available to agriculturists presenting the following complement of characteristics. It gives a practically complete efficiency against all externally carried cereal seed pathogens: Septoria, Fusarium, Helminthosporium, Tilletia, Ustilago hordei and U. avenae. It also has an efficiency varying from 95 to 100% on internally carried smuts: Ustilago tritici and U. nuda. It has a virtual absence of toxicity for man, domestic animals, wild life and crop.

The remarkable consistency of the field results with this combination recorded over three years should be noted. The originality of this combination lies in a very interesting synergism between the two components, one of which is among the first systemic fungicides to appear in the history of crop protection.

Fig. 3

Comparing the efficiencies of the combination of carboxin with copper oxyquinolate and of the organo-mercurial reference product on eight principal cereal seed pathogens



*NB. Trials carried out in Sweden showed that the activity of the combination against Fusarium nivale was in the region of 100%.

APPENDIX

Some preliminary and more limited work carried out with carboxin, used either alone or in combination with various fungicides, gives an indication of its possible use in the protection of other types of seed.

Sugar beet Copper oxyquinolate, used at 120 g/100 kg, is already considered to be one of the best fungicides for the protection of sugar beet seed against *Phoma betae*, *Pythium ultimum*, *Cercospora beticola*. The combination of 400 g/100 kg of carboxin with 120 g/100 kg of copper oxyquinolate proves to be extremely efficient against these three most frequently encountered pathogens on sugar beet. In two tests, this combination ensured a protection comparable to that supplied by 800 g/100 kg formulation of methoxyethylmercury silicate (1.5%).

Sorghum and maize Captan and thiram are the most commonly used fungicides for the disinfection of sorghum and maize seed. This is due, not only to the excellent fungicidal activity of these two substances, but also to their effect on the germination of the seed, which makes the emergence of the seedlings more rapid and vigorous. This effect is even more evident when germination and emergence conditions are difficult (Mezgovorov, 1965 and Melet, 1967). This was precisely the case in a field trial which was carried out with sorghum hybrid seeds intended for multiplication. The results were as follows (Table 4):

Table 4

Disinfection of sorghum seed

Product	% emergence 50 days after sowing
Untreated	14.3
Thiram (TMTD) 180 g/100 kg	30.3
Carboxin 100 g/100 kg + Cu. oxyquinolate 30 g/100 kg	41.3
Carboxin 90 g/100 kg + TMTD 90 g/100 kg	45.5

This trial shows the significantly superior activity of carboxin combined with copper oxyquinolate and with TMTD compared with TMTD alone applied at its normal rate of use.

Similar trials were carried out on maize seed and showed an equal, though not better, efficiency of the two combinations compared with TMTD.

Market garden crops (Beyries, 1969)

Radishes Radish seeds were sown in a soil which had been artificially contaminated with *Rhizoctonia solani*. The results obtained show that coating the seed with 11.25 g/kg carboxin offered a significantly (5%) superior protection to that given by incorporating in the soil 20 g/m^2 of a product which contains 30% pentachloronitrobenzene (PCNB).

Beans Similar trials to those mentioned above showed that bean seeds sown in a soil naturally contaminated with a complex of Pythium de Baryanum, Phythium ultimum and Rhizoctonia solani, were efficiently protected against all these parasites by the combination of carboxin (1.5 g/kg) with maneb 80% (2 g/kg).

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LABORATORY AND FIELD TRIALS IN THE UNITED KINGDOM WITH THE SYSTEMIC FUNGICIDE CARBOXIN

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Summary Extensive laboratory and field tests have been carried out with carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) as a dry seed treatment against a range of seed-borne pathogens of cereals. These tests indicate that carboxin provides excellent control of Ustilago nuda f. sp. tritici and hordei. Some activity has also been demonstrated against Helminthosporium gramineum and Fusarium graminearum. Combination of carboxin with a mercury based dressing is necessary for the effective control of Pyrenophora avenae, Fusarium spp. and Septoria nodorum.

INTRODUCTION

Considerable concern has been shown over the increase in incidence of wheat and barley loose smut in the United Kingdom over the past decade. In the mid 1950's a survey of commercial samples of barley received at the Official Seed Testing Station, Cambridge, (Marshall, 1959) showed that nearly all samples of susceptible varieties contained loose smut infected grains and that these samples were carrying an overall average of 1.6% infected embryos. Samples of "resistant" varieties contained very little smut; only 14% of the Proctor samples received contained loose smut and the overall average infection was less than 0.04%. Since then the barley acreage has trebled and the high yielding, loose smut susceptible, 'open flowering' barley varieties now constitute three quarters of the total number of barley varieties grown in Great Britain. Hewett (1968) recently reported provisional results showing that of 30 Proctor samples examined by the O.S.T.S. in 1968 - 69, 15 contained loose smut and the overall infection of loose smut averaged 0.12 thus indicating a considerable increase in inoculum potential.

In wheat (where the flowering behaviour is of less significance) a new physiologic race (C4) virulent on the previously smut-resistant winter variety Cappelle-Desprez has become widespread and of major importance on this and other varieties. The control of loose smut in barley and wheat has until recently been accomplished only by the use of hot water treatments or by crop inspection and rejection. This paper reports results obtained in the United Kingdom during the development of carboxin as an alternative control measure.

METHOD AND MATERIALS

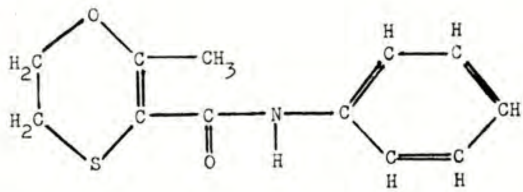
The systemic fungicide carboxin has been referred to in literature as D735 and as DCMO. It was first synthesised in Canada by von Schmeling and Kulka (1966) and was shown to have a specific and selective toxicity to Basidiomycetes (Edgington et al., 1966).

Physical and chemical properties of carboxin

Chemical name: 5,6 dihydro-2 methyl-1,4 oxathiin-3-carboxanilide.

Empirical formula: $C_{12}H_{13}O_2NS$

Structural formula:



Molecular weight: 235 Physical state: Solid

Melting range: Two crystal structures; A 91.5 - 92.5°C; B 98.0 - 100°C

Solubility: g solute/100 g solvent @ 25°C:

Distilled water: 0.017, benzene: 0.5, dimethyl sulfoxide: 150, acetone: 60, methanol: 21, ethanol: 11.

Formulations used. Carboxin was formulated for trials as 75% w/w dry powder seed dressing and as a 55% w/w dry powder seed dressing containing 1% organically combined mercury. It was also formulated as a 36% w/v liquid seed dressing and as a 40% w/v liquid seed dressing.

Toxicology. Acute toxicities of carboxin are shown in Table 1.

Table 1

Carboxin - acute toxicities

Animal	Application route	Acute LD50 value mg/kg
Rats	oral	3,200
Rabbits	percutaneous	8,000
Female mice	oral	6,200
Grey and red partridge	oral	2,000

The acute oral toxicity to female mice of 55% w/w carboxin formulated with 1% organically combined mercury is 3,800 mg/kg.

Residue data. Samples were taken for residue analysis from two winter wheat trials, a winter barley trial and a spring barley trial. Samples at the late tillering stage and harvest samples, divided into grain and straw, were taken from all four trials. The residues were assessed at the Huntingdon Research Centre. The results are given in Table 2 as mean values in ppm. The residues in the late tillering samples are calculated for 98% recovery in barley and 102% recovery in wheat, at a 1.0 ppm level. Recoveries from grain and straw at 2.0 ppm level were 78% and 68% respectively.

Table 2

Carboxin residues (ppm) found in cereals treated with 1.5 oz a.i. carboxin

Cereal	Variety	Date of sowing	Residues Late tillering*	Harvest+ grain	Harvest+ straw
Wheat	Capelle-Desprez	1/12/67	0.00	0.00	0.00
"	" "	21/ 3/68	0.05	0.00	0.00
Barley	Maris Otter	4/12/67	0.00	0.00	0.00
"	N.I.A.B coded variety	21/ 3/68	0.00	0.00	0.00

* Assessed at 179 days after sowing for winter sown trials and 69 days after sowing for spring sown trials.

\+ Assessed at 284 days after sowing for winter sown trials and 175 days after sowing for spring sown trials.

These results are in agreement with findings in the U.S.A. which confirm that there is no detectable residue in the seeds of wheat and barley and that the negligible residue tolerance of 0.2 ppm is reached within 40 days of sowing.

Trials Methods. For laboratory tests seed treatments were applied by shaking the seed and the treatments in a bottle for five minutes.

For field trials the powder seed treatments were applied to the seed in a tumbler type seed dresser. The liquid formulation was applied together with a liquid organo-mercury formulation by a Mist-O-Matic seed dressing machine.

Adhesion was assessed by gas-chromatography, the actual loading found being compared with the theoretical loading.

In field trials seed was selected with a high level of loose smut infection. This was sown with standard drills, plot sizes being 8.5 ft wide and between 40 and 100 ft long. Trials were replicated in randomized block or Latin square designs with four replications. The plots were assessed initially for emergence in order to detect any phytotoxic properties of the treatments. At ear emergence the number of smutted ears/plot was counted. Wherever possible yields were assessed by combining the plots individually and weighing the grain.

In order to assess the efficiency of treatments against those diseases normally controlled by organo-mercurial seed treatments, stocks of seed infected with leaf spot of oats (Pyrenophora avenae) leaf stripe of barley (Helminthosporium gramineum), glume blotch of wheat (Septoria nodorum) and brown foot rot (Fusarium spp.) were obtained from England, Scotland and Sweden. Wherever stocks of seed were available large scale field trials were laid down in the same way as for loose smut.

Where infected seed was limiting small hand-sown trials were laid down or Hiltner health tests utilizing molochite clay (Hiltner, 1917) were carried out.

Seedlings counts were made at emergence to assess disease control at the seedling stage. Later counts of diseased plants and/or ears were made at ear emergence. Plot yields were assessed wherever possible.

RESULTS

Initial work in 1967/68 was to establish the optimum rate of useage of carboxin for the control of loose smut in barley, wheat and oats. The results of four field trials carried out at Wheathampstead on chalk loam soil using plot sizes of 8.5 ft x 100 ft are given in Tables 4 to 6 (a plot size of 8.5 ft x 40 ft was used to obtain results presented in Table 7). Adhesion investigations were carried out before sowing and the results are given in Table 3.

Table 3

Carboxin - adhesion of treatments used in 1967/68 loose smut trials.

Treatment	Cereal	Rate	% adhesion
Carboxin 75%	Barley	2 oz/bushel	49.5
Carboxin 75%	Wheat	2 oz/bushel	60.2

The 75% carboxin formulation used, except where otherwise stated, was formulated as a wettable powder rather than as a seed treatment which probably accounts for the low adhesions obtained. These results and others show that carboxin adhers better to wheat than to barley seed.

Table 4

Loose smut control in winter barley (Maris Otter) by treatment with carboxin 75%.

Sowing date	Sowing rate (lb/ac)	Treatment rate (oz/bushel)	Emergence plants/ft row	Smutted ears/plot	Yield (cwt/ac)
4/12/67	145	3	13.3	0.75	32.0
"	"	2	14.0	0.0	34.4
"	"	1	14.4	1.0	34.7
		Control (untreated)	15.3	64.7	34.1
L.S.D. 5% (Latin square)			N.S.	22.6	N.S.

Table 5

Loose smut control in spring barley (Zephyr) by treatment with carboxin 75%.

Sowing date	Sowing rate (lb/ac)	Treatment rate (oz/bushel)	Emergence tillers/ft row	Smutted ears/plot	Yield (cwt/ac
1/4/68	165	3	39.4	0.0	29.9
"	"	2	35.5	0.25	29.8
"	"	1	37.3	0.0	29.4
"	"	Control (untreated)	39.8	71.0	31.3
L.S.D. 5% (Latin square)			N.S.	7.1	N.S.

Table 6

Loose smut control in winter wheat (Capelle-Desprez) by treatment with carboxin 75%.

Sowing date	Sowing rate (lb/ac)	Treatment rate (oz/bushel)	Emergence plant/ft row	Smutted ears/plot	Yield (cwt/ac)
1/12/67	185	3	11.4	4.25	25.7
"	"	2	10.7	1.0	25.7
"	"	1	11.9	1.0	26.1
"	"	Control (untreated)	9.4	60.5	25.8
L.S.D. 5% (Latin square)			N.S.	-	N.S.

Table 7

Loose smut control in spring oats by treatment with carboxin 75% and carboxin 50%.

Sowing date	Sowing rate (lb/ac)	Treatment	Treatment rate (oz/bushel)	Emergence tillers/ft row	Smutted ears/plot
5/4/68	213	Carboxin 75%	2	33.3	0
"	"	Carboxin 50%	2	32.2	0
"	"	1% organo-mercury	2	32.9	19.2
"	"	Control (untreated)	-	33.9	111.2
L.S.D. 5% (Randomized block)				N.S.	11.5

The results show that carboxin 75% powder treatments even when applied at 3 oz/bushel had no significant effect on the emergence of wheat, barley or oats and provided almost complete control of loose smut in winter barley, spring barley and winter wheat at rates down to 1 oz/bushel. Both 75% and 50% powder seed treatments of carboxin provided complete control of loose smut of oats (*Ustilago avenae*) when applied at 2 oz/bushel.

A Hiltner health test (Table 8) and a small plot field trial (Table 9) demonstrated that carboxin has some activity against brown foot rot of oats (*Fusarium graminearum*), and leaf stripe of barley (*Helminthosporium gramineum*) respectively.

Table 8

Results of Hiltner health test showing activity of carboxin against brown foot rot (*Fusarium graminearum*) in Astor spring oats.

Treatment	Rate	% Germination	% Pre-emergence blight	% emerged diseased plants	% emerged healthy plants
Carboxin 36% (liquid dressing)	2 fl oz/bushel	94.0	5.5	2.0	86.5
1% organo-mercury (powder dressing)	2 oz/bushel	95.5	5.0	2.0	86.5
Control (untreated)	-	93.0	8.5	7.0	77.5

Table 9

Results of trial showing activity of carboxin against leaf stripe of barley (*Helminthosporium gramineum*)

Treatment	Rate	% infected plants	Angular Transformation
Carboxin 75%	3 oz/bushel	3.3	10.3
Carboxin 75%	2 oz/bushel	4.0	9.9
Carboxin 75%	1 oz/bushel	4.8	12.1
Carboxin 50%	2 oz/bushel	6.0	13.4
1% organo-mercury	2 oz/bushel	0.5	2.9
Control (untreated)	-	15.8	23.2
L.S.D. 5% (Randomized block)		-	7.0

Variety: Swedish (unspecified). Plot size: 25 ft x 5 ft (5 rows)
Sowing rate: 1 g seed/10 ft. Layout of trial: Randomized block design.

On the basis of the results obtained in 1967/68 the formulation selected for further work was a powder seed treatment containing 55% carboxin in combination with 1% organo-mercury applied at 2 oz/bushel.

During 1968/69, field trials were carried out in Wales (on clay loam soils and using a randomized block design) to check the activity of this formulation against loose smut in barley (*U. nuda*), glume blotch (*S. nodorum*), brown foot rot (*Fusarium* spp.) and a mercury tolerant race of leaf spot of oats (*P. avenae*). In all trials a 1% organo-meroury powder dressing was used as a standard treatment.

Results of further adhesion investigations are shown in Table 10.

Table 10

Carboxin - adhesion of treatments used in 1968/69 seed-borne disease trials.

Treatment	Rate	% adhesion of carboxin
55% carboxin + 1% organo-mercury powder dressing (barley)	2 oz/bushel	75.7
55% carboxin + 1% organo-mercury powder dressing (wheat)	2 oz/bushel	76.1

A satisfactory adhesion of carboxin is obtained when it is formulated as a seed treatment. The results of four trials are given in Tables 11 to 14.

Table 11

Trial against loose smut and brown foot rot in spring barley.

Treatment	Rate	Emergence plants/ft row	Healthy seedlings/ ft row	Smutted ears/plot
55% carboxin + 1% organo-mercury	2 oz/bushel	19.3	19.2	0.0
1% organo-mercury	2 oz/bushel	18.7	18.6	181.5
Control (untreated)	-	16.6	15.4	369.8
L.S.D. 5%		N.S.	N.S.	118.8

Variety: Zephyr. Location of trial: Glamorgan. Sowing date: 18/4/69.
Sowing rate: 126 lb/ac. Plot size: 8.5 ft x 50 ft.

Table 12

Trial against glume blotch in winter wheat.

Treatment	Rate	Emergence plants/ft row	Healthy seedlings/ft row	Ears/ft row	Healthy ears/ ft row
55% carboxin + 1% organo-mercury	2 oz/bushel	13.7	13.7	16.4	14.3
1% organo-mercury	2 oz/bushel	14.2	14.2	17.2	15.7
Control (untreated)	-	13.4	11.2	15.2	11.0
L.S.D. 5%		N.S.	N.S.	N.S.	2.8

Variety: Cappelle-Desprez. Location of trial: Denbighshire. Sowing date: 28/3/69
Sowing rate: 224 lb/ac. Plot size: 8.5 ft x 75 ft.

Table 13

Trial against mercury tolerant leaf spot of oats.

Treatment	Rate	Emergence plant/ft row	% diseased seedlings	Angular trans-formation	Healthy seedlings/ ft row	Yield cwt/ac
55% carboxin + 1% organo-mercury	2 oz/bushel	12.0	7.0	15.3	11.1	45.3
1% organo-mercury	2 oz/bushel	11.6	9.5	17.8	10.5	42.1
Control (untreated)	-	9.8	15.7	23.3	8.3	41.1
L.S.D. 5%		N.S.	-	4.7	N.S.	N.S.

Variety: Forward. Location of trial: Denbighshire. Sowing date: 28/3/69.
Sowing rate: 140 lb/ac. Plot size: 8.5 ft x 75 ft.

Table 14

Trial against glume blotch and brown foot rot in spring wheat.

Treatment	Rate	Emergence plants/ft row	Healthy seedlings/ft row	Ears/ft row	Yield cwt/ac
55% carboxin + 1% organo-mercury	2 oz/bushel	21.7	21.6	27.4	40.5
1% organo-mercury	2 oz/bushel	18.8	18.8	25.8	41.9
Control (untreated)	-	16.0	13.4	23.4	37.0
L.S.D. 5%		3.0	5.1	N.S.	4.6

Variety: Kloka. Location of trial: Glamorgan. Sowing date: 18/4/69.
Sowing rate: 168 lb/ac. Plot size: 8.5 ft x 50 ft.

It will be seen from Table 11 that the full activity of carboxin when formulated with organo-mercury is maintained against loose smut in spring barley. There are indications from the assessments of brown foot rot that the combined formulation is more efficient than the straight organo-mercurial treatment.

Against glume blotch in wheat (Table 12) both the combined formulation and the straight organo-mercurial treatment significantly increased the number of healthy ears/ft row.

In the trial against partially mercury tolerant leaf spot of oats (Table 13) there was a significant decrease in the percentage of diseased seedlings with both the combined and straight organo-mercury dressings. There are indications that the addition of carboxin to an organo-mercury dressing increases the efficiency of control.

The Kloka wheat (Table 14) was severely infected with glume blotch and brown foot rot; both treatments significantly improved the establishment of healthy seedlings. This is reflected in the ear count, although no glume blotch symptoms appeared at the ear stage, and in the final yield analysis.

Table 15

Trial against loose smut in spring barley.

Treatment	Rate	Emergence plant/ft row	Smutted ears/plot	Yield cwt/ac
55% carboxin + 1% organo-mercury powder dressing	2 oz/bushel	17.3	0.0	26.1
40% carboxin liquid dressing + 2% organo-mercury liquid dressing	3 fl oz/bushel 1 fl oz/bushel	19.2	1.0	26.2
Control (untreated)	-	17.5	197	27.6
L.S.D. 5%		N.S.	56.4	N.S.

Variety: Zephyr. Location of trial: Wheathampstead. Sowing date: 17/4/69.
Sowing rate: 168 lb/ac. Plot size: 8.5 ft x 100 ft. Soil type: Chalk loam.
Layout of trial: Randomized block design.

One trial was laid down as a preliminary assessment of the efficiency of carboxin as a liquid formulation against barley loose smut. Carboxin was applied as a 40% formulation at 3 fl oz/bushel with a straight liquid organo-mercury treatment. Table 15 shows that no despression of plant emergence occurred and that a control of loose smut was obtained comparable to that obtained with a carboxin powder dressing.

Following the marketing of 55% carboxin + 1% organo-mercury powder seed dressing in this country in 1968 records were kept of field inspections for loose smut on certified seed crops which had been treated with this mixture. In Table 16 the mean number of smutted ears/10 quadrats for each variety is compared with the average reject number as laid down under the British Cereal Seed Scheme.

Table 16

Occurrence of loose smut in commercial certified seed crops treated with 55% carboxin + 1% organo-mercury powder seed dressing @ 2 oz/bushel.

Crop	Variety	Number of crops	Total acreage	Average number of smutted ears/10 quadrats +	Average reject number
Barley	Zephyr	68	1695	5.2	56
	Vada	18	290	2.5	45
	Sultan	8*	293	7.6	74
	Malta	56	169	3.0	46
Wheat	Cappelle-Desprez	5	102	3.2	42
	Joss Cambier	105	2740	1.3	42

* One crop was rejected + quadrat size = 1 yd x 24 yd

The figures show that of a total of 260 crops grown for certified seed involving 5289 acres of wheat and barley only one crop failed the standard laid down by the British Cereal Seed Scheme.

Table 17

Carboxin 55% + 1% organo-mercury powder seed treatment @ 2 oz/bushel - germination results with representative varieties of wheat, barley and oats.

Crop	Variety	Moisture content %	Storage period (weeks)	% Germination* In soil Normal	In soil Abnormal	In sand Normal	In sand Abnormal
Wheat	Kloka	15.4	0	92 (89)	2 (6)	92 (90)	2 (3)
"	"	14.0	12	97 (84)	0 (3)	90 (87)	3 (0)
"	Cappelle-Desprez	15.5	0	94 (81)	2 (7)	91 (79)	2 (11)
"	Champlein	16.4	0	87 (86)	7 (10)	85 (85)	5 (3)
Barley	Proctor	14.4	0	98 (98)	1 (0)	89 (94)	5 (0)
"	"	13.3	16	94 (92)	1 (1)	93 (96)	2 (0)
"	"	13.5	24	95 (90)	0 (5)	97 (93)	0 (0)
"	Maris Otter	16.0	0	73 (66)	4 (7)	71 (59)	6 (8)
Oats	Maris Quest	14.6	0	98 (97)	1 (5)	95 (97)	0 (1)
"	Astor	16.2	0	69 (65)	2 (3)	63 (79)	2 (1)

* Figures for untreated control samples given in brackets.

Extensive germination tests to investigate possible phytotoxic effects of carboxin in combination with organo-mercury have been carried out with representative varieties of wheat, barley and oats. From the figures shown in Table 17 it can be seen that the carboxin seed treatment presented no hazard to germination even after storage periods of up to 24 weeks.

DISCUSSION

The increasing concern about the incidence of loose smut of barley and wheat in the United Kingdom and the inadequacy of existing methods of control in 1966 to contain the deteriorating situation gave urgency to the investigation and development of carboxin as an alternative control to hot water treatment. The results reported in this paper show that in the field carboxin as a 75% powder dressing at rates as low as 1 oz/bushel affords an almost complete control of loose smut of wheat, barley and oats.

The combination of 55% carboxin with 1% organo-mercury as a seed treatment does not affect the efficiency of carboxin in controlling loose smut. Similarly, there is no diminution in the control of glume blotch, brown foot rot and leaf spot of oats against which organo-mercurial seed treatments are used as a control measure. In fact, there are indications of an additive effect between carboxin and organo-mercury in the control of these diseases.

The adhesion data given in Table 10 combined with the germination results given in Table 17 demonstrate that the use of a carboxin + organo-mercury formulation as a seed treatment is feasible and that there are no recordable phytotoxic effects. The residue data given in Table 2 shows conclusively that carboxin, when used as a seed treatment, is not a hazard in terms of residues in the mature crop.

The figures given in Table 16 demonstrate that the efficiency of carboxin as a seed treatment against loose smut when formulated with organo-mercury is as high in commercial practice as in experimental samples.

One trial was carried out with a liquid formulation of carboxin against loose smut of barley (Table 15). The results indicate a considerable promise of carboxin as a liquid seed treatment and further work is envisaged with this type of formulation. In addition, formulations of carboxin and organo-mercury with lindane as a means of controlling wireworm are being investigated.

It is concluded that carboxin, when formulated with organo-mercury as a seed treatment, affords an excellent control of loose smut of wheat, barley and oats without a decrease in the effectiveness of control of those other seed-borne pathogens against which organo-mercurial treatments are used as a control measure.

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QUANTITATIVE ESTIMATION OF SOIL-BORNE INOCULUM OF THE TAKE-ALL FUNGUS (OPHIOBOLUS GRAMINIS (SACC.) SACC.)

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Summary A maximum likelihood estimation of the number of infective units (λ) of Ophiobolus graminis inoculum in soil can be obtained from host-infection tests using dilutions of either whole soil or the organic debris extracted from it. Details of procedure, suggested test conditions and experimental examples are given. Estimates of λ were small (e.g. 5 per 150 cm^3 of a sandy loam immediately after harvesting spring wheat and 1 per 150 cm^3 in the February following) and in 3-week tests on whole soil varied with the number of seedlings/pot (optimum = 2/pot). For land growing spring barley sampled in May, estimates were greater with dilutions of extracted organic debris than of whole soil. Soil from within the barley rows had 0.59 infective units/150 cm^3 and soil from between rows only 0.37. Practical application of the method is discussed.

INTRODUCTION

The take-all fungus is a specialised root parasite that exists either in an ascendant parasitic phase or in a declining saprophytic phase (Garrett, 1936). During cereal monoculture the period between susceptible crops is brief and the control of take-all by rotating cereals with non-susceptible crops succeeds because inoculum in the soil decreases during the prolonged phase of saprophytic survival. Until satisfactory systemic fungicides are developed there is no adequate means of controlling the parasitic phase (soil fumigants can kill the fungus in its saprophytic phase but have limitations of cost and effectiveness (Salt & Widdowson, 1968)) and resistant varieties of wheat and barley are unknown. In the past, experiments have had to be assessed on yields and take-all indices. These are useful for relating yield and disease, but more comprehensive methods, applicable at other stages, are needed to evaluate control measures precisely. This paper describes how to estimate the number of infective units of O.graminis inoculum in soils. The units are thought to be colonised host debris (Hornby, 1969b) and it is assumed that each discrete fragment of colonised debris behaves as a unit. This concept and the use of a well-known statistical technique make it possible to estimate quantitatively populations of O.graminis in soil.

METHOD AND MATERIALS

The basis of the method is the estimation of the number of infective units (λ) in soil from positive and negative tests (i.e. infected and uninfected host seedlings) in a dilution series (called variously the dilution method (Fisher and Yates, 1963), most probable number method or maximum likelihood method). The method has been much used with bacteria and viruses, but little in the study of fungus diseases. Maloy & Alexander (1958) described its use for fungus pathogens in soil and so I will confine myself to details pertinent to O.graminis. Fig.1 gives an outline of the procedure.

Assumptions The infective units are assumed to be randomly distributed in the test soil and to follow a Poisson distribution in replicate samples. Therefore, the soil must be mixed thoroughly, or, when extracted organic debris is used, this must be mixed well with sand. It is assumed that infection results when one or more infective

Fig. 1

An outline of the experimental procedure for estimating the number of infective units (λ) of O.graminis inoculum in soil

SOIL SAMPLE

ALTERNATIVE 1 (Determination on whole soil) → SOIL MIXED

ALTERNATIVE 2 (Determination on organic debris) → EXTRACTION OF DEBRIS FROM SOIL

↓

PREPARATION OF DILUTION SERIES
(Dilution by volume, sand diluent)

↓

HOST-INFECTION TEST
(Cappelle-Desprez wheat)

↓

ASSESSMENT
(a. Presence of infection indicated by root lesions and runner hyphae
b. Partial confirmation by rotting roots until perithecia form (Hornby, 1969b))

↓

ESTIMATION OF λ
(Most probable number tables (Buchanan & Fulmer, 1928), dilution method tables (Fisher & Yates, 1963) or solve the maximum likelihood equation (Kleczkowski, 1968))

units occur in a pot. Accordingly, the test must last long enough for the host's roots to search the soil and the environment must favour infection. λ will be underestimated unless these assumptions are fulfilled.

Soil samples Soil used to test the method was collected as 15.2 cm deep x 5.1 cm diam cores at regular intervals on traverses of the sampling sites.

Preparation of a whole soil dilution series This is easy, provided soils are not wet and sticky. Cores are pooled and mixed thoroughly without discarding stones or purposely fragmenting plant debris. A number (n) of pots containing equal volumes (v cm^3) of soil are prepared with whole soil and the remainder of the soil is used for serial dilutions with coarse sand on a volume basis. At each dilution n pots each containing v cm^3 of the diluted soil are prepared.

Preparation of a debris dilution series The extraction procedure has been described (Hornby, 1969b). The volume of the processed soil should be noted so that the results can be related to whole soil. Debris of 700µ and smaller can be tested in polyvinyl chloride (P.V.C.) tube units ($v \approx 2$ cm^3) (Hornby, 1969b), otherwise all debris can be pooled and mixed with sand and then diluted. In either method serial dilutions with sand are prepared as above.

The host-infection test (Hornby, 1969b). The indicator plant is winter wheat (Cappelle-Desprez) and pre-germinated grains are planted in pots. Ungerminated grains are placed in the P.V.C. tube units and in this method extra units should be prepared to allow for grains failing to germinate. In the whole-soil experiments $v = 150$ cm^3 and two seedlings/pot gave the best estimate of λ (Table 2). The following test conditions are now used routinely: 18-20°C, 16 h light (warm white fluorescent tubes, max. intensity 1500 fc) and 8 h darkness for a period of 3 or 4 weeks. The soils and mixtures are watered to saturation at 0,6,12,17,21 and 25 days.

Assessment After the growing period, the roots are washed and examined under a dissecting binocular for lesions and runner hyphae. When any of the roots from a particular pot bear lesions, that unit is scored positive. Runner hyphae alone are ignored because of the possible confusion with other fungi (Scott, 1967). Root systems of negatively-scored plants may be rotted (Hornby, 1969b) and if O.graminis perithecia develop, they are reassessed as positive.

The estimate of λ For increased accuracy in the estimation of λ a small dilution factor (a) and a large number of replicates (n) are preferable (Cochran, 1950). However, space, time and facilities dictate the size of the experiment and it was found most convenient to use a 2-fold dilution and 5 replicates in routine experiments. The methods for obtaining the estimate are noted in Fig.1. Most probable number tables are easy to use, but limited to certain a and n values; Fisher & Yates' (1963) method is approximate and uses only 88% of the information in the experiment. Most of the data in this paper were analysed by a Rothamsted computer programme, which solves the maximum likelihood equation (see equation 44, Kleczkowski, 1968).

SOME EXAMPLES AND RESULTS

1. An experiment was done using whole Woburn soil collected shortly after harvest of a crop of spring wheat. Twelve cores (each approximately 310 cm^3) from a traverse of the site were pooled and mixed thoroughly. The soil was diluted with sand in a ten-fold dilution series (a = 10) down to 0.0001. At each dilution, there were 5 replicate pots (n = 5) each containing 150 cm^3 (v) of medium. A single seedling was grown in each pot for 3 weeks and then washed out and inspected for root lesions. The roots were then kept moist for 6 weeks in the light and the rotted roots checked for perithecia. Table 1 shows the results and estimates of λ .

The extra information from the perithecial assessment increases the estimate of λ , but until more is known about the strains that develop as perithecia on roots without lesions, an estimate of the pathogenic population is probably best restricted to lesions. The numbers refer to v, e.g. 5 infective units/150 cm^3 of whole soil and are noteworthy for their smallness in relation to populations of other root-infecting fungi (Baker & McClintock, 1965).

2. A comparison was made among host-infection tests where the root to soil volume ratio was changed by increasing the number of seedlings/pot. The soil came from the Woburn site during fallow in late February. The duration of the test was 3 weeks and a = 2, n = 5 and the greatest dilution was 0.0625. Root volumes were estimated by displacement of water and λ was estimated by solving the maximum likelihood equation using a computer (Table 2). Two seedlings/pot gave the largest estimate. The small estimate for 8 seedlings/pot may have resulted from experimental error or the difficulty of finding lesions on tangled root systems.

3. This example is from the results of an experiment with soil collected during May from a field of spring barley at Woburn. λ was estimated on whole soil and on extracted debris greater than 707µ (U.S. standard mesh no.25). A distinction was made between soil sampled in crop rows and between rows. Barley tops were removed from soil samples taken in the rows and 253.2g (wet weight) of debris were extracted from 5871 cm^3 of soil. This was mixed with sand to provide a known volume of test medium for the dilution series. The 113g (wet weight) of debris from an equal volume of soil sampled between rows was treated similarly. This information was required to convert the estimates back to whole soil. All test media were diluted to 0.0625 by sand in 2-fold dilutions series, n = 5, v = 150 cm^3. The estimates of λ given in Table 3 were obtained by solving the maximum likelihood equation by computer. The debris-in-sand method gave the greatest estimates, despite the fact that only coarse

Table 1.

Results of host-infection tests on a dilution series of Woburn soil and estimates of the number of infective units (λ) of O.graminis inoculum

Dilution	No. of replicates	No. positive for: Lesions	Perithecia	Total positives (Lesions + perithecia)
1.0	5	5	2	5
0.1	5	2	1	3
0.01	5	0	1	1
0.001	5	0	0	0
0.0001	5	0	0	0
From tables*		λ = 5.0	0.9	11.0
95% confidence limits+		(1.5-16.5)	(0.3-3.0)	(3.3-36.3)
By Fisher & Yates' (1963) method		λ = 4.5	1.1	11.4

* Buchanan & Fulmer (1928)

\+ Cochran (1950)

Table 2

The effect of different root:soil volume ratios on the estimated number of infective units (λ) of O.graminis inoculum in soil

No. of seedlings per pot+	Mean root volume/pot (ml)	Soil:root volume at lifting	Estimate of λ/150cm^3 soil and 95% confidence limits based on: Lesions	Lesions + Perithecia
1	0.44	340.9	0.80(0.38-1.71)	0.80(0.38-1.71)
1*	0.51	235.3	0.36(0.09-1.44)	0.59(0.19-1.83)
2	1.00	150.0	1.08(0.56-2.12)	1.08(0.56-2.12)
4	1.72	87.2	1.06(0.54-2.06)	1.06(0.54-2.06)
8	2.72	55.1	0.39(0.14-1.04)	0.63(0.28-1.40)

\+ Plastic pot, 150 cm^3 capacity

* Small plastic cup of 120 cm^3 capacity, figures corrected to 150 cm^3

material was used. The results support the idea that the fungus is most concentrated in the crop rows (Hornby, 1969b).

Table 3

Estimates of the number of infective units (λ) of O.graminis inoculum by the whole soil method and the debris-in-sand method using soils from in crop rows and from between rows

λ(with 95% confidence limits) per 150 cm^3 whole soil

Soil sampled in rows		Soil sampled between rows	
Whole soil	Extracted debris	Whole soil	Extracted debris
0.14(0.01-0.97)	0.59(0.26-1.33)	0	0.37(0.13-1.01)

DISCUSSION

This paper is restricted to describing the method of estimating soil-borne inoculum of the take-all fungus; the factors known to influence the host-infection tests (mentioned briefly in Hornby, 1969a) are not discussed, but will be the subject of a future publication. The suggested test conditions given here are, however, a synthesis of what is currently known.

The estimates of λ are interpreted most easily when made on soils in which the fungus is surviving saprophytically (e.g. fallow or growing non-susceptible break crops). Estimates repeated in time would indicate the decline of the fungus population. In the presence of a living susceptible crop the estimates are difficult to interpret, as a proportion of the test infections arise from fungus existing parasitically at the time of sampling. The concept of the infective unit is also confused by detaching infected living root fragments with the coring tool. However, recording 'λ' during crop growth may be a valuable measure of spread of the fungus on living roots. A reproducible set of test conditions must be used when comparisons in time are required. Comparisons among soils types will be most reliable when made on extracted debris, thereby decreasing the different effects of the parent soils.

An immediate practical application of the method could be in evaluating possible control measures for take-all, where it could be used to supplement disease ratings and yields to give a more complete picture of the treatment effects. As the parasitic spread of the fungus finally determines the amount of disease in a crop, knowing the amount of inoculum in a soil before sowing is not necessarily useful in disease prediction. For this reason, the method is unlikely to be more trustworthy as an advisory technique than previous host-infection methods (e.g. the "tumbler method", until recently used by the N.A.A.S.)

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CHEMICAL CONTROL OF LEATHERJACKETS (LARVAE OF TIPULA spp) IN SPRING CEREALS WITH SPECIAL REFERENCE TO ALTERNATIVES TO PERSISTENT ORGANOCHLORINE INSECTICIDES

A Contribution from the Closed Conference of Advisory Entomologists
Summarized by J.M. Rayner
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Summary Trials of chemicals for the control of leatherjackets have been carried out on twenty sites over the past seven years. Results from some of these suggest that DDT, aldrin and dieldrin sprays can give good control, although sometimes inadequate. Gamma BHC spray at the usual recommended rate was not satisfactory, and even at twice the rate was a little inferior to the other organochlorine insecticides. When gamma BHC was used with bran bait, good control was consistently obtained.

The best organo phosphorus material, when used as a spray, was fenitrothion but even this gave variable results. This material was as good as gamma BHC, when used with bran bait.

Other materials showing promise were parathion granules, methiocarb as a spray and methiocarb and bromophos with bran bait.

INTRODUCTION

The organochlorine insecticides, DDT, BHC, and aldrin, have become well established for the control of leatherjackets in spring cereals, applied either as sprays or with bait. In view of the desirability of reducing or eliminating the use of the more persistent materials, DDT and aldrin, and eventually perhaps, of BHC, a series of standardised trials was carried out by the Leatherjacket Working Party of the Closed Conference of Advisory Entomologists from 1965 to 1969. The object of the trials was to examine the effectiveness of alternative insecticides, and to clarify the uncertainty existing as to the effectiveness of BHC sprays. The results of these trials are reported here, with results of some earlier trials carried out by individual advisory entomologists.

METHOD AND MATERIALS

The trials were sited on crops of spring barley or wheat, in most cases following permanent grass or ley. Sites were located in fields concerning which advice on leatherjacket control had been requested, or on fields deliberately examined for leatherjackets and found to have suitably high populations. Treatments were applied at plant growth stages 1 to 4, either before or after significant damage had occurred, but in three instances before crop emergence.

Each trial area was sampled for leatherjackets immediately before application of treatments, using a 4 in diameter sampling tool, to a depth of 2 to 4 in. Not fewer than 20 samples were taken from each trial area. Leatherjackets were extracted by hand sorting, or by washing, using a modified Salt and Hollick method, or the 'Bristol' washing machine (Mayor and Browne, 1964). In two trials, the soil in seven one foot squares, or 20 one foot lengths of drill row, per plot was hand sorted in situ; figures for these sites have been raised by 50% as an approximate adjustment for the likely lower efficiency of this method.

The trials were laid out in two to five randomized blocks, or occasionally unreplicated. The standard size of plot was 10 yd x 10 yd but some sites had plots with a minimum dimension as little as 5 yd.

Treatment effects were assessed by soil sampling and examination as for pre-treatment population assessment, taking 10 to 30 samples per plot, mostly within 2 to 4 weeks of treatment. Hand sorting of soil in the field was used at two sites, as for pre-treatment population estimates. On a proportion of sites yield was assessed; on some by cutting 20 one yard lengths of row per plot, on others by cutting a 10 yd by 1 yd strip in the centre of each plot. The samples were threshed in a laboratory thresher.

Further site details are listed in Table 1, and treatments applied, in Tables 2 and 3.

RESULTS

Table 2 presents the results of the main treatments; Table 3 contains those applied at one site only, or otherwise of less interest. The assessments of post treatment leatherjacket populations are expressed as thousands of leatherjackets per acre in the control plots, and as the percentage reduction in the treated plots, relative to the control plot population, at the time of assessment, at the same site.

Yield was assessed as cwt. per acre at 85% dry matter content; the effects of treatment on yield are discussed later.

Table 1.

Chemical control of leatherjackets in spring cereals
Details of sites

Year	Site No.	Location	Soil Type	Cereal Variety (barley unless stated otherwise)	Sowing date	Treat-ment date	Pre Treat-ment leather-jacket population 000's/ac
1963	(1)	Morpeth, N'land	-	Rika	Early April	12 May	229 (H)
1964	(2)	Morpeth, N'land	-	Pallas	-	6 May	114
1965	(3)	Longtown, Cumberland	Med/heavy loam	Proctor	24 April	27 April	475
	(4)	Bradley, Staffs	Medium loam	Kloka (wheat)	31 March	15/21 April	142
1966	(5)	Westlinton, Cumberland	Light Loam	Proctor	14 April	28 April	456
1967	(6)	Great Orton, Cumberland	Med/light loam	Vada	3 April	1 May	287
	(7)	Cornsay, Co.Durham	Medium loam	Vada	Late April	18 May	278 (H)
	(8)	Elham, Kent	Clay-with-flints	Zephyr	15 March	2 May	168
	(9)	Wivelsfield, Sussex	Weald Clay	Proctor	29 March	27 April	313
	(10)	Ansley, Warwicks	Sandy loam	Zephyr	Early March	19 April	250
	(11)	Axmouth, Devon	Silty loam	Kloka (wheat)	17 April	9 May	150
	(12)	Bude, Corn-wall	Silty loam	Proctor	22 March	21 April	167
1968	(13)	Morpeth, N'land	Medium loam	Julia	Early April	23 April	550
	(14)	Castle Eden, Co.Durham	Medium loam	Zephyr	29 April	6 May	280
	(15)	Wivelsfield, Sussex	Weald Clay	Proctor	29th March	18 April	1325
	(16)	Stonnall, Staffs	Med/heavy loam	Zephyr	13 March	4 April	750
	(17)	Axmouth, Devon	Silt loam	Maris Badger	28 March	10 April*	625
1969	(18)	Dalston, Cumberland	Light loam	(barley)	8 April	16 May	294
	(19)	Itchingfield, Sussex	Clay loam	Impala	3 April	22 April	288
	(20)	Breedon, Leics.	Medium loam	Sultan	re-drilled 14 May	15 May	750

H - hand sorted and adjusted by + 50%

* - pre-sowing treatment 20 March

Table 2

Chemical Control of Leatherjackets in Spring Cereals. Results of assessment of post-treatment leatherjacket populations (a)

Quantities of insecticide expressed as oz active ingredient per acre

% Control (Difference between treatment population and control plot population expressed as % of the latter)

Site No.	Control plot popn. 000's/ac	Gamma BHC bran bait 4oz	bran crumbs 4oz	extruded pellets 4oz	coll. spray 8oz	coll. spray 16oz	e.c. spray 8oz	e.c. spray 16oz	DDT e.c. spray 16oz	Aldrin e.c. spray 12oz	Fenitrothion bran bait 8-10oz	Fenitrothion e.c. spray 16-20oz	Chlorfenvinphos bran bait 8-10oz	Chlorfenvinphos e.c. spray 16-20oz	Parathion 10% granules 16oz	Methiocarb 4% pellets 3oz
(1)	157															
(2)	131	81			52				52							
(3)	475										90	47	100*	90**	100***	
(4)	142	82			0					65		82/				
(5)	167		90	60												
(6)	325	83	80								80	76	86	50		
(7)	216	44	45										28			
(8)	154	68	65	32			11				78	22	73	3		
(9)	354		72	71	5						75	53				
(10)	150	78	78	64						22	75	81	70	67		
(11)	70	77		24							88	18	0	71		
(12)	58	78	67								67	11	56	11		
(13)	270				30	65	39	52				82				
(14)	242	100	79													
(15)	1325///	76			68	71	58	58	55	80		79				
(16)	567				41	74	40	72				70				
(17)	533				25	41	33	57				92				
(18)	167			60	15	55						60			65	35
(19)	215			56	5	60						0//			89	19
(20)	565			68	18	53									79	53

* 14oz a.i./ac ** 29oz a.i./ac *** 32oz a.i./ac / 10oz a.i./ac // 8oz a.i./ac

/// pre-treatment population - crop destroyed by leatherjackets which moved out of control plots before post-treatment assessments made. The true figures for % control for this site are therefore somewhat lower than those given.

Table 3

Chemical control of leatherjackets in spring cereals
Results of assessment of post-treatment leatherjacket populations (b)

Site No.	Insecticide	Formulation	Method of Application	Rate a.i./ac	% control
(1)	Dieldrin	e.c.	spray	6oz	71
(2)	Diazinon	-	bran bait	16oz	43
	"	e.c.	spray	16oz	33
	DDT	-	bran bait	5oz	90
	Thionazin	-	" "	16oz	81
	"	e.c.	spray	16oz	33
(3)	Thionazin	-	bran bait	8oz	79
	"	e.c.	spray	16oz	79
	"	granules	-	16oz	74
	Chlorfenvinphos	"	-	3lb 10oz	100
	Parathion	-	bran bait	0.8oz	100
	"	e.c.	spray	1.6oz	100
(4)	Bromophos	-	bran bait	4oz	82
	"	e.c.	spray	8oz	0
	Mecarbam	-	bran bait	19oz	53
	'Paris Green' (copper aceto-arsenite)	-	" "	16oz	65
(11)	Trichlorphon	-	bran bait	8oz	53
(12)	Trichlorphon	-	bran bait	8oz	33
(17)	Aldrin	e.c.	spray (pre-sowing)	24oz	88
(18)	Methiocarb	w.p.	spray	16oz	70
(20)	Methiocarb	w.p.	bran bait	16oz	77

DISCUSSION

Sprays or baits containing DDT, gamma BHC, aldrin, dieldrin and Paris Green are generally accepted as being effective for leatherjacket control, although the effectiveness of gamma BHC has been called in question from time to time. White (1963) quoted results from his own and other authors' work which are summarized in Table 4,(where actual figures are not given in White's paper, they have been supplied by him).

Table 4

Chemical Control of leatherjackets in Spring Cereals -
Percentage reduction in leatherjacket populations in trials referred to in White (1963)

Author	Crop	Paris Green bait 16oz	Gamma BHC bait 8oz	Gamma BHC spray 5oz	DDT spray 16-48oz	Aldrin spray 12oz	Dieldrin spray 6oz
Packard and Thompson (1921) in Rodriguez (1953)	not stated	72(rate?)	-	-	-	-	-
Rodriguez (1953)	Lespedeza	-	-	65	46(48oz)	-	-
White (1963)	?spring cereal	-	90	-	90(24oz)	-	-
White (1963)	spring barley	-	-	-	-	85	100
White (1963)	" "	-	-	-	-	70	-
White (1963)	" "	-	-	-	-	96	-
La Croix and Newbold (1968)	grass	66	-	-	97(16oz)	-	-
" "	spring barley	36	-	-	84 "	-	-
" "	grass	-	-	-	73 "	-	-

Although the first two sets of figures above refer to trials under American conditions, these figures do at least indicate the level of control achieved with existing materials, and which is apparently acceptable in practice.

The figures in Tables 2 and 3 show that DDT and BHC have not performed so well as sprays in the N.A.A.S. co-operative trials, but the other materials have generally given similar results to those in Table 4.

It is particularly interesting to note that gamma BHC sprays, (Table 2), at the customary rate of 8 oz a.i./acre, have given results which are very variable and only occasionally satisfactory. This suggests that reports of unsatisfactory control in practice are well founded. There is apparently no difference in performance of the two formulations used as sprays (colloidal and e.c.), but increasing the rate of application to 16 oz., a.i./acre results in a distinct, but inadequate, improvement with both formulations.

When used with bran bait, at only 4 oz. a.i./acre, gamma BHC gives consistently good control. This degree of control is almost equalled by the bran crumb bait (a readymade, loosely pelleted formulation). The extruded pellets used in 1966 and 1967 were based on wheat meal; being larger and more dense, they gave fewer pellets per unit of treated area than the bran crumbs, and were distinctly inferior in performance. The extruded pellets used in 1969 were based on bran and were smaller than the wheatmeal pellets; in performance they appear to be intermediate between the other two types.

Fenitrothion used with bran bait gives consistently good control comparable with gamma BHC and bran, but again, the spray, at twice the rate of a.i./acre gives variable results, although quite often good.

Chlorfenvinphos bait and spray are variable in their results; thionazin (Table 3) performs likewise as a spray, and is too toxic for use with bran bait.

Of the remaining materials, parathion spray and bait, although effective, are too toxic to consider seriously, but the granule is much safer to handle and evidently effective. This good performance is confirmed by La Croix and Newbold (1968). If this material can be shown to give consistently good results it will be superior to any non-organochlorine spray so far investigated, and more convenient than a farm mixed bait.

Thionazin granules are no longer available, and chlorfenvinphos granules (at 3 lb 10 oz a.i./acre) are too expensive; bromophos (one site only) shows promise when used with bran bait, as also does methiocarb as a spray and with bran bait.

It is worth emphasizing how frequently the use of an insecticide with bran bait gives a superior control compared with the same material used as a spray. Gamma BHC and fenitrothion when used with bran bait give remarkably consistent control.

The detailed effect of treatments on yield is dealt with elsewhere (Rayner 1970). It is there shown that for a field with a pre-treatment population of 500,000 leatherjackets per acre, the yield response in cwts per acre resulting from the control of leatherjackets, is numerically equal to 0.09 x the percentage reduction in leatherjackets as given in Tables 2 and 3. For other populations the response is proportionate. It is also shown that a crop of spring barley with a leatherjacket population (for example) of 350,000 per acre, on average would be expected to suffer a yield loss due to leatherjackets of 12 cwt/acre. The expected yield response for an insecticide giving 100% control is $\frac{350}{500}$ 0.09 x 100 = 6 cwt/acre. Thus only a proportion of the total yield loss is recoverable with the best of post emergence treatments. Assuming a mean crop yield in the absence of leatherjackets, of say, 29 cwt/acre, the final result of controlling even a moderate leatherjacket population is a mere 23 cwt crop. Individual crops will vary greatly from this figure, but it remains that a substantial proportion of treated crops will not yield satisfactorily. Pre-sowing treatments may be the answer, but this requires advance knowledge of damaging leatherjacket populations to be justifiable.

In conclusion, it seems that the most satisfactory alternatives to the persistent organochlorine materials are gamma BHC with farm mixed or readymade bran baits with a crumb structure, fenitrophion with a bran bait, parathion 10% granules, and possibly methiocarb as a spray, and methiocarb and bromophos with bran bait. Fenitrothion spray at 16 oz a.i./ac is fairly satisfactory but gamma BHC at the same rate is rather less so.

These results demonstrate the value of a series of trials with any one material in showing up the variation in performance from site to site.

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EFFECT OF COMBINED SEED TREATMENTS OF CHLORMEQUAT AND ORGANO-MERCURY ON FOOT ROT AND WINTER GROWTH OF OATS

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Summary A combination of an organo-mercury dust fungicide and a dust formulation of the growth regulant chlormequat, when used as a seed treatment for oats, enhanced the survival of seedlings growing under natural and simulated winter conditions. Treatment of seed of ten oat cultivars increased the frost hardiness of some cultivars and protected the mesocotyl and stem base tissues from attack by soil fungi. The treatment also increased root length in some cultivars but had no significant effect on seedling leaf area. The use of either chemical alone was not as effective in these respects as the combined treatment. Chlormequat alone tended to increase the numbers of seedlings showing disease symptoms on mesocotyl and stem base tissues. In a field experiment a combined treatment of organo-mercury and a seed soak in a liquid formulation of chlormequat was found to have the same beneficial effect on seedling survival, disease and seedling dry weight as the combined treatment with dust formulations of these chemicals. Both combined treatments increased the number of fertile tillers per drill in some cultivars.

INTRODUCTION

Oat seedlings growing under winter conditions may be attacked by soil fungi that are not commonly considered to be cereal pathogens. An attack on the mesocotyl or sub-crown internode tissues of oat seedlings by fungi commonly regarded as soil saprophytes can result in considerable reduction in seedling vigour (Rawlinson, 1967. Rawlinson and Colhoun, 1969 b). This paper reports part of an investigation into factors influencing the infection of seedlings by such fungi. An attempt is being made to determine whether predisposition of seedling tissues, caused by their growth under adverse environmental conditions, is mainly responsible for infection or whether the fungi are able to become aggressive under these conditions (Patrick and Toussoun, 1965). Following reports that the growth regulant chlormequat (2-chloro-ethyltrimethylammonium chloride) enhances winter hardiness (Jung, 1965. Wunsche, 1966) and shortens internode length in wheat (Humphries, 1968) this chemical was used as a seed treatment in an attempt to modify seedling metabolism and morphology and perhaps thereby modify predisposition to fungal attack. The work was also designed to investigate the effectiveness of chlormequat in combination with a conventional organo-mercury fungicide as a seed treatment in promoting healthy, winterhardy oats since this prospect would be of some interest to oat breeders (Jenkins, 1967).

METHOD AND MATERIALS

Soil. The growth cabinet experiment was made in a loam of pH 6.0 having a maximum waterholding capacity (M.W.H.C.) of 36%. The soil had not carried a cereal crop for at least 15 years and so the viable population of soil-borne cereal pathogens was expected to be low. The field experiment was made in a loam of pH 8.0 having a M.W.H.C. of 42%. The experiment was carried out at the School of Agriculture Field Station, Cambridge on land which had not carried a cereal crop for at least ten years.

Seeds. Samples of oat cultivars Novosadsky, T.O. 4290, Powys, Pennant, Peniarth, Maris Quest, Cimarron, Grey Winter, Feltwell and Blenda were obtained from the Plant Breeding Institute, Cambridge. Samples of cv. Novosadsky, Peniarth and Blenda were found to be free of known pathogenic fungi when examined by the Ulster method (Muskett and Malone, 1941). When examined by the same method 15% of a sample of cv. Grey Winter was found to be contaminated by *Helminthosporium avenae* but the remaining cultivars were only contaminated by less than 5% with this fungus. T.O. 4290 was the only cultivar contaminated by *Fusarium nivale* (3%).

Chemical treatment of seed. The organo-mercury dry seed dressing (1.5% mercury) was used in all experiments at the recommended rate (equivalent to 0.30g per 100g seed). A 65% a.i. dust formulation of the plant growth regulant chlormequat was used in all experiments at a rate equivalent to 2g a.i. per 100g seed. In the field experiment seed was also soaked overnight at 10°C in a liquid formulation of chlormequat providing 2g a.i. per 100g seed. After soaking, the seed was allowed to dry on sterile filter paper before being sown. A combined seed treatment was given in which the seed was first shaken with the organo-mercury in a clean glass vessel on a mechanical shaker for 5 min. then treated in the same manner with chlormequat dust. In the field experiment where the liquid formulation of chlormequat was used either alone or in combination with organo-mercury the seed was soaked in chlormequat, dried and then sown or subsequently treated with organo-mercury and then sown. All unnecessary handling of treated seed samples was avoided.

Growth cabinet experiment. Treated and untreated seeds of ten oat cultivars were sown in rectangular plastic boxes (10 x 20 x 7 cm deep) containing 1 kg soil. Using a template 50 seeds were sown in each box at a depth of 2 cm. Five seeds of each cultivar were sown in random order, one cultivar to each of the ten rows per box. Nine replicate rows constituted a unit for each seed treatment; each box containing seed of all cultivars was given any one treatment. The moisture content of the soil was adjusted to 50% M.W.H.C. prior to sowing and the boxes were weighed subsequently on alternate days when water was added to bring the weight up to that recorded at sowing time. The boxes were maintained at an alternating temperature regime of 5°C at night and 15°C during the day until the seeds had germinated. The boxes were then arranged in a split plot design in a low temperature growth cabinet (Thomas, 1966) which was thermostatically controlled to provide a day/night regime of 10/6°C air temperature. Daylength was 10h and illumination was from fluorescent tubes and pigmy tungsten bulbs giving 350 f.c. intensity. The light quality closely resembled natural daylight in spectral composition.

At five weeks after sowing the seedlings were subjected to conditions of frost by lowering the normal night temperature to -2°C for 1h. The temperature was then further lowered to -6°C for 4h, after which time it was allowed to return to normal night temperature again for 7h until the onset of light and the coincident temperature of 10°C. One week after this period of frost the seedlings were given another freezing as before, except that the lowest temperature achieved was -8°C. After a further week the seedlings were given a final freezing at -10°C. Six days after each freezing regime each seedling was examined in order to assess its reaction to frost. Seedlings with shrivelled or collapsed leaves, or which had died, were recorded as suffering frost damage. The surviving seedlings were grown on for six weeks and then removed from the cabinet.

At the end of the experiment a count was made of the number of seedlings which survived freezing and of those showing symptoms of disease and decay of the stem base. They were then carefully removed from the soil by washing. Each seedling was examined and the following measurements recorded, height (from seed to tip of longest leaf), root length (from seed to tip of longest root), leaf area and presence of disease symptoms and lesions on mesocotyls or sub-crown internodes.

Field experiment. Seeds of five oat cultivars, Peniarth, Powys, Maris Quest, Feltwell and Blenda, treated with dust and liquid formulations of chlormequat, with organo-mercury and with combinations of these chemicals were sown in three plots. Each plot consisted of 180 drills 1.3m long and 30 cm apart. Seeds were sown at the rate of 4g per drill at a depth of 5 cm. In each plot six replicate drills of each cultivar, given each seed treatment, were sown at random throughout the plot on 20 November 1968. On 18 March, 1969 one plot was examined. The seedlings were carefully removed from the soil and counted and the numbers of seedlings per drill that showed symptoms of disease and lesions on mesocotyl or sub-crown internode was recorded. Finally the dry weight of seedlings in each drill was measured. On 1 April, 1969 the second plot was examined and the mesocotyls of ten seedlings from each drill were examined by isolation techniques for the presence of fungi (Rawlinson and Colhoun, 1969 a). The third plot was harvested on 11 August, 1969 when the number of ripe heads of grain and total number of ripe heads plus unripe tiller-heads per drill was counted. The yield of grain per drill was not recorded because of premature shedding in many drills.

Statistical analysis of results. Measurements of plants in growth cabinet and field experiments were made for individual plants and the results subjected to statistical analysis on a one-way computer programme. Values for measurements have in all cases been corrected to the nearest whole number for clarity in presentation. When the difference between values for untreated plants and plants given any one seed treatment is statistically significant the following notation is employed:- + = $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. Values for standard error are included in some tables.

RESULTS

Growth cabinet experiment - simulated winter conditions

Seedling survival. Mean figures over all seed treatments for survival counts in each cultivar and mean survival counts over all cultivars for each treatment are given in Table 1. Treatment with chlormequat and chlormequat + organo-mercury both increased the number of surviving seedlings. The highest survival counts were achieved after treatment with the combined seed dressing. The organo-mercury when used alone had no effect on mean survival counts.

Table 1.

Effect of seed treatments on seedling survival

Cultivar	Mean survival count over all treatments	Seed treatment	Mean survival count over all cultivars
Peniarth	44 ± 1		
Cimarron	42 ± 1		
Maris Quest	42 ± 1	Chlormequat + organo-mercury	44 ± 1
Novosadsky	41 ± 1		
Pennant	40 ± 1	Chlormequat	41 ± 1
Grey Winter	40 ± 1		
T.O. 4290	38 ± 1	Organo-mercury	38 ± 1
Feltwell	36 ± 1		
Blenda	36 ± 1	Untreated	38 ± 1

Seedling height, root length and leaf area. Treatment of seed with organo-mercury alone had no effect on seedling height in any cultivar (Table 2). However, root length was reduced in cv. Powys and T.O. 4290, and leaf area was reduced in cv. Powys, Pennant, Cimarron, Blenda and Feltwell. Treatment with chlormequat alone had no effect on root length in any cultivar but reduced seedling height in cv. Blenda, Feltwell and Grey Winter and reduced leaf area in cv. Pennant and Feltwell. The combined seed treatment had no effect on leaf area in any cultivar but increased seedling height in cv. T.O. 4290 and reduced it in cv. Blenda and Feltwell. The combined seed treatment was the only treatment to give increased root length in any cultivar; root length was increased in cv. Novosadsky, Cimarron, T.O. 4290, Feltwell and Grey Winter.

Table 2.

Effect of seed treatments on seedling height (H, cm) root length (R, cm) and leaf area (L, cm^2).

Cultivar	Seed treatment											
	Untreated			Organo-mercury			Chlormequat			Chlormequat+organo-mercury		
	H	R	L	H	R	L	H	R	L	H	R	L
Peniarth	13	11	4	13	9	4	12	11	4	13	12	4
Maris Quest	15	10	6	15	9	5	13	11	6	13	11	6
Novosadsky	16	14	5	14	11	4	15	14	4	14	17*	4
Powys	15	11	5	14	8*	4*	13	11	4	14	13	4
Pennant	15	11	7	14	8	4**	13	10	5*	14	12	5
Cimarron	16	14	9	15	12	7*	14	13	8	14	19**	8
T.O. 4290	15	14	8	16	11*	7	15	13	8	17*	22***	10
Blenda	18	9	8	17	6	6*	16*	10	7	16**	10	7
Feltwell	19	14	8	18	14	6*	16**	16	6*	16**	18*	6
Grey Winter	18	13	5	18	12	4	15**	14	4	16	23***	5

Seedling disease. The number of seedlings showing symptoms of disease and lesions on the stem base was recorded at the end of the experiment before the seedlings were removed from the soil. Because of the low number of diseased seedlings compared to healthy seedlings in any one cultivar none of the seed treatments was found to have any statistically significant effect on the number of diseased seedlings. However, by inspection, the data given in Table 3 indicates that the mean percentage number of diseased seedlings over all cultivars was reduced by the combined seed treatment and by organo-mercury but was increased by treatment with chlormequat alone.

After the seedlings has been removed from the soil and washed the number showing symptoms of disease and lesions on the mesocotyl was recorded. Because of the shallow depth (2 cm) at which the seed was sown and the consequent low number of mesocotyls produced by any one cultivar it was again not possible to analyse statistically each cultivar separately for its response to chemical treatment. Thus the effect over all cultivars given any one seed treatment was analysed (Table 4). These data indicate that both the organo-mercury treatment and the combined treatment reduced the proportion of seedlings with diseased mesocotyls, the latter treatment being most effective in this respect. By inspection the data indicate that chlormequat increased the number of seedlings with diseased mesocotyls although, because of the low number of seedlings involved, the increase was not statistically significant.

Table 3.

<u>Effect of seed treatments on percentage number of seedlings with disease symptoms on stem base.</u>

Cultivar	Seed treatment Untreated	 Organo-mercury	 Chlormequat	 Chlormequat + organo-mercury	
T.O. 4290	30	22	44	9	
Pennant	17	9	34	7	
Grey Winter	16	0	29	0	
Cimarron	14	0	2	0	
Peniarth	14	0	11	7	
Blenda	13	2	20	2	
Maris Quest	11	0	18	0	
Powys	9	0	13	4	
Novosadsky	7	7	14	2	
Feltwell	5	16	28	7	
Total number seedlings examined	428	446	430	441	Chi^2 = 9
Total number healthy seedlings	370	421	340	424	
Total number diseased seedlings	58	25	90	17	
Mean % diseased seedlings	13	6	21	4	

Table 4.

<u>Effect of seed treatments on mean percentage number of seedlings of all cultivars with healthy unlesioned mesocotyls.</u>

	Seed treatments Untreated	 Organo-mercury	 Chlormequat	 Chlormequat + organo-mercury
Total number mesocotyls	80	14	15	15
Number healthy mesocotyls	16	9	1	14
% healthy mesocotyls	20	64	7	93
Chi^2 treatments cf. untreated	-	6*	1	12***

Seedling frost resistance. The extent of damage to seedlings caused by the freezing temperatures employed in the experiment varied from shrivelled and collapsed leaves to complete seedling kill; these categories of damage were assessed and recorded separately but are presented collectively for convenience as frost damage in Table 5. The first freezing (-6°C) did not give sufficient damage to allow seedlings to be differentiated according to their reaction to frost. Damage at this temperature was merely sufficient to indicate the susceptibility to frost damage of cv. Blenda. Frost damage after the third freezing (-10°C) was too extensive to give good differentiation between treatments. The data presented in Table 5 are for frost damage occurring after the second freezing (-8°C). When assessed over all cultivars, all the treatments reduced the percentage number of seedlings suffering frost damage, the greatest reduction occurring with the combined seed treatment.

Table 5.

Effect of seed treatments on numbers of undamaged (O) and frost-damaged (D) seedlings after 4h exposure to -8°C.

Cultivar	Seed treatments							
	Untreated		Organo-mercury		Chlormequat		Chlormequat + organo-mercury	
	O	D	O	D	O	D	O	D
Peniarth	45	0	45	0	43	1	43	0
Cimarron	44	0	45	0	44	0	45	0
T.O. 4290	40	0	44	0	38	0	43	0
Powys	43	1	44	1	45	0	45	0
Maris Quest	43	2	45	0	45	0	44	0
Novosadsky	41	2	44	1	43	1	45	0
Pennant	40	1	45	0	42	0	45	0
Grey Winter	35	9	41	3	42	2	42	3
Feltwell	29	10	35	8	37	5	41	1
Blenda	19	26	13	32	25	19	31	12
Chi^2 treatments	130***		220***		117***		85***	
Mean % frost-damaged seedlings	13		11		7		4	

Data for frost damage occurring in untreated seedlings of each cultivar exposed to the third freezing (-10°C) are given in Table 6. These data indicate that cv. Feltwell, Grey Winter and Blenda were the least frost hardy of the ten cultivars used. It is in these three cultivars that the combined seed treatment most markedly reduced frost damage; seed treatment had little effect on the frost resistance of other more hardy cultivars.

Table 6.

Percentage number of untreated seedlings damaged by frost after 4h exposure to -10°C.

	Cultivar										
	Novosadsky	Pennant	Peniarth	T.O. 4290	Powys	Cimarron	Maris Quest	Feltwell	Grey Winter	Blenda	
Number undamaged	35	34	33	28	30	28	25	18	17	11	Chi^2 = 60
Number damaged	7	8	11	12	14	15	20	21	27	34	
% damaged	17	19	25	30	32	35	44	54	61	75	
Frost resistance	Good				Moderate			Poor			

A statistical analysis of data for frost damage and for number of diseased seedlings revealed that there were no significant treatment/cultivar interactions. Thus frost damage was not positively correlated with symptoms of disease on stem bases and mesocotyls in any cultivar given any seed treatment.

Field experiment - winter 1968-69.

Seedling survival. The ability of seedlings to survive over winter was measured by counting the number of seedlings per drill on 18 March 1969 and by measuring the total dry weight of seedlings per drill. The dust (D) and liquid (S) formulations of chlormequat when used alone as seed treatments both tended to enhance the survival of cv. Blenda but decreased that of cv. Feltwell and had no effect on other cultivars (Table 7). Both the combined seed treatments and the organo-mercury treatment significantly increased survival in all cultivars except Feltwell.

Table 7.

Effect of seed treatments on (1), percentage number of diseased seedlings (2), mean number of seedlings and (3), mean dry weight (mg) of seedlings per drill.

Seed treatment	Character measured	Peniarth	Powys	Cultivar Maris Quest	Feltwell	Blenda
Untreated	1	32 ± 3	23 ± 4	27 ± 4	22 ± 3	33 ± 4
	2	21 ± 3	25 ± 4	17 ± 2	42 ± 3	20 ± 4
	3	622 ±134	938 ±180	595 ±98	1976 ±185	790 ±111
Chlormequat (D)	1	33 ± 3	40 ± 4*	50 ± 4*	38 ± 3*	28 ± 4
	2	24 ± 3	20 ± 4	20 ± 2	37 ± 3	31 ± 2**
	3	772 ±134	642 ±165	783 ±98	1489 ±185$^+$	956 ±111
Chlormequat (S)	1	34 ± 3	44 ± 4*	31 ± 4	23 ± 3	20 ± 4
	2	21 ± 3	16 ± 4	14 ± 2	31 ± 3*	34 ± 2***
	3	706 ±134	608 ±165	474 ±98	1291 ±185*	1203 ±111*
Organo-mercury	1	9 ± 3***	5 ± 4*	7 ± 4**	2 ± 3***	24 ± 4
	2	55 ± 3***	52 ± 4***	45 ± 2***	51 ± 3$^+$	30 ± 2**
	3	1862 ±134***	1981 ±165***	1930 ±98***	2186 ±185	848 ±111
Chlormequat + organo-mercury (D)	1	4 ± 3***	7 ± 4**	13 ± 4$^+$	3 ± 3***	14 ± 4**
	2	45 ± 3***	52 ± 4***	41 ± 2***	48 ± 3	33 ± 2***
	3	1764 ±134***	1991 ±165***	1742 ±98***	2062 ±185	1073 ±111$^+$
Chlormequat + organo-mercury (S)	1	4 ± 3***	4 ± 4**	9 ± 4*	3 ± 3***	9 ± 4**
	2	41 ± 3***	57 ± 4***	46 ± 2***	48 ± 3	34 ± 2***
	3	1429 ±134***	1983 ±165***	1648 ±98***	2075 ±185	1020 ±111

Seedling disease. Treatment of seed with chlormequat (D) and chlormequat (S) increased the percentage number of diseased seedlings per drill in cv. Powys, and with chlormequat (D) also in cv. Maris Quest and Feltwell (Table 7). Treatment with organo-mercury reduced the percentage of diseased seedlings in all cultivars except Blenda. Both the combined seed treatments reduced the percentage number of diseased seedlings per drill in all cultivars.

When the mesocotyls of seedlings were examined on Modified Czapek Dox agar for the presence of fungi those most frequently isolated were, in descending order of frequency, *Fusarium nivale*, *Helminthosporium avenae*, *Botrytis cinerea*, *Aureobasidium bolleyi*, *A. pullulans*, *Rhodotorula sp.* *Phoma sp.* and *Fusarium spp.*

Table 8 shows the effect of seed treatments on the contamination of mesocotyls by the most frequently isolated fungi. All treatments reduced contamination of mesocotyls but the combined seed treatments were most effective in this respect. None of the treatments significantly reduced either the length of mesocotyls or the number of seedlings with mesocotyls.

Table 8.

Mean percentage number of mesocotyls of five oat cultivars contaminated by species of fungi.

Seed treatment	Total fungi	Species isolated: Fusarium nivale	Helminthosporium avenae
Untreated	57	25	14
Chlormequat (D)	34	18	4
Chlormequat (S)	31	15	6
Organo-mercury	29	12	8
Chlormequat (D) + organo-mercury	21	8	5
Chlormequat (S) + organo-mercury	15	3	7

Table 9.

Effect of seed treatments on mean number of (1) ripe heads of grain and (2) total heads (including unripe tiller-heads) of grain, per drill.

Seed treatment	Character measured	Peniarth	Powys	Maris Quest	Feltwell	Blenda
Untreated	1	30 ± 5	27 ± 5	27 ± 5	40 ± 4	33 ± 3
	2	54 ± 9	44 ± 9	35 ± 6	46 ± 5	54 ± 5
Chlormequat (D)	1	23 ± 5	29 ± 5	21 ± 5	42 ± 4	26 ± 3
	2	36 ± 9	50 ± 9	24 ± 6	54 ± 5	52 ± 5
Chlormequat (S)	1	28 ± 5	25 ± 5	30 ± 5	45 ± 4	35 ± 3
	2	43 ± 9	37 ± 9	35 ± 6	52 ± 5	57 ± 5
Organo-mercury	1	36 ± 5	58 ± 5***	51 ± 5**	52 ± 4*	30 ± 3
	2	59 ± 9	97 ± 9***	57 ± 6*	69 ± 5**	48 ± 5
Chlormequat (D) + organo-mercury	1	49 ± 5**	57 ± 5***	41 ± 5⁺	49 ± 4	28 ± 3
	2	83 ± 9*	100 ± 9***	52 ± 6⁺	66 ± 5*	52 ± 5
Chlormequat (S) + organo-mercury	1	49 ± 5*	55 ± 5***	38 ± 5	49 ± 4	30 ± 3
	2	80 ± 9*	88 ± 9**	43 ± 6	60 ± 5⁺	50 ± 5

Mature plant heads of grain. No seed treatment had any significant effect on the number of heads of grain per drill in cv. Blenda (Table 9). Neither formulation of chlormequat affected the number of heads of grain per drill. Treatment with organo-mercury increased the total number of heads and the number of ripe heads per drill in cv. Powys, Maris Quest and Feltwell. Both combined treatments increased the total number of heads and the number of ripe heads in cv. Peniarth and Powys and the total number of heads in Feltwell. In addition, the combination of chlormequat dust and organo-mercury increased the total number and the number of ripe heads in Maris Quest whereas the combination of liquid chlormequat soak and organo-mercury had no effect in this cultivar. When assessed visibly at harvest, without the aid of measurements, no treatment was seen to have any marked effect on plant height. An identical field experiment, done on land with a normal history of cereal crops, gave results similar to those just described.

DISCUSSION

In a growth cabinet experiment, simulating winter conditions, a combined dust treatment of chlormequat and organo-mercury applied to the seed of ten oat cultivars increased seedling survival. This treatment also increased root length and frost resistance in some cultivars and reduced the numbers of seedlings showing symptoms of disease on stem base and mesocotyl tissues. Similar beneficial effects of the combined treatment were recorded when a dust and a liquid formulation of chlormequat were each combined with organo-mercury and used to treat the seed of five oat cultivars in a field experiment made during the severe winter of 1968-69. In this experiment isolations of fungi were made from mesocotyls and the results confirmed that the reduction in number of diseased mesocotyls was accompanied by a reduction in number of fungi isolated. At harvest both the combined seed treatments were found to increase the number of fertile tillers per drill in all cultivars except cv. Blenda. In both the growth cabinet and field experiments treatment of seed with chlormequat plus organo-mercury produced a combination of beneficial effects on seedlings and mature plants that was not achieved by using either chemical separately.

Chlormequat when used alone tended to increase the number of seedlings with disease symptoms. It is interesting that a possible increased susceptibility to fungal diseases after treatment with chlormequat has been reported for wheat (Vez and Spoorenberg, 1967. Dilz et al., 1965) but not previously for oats. The increased numbers of diseased seedlings after treatment with chlormequat cannot be due in these experiments to the culm shortening effect usually offered to explain the susceptibility of wheat to Septoria and Fusarium diseases (Bockman, 1968).

Linser et al. (1961) suggested that treatment of oats with chlormequat before sowing allows too little to be absorbed to be effective. Moreover Linser and Bohring (1967) state that oats do not readily take up chlormequat from soil. It is therefore suggested that any effects of chlormequat, when used in the combined seed treatment, in these experiments may have been due to the presence of organo-mercury delaying microbial degradation of the chlormequat in soil. The low temperatures employed may also have allowed its greater persistence in soil (Kuhn, 1964).

In the field experiment neither the dust nor the liquid chlormequat treatment alone had any effect on the number of heads of grain per drill. In contrast, Humphries et al. (1967) working with wheat reported that chlormequat increased tiller survival and related this to a larger root system which functioned more efficiently under certain soil conditions. In the growth cabinet experiment only the combined seed treatment increased root length in some cultivars. This may also have occurred in the field and perhaps accounted for the increased tiller survival. Appleby et al. (1966) reported increases in yield of wheat following seed treatment with chlormequat. However, in the present experiment increases in panicle number, which may reflect actual yield, did not occur with chlormequat alone but only in the organo-mercury and combined treatments.

In the growth cabinet experiment both chlormequat and the combined treatment increased the frost resistance of the less hardy oat cultivars. A similar effect on the winter hardiness of wheat has been reported by Jung (1965) and Wunsche (1966). The results presented indicate a genuine increase in hardiness rather than an indirect effect related to disease. The extent to which both treated and untreated tissues became diseased was independent of frost damage. Moreover, in neither the growth cabinet nor field experiment did any seed treatment significantly reduce either the length or the occurrence of the mesocotyls region of seedlings. It therefore seems likely that the increase in frost resistance conferred by seed treatment was related to changes in seedling anatomy or metabolism.

If it can be confirmed that the extent to which the seedlings become diseased is independent of frost damage then it would appear that seedling infection is due to the increased aggression of certain soil fungi under adverse environmental conditions. Previous data for the isolation of fungi from samples of the same soil as that used in the growth cabinet experiment and from oat seedlings growing under similar low temperature conditions (Rawlinson and Colhoun, 1969b) showed that the fungi most frequently isolated under these conditions were soil saprophytes. By contrast, however, in the present field experiment isolations of fungi from seedlings revealed that the low temperature pathogen Fusarium nivale was the most frequently isolated fungus and was probably mainly responsible for the disease symptoms on seedling mesocotyls. The high frequency of occurrence of F. nivale is interesting since only one seed sample (T.O. 4290) was contaminated (3%) with this fungus. Much of the inoculum of F. nivale must have been derived from soil (Rawlinson and Colhoun, 1969a).

In the present field experiment there was an apparent paradox between the effects of chlormequat seed treatment on the number of seedlings showing symptoms of disease and the number of mesocotyls yielding fungi. Chlormequat treatment increased the number of seedlings with diseased mesocotyls but decreased the number of mesocotyls contaminated by fungi. Since chlormequat itself does not stimulate the growth of F. nivale (Pommer, 1967) one may postulate that it affects the metabolism of mesocotyl tissues making them susceptible to attack by this fungus and perhaps other soil fungi. Thus low levels of fungal contamination may result in high levels of symptom expression. The hypothesis that chlormequat may affect the susceptibility of seedling tissues to fungal attack and to frost damage is receiving further attention. It is suggested that seed treatment with chlormequat may affect the soluble sugar or total carbohydrate status of mesocotyl tissues and thereby make them susceptible to fungal attack (Horsfall and Dimond, 1957). At the same time any alteration in level of sugars, polysaccharides or consequent osmotic pressure of tissues may be related to increased frost resistance (Marth, 1965, Scarth, 1944).

In the growth cabinet and field experiment the apparent undesirable effect of chlormequat on the occurrence of disease symptoms in seedlings was controlled by the addition of organo-mercury to the seed. Thus the combined treatment would seem to allow the most beneficial effects of both chemicals to be expressed. Since the dust formulation of chlormequat is hygroscopic and perhaps unsuitable for practical use as a seed treatment (Caldicott and Lindley, 1964) the soak treatment would seem to be a practical alternative method giving similar results and worthy of further investigation.

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MONITORING FOR INSECTICIDE RESISTANCE IN ROOT FLIES

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Summary. Resistance to cyclodiene insecticides has been found in England in cabbage root fly, carrot fly, bean seed fly and onion fly. Resistant individuals can be detected with topically applied discriminating doses. Most, but possibly not all of the species, are sufficiently mobile so that sampling at 2 to 3 mile intervals gives a reliable indication of the incidence of resistant flies. The current distribution of resistant flies is reviewed.

INTRODUCTION

Resistance to cyclodiene insecticides in cabbage root fly (*Erioischia brassicae*) was first reported in England by Coaker *et al* (1963) and subsequent monitoring has been reported by Gostick & Baker (1966, 1968a). Similar resistance has also been found in carrot fly (*Psila rosae*) by Wright & Coaker (1968) and Gostick & Baker (1968b); in bean seed fly (*Delia platura*) by Gostick and Baker (1966); and recently in onion fly (*Delia antiqua*) by Gostick (unpublished). Wheat bulb fly (*Leptohylemyia coarctata*) and large narcissus fly (*Merodon equestris*) have also been tested, but resistance has not yet been found in these species.

The main object of monitoring resistance is to give advance warning to growers that resistance is developing, since treatment with alternative insecticides is useless after damage has become obvious in a crop.

TECHNIQUES

Stage tested. Although it is the larval stage of these species that attacks the roots of plants, this is not usually the most convenient stage to test for resistance. It is easier to apply an accurate dose of insecticide to an adult fly, and since many samples can only be tested as flies after being obtained in the pupal stage, most of the tests have been done on adults in order to make results of individual tests more comparable.

Testing. Where resistance is principally monofactorial, as apparently in all the cases of resistance in root flies found in England, resistant and susceptible individuals are more easily distinguished than in multifactorial resistance. It is possible to determine a discriminating dose which will kill all the individuals in a reference susceptible strain and allow the survival of resistant individuals. A discriminating dose of 0.2 µg dieldrin per fly has satisfactorily detected dieldrin-resistance in cabbage root fly, carrot fly and bean seed fly. Work on a discriminating dose of dieldrin for onion fly is still in progress. Doses of insecticide were applied topically from microcapillaries (Hewlett & Lloyd, 1960). A standard FAO topical-application test for insecticide resistance in root flies will be published soon (FAO, 1969). This will allow valid comparisons between the results of workers in different centres, and thus avoid the need for each laboratory to maintain reference strains of all the species that it may have to test.

Collection of samples. The stage which is most easily collected differs with the species. When operating a resistance warning scheme it is necessary to collect adequate samples in areas where resistance is not yet known, with the least possible damage to crops.

With cabbage root fly it is easy to collect eggs in early summer or pupae later in the season, and breed them through to the adult stage in the laboratory. The carrot fly is best collected as larvae in infested carrots, and allowed to pupate in the laboratory at 18-20° C; adults for testing then emerge after a short pupal period. The other species may also be collected as pupae.

The number of samples which are needed to assess the distribution of resistance in an area depends on the biology of the species. Where the species is known to be mobile, only a few samples are required to show the distribution of resistance. Other species may be relatively immobile and discrete populations may exist only a few hundred yards apart. When resistance first occurred in England, much of the necessary information on the movement of the species was lacking. Equally, since resistance develops in the presence of insecticide selection pressure, the first places to look for resistance are those areas where an insecticide has been used most intensively against the pest under investigation. The current M.A.F.F. pesticide-usage surveys should provide information on this aspect for the future.

Our usual approach has been to start at sites where failure to achieve insecticidal control has been noticed in the field, or in areas important for crops at risk to the pest involved and to take samples across the area from sites up to 3 or more miles apart. With cabbage root fly this has given a satisfactory indication of the distribution of resistant flies (Gostick & Baker, 1968a). Carrot fly can be similarly assessed but it remains to be seen whether this spacing of sampling points is suitable for onion fly.

Wherever possible at least 200 flies from each site should be tested in order to get a good estimate of the percentage of resistant flies present and, of more importance, to avoid missing small numbers of resistant flies which could breed to much larger proportions in subsequent generations. In practice however, this is not always possible. The percentages in Tables 1 and 2 are based on samples of 33 to 298 flies (average 113).

CURRENT POSITION

Cabbage root fly. High percentages of resistant flies have been reported previously in North Oxfordshire by Coaker et al (1963), and in north Gloucestershire, Worcestershire, Bedfordshire and east Kent by Gostick & Baker (1966, 1968a). Recent results from Worcestershire and Kent are shown in Tables 1 and 2.

Percentages of resistant flies have apparently not altered greatly in these areas recently. In Lincolnshire no resistant flies were detected up to and including 1968 when flies from 9 sites were tested. In 1969 some flies from two sites were found to be resistant (2 out of 113 from Kirton and 1 out of 53 from Bourne). Also single resistant flies have been found in one sample from Norfolk and one sample from Cornwall.

Table 1

Tests for resistant cabbage root fly from Worcestershire

% resistant flies

	1965	1967	1969
Harvington	(32)		28
Hazelor		31	13
Birlingham	6		7
Ombersley		6	15
Lincombe		(9)	32

Figures in brackets, flies collected as pupae; others collected as eggs.

Table 2

Tests for resistant cabbage root fly from west Kent

% resistant flies

	1967	1968
Strood	2	0
Gravesend	(6)	20
Swanley	1	2

Carrot fly. Tests made in 1969 have confirmed previous findings that resistant flies are widely established in Lincolnshire, Norfolk and Cambridgeshire.

Bean seed fly. No new cases of resistance have been found recently.

Onion fly. Resistant flies were found in 1969 in Bedfordshire. The extent of their distribution is still being investigated.

The rate of development of cyclodiene resistance in cabbage root fly, carrot fly and bean seed fly seems to have decreased recently, probably in association with the increased use of alternative organophosphorus insecticides. Resistance to these insecticides has not yet been detected in Britain, nor in north America where cyclodiene resistance was found several years before it occurred here. Some monitoring will be advisable after organophosphorus insecticides have been in use for about five years and at present tests are made on cabbage root flies from the area where organophosphorus insecticides were first used against them in 1963 and have been used every year since.

Acknowledgments

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DIFFERENCES IN SUSCEPTIBILITY OF DIFFERENT STRAINS OF CHRYSANTHEMUM LEAF MINER (PHYTOMYZA SYNGENESIAE) TO BHC AND DIAZINON

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Summary Strains of leaf miner were collected from year-round chrysanthemum nurseries and sub-cultured on to cos lettuce. The tolerance of the mature larvae was compared by dipping leaf discs, containing the larvae, in different concentrations of insecticide. The resultant log-dosage probit lines revealed large differences in the susceptibility of different strains.

INTRODUCTION

The chrysanthemum leaf miner (*Phytomyza syngenesiae* Hardy) is a constant threat to year-round chrysanthemum culture especially where, in order to utilise biological control of aphids and red spider mites, the normal spray programme of aphicides and acaricides has been relaxed. It is, perhaps, timely to draw attention to a new synonymy of this pest outlined in a little known paper by Griffiths (1967). For many years the species was widely known as *P. atricornis* but studies on the patterns of larval frass deposition in the mines (Hussey & Gurney, 1962; Trehan & Sehgal, 1963) suggested that different strains existed. Griffiths was able to recognise several different, but closely related, species and claimed that *P. syngenesiae* was the only one to attack chrysanthemums. Our own studies confirm this view so the familiar name *atricornis* must be sunk in *syngenesiae*.

During 1965 and 1966 several cases of apparent tolerance to BHC were reported in the larvae of chrysanthemum leaf miner. At first these reports were treated with scepticism as this pest, once established, is notoriously difficult to control. This difficulty arises partly from the indifferent coverage of the foliage so often achieved by high-volume sprays on densely planted beds of year-round chrysanthemum and partly due to the fact that the eggs and pupae within the leaf-tissues are naturally tolerant to most pesticides.

However, Gould (personal communication) made some preliminary tests which strongly suggested that resistance to BHC had been induced in some strains. With the co-operation of the N.A.A.S. entomologists mined leaves were collected from nurseries in various parts of the country.

Further, it is of interest to record that a glasshouse experiment made at the GCRI in 1958 showed that 0.1% malathion and .01% BHC achieved 100% mortality on cv. Fred Shoesmith, though in a similar trial in 1966 very little control was achieved.

METHOD AND MATERIALS

Successful transference of leaf miners from one chrysanthemum to another was difficult, presumably because of differential varietal susceptibility (Hussey &

Gurney, 1962), so flies reared from the collected leaves were caged over cos lettuce. The cos lettuce were grown in large cages each containing twelve plants. The cages (3' x 2' x 1') were made of polythene sheet with a hinged front covered with nylon gauze. No.1 dental rolls, which were moistened daily with sugar solution, were inserted through the plastic sheets. All the strains produced sufficient larvae for testing within one generation, thus overcoming the common problem of affecting the tolerance level by bulking large numbers of test insects in the absence of selection pressure.

Any bio-assay studies on leaf miners are complicated by the fact that the 'target' stage is hidden within the leaf-tissues by the intact epidermal layer. It would obviously be preferable to remove the larvae from the mines for testing purposes but all attempts to maintain untreated larvae alive, even for 24 hrs, failed. This difficulty stemmed from the inability of the larvae to feed, which they do almost continuously, rather than from difficulties in providing them with suitable environmental conditions. Third instar larvae were selected for testing and were cut from the leaf in the centre of a $\frac{1}{2}$ in. leaf disc. The choice of the thin-leaved cos lettuce simplified observation of larval growth.

Thirty discs were dipped, for ten seconds, in each of five concentrations of BHC or diazinon ranging from 0.00001% to 0.1%. After dipping, surplus insecticide solution was drained from the discs with filter-paper. All the control larvae remained alive for at least 48 hr if the discs were then set out on continuously moistened blotting paper.

Preliminary experiments showed that larvae succumbed more rapidly to BHC than diazinon so the mortality assessments were made at 24 hr and 48 hr respectively. The experiments could not all be made simultaneously as samples could only be collected in the N.A.A.S. regions as they became available. The series of tests were therefore made between April and October.

RESULTS

The experiments showed that a strain obtained from the same house at GCRI in which BHC tolerance occurred in 1966 remained highly resistant (Fig. 1) - the commercial rate of BHC (.01%) only achieving 20% mortality. On the other hand, a strain collected from weeds in the Institute grounds, together with two strains from commercial chrysanthemum crops, was very susceptible to BHC.

In the case of diazinon (Fig. 2) the regression lines again signify that the populations were highly heterogenous, but in only two strains, that from GCRI and one from Wye would many larvae have survived at the commercial concentration.

DISCUSSION

The most striking feature of these probit regression lines is their general flatness. Obviously the most casual insecticidal usage could select resistant individuals from such a wide range of susceptibility. As these collections were made without regard to control difficulties, the slopes of the ld-p lines possibly represent different stages in the selection of resistant strains. This is particularly evident in the BHC data where the ld-p of the resistant GCRI chrysanthemum strain is steeper than the remainder. Hoskins & Gordon (1956) showed that when selection operates on an almost purely susceptible strain the L.D.50 would become higher and the ld-p line decrease in slope as the proportion of resistant individuals rises. If selection continues the slope would again become steeper for

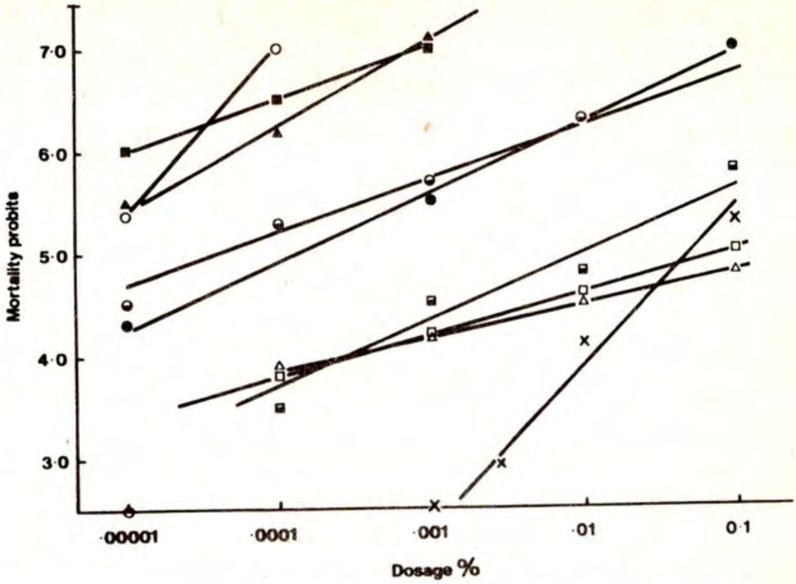

Fig. 1. Susceptibility of different strains of leaf miner larvae to BHC

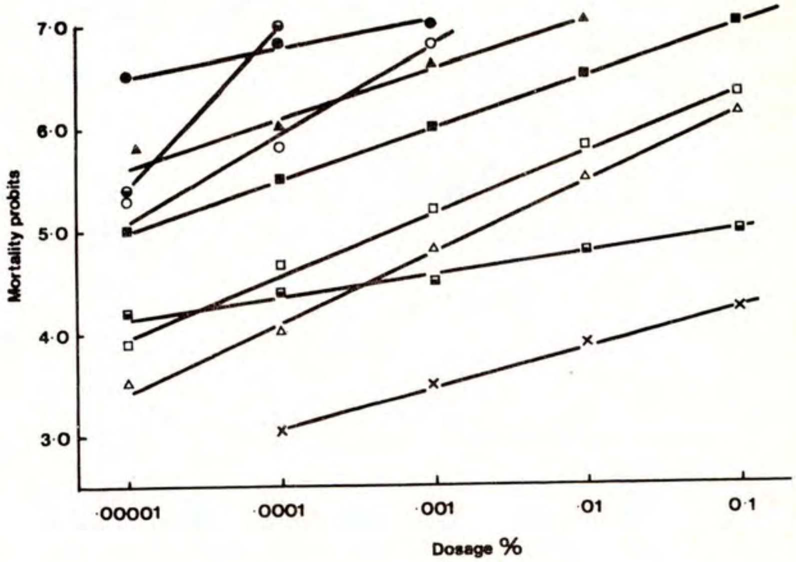

Fig. 2. Susceptibility of different strains of leaf miner larvae to diazinon
(×) G.C.R.I. glasshouse 1 (◪) Wye A. 2 (△) Cranleigh, Surrey 3
(□) Petworth 4 (●) Newcastle 5 (■) York 6 (○) G.C.R.I. weeds 7
(▲) Wye B. 8 (◒) Evesham 9

the population would consist almost entirely of resistant individuals. This trend can be seen in the ld-p lines (Fig. 1) of the strains under test and could represent different stages in the selection process.

All the strains were highly heterogeneous with respect to diazinon. As with BHC this is probably due to the long exposure times which had to be used in these tests for, in such circumstances, the amount of toxicant picked up by the larvae is not proportional to the concentration used. There may also have been some variability in penetration of the insecticides into differently orientated mines. It is possible that more critical studies could be made by an application method in which the toxicant is injected through the leaf-epidermis on to the larvae. Nevertheless, despite the limitations of the present technique, it has demonstrated considerable differences in chemical susceptibility of P. syngenesiae from various sources. More than half the strains tested required significantly more toxicant to kill them than the 'wild' strain collected from weeds in the Littlehampton district. It may also be significant that the relation between the respective ld-p lines of all the strains to BHC and diazinon is similar. It is, therefore, tempting to suggest that their tolerance to these chemicals may be a case of cross-resistance. In practical terms, the normal commercial rate of diazinon would kill only 50% or less of the larvae of two of the strains and, in the case of BHC, of four of the nine strains tested.

Although these tests demonstrated clear differences in tolerance to BHC and diazinon, they did not necessarily agree with the practical impressions formed by the growers in their efforts to control the glasshouse populations. For instance, vast numbers of strain 9 from Evesham had survived five BHC applications while no difficulty was being experienced in controlling the Petworth strain 4. These discrepancies can be accounted for either by poor application or by the fact that test populations, raised from a small sample of the total population, are themselves atypical of the whole. Another variable factor where comparisons were made over a six-month period, is the state of vegetative growth of the lettuce plants. The strains were tested serially (1-9) on receipt and there was a tendency (Figs. 1 & 2) for the earlier tests to indicate a greater tolerance than the later samples. However, only strains 8 and 9, tested in September and October respectively, were really atypical, as the remaining strains were tested in May and June. The 'wild' strain 7 was tested twice, in June and October, with almost identical results.

More tolerance appears to have developed in the southern strains, probably due to the generally higher temperatures inducing rapid development of the survivors from chemical treatments. This, in turn, would require further treatment and continued selection pressure.

Development of resistance to the most widely accepted controls naturally increases the need for other effective chemicals. From a comparison of seventeen new insecticides for aphid and mite control (Worthing, 1969) only aldicarb and methomyl (100mg/9 in.pot) were effective against strain 1.

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METHODS FOR MONITORING RESISTANCE IN APHIDS

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Summary. The distribution of resistance in aphids is summarised. Due to the wide range of insecticides in use and their differing modes of action, several methods have been devised to test for resistance. These include simple field application to check on reported failure of routine sprays, and laboratory techniques which may be used for direct application to the aphid or to provide a residual deposit of known magnitude. Where possible the sensitivity of the method is indicated, together with the factors liable to cause variability in results.

INTRODUCTION

Resistance to an insecticide by aphids was first reported by Boyce (1928), who artificially selected tolerance to hydrocyanic acid in the melon and cotton aphid (*Aphis gossypii*), but it was not discovered in the field until the walnut aphid (*Chromaphis juglandicola*) became resistant to parathion in California (Michelbacher *et al*., 1954). Shortly afterwards, resistance to organophosphates was reported in the peach-potato aphid (*Myzus persicae*) by Anthon (1955) and Klostermeyer *et al*. (1956), and in the spotted alfalfa aphid (*Therioaphis maculata*) by Stern and Reynolds (1958). Although the latter developed 100-fold resistance within four years (Stern, 1962), the problem of resistance in aphids was not yet regarded as of great importance, due to its sporadic and local appearance and its rapid decline when selection pressure was removed.

More recently, resistance to organophosphates in the damson-hop aphid (*Phorodon humuli*) appeared almost simultaneously in England (Anon., 1966), Germany (Hierholtzer, private communication) and Czechoslovakia (Hrdy and Zeleny, 1968). Resistance has also been reported in *A. gossypii* to BHC and organophosphates in Peru, U.S.A., West Germany and Zambia; in rosy apple aphid (*Dysaphis plantaginea*) to organophosphates in Switzerland; in green apple aphid (*Aphis pomi*) to organophosphates in Switzerland and U.S.A.; and in woolly aphid (*Eriosoma lanigerum*) to BHC in Australia (Anon., 1965, 1968).

Since the first reports, resistance to organophosphorus insecticides, particularly in *M. persicae*, has become widespread and of economic importance. Resistance in this aphid has been reported from U.S.A., Brazil, Venezuela, Norway, Sweden, Finland, West Germany, Italy, Spain, France, Great Britain, Ceylon and China (Anon., 1965, 1968; Baranyovits and Gosh, 1969). In Great Britain resistance, of economic importance, in *M. persicae* is limited to glasshouse chrysanthemums, but in Italy, France and Spain it is regarded as a threat to peach growing. The indications are that continued use of insecticides will result in a rapid and widespread increase in aphid resistance.

Knowledge of the geographical distribution, the degree of resistance and the nature of possible cross resistance is essential to deal with this problem. Such information can be obtained only with the help of simple, reliable test techniques, some of which are described below.

METHODS

Field application. These techniques may be regarded as careful treatment of the field population in its natural environment, to test for resistance when an insecticide treatment has failed. This may be done by the normal application methods or by small-scale hand-spraying (Anthon, 1955; Stern and Reynolds, 1957, 1958). Such methods can supply valuable information, especially if followed by laboratory tests, but suffer from the disadvantage that no direct comparison with a normal strain of aphids can be made.

Topical application. The technique has been described by Needham and Dunning (1965), in which insecticides are applied in acetone solution by a microsyringe (Arnold, 1965, 1967). Table 1 shows the results of a comparison between clonal cultures of resistant and susceptible *M. persicae*. The smallest difference in susceptibility that can be detected, that is the sensitivity, may be expressed as a factor for the least significant difference in the median lethal dose. With topical application this is 1.5 to 3 depending on the numbers of aphids used in the comparison. The main disadvantage of the technique is that individual treatment and handling makes the dosing of large numbers of aphids a formidable task, particularly if many samples have to be tested. If small numbers are used there is a loss of sensitivity.

Table 1

Sensitivity of comparisons of susceptibility to dimethoate in clonal cultures of peach-potato aphid by topical application

	Aphid sample	No. of aphids used for each regression line	LD50 % w/v (0.25 µl/aphid)	Factor for least significant difference
Test I	Susceptible	3 replicates of 10 at each of 5 doses	0.00078 ± 0.000113	1.5
	Resistant		0.42 ± 0.0454	
Test II	Susceptible	3 replicates of 10 at each of 4 doses	0.00010 ± 0.000014	2.9
	Resistant		0.10 ± 0.01978	

Residual deposit and direct spraying. Residual deposits may be applied to leaves or other surfaces in a Potter-Tower (Potter, 1952), by leaf dipping, or by hand sprayer. When leaf discs were sprayed in a Potter-Tower, allowed to dry, and the aphids then confined on the treated surface with 'Fluon'* coated glass rings (Needham and Dunning, 1965), the sensitivity was found to be of the same order as obtained by topical application (Table 2); that is, differences of between 2- and 3-fold could be detected with single tests. This can be reduced if the results of several tests are combined.

Residual deposits may also be obtained by dipping cut shoots, leaves or whole plants and allowing them to dry before the test insects are transferred (Mello *et al.*, 1967). The advantages of this technique are its simplicity and that it provides the aphid with a natural contact surface and food. Disadvantages are that the chemical is unevenly distributed on the leaf and the deposit varies with the plant species, the surface structure, and the age and condition of the leaves. Different chemicals and formulations will also produce considerable variations on

*'Fluon', a polytetrafluoroethylene (PTFE) dispersion, I.C.I., Welwyn Garden City, Herts.

identical leaf surfaces. For example, more chemical is retained on waxy leaves when oily substances (parathion, demeton-S-methyl, etc.) are used than will be the case with non-oily chemicals (menazon, DDT, BHC, etc.). Increasing the amount of wetter can achieve very good wetting and give the impression of good retention, but this procedure introduces an unknown factor to the test. Materials such as Lissapol NX, if used above 0.03%, can destroy the structure of the leaf wax, resulting in an increased uptake by the leaf and an oral poisoning effect which certain insecticides would not normally possess in their standard formulations (Baranyovits, unpublished). The deposit can be considerably reduced by the addition of extra wetter as is shown in Table 3.

Table 2

Comparison of susceptibility to residual deposits of dimethoate between field collected samples of peach-potato aphid and after rearing for two generations

Test	Aphid sample	No. of aphids used for each regression line	LD50 % w/v (2mg wet spray/cm^2)	Factor for least significant difference at LD50
Field aphids	6	2 replicates of 10 at each of 4 doses	0.0063 ± 0.0020	2.7
	34	"	0.0089 ± 0.0015	
Cultured aphids	6	"	0.0112 ± 0.0023	1.9
	34	"	0.0148 ± 0.0012	

A Potter-Tower, slightly modified to deliver coarser droplets, will operate with all commercial formulations. The use of solvents, combined with a very fine spray-mist, can cause rapid loss of active ingredient, due to evaporation, with very volatile aphicides (mevinphos, diazinon, etc.) in contrast to non-volatile aphicides (menazon, azinphos-methyl, DDT). Solvents can also increase penetration through the insect cuticle or leaf wax. Such factors may enhance or diminish the toxicity of insecticides.

Table 3

Effect of additional wetter on retention of amiton on Valencia oranges

Fruit	Average weight of the fruit	Average retention of spray liquid	Residue a.i.	Average retention of spray plus Synthrapol (2 oz/100 gal)	Residue a.i.
Mature	141 g	0.66 g	135 γ	0.26 g	58 γ
Half-grown	104 g	0.50 g	102 γ	0.25 g	51 γ

A Potter-Tower can be used to spray aphids on cut leaves or inert surfaces and to produce accurate deposits for residual tests (Needham and Dunning, 1965). The leaf, as a treated surface, has the advantage of providing the aphid with food and moisture but a more accurate and even deposit is obtained on an inert surface, such as glass. Table 4 illustrates results with susceptible (Rothamsted) and medium resistant strains (Jealott's Hill strain developed by using 'Vapona' strip in the greenhouse) of M. persicae using the modified Potter-Tower technique. When it is desired to assess only contact action the aphids can be transferred, after spraying, to untreated leaves.

Table 4

Mortality (%) after 6 hr when two strains of peach-potato aphid on turnip leaves were sprayed in Potter-Tower (0.0015 ml/cm^2)

Treatment	Concentration a.i.	Rothamsted strain S	Jealott's Hill strain R
Demeton-S-methyl	0.0025%	100%	10%
" "	0.0050%	100%	40%
" "	0.0100%	100%	96%
'Pirimor' (PP062)	0.0025%	100%	100%
" "	0.0050%	100%	100%

When a Potter-Tower is not available a small hand sprayer, delivering a fine spray, may be used. It is, however, not recommended as a standard technique as the amount of spray deposit will vary with the equipment and the operator.

The residue and direct spraying techniques have one important advantage over topical application, in that the insects do not have to be treated individually. It is thus possible to use more individuals in the tests or to examine a larger number of samples in a given time. With residue methods, the treated surfaces can be prepared in advance of sample collection and the aphids placed directly on them, thus avoiding repetitive handling and damage to the aphids. However, it is not possible, without conducting an analysis on the aphids, to determine the dose obtained by each individual.

Oral toxicity. In addition to the techniques discussed above, oral toxicity may occur due to the systemic activity of some insecticides in plants. To test for this action cut shoots may be immersed in aqueous formulations of insecticide or the liquid may be watered on to the soil of potted plants. It is difficult without careful analysis of the aphids with the aid of radioactive tracers, to make this technique quantitative. The uptake of insecticide by plants may vary, as may the feeding behaviour of the aphids.

Aphid material. The fact that most aphid species are easily upset when transferred from their original host plant and cultured on a different one in the laboratory, indicates that testing should be done on field-collected material wherever this is possible. Table 2 shows the effect on susceptibility of the peach-potato aphid, collected from mangold clamps, when cultured on Chinese cabbage for two generations under glasshouse conditions. The field material was slightly more susceptible than the cultured aphids. This raises the question of the value of laboratory-maintained cultures of susceptible aphids, with which to compare field samples, in order to assess the degree of resistance, as it has been reported that resistance in aphids can soon be lost when selection pressure is removed (Anthon, 1955; Dunn and Kempton, 1965; Georghiou, 1963). This does not appear to occur with the damson-hop aphid as a culture, originating from Wye, Kent, in 1966 (Table 5), has been maintained in the parthenogenetic phase for three years without any detectable change in its response to demeton-S-methyl. However, a marked increase in susceptibility was observed after these aphids had passed through a sexual phase in 1968-9.

Table 5

Mortality (%) of damson-hop aphid when sprayed in Potter-Tower with demeton-S-methyl

Culture	ppm a.i. w/v								
	1.5	3.0	6.0	12.5	25	50	100	200	400
Wye 1966	-	-	-	6	17	69	93	-	-
" 1967	-	-	-	5	31	58	86	-	-
" 1968	-	-	-	4	46	55	100	-	-
" 1969*	-	0	29	70	97	100	-	-	-
Yorks 1969	11	30	69	97	100	-	-	-	-
Kent 1969	-	-	-	-	-	23	78	92	100

* After sexual phase, 1968-9

Measurement of resistance. Expressing resistance in terms of differences between LD50 values can be misleading when populations are heterogeneous, since a small number of resistant individuals may escape detection. It is better to express differences in susceptibility at LD95, or higher, in order that those surviving the high doses of insecticide are included in the assessment. For detection of resistance in samples from the field it may be satisfactory to use only one or two discriminating doses of insecticide, above those required to kill 95% of susceptible aphids, in order to demonstrate that resistant individuals are present in the population.

The response of the damson-hop aphid to demeton-S-methyl is shown in Table 5, where a sample from wild hop in Yorkshire is compared with the average of five samples from cultivated hops in Kent. Doses of 50, 100, 200 and 400 ppm are used for typing field populations and survival at 100 ppm is taken as representing the presence of resistant individuals.

DISCUSSION

An ideal technique should test for all modes of action of the aphicides in common use, viz., contact, fumigant and systemic or oral effects. Furthermore, it should employ simple equipment which can be used in field laboratories possessing minimal service facilities. None of the techniques fulfil all these conditions, since accurate quantitative estimates of resistance can be obtained only with precision equipment and controlled environmental conditions. Thus, methods suitable for use in field laboratories, such as dipping or hand spraying, can be expected to do little more than detect the presence of resistant individuals in field populations.

Detailed studies in the research laboratory can be undertaken with topical application by microapplicator or precision spraying in the Potter-Tower. These methods are of equal sensitivity (Tables 1, 2), hence the choice will depend on other factors. Topical application permits more accurate measurement of the dose, but is laborious. The Potter-Tower provides reproducible deposits, which need not vary by more than 5%, on leaves or inert substrates. It can also be used for a less accurate form of topical application if the aphids are transferred to a clean substrate immediately after treatment.

Irrespective of the technique, evidence for resistance involves comparison with the response of a 'susceptible' population. Reference cultures are not always available in field laboratories, hence calibration may be necessary before field populations are monitored, as has been proposed by Needham and Dunning (1965) for the peach-potato aphid on sugar beet.

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