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INTERACTIONS OF PYRIMIDINE FUNGICIDES WITH SOIL AND THEIR INFLUENCE ON UPTAKE BY PLANTS

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Summary Adsorption and desorption of ethirimol and dimethirimol by a range of soils and clays was measured under various conditions. Ethirimol and dimethirimol were moderately strongly adsorbed compared with other pesticides, ethirimol being more extensively adsorbed than dimethirimol. Adsorption was greatest with soils containing much organic matter and with acid soils where a large proportion of the chemical is present in the protonated form. Desorption was slower than adsorption but adsorption appeared largely reversible if sufficient time was allowed.

Relative mobilities in laboratory columns of different soils and control of mildew on barley grown in contrasting soils uniformly treated with ethirimol were consistent with differences in adsorption. However the relationship between adsorption and uptake of ethirimol by barley from radiolabelled seed dressings in different soils was less simple, indicating that other factors modify the influence of adsorption with localised applications. Direct observations of the distribution of ^{14}C labelled ethirimol in various soils by impregnation with resin and autoradiography suggest that structural and textural factors may be particularly important.

INTRODUCTION

The interactions of the systemic fungicides ethirimol (5-butyl-2-ethylamino-4-hydroxy-6-methylpyrimidine) and dimethirimol (5-butyl-2-dimethylamino-4-hydroxy-6-methyl pyrimidine) with soil are of interest because these chemicals are applied mainly as soil drenches or as seed dressings to give extended control of powdery mildew on cereals and cucurbits. Among these interactions, adsorption and desorption are of major importance because they can influence movement and also availability to plants and soil microorganisms.

Adsorption is influenced considerably by soil type. Previous studies with various pesticides have shown that the soil property which generally has the greatest effect on the extent of adsorption is organic matter content, although clay content, cation exchange capacity and acidity have also been shown to have some importance (Goring, 1967; Knight *et al.*, 1970; Osgerby 1970). Soil pH might be expected to be particularly important for ethirimol and dimethirimol which are weak bases (pK_a 5·3 and 4·8) and which like some triazine herbicides (Weber, 1970) are therefore protonated in acid soils, especially near negatively charged surfaces where hydrogen ion concentrations are greater than in the bulk of the soil solution. The cationic species present under these conditions can compete for cation exchange sites, thus tending to increase adsorption in acid soils.

This paper reports investigations into adsorption and desorption of ethirimol

and dimethirimol by soils and clays, and related studies on uptake of ethirimol by barley following uniform and localised applications to soil. Direct observations of redistribution in soil following localised applications are also described which illustrate structural and textural factors that may modify the importance of adsorption under natural conditions.

METHOD AND MATERIALS

Soils

Table 1 shows properties of the soils used in the various parts of these investigations.

Table 1

Soils	Sand %	Silt %	Clay %	Organic matter %	pH	Cation Exchange capacity, m.equiv/100g
Limekiln	30	18	52	5·8	8·0	21·4
Frensham	82	7	11	2·3	7·8	7·2
Bagshot	80	9	11	5·1	4·8	28·0
Whiskers	60	17	23	4·1	7·0	17·0
Steyning	35	35	30	5·2	6·1	30·8
Oakham	27	29	44	5·9	6·0	25·0
Pear tree meadow (unlimed)	41	27	32	5·2	8·2	28·2
Pear tree meadow (limed)	52	24	24	5·8	6·5	20·2
Christmas Hill	peaty sand			27·0	7·8	50·0
Holme Fen	light peat			83·0	6·5	141
Acid peat	light peat			84·8	4·8	104
Darlows	fen peat			86·5	4·0	140

Adsorption and Desorption - Amounts adsorbed were determined from the change in solution concentration when weighed portions of soil or clay were shaken with aqueous solutions of fungicide having a range of initial concentrations (10–100 μg/ml for ethirimol, 10–1000 μg/ml for dimethirimol) using a ratio of adsorbent to solution of 1 to 20 in most experiments. Preliminary experiments established that equilibrium was acheived within 48 hours. After equilibration, the suspensions were centrifuged and the concentration of fungicide determined in portions of the supernatant by UV analysis after acidification to pH 1 or in some cases where ^{14}C labelled chemical was used, by liquid scintillation counting.

The influence of dissolved salts was investigated by measuring adsorption by a range of soils from solutions of ethirimol in 0·01M $CaCl_2$ and also by adding different amounts of sodium chloride to the equilibrating solutions. The effects of pH were investigated with bentonite using a range of contrasting buffer solutions.

Desorption was investigated by removing all or part of the equilibrium solution after centrifuging and replacing with more dilute fungicide solutions. The suspensions were re-equilibrated and amounts desorbed calculated from the change in solution concentration.

Adsorption and desorption isotherms were obtained by plotting amounts adsorbed (μg/g) against the final equilibrium solution concentrations (μg/ml).

Leaching in Soil Columns - Movement in laboratory prepared columns of soil was investigated using the method of Harris (1967). Fungicide was applied to the lower surfaces of 15 cm vertical columns of 2 mm sieved soil and was leached upwards through the column as water was drawn up from a reservoir at the base and evaporated from the upper surfaces. After 3 days, the columns were sectioned and the relative quantity of fungicide in each section determined by bioassay with cucumber seedlings. Mobility factors were calculated as described by Harris (1967). On the scale used, a factor of 6 indicates complete movement to the top of the column and a factor of 1 indicates no movement from the point of application.

Uptake of Ethirimol by Barley from Uniformly Treated Soil - Uptake from uniformly treated Frensham and Bagshot soils was compared by bioassays in which control of mildew on inoculated plants was assessed.

Ethirimol solutions with a range of concentrations (0·05 to 1·28 μg/ml for Frensham, 0·57 to 14·3 μg/ml for Bagshot) were uniformly mixed with sieved air-dry soil in the ratio 1 ml solution to 10 g soil using a food mixer; each treatment was replicated three times. The mixed soil was allowed to equilibrate for a further 4-5 days in polythene bags before transferring to 15 cm diameter plant pots which were fitted with vertical glass tubes reaching to the base to allow water to be supplied below the soil surface. 12 Barley seeds were sown as uniformly as possible 1·5-3 cm deep in each pot and the soil covered with sand to reduce evaporation from the surface. The pots were watered daily in the greenhouse and at the 2-leaf stage the plants were inoculated with mildew. Inoculation was repeated at approximately 2-week intervals and mildew assessed as the percentage of the leaf area infected in 3 replicate plants from each pot. The soil concentrations required to control 80% of the infection on control plants (LC 80) were calculated from the results.

Uptake of Ethirimol from Seed Dressings - Uptake of ethirimol by barley after application as radiolabelled seed dressings was investigated in pot experiments with 5 contrasting soils: Frensham, Bagshot, Whiskers, Steyning and Darlows. To simulate typical field conditions as far as possible, the soils were coarsely shredded (1·5 cm sieve) and packed lightly into tall parallel-sided plant pots (length 40 cm, diameter 15 cm) so that the lower half of the soil was denser than the upper half. The resulting bulk densities in the two halves of the pots were 1·3 and 1·1 g/cm^3 for the mineral soils and 0·67 and 0·62 g/cm^3 for Darlows.

Barley seeds, each coated with approximately 70 μg ^{14}C labelled ethirimol were planted in a circle (diameter 10 cm) spaced at the commercial rate of 10 seeds/ft (33 seeds/m). Each treatment was replicated three times.

The pots were placed in individual sand/water reservoirs, which supplied water to the base of the soil, and kept outside exposed to normal weather conditions. After 4 weeks growth, plants were harvested, dried and milled in a small hammer mill to give a fine powder.

The radioactivity present in samples of the milled plant material was determined by oxygen flask combustion. The $^{14}CO_2$ liberated was absorbed in phenylethylamine and assayed by liquid scintillation counting.

Direct Observation of Ethirimol Distribution in Soil Cores by Impregnation with Resin and Autoradiography - Movement of radiolabelled ethirimol from seed dressings was examined over a period of 12 weeks in laboratory cores of Frensham, Bagshot, Limekiln and Steyning soils.

Weighed portions (120 g) of air-dry soil were packed lightly into small open-ended tins (diameter 7·5 cm, depth 5 cm) and allowed to take up water from a

saturated sand table. Two barley seeds dressed with radioactive ethirimol were planted in each tin and the soil moisture tension adjusted to 100 cm. Water was applied to the soil surface at a pre-determined weekly rate equivalent to the long term mean rainfall over the spring and summer growing seasons at Jealott's Hill. The cores were maintained in a cold room at 5-10°C which effectively prevented germination and kept evapotranspiration to a minimum.

At various time intervals, cores were prepared for autoradiography by freezing in solid carbon dioxide and removing from the tin by thawing the outer edges of the core, leaving the core itself intact. The core was then freeze-dried at between -5 and -10°C for up to 3 weeks and then transferred to an oven at 50°C for not more than 48 hours to ensure complete drying. Preliminary experiments established that no apparent redistribution of the chemical occurred during drying.

The cores were then impregnated under vacuum by slowly adding araldite resin MY 778 (100 parts) containing hardeners HY 951 (3 parts) and HY 992 (10 parts). After impregnation the cores were allowed to stand at room temperature overnight and curing completed in an oven at 100°C for 24 hours. After cooling, the cores were sectioned along a diameter through the seeds using a diamond impregnated circular saw and placed on X-ray film for 5 days to obtain autoradiographs showing the distribution of radioactivity in the soil. This technique will be described more fully in a forthcoming publication.

RESULTS AND DISCUSSION

Adsorption and Desorption - Adsorption isotherms for ethirimol and dimethirimol were generally slightly curved, with adsorption increasing almost linearly with concentration. Such data are conveniently described by the empirical Freundlich equation $x/m = KC^n$ where x/m = amount adsorbed per unit weight of soil ($\mu g/g$), C = equilibrium solution concentration ($\mu g/ml$), K and n = constants. However comparison of different soils is difficult using this relationship because it contains 2 independent variables. As the curvature is small a good approximation is obtained using the straight line relationship $x/m = K_dC$ where K_d = distribution coefficient. Table 2 gives values of K, n and K_d for the best-fitting relationships describing adsorption of ethirimol and dimethirimol from aqueous solutions. Although the Freundlich equation gave a significant improvement in fit in some cases, the straight line accounted for at least 85% of the variance in most experiments.

Table 2

Soil	Ethirimol			Dimethirimol		
	K_d	K	n	K_d	K	n
Frensham	4·8	5·8	0·89	2·3	3·8	0·91
Bagshot	32·2	53·4	0·75	7·1	50·2	0·61
Whiskers	29·5	25·5	1·07	7·4	23·8	0·68
Pear tree meadow(unlimed)	43·7	24·7	1·23	10·4	63·0	0·66
Steyning	71·0	80·9	0·91	4·7	14·9	0·78
Oakham	16·5	15·8	1·02	6·4	18·2	0·64
Darlows	372·8	502·2	0·79	108·9	404·6	0·69
Christmas Hill	48·2	98·8	0·64	-	-	-
Limekiln	15·5	12·4	1·83	2·9	1·4	1·19

Compared with other pesticides ethirimol is fairly extensively adsorbed and in all cases where comparison is possible adsorption was greater for ethirimol than dimethirimol. This may be associated with the differences in the substituents on the extracyclic nitrogen or with the slightly higher pK_a for ethirimol which results in its being protonated at slightly greater pH values. Comparison of the adsorption constants in Table 2 with the soil properties in Table 1 suggests that adsorption is greatest in acid soils or soils containing much organic matter. The importance of organic matter is supported by measurements of adsorption of dimethirimol by a more extended range of peats. Adsorption was generally very much greater than with mineral soils. The Freundlich or straight line relationships did not fit the data well but for comparison with previous values, K_d values from the best fitting straight lines lay in the range 80–120. The results are shown in Figure 1.

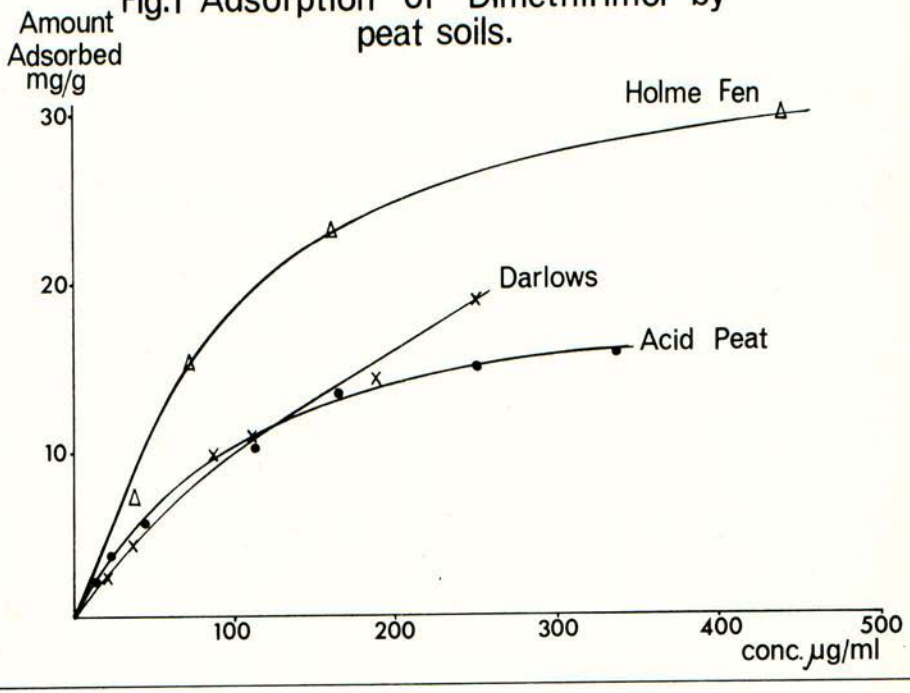
Because of the difficulties of controlling the pH in soil suspensions, more information about the influence of acidity was obtained by investigating adsorption of dimethirimol by bentonite over a range of pH values using different buffer solutions. As with peat soils, adsorption was considerable and the data do not fit the mathematical relationships well. Results are shown in Figure 2 together with those for other clays. Adsorption by bentonite increases markedly as pH decreases and at the lowest pH (3·1) reaches an approximately limiting value at a solution concentration of about 100 $\mu g/ml$. For comparison with the other adsorbents, K_d values ranged from about 20 at pH 8 to over 2000 at pH 3·1. The largest increase occurs between pH 6·9 and 5·3 which is consistent with the hypothesis that it is associated with an increase in the proportion of cationic species present. This increase occurs most rapidly at pH values in the region of the pK (i.e. 4·8 for dimethirimol) but would be expected to occur at somewhat larger pH values in soil suspensions because the actual hydrogen ion concentrations near the surfaces where adsorption occurs are larger than those measured in the bulk of the solution.

There are also large differences between amounts adsorbed by different clays at any given pH. These may be attributed to differences in the values for surface area and cation exchange capacity which are largest for bentonite and least for kaolinite.

Comparison of adsorption by soil from two adjoining plots of the same field (Pear Tree Meadow) only one of which had received heavy dressings of lime provides further evidence for the importance of acidity. These results are included in Table 3.

If pyrimidine fungicides are adsorbed partly as cations, the presence of other cations which can compete for cation exchange sites would be expected to influence the extent of adsorption. This was investigated by including various salts in the equilibrating solutions. Adding sodium chloride over the range 0·1 – 1·0 M had no significant effect on the adsorption of dimethirimol by bentonite but comparison of Table 3 with Table 2 shows that adsorption of ethirimol by soil was reduced when solutions were made up in 0·01 M $CaCl_2$ compared with distilled water suggesting that calcium is a more effective competitor as would be expected from previous ion-exchange studies.

Fig.1 Adsorption of Dimethirimol by peat soils.



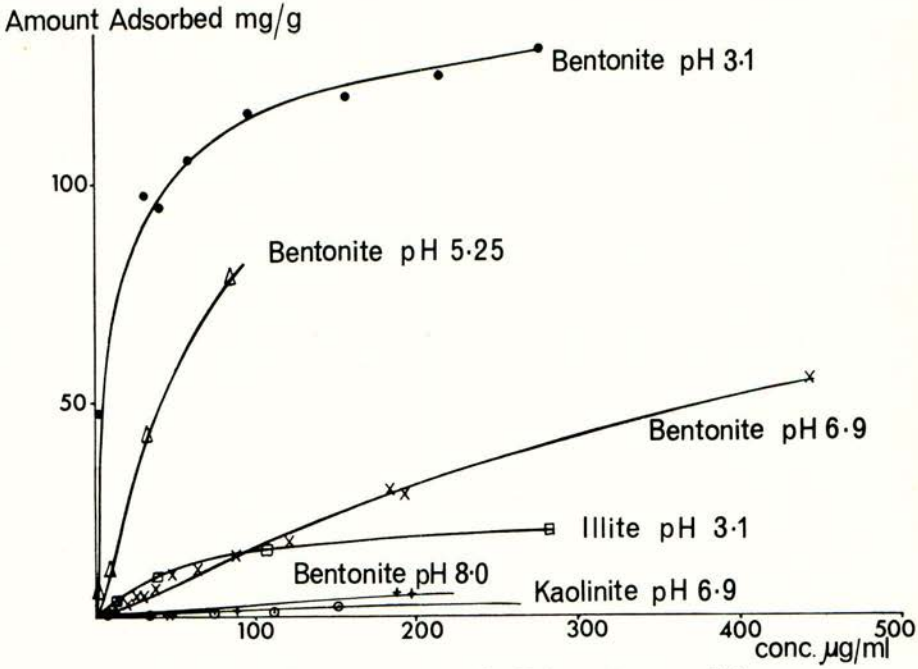


Fig 2. Adsorption of Dimethirimol by clay minerals.

Table 3

Constants for Adsorption of Ethirimol from Solution in 0·01 M $CaCl_2$

Soil	K_d	K	n
Frensham	3·9	3·0	1·08
Bagshot	12·4	16·1	0·92
Whiskers	23·3	28·5	0·89
Pear Tree Meadow (limed)	4·9	3·5	1·15
Pear Tree Meadow (unlimed)	11·9	9·5	1·17
Steyning	36·6	24·6	1·55
Oakham	4·64	12·02	0·79
Darlows	71·3	123·0	0·74

The significance of adsorption depends greatly on the extent to which it is reversible. Desorption isotherms for aqueous solutions of dimethirimol with Oakham soil and for solutions of ethirimol in 0·01 M $CaCl_2$ with Bagshot and Darlows soil are shown in Figure 3 together with the corresponding adsorption isotherms. Under these conditions where approximately 48 hours was allowed for equilibration it is clear that adsorption was not completely reversible. However this could be due to a time effect if desorption was slower than adsorption, so that the effect of time on desorption was investigated by comparing the distribution of ethirimol between soil and solution after equilibrating portions of Whiskers and Steyning soil with 0·01 M $CaCl_2$ for periods of 1 and 4 weeks following initial adsorption with solutions of 10 and 100 µg/ml. Table 4 shows the results, expressed as K_d values.

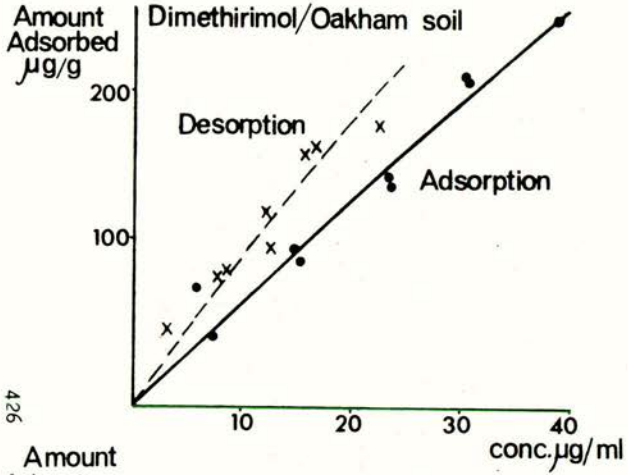
Table 4

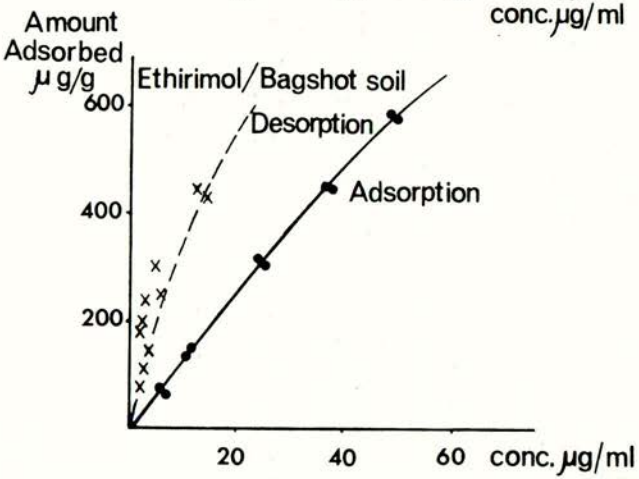
Effect of time on Desorption (solutions in 0·01 M $CaCl_2$)

Soil	Initial concentration µg/ml	1 week K_d	4 weeks K_d
Steyning	10	42·2	33·4
	100	77·0	52·9
Whiskers	10	35·2	14·5
	100	51·2	35·3

These results show that further ethirimol is slowly released to the solution as the equilibration time increases, and that adsorption is largely reversible if sufficient time is allowed.

Movement in Soil Columns - Table 5 shows values for mobility factors for dimethirimol and ethirimol in various soils from the experiments with Harris columns. Some values for Monuron are included for comparison.





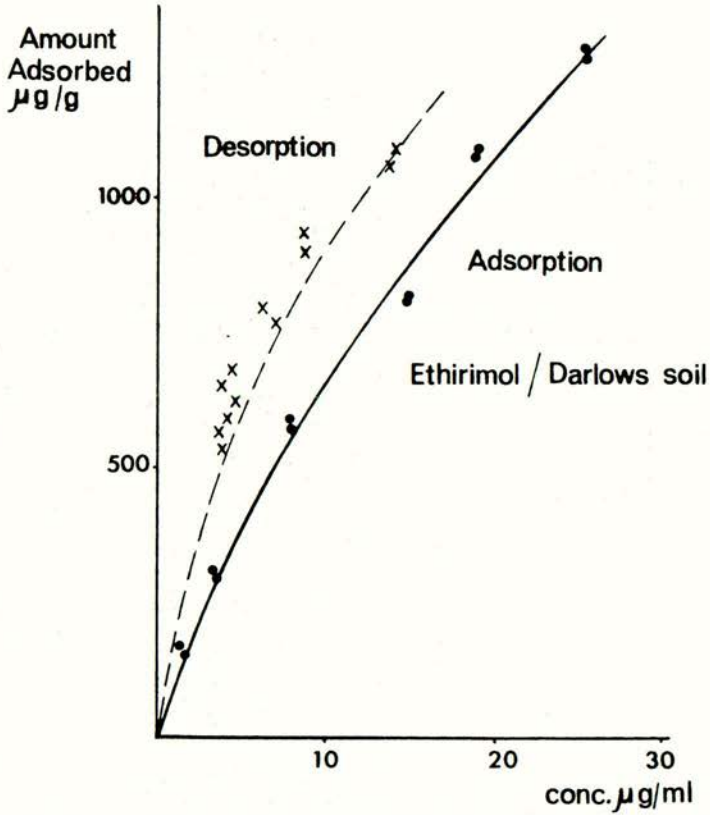


Fig.3. Desorption of ethirimol and dimethirimol.

Table 5

Harris Mobility Factors in Various Soils

Soil	Mobility Factor		
	Dimethirimol	Ethirimol	Monuron
Frensham	3·0	2·8	-
Whiskers	1·2	1·0	2·6
Limekiln	2·1	1·7	2·7
Darlows	1·0	1·0	1·0

The relative mobilities are consistent with the differences in adsorption shown in Table 2 so that for this carefully controlled laboratory system mobility can be largely predicted from adsorption.

Uptake of Ethirimol by Plants from Uniformly Treated Soil - The performance of ethirimol in different soils was first investigated in the simplest case in which the fungicide was uniformly incorporated into the soils. Table 6 shows LC 80 values for control of mildew in Frensham and Bagshot soils at various time intervals after planting. For comparison K_d values for adsorption from $CaCl_2$ solution are shown. These values are probably the most appropriate because 0·01 M $CaCl_2$ may be regarded as approximately simulating the composition of the soil solution.

Table 6

Soil	K_d	LC 80 for various intervals after planting (µg/g dry soil)			
		18 days	27 days	39 days	Mean
Frensham	3·9	0·50	0·36	0·30	0·39
Bagshot	12·4	2·19	3·02	1·38	2·20

The relative effectiveness in the two soils throughout the experiment agrees reasonably well with the differences in adsorption, as would be expected if adsorption was reversible and simply reduced the concentrations available to the roots in the soil solution. Exact agreement would not be expected because K_d values are averages for adsorption over a relatively large concentration range. Similar results were obtained in other studies with uniformly incorporated systemic insecticides (Graham-Bryce, 1967, Graham-Bryce and Etheridge, 1970).

In practice however, distributions are rarely uniform and the effects of adsorption may be modified by other factors. The relationship between adsorption and uptake following more localised application is shown by results from the experiments with radiolabelled seed dressings. Table 7 shows mean values for quantities taken up by barley from different soils after 4 weeks. K_d values for adsorption from $CaCl_2$ solutions are included for comparison.

Table 7

Soil	K_d	Ethirimol taken up μg/plant
Frensham	3·9	1·58
Bagshot	12·4	1·19
Whiskers	23·3	0·70
Pear Tree Meadow	11·9	0·62
Darlows	71·3	0·29

Although there is a broad trend for uptake to decrease with increasing adsorption, there are exceptions to this if individual soils are compared, and in particular the results for Frensham and Bagshot contrast sharply with those obtained with uniform incorporation. The range of quantities taken up from different soils is also considerably less than the range of corresponding K_d values indicating that the influence of adsorption is damped by other factors. A practical consequence of this is that performance of seed dressings should be far less dependent on soil type than that of incorporated treatments, or than would be predicted from considerations of adsorption alone.

The nature of some of the factors which modify the influence of adsorption is suggested by the direct observations of ethirimol distribution using the resin impregnation method. Figure 4 shows some examples of how the distribution changed with time in 4 contrasting soils. Comparison of figure 4 with tables 2 and 3 shows that there is no simple relationship between adsorption and movement. Thus although there was probably most movement in Frensham soil where the chemical appeared to be readily leached as a roughly conical funnel beneath the source, there was also significant movement in Whiskers soil where adsorption was much greater. Movement was less in Steyning and least in Limekiln soil where the chemical remained localised close to the source although adsorption was limited with this soil.

Further consideration of the distribution patterns found with these different soils suggests that the influence of adsorption on movement is modified particularly by structural and textural properties. Frensham is a sandy soil with little tendency to form aggregates. After application, the chemical would be accessible to leaching in relatively large open pores and the free movement of water through this relatively simple pathway causes the chemical to be leached in the roughly conical pattern shown. In contrast Limekiln and Steyning are much heavier textured soils which are usually well aggregated. The pore system is much more complex and water movement slower. Chemical can be taken up into fine pores within aggregates from which it is only released back to the main channels of water flow with difficulty. In this situation leaching would be very much reduced, irrespective of adsorption onto surfaces within the structural aggregates. Not only the equilibrium adsorption value, therefore, but also the accessibility of the adsorbing surfaces and the rates at which adsorption equilibria are achieved must be considered.

Such interactions between structural properties and adsorption are also likely to be involved in determining the relative amounts of ethirimol taken up from seed dressings in different soils (Table 7). Other factors such as the relative distributions of chemical and active roots are almost certainly involved also.

Although therefore laboratory adsorption measurements provide a reliable guide

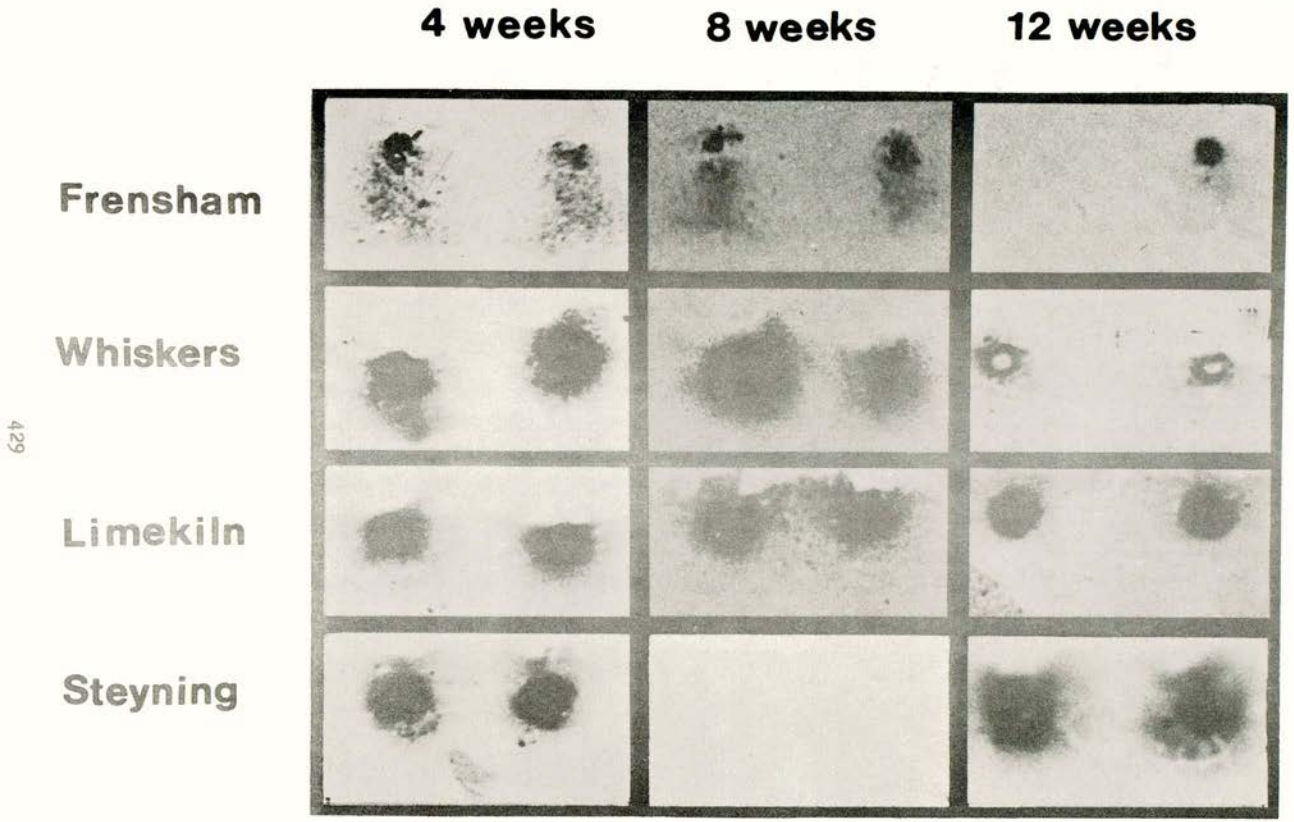


Fig. 4

Autoradiographs of ethirimol movement from seed dressings

to performance in certain well defined cases, these results illustrate the problems of extrapolating to more complex situations and the difficulties of designing laboratory tests which will indicate performance in the field.

Acknowledgments

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SOME ASPECTS OF THE METABOLISM AND TRANSLOCATION OF THE PYRIMIDINE FUNGICIDES

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Summary Dimethirimol and ethirimol are translocated with the transpiration stream. In non-woody plants, the fungicides rapidly accumulate in the leaves and are concentrated in the leaf margin. In woody plants so far examined, there is little movement of the fungicide from the region of the veins.

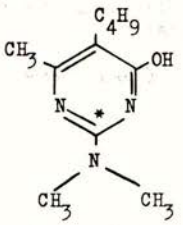
Both compounds are metabolised by progressive N-dealkylation followed by conjugation as glycosides and perhaps phosphates. Oxidation of the 5-n-butyl group to afford hydroxybutyl derivatives also takes place. These metabolic changes give rise to a complex series of metabolites. Some of the metabolites are, themselves, fungicidally active.

INTRODUCTION

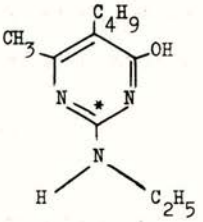
Dimethirimol (5-n-butyl-2-dimethylamino-4-hydroxy-6-methyl-pyrimidine) is a systemic fungicide (Elias et al 1968, Geoghegan 1969) used for the control of powdery mildew (Sphaerotheca fuliginea) on a range of cucurbit crops. The related compound ethirimol (5-n-butyl-2-ethylamino-4-hydroxy-6-methyl-pyrimidine) is used to control powdery mildew (Erysiphe graminis) of cereals, especially barley (Bebbington et al 1969 and Geoghegan 1969). Although ethirimol has intrinsic activity against powdery mildew (Podosphaera leucotricha) of apple, when root applied it has no activity. Both compounds show a remarkable specificity in that they are active against the powdery mildews only. They exhibit extremely low toxicity towards other fungi and mammals, (Bebbington et al 1969).

In this paper we will discuss how translocation and metabolism affect the performance of these pyrimidine fungicides.

Fig. 1



dimethirimol



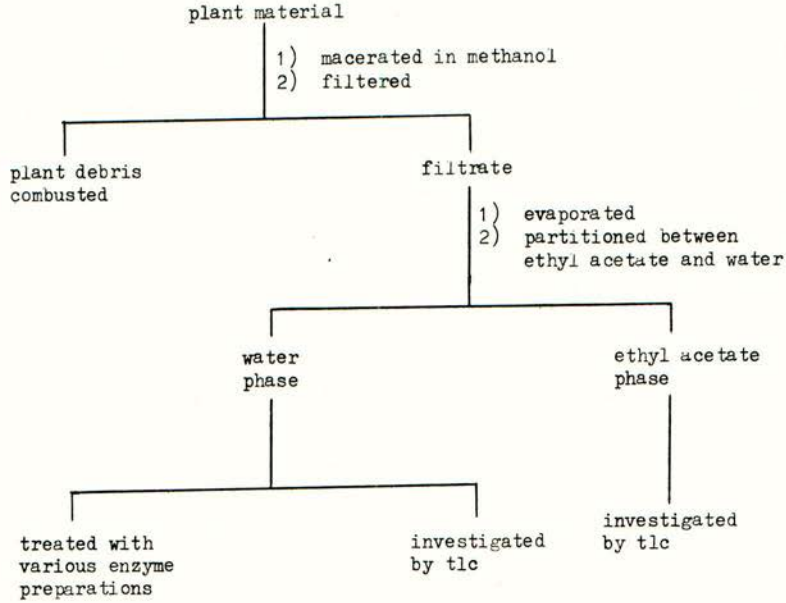
ethirimol

METHOD AND MATERIALS

^{14}C-Dimethirimol and ^{14}C-ethirimol, labelled at C-2 (asterisked in Fig. 1) in the pyrimidine ring were used for uptake, translocation and metabolism studies.

After root feeding in water culture, or treatment by foliar application, plants were freeze-dried, prior to autoradiography. The translocation pattern was determined from these autoradiographs.

For metabolism studies, plants were extracted and the extracts worked up according to the following scheme.



Tlc was carried out on silica gel F_{254} plates using various solvent systems to separate the metabolites. Most of these systems used mixtures of methanol (10-35%) in chloroform, but other systems were used for two-dimensional chromatograms.

Enzymic hydrolyses of the water-soluble metabolite fractions were carried out at 37° using β-glucosidase and acid and alkaline phosphatases.

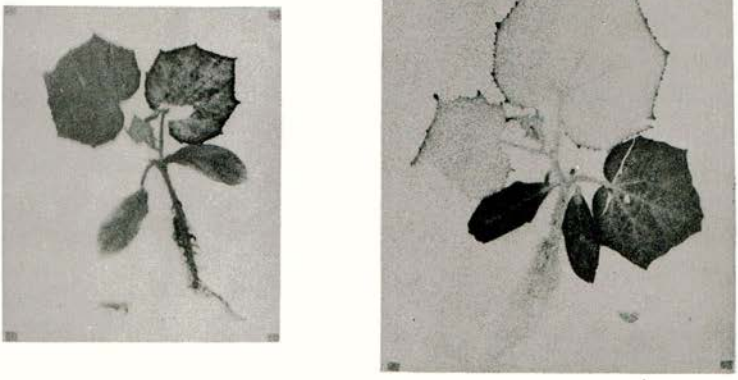
Synthetic reference samples of compounds 2, 3, 4, 5, 6 and 7 were available (see Fig. 5).

DISCUSSION AND RESULTS

Translocation

In order to give protection against foliar diseases a root-applied systemic fungicide must be translocated from the vascular system into the leaf tissue.

Fig. 2
Whole plant autoradiographs showing
translocation of dimethirimol in cucumber seedlings
(single-dose root feeding)



1 day

6 days

Fig. 3
Whole plant autoradiographs showing
translocation of ethirimol in wheat seedlings
(single-dose root feeding)

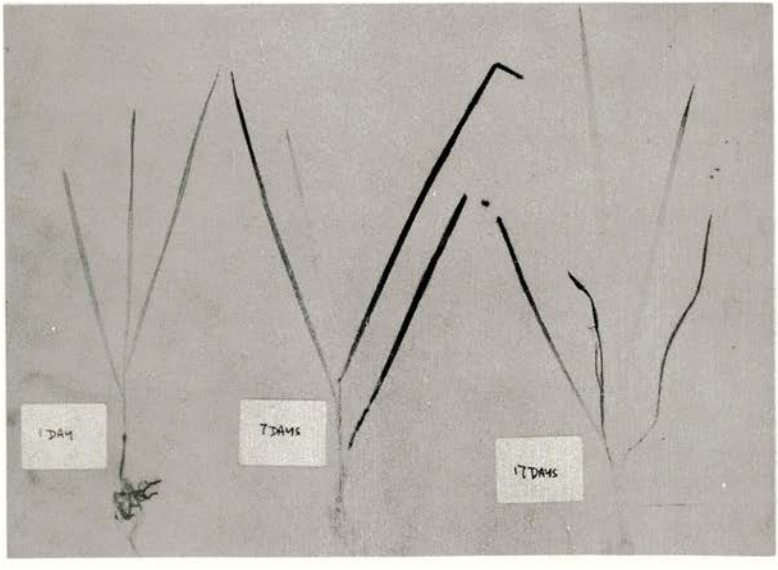
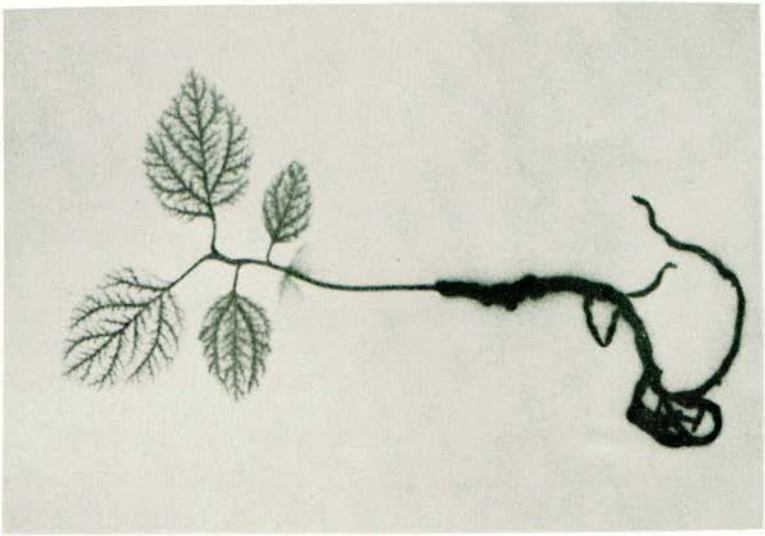


Fig. 4
Whole plant autoradiograph showing
translocation of ethirimol in apple seedling
(root-feeding, 2 days)



When dimethirimol is introduced as a single dose via the roots of cucumber plants, it is initially translocated generally throughout the plant. After a few days, there is some concentration in the leaf margin. The new growth contains very little of the fungicide and is therefore susceptible to attack by powdery mildew. Following this single application of dimethirimol, fungal invasion when it takes place, follows the fungicide concentration gradient within the leaf, starting at the base of the leaf and spreading to the leaf margins. Under field application conditions, dimethirimol is available in the soil for continued uptake into the plants so that disease protection is prolonged. A similar pattern of translocation is seen following root application of ethirimol to cereals.

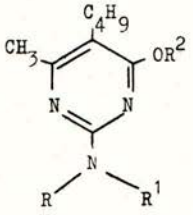
This type of translocation should be compared to the behaviour of the pyrimidine fungicides in a woody plant. Apple seedlings, root-fed with ethirimol, show a very different pattern of translocation. Most of the chemical is confined to the veins. Thus ethirimol, although it has intrinsic activity against apple powdery mildew (Podosphaera leucotricha), would not be expected to have much systemic activity against this fungus. This, in fact, is the case. (A more detailed study of the translocation of the pyrimidine fungicides in woody plants has been carried out by Dr M C Shephard).

Following foliar application, the pyrimidine fungicides are translocated

acropetally from the site of application. There is no basipetal movement within the treated leaf and, therefore, no inter-leaf movement. Thus, only existing foliage will be protected by spray treatment. A spray programme is required to protect new foliage from fungal attack.

Metabolism

Fig. 5



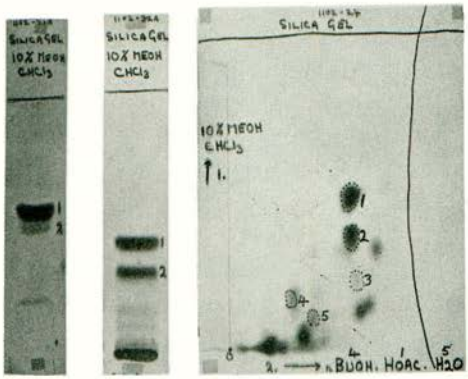
dimethirimol	$R = R^1 = CH_3$, $R^2 = H$
ethirimol	$R = C_2H_5$, $R^1 = R^2 = H$
metabolite 2	$R = CH_3$, $R^1 = R^2 = H$
" 3	$R = R^1 = R^2 = H$
" 4	$R = R^1 = CH_3$, R^2 = glucosyl
" 5	$R = CH_3$, $R^1 = H$, R^2 = glucosyl
" 6	$R = C_2H_5$, $R^1 = H$, R^2 = glucosyl

Metabolism is a second factor affecting the performance of systemic fungicides. The rate at which the fungicide is metabolised and the nature of its metabolites will contribute to its overall biological activity.

Dimethirimol is rapidly transformed into a complex series of related products in cucumber leaves. Initially, a fungicidally-active metabolite, the desmethyl derivative (2) is formed. Further demethylation to the fungicidally-inactive amino-compound 3 occurs. Most of the other metabolites are polar and water-soluble. They include the glucosides, 4 and 5 of dimethirimol and 2 respectively but no others have been positively identified. The presence of phosphates of dimethirimol, 2, 3 and dimethirimol glucoside (4) is strongly suspected since treatment of the water-soluble fraction of a plant extract with phosphatase enyzmes liberates significant amounts of all the above compounds. Compounds 4 and 5 are also active against cucumber powdery mildew.

An essentially similar metabolic pathway occurs with ethirimol. In barley or wheat plants, dealkylation to inactive compound 3 occurs. Ethirimol conjugates and conjugates of 3 are also present. Ethirimol glucoside (6) has been positively identified. It is biologically active. Acid phosphatase hydrolysis of the water-soluble fraction has yielded ethirimol glucoside, ethirimol and its dealkylated derivative. Phosphate derivatives of all three are therefore postulated as metabolites. A hydroxy-butyl analogue (7) of ethirimol, probably occurring as a glucoside, has been confirmed as a metabolite. It is not fungicidally active.

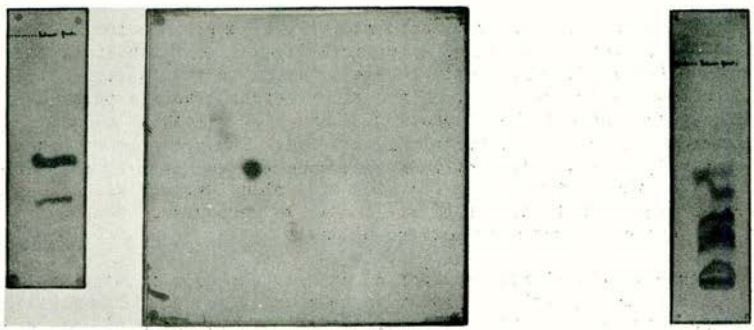
Fig. 6
Autoradiographs of tlc plates showing
metabolites of dimethirimol from cucumber plant



1. DIMETHIRIMOL
2. DESMETHYL 1
3. DIDESMETHYL 1
4. GLUCOSIDE OF 1
5. GLUCOSIDE OF 2

1 day 3 days 6 days

Fig. 7
Autoradiographs of tlc plates showing
metabolites of ethirimol from barley plants
after 7 days



ethyl acetate-soluble
metabolites

water-soluble
metabolites

Acknowledgements

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Proc. 6th Br. Insectic. Fungic. Conf. 1971

THE ACTIVITIES OF SOME SYSTEMIC FUNGICIDES AGAINST POWDERY MILDEW FUNGI

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Summary The biological activities of some benzimidazole, pyrimidine and piperazine fungicides were investigated in vitro and in vivo with powdery mildew fungi on blackcurrants and marrows, with the object of elucidating the roles of the pathogen and the host plant in determining the observed fungicidal activity. In general, for a given fungicide, activity of the same order was obtained in vitro with both pathogens. However, this activity was rarely paralleled by that in vivo. Some of these discrepencies are explainable in terms of time of action of the fungicide whilst unexpected vapour phase activities in vitro were possibly due to the release of highly volatile, fungicidally active breakdown products. Bioassays of fungicides on leaves following foliar or root application will not necessarily rank systemic activities in the same order.

INTRODUCTION

The past few years have seen the introduction of several systemic and leaf translocatable fungicides for which the control of powdery mildew fungi has been claimed. Work at Long Ashton has indicated the need for a foliar fungicide to be re-located on or within the leaf surface by interaction with leaf surface constituents, vapour phase activity or by a combination of both. Since it is well known that some systemic fungicides show marked specificities possible host/fungicide and pathogen/fungicide interactions were investigated with a view to elucidating the mechanisms of these specificities.

Our approach was to attempt to examine the toxicities of the compounds, firstly in the absence of the host i.e. in vitro, and then in vivo, since it was felt that possible differences in inherent toxicities of compounds for a particular fungal species might be an important factor contributing to their efficacy.

Few, if any, successful methods for bioassay of powdery mildew fungicides in vitro have been described (Zaracovitis, 1964). This is probably due to inherent difficulties in handling these obligate parasites which will not usually germinate in liquid water although a high relative humidity is often essential for maximum germination. However, considerable success with the bioassay in vitro of alkyl-dinitrophenol fungicides prompted us to use this technique for the present work.

MATERIALS AND METHODS

Benomyl*, triarimol**, thiophanate***, thiophanate methyl***, NF48*** [2-(3-methoxycarbamylthioureido)-aniline], triforine* (Cela W524), ethirimol* dimethirimol* and "Milcurb"+ were obtained either as gifts from the suppliers or were extracted from the commercial formulation and purified by recrystallisation. Methyl and ethyl benzimidazol-2-ylcarbamates were prepared from the appropriate thiophanate or NF48 by the method of Selling et al. (1970) followed by recrystallisation of the crude esters from ethanol.

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*, pure, recrystallised; **, technical, 100%; ***, 95-100%; +, formulated as a 10% w/v solution of the hydrochloride.

Biological assays were carried out using the following powdery mildew fungi: Sphaerotheca fulginea (Schlecht) Poll. grown on vegetable marrows; Sphaerotheca mors-uvae (Schw.) Berk. grown on rooted black currant cuttings (cv. Wellington XXX) and Oidium begoniae Oudem grown on tuberous yellow begonias.

Methods for the determination of protectant activity in vivo (Clifford et al., 1970), vapour phase activity in vivo (Clifford and Watkins, 1971) and vapour phase activity in vitro (Clifford et al., 1970) have already been published.

Trans-laminar zonal activity tests

Materials were formulated by dissolving a weighed quantity of substance in acetone (0.25ml) and pipetting into this well-stirred solution 0.03% aqueous "Ethylan C.P." wetting agent (4.75ml). This gave 5 x 10^{-3}M solutions or well-dispersed suspensions containing 5%v/v acetone. Aliquots (0.001ml) of this formulation were placed as single drops on the abaxial surface of marrow leaves using an Agla microsyringe and allowed to dry overnight. Four drops (each containing ca 2µg a.i.) were placed on each leaf. The plants were heavily inoculated with powdery mildew conidia and incubated in the glasshouse for 7-10 days. In some cases, marked zones of inhibition of the fungus on the adaxial surface were observed these being characteristically directed towards the leaf margin.

Protectant activity tests in vitro

Clean glass microscope slides were sprayed in a settling tower (Hislop, 1966) with solutions or dispersions of the appropriate concentration of the fungicide in 20%v/v aqueous acetone and the deposit allowed to dry. The slides were then dusted with conidia in a small settling tower and incubated individually at 22°C for 18h. in glass petri dishes lined with moist filter paper to give a water saturated atmosphere. Control slides, incorporated into each test, usually showed at least 70% germination of conidia. Approximate ED_{50} values were calculated by plotting germ tube inhibition (as a percentage of the control) against fungicide concentration on logarithmic probability paper. Two hundred turgid conidia on each of three replicate slides were examined.

RESULTS AND DISCUSSION

For convenience, the compounds tested have been classified according to the basic structures of the known active species. Thus Table 1 records fungicidal activities of benomyl, the thiophanates and NF48 together with the benzimidazole carbamic acid esters which are breakdown products of these compounds. Table 2 deals with the pyrimidines dimethirimol, its hydrochloride ("Milcurb"), ethirimol, triarimol and the piperazine fungicide triforine (Cela W524).

Compounds in the first series revealed remarkable uniformity in showing no translaminar, zonal or vapour phase activity in vivo yet outstanding protectant activity. The marked difference in protectant activities in tests in vivo and in vitro are explicable in terms of the site of action of these fungicides since primary germ tube development was not inhibited. Furthermore, tests in vitro with Oidium begoniae clearly showed that thiophanate and NF48 completely failed to inhibit appressorial development. With Sphaerotheca fuliginea on marrows (both in vivo and in vitro) appressorial development was difficult to differentiate from the primary germ tube. However, evidence was obtained that thiophanate methyl and NF48 frequently failed to inhibit appressorial development in vivo. It is clear therefore, that this group of fungicides acts at some stage after development of the primary germ tube and possibly also of the appressorium; haustoria were not observed. Thus in some respects, our results differ from those of Schlüter and Weltzien (1971) who reported these compounds to be appressorial inhibitors, although it is possible that this difference is merely one of degree.

Table 1.

Comparison of in vivo and in vitro activity of some benzimidazole compounds against S. fuliginea and S. mors-uvae.

Fungicide	MARROW MILDEW						BLACKCURRANT MILDEW		
	Protectant activity $10^5 ED_{50}$(M)		Vapour activity[a]		Zonal activity[a]	Translaminar zonal activity[a]	Protectant activity $10^5 ED_{50}$(M)		Vapour activity[a]
	in vivo	in vitro	in vivo	in vitro			in vivo	in vitro	in vitro
Benomyl	0.4	b	0	**	0	0	0.1-1.0	b	b
"Benlate"	b	**	b	b	b	b	b	>100	**
Thiophanate	1.5	>100	0	***	*	0	ca. 1	>100	*****
Ethylbenzimidazol-2yl-carbamate	ca. 5	>100	0	0	0	0	b	>100	0
Thiophanate methyl	0.6	>100	0	***	0	0	1-10	>100	*****
Methylbenzimidazol-2yl-carbamate	1.6	>100	0	0	0	0	b	>100	**
NF48	0.1	100	0	**	0	0	1-10	>100	***

a, ***** denotes very high activity, * denotes very low activity; b, not tested; c, NF48 is 2-(3-methoxycarbamyl-thioureido)-aniline.

The patterns of activity shown by members of this group *in vitro* against *S. fuliginea* and *S. mors-uvae* are interesting in that all materials were inactive in the protectant tests but some showed high activity in the vapour test. The conditions of these tests were such that breakdown of the thiophanates to benzimidazole derivatives would be favoured, but the benzimidazolyl carbamates *per se* showed no vapour phase activity *in vitro*. It is suggested that the vapour phase activity observed *in vitro* with the thiophanates, NF48 and benomyl may be attributable to highly volatile decomposition products which might include carbonyl sulphide, hydrogen sulphide, methylamine, carbon dioxide and carbon disulphide. Evidence for the presence of a toxicant other than the thiophanate or benzimidazole derivative in vapour tests *in vitro* is provided by the fact that these compounds were either haustorial or appressorial inhibitors whereas activity in these tests infers that germ tube inhibition was taking place. In the protection tests *in vitro*, the dose per unit area on the slide was relatively low and the deposit was non-continuous. Hence, the level of highly volatile decomposition products in the vicinity of any one spore would be low. On the other hand, the vapour phase activity test *in vitro* employed a high, locally concentrated deposit which would give rise to an equally concentrated vapour zone on the upper slide. The observed lack of vapour phase activity *in vivo* with the thiophanates probably resulted from failure of volatile, fungicidally active breakdown products to penetrate farther in a horizontal direction than the boundary of the aluminium disc used to separate the deposit of fungicide from the leaf. Also, there would be a slower rate of breakdown of the thiophanate under the drier ambient conditions on this disc compared with those on the leaf surface and in the vapour phase activity test *in vitro*.

Thus, whilst these compounds are known to show systemic activity against *S. fuliginea* on marrows as a result of root uptake (Doma *et al.*, 1971) our results show that their application to marrow leaves tends to result in control which approximates more nearly to conventional protectant than to systemic activity, although we observed significant translaminar activity when they were applied as *sprays* to the abaxial surface.

The substituted pyrimidines as a group contrast markedly with the benzimidazoles in that they showed considerable zonal activities (Table 2) when applied to adaxial or abaxial surfaces of marrow leaves and some also had vapour phase activity *in vivo* and *in vitro*. These compounds showed high protectant activity *in vitro*, for with the exception of triarimol, they inhibited development of primary germ tubes (cf. Bent, 1970). Similarly, primary germ tube development was not observed when the hydroxypyrimidines were used *in vivo*. Triarimol, however, differs from the hydroxypyrimidines in that it was inactive in protection tests *in vitro* yet highly active *in vivo*. Furthermore, it showed no vapour phase activity *in vitro* but considerable activity of this type *in vivo*. Again, these differences are explicable in terms of the stage at which inhibition of fungal development occurs. Triarimol did not inhibit primary germ tube development in *S. fuliginea* *in vivo* or *in vitro* or appressorial development of *Oidium begoniae* *in vitro*. It did permit some development of secondary germ tubes on leaves sprayed with concentrations of the fungicide which gave total protection, and observations with these latter leaves indicated some haustorial development.

Dimethirimol is formulated commercially as its hydrochloride ("Milcurb") and the much improved zonal and protectant activities *in vivo* evident with this formulation compared with dimethirimol itself were presumably associated with preferential absorption of charged molecules into and possibly through the leaf. However, the improved vapour phase activity *in vitro* with "Milcurb" may be due to the release of hydrogen chloride and not to vaporisation of the hydroxypyrimidine moiety. Dimethirimol and ethirimol have been reported as being intrinsically active against powdery mildews in general, and their specificity has been attributed to the possession of optimum uptake characteristics for cucurbits and cereals respectively. Our results with these compounds in protectant tests *in vitro* against both of our pathogens were contradictory to those obtained *in vivo* and also to those

Table 2.

Comparison of in vivo and in vitro activity of some pyrimidine compounds and one piperazine derivative against S. fuliginea and S. mors-uvae

Fungicide	MARROW MILDEW						BLACKCURRANT MILDEW		
	Protectant activity $10^5 ED_{50}$(M)		Vapour activity[a]		Zonal activity[a]	Translaminar zonal activity[a]	Protectant activity $10^5 ED_{50}$(M)		Vapour activity[a]
	in vivo	in vitro	in vivo	in vitro			in vivo	in vitro	in vitro
Dimethirimol	1.7	2.3	0 to *	* to **	***	**	>10	1.0	**
"Milcurb"	<0.6	b	*	*****	*****	*****	b	b	b
Ethirimol	2.6	0.07	0	* to **	0 to *	0 to *	>10	<0.1	*
Triarimol	0.1	>100	***	0	***	** to ***	0.1-1.0	>100	0
Triforine	0.3	1.5	0	*	0	0	>10	>10	*

a, ***** denotes very high activity, * denotes very low activity; b, not tested.

of Bent (1970) with cucumber mildew, in that ethirimol showed superior activity to dimethirimol. The reversed order of activity observed in translaminar and zonal tests in vivo emphasises the importance of the role of the host plant in the control of fungal diseases by fungicides.

Triarimol was unique in that, of all the compounds tested, it showed marked vapour phase activity in vivo but none in vitro. Vapour phase activity in vitro was not expected for this compound since it is very probably a haustorial inhibitor: the vapour phase activity in vivo can be attributed either to an effect of a volatile moiety on mycelial rather than germ tube development (secondary germ tubes were observed), or to absorption by the leaf and uptake by the fungus via the haustoria.

With the piperazine derivative triforine, secondary germ tubes were frequently observed in vivo indicating that activity was taking place at the haustorial level. However, the compound was also fairly active in protectant and vapour tests against S. fuliginea in vitro but showed poor activity in protection tests in vitro against S. mors-uvae. Thus more than one toxicant might be involved here. Our results suggest that this fungicide is highly active in protectant tests in vivo but shows little systemic activity when applied to foliage.

This investigation, which was completed before we were aware of the work of Schlüter and Weltzien (1971), essentially confirms their observation that these compounds act at specific times in the early development of the pathogen.

We have not obtained from this examination any indication of significant differences in the activity of the fungicides against our two pathogens but there was some evidence that the chemical nature of the fungicide could markedly influence movement of the material into and within leaves of the host plant. The results from translaminar zonal activity tests clearly show, however, that there is a need for care in interpreting the term "systemic fungicide" since a number of the fungicides examined in the present work could not be classed as systemic on the basis of foliar as opposed to root application. However, some materials which showed no translaminar zonal activity did exhibit some protection when the whole abaxial surface of the leaf was sprayed.

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DEVELOPMENT IN THE USE OF CARBOXIN AND MERCURY AS SEED DRESSINGS

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D. Gunary
Rothwell Plant Breeders Ltd., Rothwell

Summary Extensive laboratory and field tests have been carried out with various formulations of carboxin as a seed dressing for the control of Ustilago nuda spp. tritici and hordei. A liquid formulation of carboxin/organo-mercury, alone and in combination with lindane and a powder formulation of carboxin/organo-mercury/lindane gave excellent control of U. nuda. There were no adverse effects on the germination and yield of barley but the liquid carboxin/organo-mercury + lindane gave reduced germination of wheat. The wheat bulb fly insecticide carbophenothion when applied in combination with carboxin/organo-mercury had no adverse effect on the control of loose smut.

INTRODUCTION

The efficiency of carboxin for the control of loose smut of wheat or barley and of carboxin/organo-mercury for the control of loose smut and other seed borne diseases of wheat and barley has been previously reported (Macer et al., 1969). The formulations mainly used were dry powder seed dressings containing carboxin 75% w/w and carboxin 55%/organo-mercury 1% w/w, although a limited reference was made to a liquid formulation of carboxin. The present paper reports results obtained in the United Kingdom during 1970-71, using a liquid formulation of carboxin/organo-mercury to perform the same function as the powder.

In addition to controlling loose smut, and other seed borne diseases, it is desirable to control such insect pests as wireworm and wheat bulb fly. A powder formulation of carboxin/organo-mercury/lindane and a liquid formulation of carboxin/organo-mercury with lindane, for the control of wireworm, was tested. For the control of wheat bulb fly carbophenothion was used with a liquid formulation of carboxin/organo-mercury.

METHOD AND MATERIALS

Formulations used	Rate of use
Carboxin 55%/organo-mercury 1% w/w (C/M powder)	4 oz/cwt
Carboxin 55%/organo-mercury 1% w/w/lindane 20% w/w (C/M/L powder)	4 oz/cwt
Carboxin 37.5%/organo-mercury 1% w/v (C/M liquid)	4 fl oz/cwt
Carboxin 37.5%/organo-mercury 0.67% w/v (C/M 0.67% liquid)	6 fl oz/cwt
Lindane 20% w/w	2 oz/cwt
Lindane 20% w/v	2 fl oz/cwt
Carbophenothion 60% w/v	4 fl oz/cwt

The organo-mercury mentioned in this report was in the form of phenyl mercury acetate. All liquids were formulated for application through the Mist-o-Matic seed dresser.

Applications of dressings

Powder dressings were applied by shaking measured quantities of seed anddressing together for a standard time. The large trials were dressed through commercial machines.

Liquid dressings were applied to measured quantities of seed by means of a modified gas-pressurised spray gun. The liquid was sprayed as a fine mist onto seed which was agitated in a trough to obtain even distribution.

Trials methods

In field trials seed was selected with a high level of loose smut infection. Sowing rate was 1 - 1½ cwt/acre using standard drills, plot sizes ranging from 4 ft x 30 ft to 6 ft x 100 ft. Trials were replicated in randomised block or Latin square designs with from 3 - 6 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 per plot was counted. Wherever possible yields were assessed by combining the plots individually and weighing the grain.

RESULTS

Effectiveness of liquid formulations

Work in 1970 was designed to evaluate two liquid Mist-o-Matic formulations of carboxin/organo-mercury, applying the same level of organo-mercury but different levels of carboxin, in comparison with the powder formulation. The results of a comparison between laboratory and commercially dressed trials with the three formulations are given in Table 1, and results of a second laboratory dressed field trial in Table 2.

Table 1

Loose smut counts in Spring Barley (Zephyr). Trial A laboratory dressed Trial B commercially dressed

	Treatment	Treatment rate per cwt	Emergence plant/ft row	Smutted ears per plot
Trial A	1. C/M 0.67% liquid	6 fl oz	8.1	0.00
	2. C/M liquid	4 fl oz	8.3	0.25
	3. C/M powder	4 oz	8.4	0.00
	4. Untreated		9.0	9.75
	L.S.D. 5% (randomised block)		N.S.	6.50
Trial B	1. C/M 0.67% liquid	6 fl oz	20.2	0.50
	2. C/M liquid	4 fl oz	21.2	3.25
	3. C/M powder	4 oz	20.2	4.25
	4. Untreated		21.0	137.00
	L.S.D. 5% (Randomised block)		N.S.	10.32

Trial A plot size 5 ft x 25 ft with 4 replicates. Sowing date 19 May 70
Trial B plot size 6 ft x 100 ft with 4 replicates. Sowing date 13 May 70

Table 2

Loose smut counts in Spring barley (Sultan). Field trial with laboratory dressed seed

Treatment	Treatment rate per cwt	Smutted ears per plot	Yield cwt/acre
1. C/M 0.67% liquid	6 fl oz	0.0	30.5
2. C/M liquid	4 fl oz	0.0	30.8
3. C/M powder	4 oz	7.4	33.2
4. Untreated		201.0	29.9

Plot size 4 x 46 ft 6 replications. Sowing date April 1970

The results indicate that the liquid carboxin applied at the lower rate of use gave loose smut control comparable with the powder formulation.

Further results with carboxin/organo-mercury on Winter wheat and Spring barley can be seen in Tables 6 and 8 respectively.

Effectiveness of liquid and powder formulations with insecticides

Laboratory germination tests were carried out on two varieties of Winter wheat and one Spring barley to determine any phytotoxicity after dressing with various powder and liquid combinations of carboxin/organo-mercury/lindane. The results are given in Table 3.

The results indicate that no treatments had much effect on the germination of Sultan barley although some of the results in sand were lower than those in soil. The combination of liquid carboxin/organo-mercury + liquid lindane, even with the lindane at half rate, gave considerable reductions in the germination of both the Winter wheats. Where either carboxin/organo-mercury powder or lindane powder were used in combination with a liquid, then very little phytotoxicity was apparent.

A further laboratory germination test comparing a powder formulation of carboxin/organo-mercury with carboxin/organo-mercury/lindane at normal and 1½ times normal rate, was carried out on eight barley varieties. The results are given in Table 4.

Table 3

Germination of Winter Wheat (Cappelle and Maris Ranger) and Spring Barley (Sultan) as shown by laboratory tests in soil and in sand.

Treatment	Treatment rate per cwt	% germination					
		Soil			Sand		
		Cappelle	M. Ranger	Sultan	Cappelle	M. Ranger	Sultan
1. lindane powder	2 oz	95.5	95.5	93.5	96.0	93.0	94.0
2. lindane liquid	2 fl oz	92.0	88.0	95.0	92.0	85.0	98.0
3. C/M powder	4 oz	91.0	98.5	98.0	96.0	97.0	96.0
4. C/M liquid	4 fl oz	94.0	93.0	93.0	92.0	91.0	96.0
5. C/M powder + lindane liquid	4 oz + 2 fl oz	90.0	94.0	93.5	90.0	79.0	90.0
6. C/M/L powder	4 oz	95.5	91.0	92.0	90.0	79.0	90.0
7. C/M liquid + lindane powder	4 fl oz + 2 oz	94.0	91.5	93.5	89.0	88.0	93.0
8. C/M liquid + lindane liquid	4 fl oz + 2 fl oz	62.0	60.0	89.5	46.0	52.0	80.0
9. C/M liquid + lindane liquid	4 fl oz + 1 fl oz	67.5	62.5	92.0	38.0	26.0	92.0
10. Untreated		96.5	98.5	96.5	97.0	98.0	98.0

Table 4

Emergence of 8 barley varieties 13 days after sowing

Variety	% emergence Untreated	C/M powder 4 oz/cwt	C/M/L powder 4 oz/cwt	C/M/L powder 6 oz/cwt
Zephyr	92.0	96.0	94.7	90.7
Julia	94.0	97.0	96.0	92.3
Vada	91.7	93.3	88.7	92.3
Mazurka	94.3	94.0	94.0	90.7
Tern	48.7	60.7	62.3	35.3
Wing	92.7	98.0	97.0	93.0
Hassan	93.3	98.0	97.0	94.0
Proctor	91.0	91.0	94.7	89.0
MEAN	89.0	91.0	90.7	84.7

The results show that the carboxin/organo-mercury/lindane powder at normal rate of use (4 oz/cwt) caused no retardation or reduction of germination of the 8 varieties tested. The application of the 1½ times normal rate caused a slight drop in final germination in some of the varieties.

A similar trial was conducted with the Spring barley varieties Akka, Crusader, Hassan and Proctor using liquid carboxin/organo-mercury + liquid lindane at both normal and twice normal rate. Assessment at 8 and 11 days after sowing showed no delay or effect on the final emergence.

Loose smut counts and yield results from two varieties of Spring barley (Sultan and Crusader) treated with carboxin/organo-mercury and lindane are given in Tables 5 and 6 respectively. The treatments in Table 6 include a commercial application of liquid lindane overdressed with liquid carboxin/organo-mercury. All treatments gave satisfactory yields and good control of loose smut.

Table 5

Loose smut counts on Spring barley (Sultan) from a laboratory dressed field trial

Treatment	Treatment rate per cwt	Smutted ears per plot	Yield cwt/acre
C/M powder	4 oz	7.4	33.2
C/M liquid	4 fl oz	0.0	30.8
C/M/L powder	4 oz	4.4	34.7
Untreated		210.0	29.9

Plot size 4 x 46 ft 6 replications. Sowing date April 1970

Table 6

Loose smut counts and yield results from two trials on barley (Crusader) commercially treated using Mist-o-Matic and Robinson seed-dressers.

Treatment	Treatment rate per cwt	Smutted ears per plot. Trial 1	Trial 2	Yield per plot cwt/acre Trial 1	Trial 2
1. C/M powder	4 oz	2	0	36.1	41.9
2. C/M liquid	4 fl oz	0	0	33.0	43.5
3. C/M liquid + lindane liquid	4 fl oz + 2 fl oz	0	0	35.1	41.8
4. Untreated		177	142	32.1	42.6

Trial 1 plot size 12 x 714 ft harvested from unreplicated one acre plots.
Trial 2 plot size 4 x 46 ft with 6 replicates. Both trials were sown March 1971.

A trial on smut infected Winter wheat (Maris Ranger) was carried out to compare a commercial Mist-o-Matic application of carboxin/organo-mercury liquid with laboratory applications of carboxin/organo-mercury alone and as an over-dressing on carbophenothion.

The results are given in Table 7.

Table 7

Loose smut and yield results from a Winter wheat (Maris Ranger) trial treated with laboratory and commercial applications of carboxin/organo-mercury.

Treatment	Treatment rate oz/cwt	Emergence plants/5 metre	Smutted ears/plot	Yield cwt/acre
1. C/M powder	4 oz	292	3.25	24.2
2. C/M liquid	4 fl oz	296	3.50	22.2
3. C/M liquid (commercially applied)	4 fl oz	293	1.50	23.9
4. C/M/L powder	4 oz	273	2.50	23.1
5. C/M liquid + trithion liquid	4 fl oz + 4 fl oz	280	1.25	22.75
6. Untreated		258	142.25	23.2

Plot size 6 x 100 ft with 4 replicates. Sowing date 16 October 1970

The above trial indicated superior loose smut control by the commercially dressed over the laboratory dressed carboxin/organo-mercury liquid.

However, the best control of loose smut was achieved by a laboratory dressing of trithion over-dressed with carboxin/organo-mercury liquid. The combined powder dressing of carboxin/organo-mercury/lindane showed improved control over the formulation without lindane.

Table 8

Emergence, loose smut counts and yield results from four trials (A,B,C and D) on Spring barley (Sultan) treated with liquid and powder carboxin/organo-mercury seed dressings

Treatment	Treatment rate oz/cwt	Emergence/5 metre of row					Smutted ears per plot					Yield in cwt/acre				
		A	B	C	D	Mean	A	B	C	D	Mean	A	B	C	D	Mean
1. C/M powder	4 oz	189	198	180	-	189.0	0.75	0.00	0.75	0.00	0.38	33.6	25.3	23.4	34.0	28.8
2. C/M liquid	4 fl oz	182	202	170	-	184.7	1.50	1.25	12.75	0.75	4.10	29.0	25.7	20.4	33.8	27.2
3. C/M/L powder	4 oz	174	206	171	-	183.7	0.75	0.25	0.75	0.00	0.44	28.0	25.7	19.8	34.6	26.9
4. C/M liquid + lindane liquid	4 fl oz + 2 fl oz	171	194	177	-	180.7	1.00	0.50	16.25	0.00	2.94	28.8	23.5	20.9	33.8	26.7
Untreated		178	201	163	-	180.7	231.75	319.25	173.0	232.75	239.2	30.6	25.5	20.4	32.7	27.3

Trials A and D were drilled at one site on the 2 and 7 April 1971 and Trials B and C at another site on the 8 April and 23 April 1971.

Plot size 4 ft x 40 ft with 4 replicates.

A further series of trials were conducted to investigate loose smut control on Spring barley (Sultan) with various formulations of carboxin/organo-mercury. A range of drilling dates at two sites four miles apart enabled the effects of rainfall on loose smut control to be studied. Results are given in Table 8.

Emergence was satisfactory for all treatments in all four trials. Loose smut control was highly effective by all powder treatments in all trials. Control by the liquid treatments in Trial C was rather less effective but still exceeded 90%.

Field data indicates that combinations of carboxin/organo-mercury with lindane had no adverse effect on yield.

DISCUSSION

The evidence presented in this report shows that the liquid formulation of carboxin/organo-mercury gave comparable control of loose smut, on both barley and wheat, to the standard powder formulation. The efficiency of liquid treatment depends on the efficiency of application as well as on biological effectiveness. It has been observed that some laboratory applications were inferior to a commercial Mist-o-Matic application whereas laboratory application of powders were more representative of commercial applications.

The combination of carboxin/organo-mercury, liquid and powder, with lindane liquid or powder had no adverse effect on the control of loose smut. With barley there was no evidence of adverse effect on germination or yield and the use of carboxin/organo-mercury/lindane powder or carboxin/organo-mercury with lindane liquid can be recommended in situations where wireworm is a problem. However, with wheat evidence is inconsistent, depression in emergence has been observed although in the one yield trial reported there was no drop in yield. Further work is being carried out.

There was no adverse effect on the control of loose smut or health of the crop by the addition of carboxin/organo-mercury to wheat already treated with carbophenothion to control wheat bulb fly. Other work not reported in this paper indicates the safety of carboxin/organo-mercury when used in combination with aldrin. Work is at present being carried out with combined liquid formulations of carboxin/organo-mercury/carbophenothion and carboxin/organo-mercury/aldrin to be applied at the rate of 6 fl oz/cwt, thus obviating the need for a two stage treatment.

The reason for the reduced control of loose smut by the liquid carboxin/organo-mercury formulations in trial C (Table 8) is not clearly understood. Further work is being carried out.

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Proc. 6th Br. Insectic. Fungic. Conf. (1971)

STRUCTURE-ACTIVITY RELATIONSHIPS IN A GROUP OF CARBOXANILIDES SYSTEMICALLY ACTIVE AGAINST BROAD BEAN RUST (UROMYCES FABAE) AND WHEAT RUST (PUCCINIA RECONDITA)

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Summary In the larger context of the development of systemic pesticides, information has been obtained on the structural characteristics required for systemic control of broad bean rust (Uromyces fabae) and wheat brown rust (Puccinia recondita) by a group of carboxanilides and a hypothesis that the cis-crotonanilido moiety is a prerequisite for activity is presented.

INTRODUCTION

The discovery of the systemic fungicidal properties of oxycarboxin, 2,3-dihydro-6-methyl-5-phenylcarbamoyl-1,4-oxathiin dioxide, von Schmeling and Kulka (1966), gave a new impetus to the search for systemic fungicides. Subsequent developments in this field have shown the tonic effect of this discovery. The work reported in brief in the present paper used the oxathiin derivative as a starting point and had the following objectives:

(1) To find out what structural modifications may be made to the parent compound without losing its hitherto unique biological properties. This information could elucidate a topography of the molecule either wholly or in part associated with the systemic fungicidal effect.

(2) To find out whether the parent compound and its relatives are active per se, or whether their activity results from modification within, or in the vicinity of, the plant.

(3) To design new compounds with similar or improved systemic fungicidal properties by making use of the information obtained.

METHODS AND RESULTS

I. Structure-activity relations on broad bean rust (Uromyces fabae)

(a) Methods

All the chemicals used in this work were synthesised at Woodstock: their purity was 95% or greater. Their activity as systemic fungicides was first assessed in two glasshouse tests, designed to measure (a) systemic activity against broad bean rust after root absorption and (b) direct and systemic activity following a foliar spray treatment:

(i) Aqueous solutions or suspensions containing 100 ppm of the test chemical were drenched onto the roots of two-week old broad bean seedlings (var. Aquadulce)

growing in a 1:1 mixture of soil and perlite in 5" pots. Two days after treatment the leaves were inoculated with a conidiospore suspension of the rust fungus (Uromyces fabae), after which plants were kept for 48 h at 100% relative humidity before being returned to ambient glasshouse conditions. Readings on disease incidence were made 10 days after inoculation.

(ii) Solutions or suspensions containing 0.25% w/v of the test chemical, plus 1% w/v of glycerol, 0.005% w/v of Triton X-100 and 5% v/v acetone, were sprayed onto the first three leaf pairs of three-week old broad bean seedlings. The treatment was followed by a period of high humidity, during which the deposit remained moist. Two days after spraying the whole plant was inoculated. Incubation was carried out and readings taken as in the previous test.

(b) General consideration of the parent molecule

An acceptable systemic fungicide must produce its effect without damaging the host plant. If the chemical per se is responsible for the fungicidal effect at the site of action it must be stable within the plant. Alternatively, the chemical may depend on metabolism by the host plant for the production of the ultimate toxicant.

A closer look at the structure of the compound we used as a starting point shows

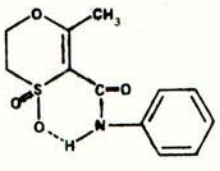


Figure 1

a number of interesting structural features, all of which could be responsible for its fungicidal activity (Fig. 1).

(a) It is an α,β-unsaturated sulphone, several of which have been described as being fungicidally active.

(b) It is an anilide, some of which are fungicides of long standing, e.g. salicylanilide.

(c) The anilide could be stabilised through H-bonding.

(d) The compound is a vinylogous cyclic sulphonate and might be active by virtue of its chemical reactivity, a common feature of conventional fungicides.

(e) The methyl group may be oxidised in the plant.

(f) Another possibility to be considered is that the whole unchanged molecule is responsible for activity. If this were the case, the question arises as to what features are responsible for its unique properties.

Having these purely chemical considerations in mind, a series of compounds was made in order to get some idea why the oxathiin parent exhibits this extraordinary combination of high fungicidal activity, translocatability and relative lack of phytotoxicity and to find out whether variations in its structure would be permissible.

Results obtained with these compounds, indications from literature and work with ^{14}C-labelled parent compound and relatives have shed some light on the complex question of structure-activity relationships.

Firstly, it was found that the oxathiin derivative, when applied to hydroponically grown broad bean plants, is not substantially changed; about 90% of unchanged labelled material could be recovered after four days. A small amount had been para-hydroxylated. Autoradiography showed that the labelled material had largely accumulated in the leaf margin. The disease control pattern closely followed the distribution pattern of unchanged material (Fig. 2).

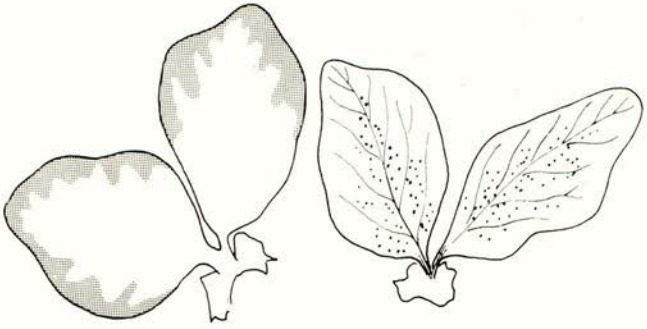


Figure 2

(c) Importance of the oxathiin ring (Table 1)

The next find concerned the sulphone group in the parent compound. Removal of the SO_2 group leads to 4,5-dihydro-2-methyl-3-phenylcarbamoyl furan (WL 22414). The compound gave good control of disease in our tests. Introduction of a second double bond gave WL 22361, which still gave good disease control. Replacement of the SO_2 group by CH_2 gave the dihydropyran derivative WL 22651 (Hoe 2989), again an active compound.

Since the nature of the ring in these compounds did not appear to be critical, open chain analogues were prepared and tested.

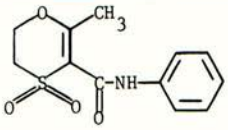
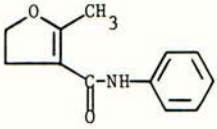
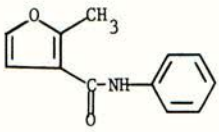
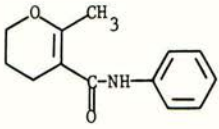
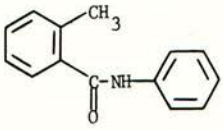
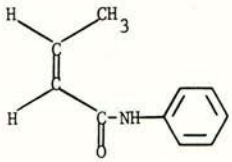
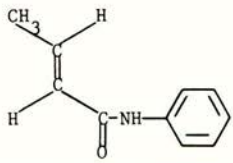
Of the two geometrical isomers of crotonanilide only the cis isomer, WL 22911, exhibited the same type of activity as that of the parent compound.

(d) Importance of the double bond (Table 2)

The next question arising was that of the importance of the double bond. When the two fully hydrogenated geometrical isomers of the pyran carboxanilide (WL 23904 and WL 23905) were tested both were found to be inactive. Likewise, saturation of the double bond in cis crotonanilide leads to the inactive butyranilide. Methylcrotonanilide (WL 22697), which by implication has a methyl group cis in relation to the carboxanilido group, is active, whereas the saturated analogue, isovaleranilide, is inactive. A triple bond leading to a linear molecule brings about loss of activity (WL 23541).

Table 1

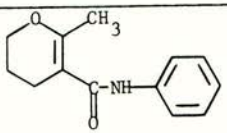
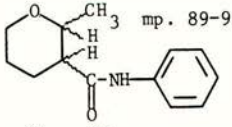
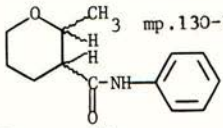
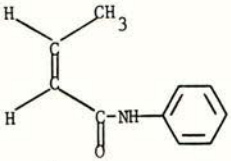
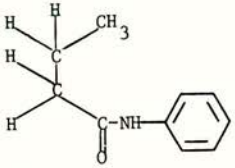
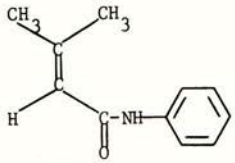
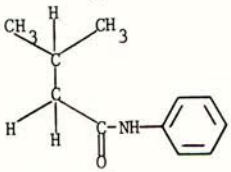
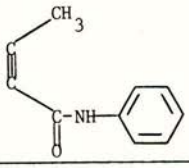
Importance of the oxathiin ring

Code No.	Structure	Foliar Appl. Direct	Foliar Appl. Syst.	Root Drench
WL 20565 Oxycarboxin		2*	2	2*
WL 22414		2*	2	2*
WL 22361		2*	2	1
WL 22651 Hoe 2989		2	2	2*
WL 22649 Mebenil		2	2	1
WL 22911		X	1*	2*
WL 22647		0*	0	0

Abbreviations:
2: 80-100% reduction in rust symptoms.
1: 50-80% " " " "
0: insignificant" " " "
*: slight to moderate necrosis.
X: leaves dead.

Table 2

Importance of the double bond

Code No.	Structure	Foliar Appl. Direct	Foliar Appl. Syst.	Root Drench
WL 22651 Hoe 2989		2	2	2*
WL 23904	 mp. 89-90°	0*	0	0*
WL 23905	 mp. 130-131°	0	0	0*
WL 22911		X	1*	2*
WL 25940		0	0	0
WL 22697		1*	1	1*
WL 23137		1	0	0*
WL 23541		0*	0	0

Abbreviations: See foot of Table 1.

(e) Replacement of the methyl group (Table 3)

In two particular anilides the importance of the methyl group was looked at in more detail. It was found that the activity of WL 22651, the methyl dihydropyran carboxanilide, was completely lost when the methyl group was replaced by H, ethyl or phenyl.

Hydroxylation of the methyl group in ortho toluanilide leads again to complete deactivation.

(f) Importance of carboxanilido function (Table 4)

On replacement of the carbonyl group in ortho toluanilide (WL 22649) by SO_2 to give ortho methylbenzenesulphonanilide (WL 24199) all systemic rust-controlling properties are lost. Conversion of ortho toluanilide and methylfurancarboxanilide into their corresponding thiono analogues leads to loss of activity. The function of the NH group appears to be more than that of a "spacer" because replacement of NH by CH_2 leads to loss of activity, although the distance between the carbonyl carbon and the 1-phenyl carbon in both compounds is about 2.5 Å.

Loss of activity is observed when the amide proton is substituted by either alkyl or carbamoyl groups.

II. Experiments on wheat brown rust (Puccinia recondita)

Several of the compounds mentioned so far were also tested against wheat rust (Puccinia recondita). A good correlation was found to exist between results obtained in the broad bean tests and those obtained in similar tests on wheat. However, phytotoxicity is an important problem. It was found that a number of substituents introduced in the anilido part of the molecule can considerably reduce phytotoxicity without causing loss of fungicidal activity. This is demonstrated in the following glasshouse test.

(a) Method

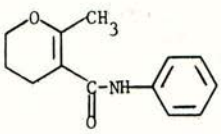
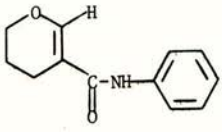
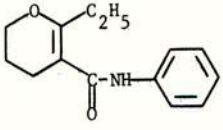
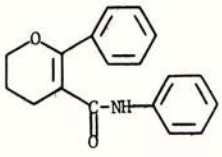
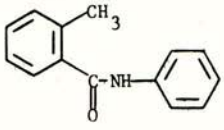
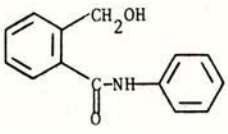
Each chemical was ground and mixed with china-clay. These dusts were lightly incorporated into the surface of soil held in plastic seedboxes (48 x 13 x 7 cm). Application was made to a 4 cm wide band at the rate of 0.25 g a.m. per metre row. Wheat seed (var. Kloka) was sown onto this band and the whole surface covered with a further 2 cm layer of soil. The boxes were kept in a cool glasshouse for five weeks, after which time the seedlings were inoculated with a dust containing spores of Puccinia recondita. After ten days' incubation (48 h at 100% R.H., then at ambient humidity) readings were made by counting the number of pustules occurring on 5 cm leaf lengths.

(b) Results

Under these experimental conditions, designed to simulate field conditions as nearly as possible, five of the eight selected compounds gave a complete or almost complete control of rust. Some of these caused varying degrees of chlorosis and growth retardation ("stunting"). The parent oxathiin (WL 20565) and the para-fluoroanilide of methyldihydropyran carboxylic acid (WL 24110) were only slightly affected. The non-fluorinated anilide (WL 22651) gave rise to severe phytotoxicity. Only the methylenedioxy derivative (WL 24479) combined high systemic fungicidal activity without concurrent phytotoxicity.

Table 3

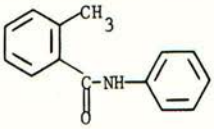
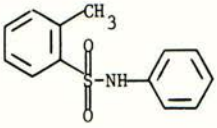
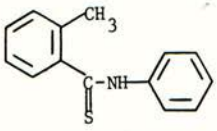
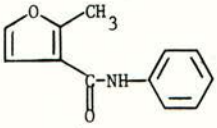
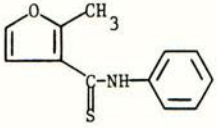
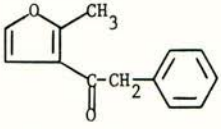
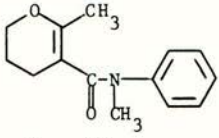
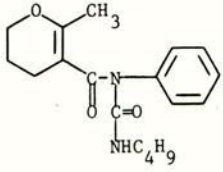
Replacement of the methyl group

Code No.	Structure	Foliar Appl. Direct	Foliar Appl. Syst.	Root Drench
WL 22651 Hoe 2989		2	2	2*
WL 23701		0*	0	0*
WL 24108		2*	0	0
WL 24784		0	0	0
WL 22649		2	2	1
WL 25795		0	0	0

Abbreviations: See foot of Table 1.

Table 4

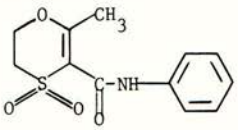
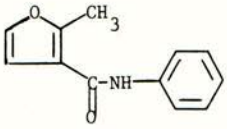
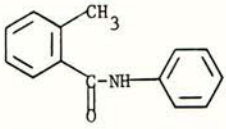
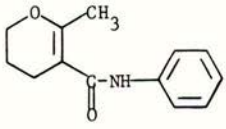
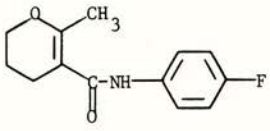
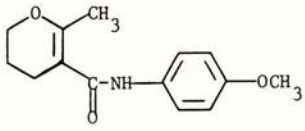
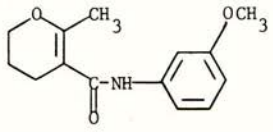
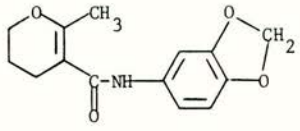
Importance of the carboxanilido function

Code No.	Structure	Foliar Appl. Direct	Foliar Appl. Syst.	Root Drench
WL 22649 Mebenil		2	2	1
WL 24199		0	0	0
WL 23679		2	0	0
WL 22361		2*	2	1
WL 24669		2*	0	0
WL 25080		0	0	0
WL 23755		1*	0	0
WL 25566		1	0	0

Abbreviations: See foot of Table 1.

Table 5

Glasshouse evaluation against wheat brown rust (Puccinia recondita)

Code No.	Structure	% Rust Control	Phytotoxicity (1)
WL 20565 Oxycarboxin		100	1
WL 22361		50	2
WL 22649 Mebenil		0	1
WL 22651 Hoe 2989		100	3
WL 24110		99	1
WL 23988		36	0
WL 24512		100	2
WL 24479		97	0

(1) Phytotoxicity: 0: No visible phytotoxicity.
1: Slight "stunting" and chlorosis.
2: Moderate " " "
3: Severe " " "

DISCUSSION

From the data presented it appears that a large number of anilides can systemically control rust diseases on two crops. The incidence of this activity is very high amongst compounds whose structure conform to the following rules:

(a) The anilides must be derived from α,β-unsaturated acids.

(b) The β-carbon atom must carry a methyl group.

(c) The double bond may form part of a planar, aromatic system as, for instance, in benzene, furan, thiazole and oxazole, or a non-planar system like dihydro-oxathiin, dihydrofuran and dihydropyran. In those systems the β-methyl group is by implication cis in relation to the carboxanilido group. If the double bond forms part of an open chain system the β-methyl group must be cis in relation to the amide function.

(d) The carboxanilido group must be primary, i.e. the nitrogen must carry a free proton. The aniline ring may be substituted; electron-donating groups appear to be more acceptable than electron-withdrawing ones.

(e) The basic structure required for activity will therefore have the following general structure (Fig. 3):

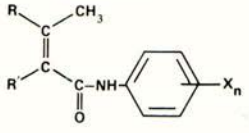


Figure 3

$R = CH_3$; $R' = H$ or $R = R'$ + double bond form a ring system.
X_n = One or more electron-donating group, or unsubstituted.

It is felt that the hitherto rare combination of systemic and fungicidal properties in the group of what may now be called "cis-crotonanilides" is related to their metabolic stability, since it appears that the unchanged molecule is responsible for fungicidal activity. In the context of the unchanged molecule, interesting observations were made by Kuhn (1937). He found that the metabolic stability of α,β-unsaturated acids is increased dramatically when the carboxyl group is converted into an amide. He also found that a methyl group on the β-carbon atom when cis in relation to the carboxamido group was not attacked, whereas in the trans position oxidation of this group took place. The active compounds discussed in this paper all have a structural arrangement similar to those found by Kuhn to have a high degree of metabolic stability.

Attempts to develop a quantitative structure-activity relationship using water solubility and partition co-efficient have been inconclusive. Therefore we conclude that in a group of systemics that are active per se metabolic stability is probably a more important parameter than simple physical parameters.

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CHANGES IN THE USE AND CHOICE OF INSECTICIDES AGAINST FOREST INSECTS IN CENTRAL EUROPE

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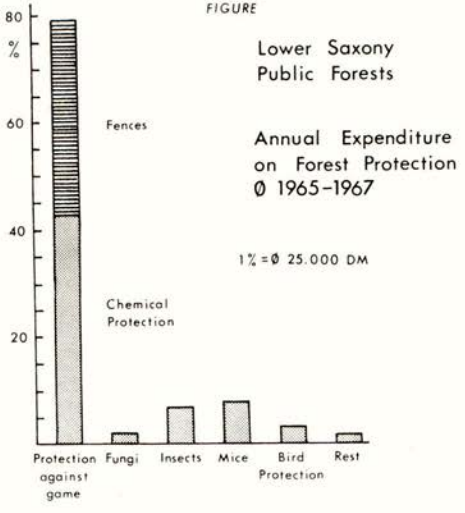
Summary In the past D.D.T. has provided a solution to a large number of more important forest insect problems in Germany. With a fuller appreciation of the ecologically undesirable effects of this chemical great efforts are now being made to select and test alternatives and to limit even their use by finer appraisal of the objectives to be achieved through their application. Examples given include the use of Bacillus thuringiensis which effects a sufficient degree of control of oak defoliators to ensure their fruiting; the use of granular formulations of systemics against Laspeyresia pactolana and Hylobius abietis; and the use of organophosphorus insecticides against Rhyacionia buoliana and Coleophora laricella. The point is made finally that certain forest practises result in inevitable rise in numbers of pests which in turn may demand increasing wide scale use of insecticides.

In the interests of a better understanding between western and central European countries it may be useful for all those engaged in forest protection to have a look at central Europe. In German forests the main problems concern protection against fungi, weeds, insects, mice and game. Their relative importance to practical forestry may be judged from published statistics of expenditure (Figure).

FIGURE



Only those familiar with the importance attached to hunting in German woods will be able fully to understand how it is that 80% of the total sum is devoted to protection against game, such as Roe, Red and Fallow Deer (Capreolus, Cervus and Dama) half being spent on fencing and half on chemical plant protectants. The remaining 20% represents expenditure upon insecticides, fungicides, rodenticides and protection against birds. The costs associated with herbicides are not included in the figure since they happen to be accounted for under silvicultural operations.

In the protection field perhaps the most difficult though fascinating problems lie among those concerned with the very many interesting insect pest species and their control. After World War II D.D.T. was by far the most widely used insecticide against a wide range of pests due to its efficacy and cheapness. These pests included caterpillars of Panolis, Bupalus, Dendrolimus, Operophtera, Rhyacionia, Tortrix viridana as well as sawfly larvae and beetles. It is true to say that since the war and up to 1968 all the larger control operations were carried out using D.D.T., whether it was applied from the air or by fogging, spraying or dusting from the ground. During this period, whilst the dangerous side-effects of D.D.T. upon the biocoenosis, particularly in respect to birds, were not fully appreciated, there was no reason to look for alternatives since life with D.D.T. was so easy! We have now therefore to do a great deal of rethinking.

In addition we have now learnt that the use of chemicals can often be avoided in situations which on the face of it appear to demand them. Firstly it is necessary for trained scientists to investigate the reasons underlying outbreak. From such investigations it may sometimes prove possible to so change the forest condition through silvicultural practise that serious damage is obviated. If, however, the danger appears to be on the increase and imminent it is necessary for an investigation to be initiated immediately to check on the health, fertility and parasitism within the population and to formulate a rough forecast of likely changes in numbers. If at that point damage appears inevitable then one of the three Forest Protection Institutes in the Federal Republic of Germany take over responsibility and will instruct the forest service on matters of control. The decision to control having been taken, the action is then confined to the minimum area possible. As to the choice of chemicals - against caterpillars organophosphorus or carbamate materials are used. Tetrachlorvinphos (Shell's Gardona or Obstabil as it is called in Germany) has been found particularly effective.

Nowadays we try to choose non-persistent insecticides without objectional residues. Best of all choices from the point of view of the biocoenosis as a whole are materials with specific action against particular insect species or families. Viruses and bacteria provide good opportunities for this approach. From our experience with Bacillus thuringiensis, for instance we know not to expect the striking effects resulting from the use of D.D.T. but instead we gain in other ways. For instance, in May 1971, our team from Göttingen carried out an operation in the famous recreation forest of Lüneburg Heath (Forstampt Göhrde). 500 g of B. thuringiensis as Thuricide H.P. (Flowablex powder) in 50 l of water were applied per ha. The caterpillar population was reduced by about 80% and this was enough to protect both flowers and leaves from severe damage. The main purpose was, in fact to ensure that the flowers of the oaks produced acorns from these famous veneer oaks. It was quite striking that whilst the larvae of Tortricidae and some species of Winter Moth (Erannis and Operophtera) had been killed, predators and parasites remained alive.

It is also worth mentioning that no harm to other animals, including birds resulted in the 126 ha concerned in the operation.

Another fascinating approach to control problems lies in the use of chemicals with specific action as opposed to broad spectrum ones. We have obtained good results with systemic organophosphorus materials, particularly when applied to the soil in granular formulation against sucking insects and larvae mining in the bark of young trees. An example of the latter type is Laspeyresia pactolana which has become a difficult and widespread problem in thicket stage spruce. Good timing is essential with this technique, and the material has to be incorporated in the soil next to the roots some weeks or even months before the larvae commence feeding. The method also provides a means of combating the depredations of Pine weevil (Hylobius abietis) in young conifer plantations.

We have also obtained other very encouraging results with organophosphorus materials for instance with Dipterex against Pine shoot moth (Rhyacionia buoliana) in autumn and with Malathion against the mining larvae of Larch case-bearer (Coleophora laricella) in mid-summer.

In the future we shall certainly be able to reduce the requirement for insecticides through advances in silvicultural practice and by encouraging natural control factors such as predators and parasites. For example in the Emsland region where the larch case-bearer (C. laricella) occurs in outbreak proportions in young thicket-stage larch it has proved possible to reduce populations by a combination of thinning practice and intensive bird management by providing them with 10-20 nest-boxes per hectare. In some cases titmice have been recorded to have removed 50% of the over-wintering Coleophora larvae which are available to them from September, throughout winter and up to April/May.

However, whilst there is now a glimpse of hope for a greater degree of integrated control in particular cases, there are still certain circumstances which can call for an increasing and large scale use of insecticides. In former days, for instance all felled stems were barked by the workers on the spot, but now many of these are left either waiting for the arrival of a mobile debarking machine or for transport to the sawmill where stationary debarking equipment is becoming more and more the rule. The logs quite frequently remain in the forest until summer or even later and so have to be treated against bark beetles with Lindane or Lindane-Carbamate mixtures. In lower Saxony between 1969 and 1970 such treatment increased by 300%, and must be considered as retrograde in our progress towards more enlightened methods. The treatment, however, is necessary if the timber is to be saved. In Germany foresters refer to the endemic population as the "iron" one, so now it could be said they have a "golden" one!

For Bark beetle populations, particularly Ips typographus and Trypodendron lineatum have risen from year to year, and the implications are clear to all German foresters who witnessed the spectacular bark beetle catastrophes following World War II.

One other subject must be mentioned. Of the 24.8 million ha in the German Federal Republic, 7 million ha are now woodland. Under the E.W.G. Mansholt plan

some 2 million ha of former agricultural land will cease to be used for its old purpose and, whether we like it or not, half will come under forest management. The task of afforestation will have to be carried out quickly before woody and herbaceous weeds can colonise the area. So a tremendous task faces the forest staff in the next few years, and for that matter the forest protection staff since we know that new forests on old agricultural land are particularly subject to damage by forest insects. Under these conditions, nothing much can be hoped for from "natural factors" because they do not exist in farmland, and we must recognise that the only alternative lies with insecticides.

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CHEMICALS FOR DUTCH ELM DISEASE

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Summary No adequate chemical control is at present available for Dutch elm disease. Recent research on the problem is discussed.

Dutch elm disease, so called because of early research carried out in Holland, is a disease of elms characterised by wilting and yellowing of the foliage and subsequent leaf fall. It is caused by the fungus Ceratocystis ulmi, which is spread from diseased to healthy trees by beetles of the genus Scolytus. The disease cycle is described in Forestry Commission Leaflet No. 19. (Gibbs, 1971). Following its discovery in 1927 it spread rapidly through most of Britain and heavy attacks involving the death of many trees occurred. However, although it was estimated that by 1960 more than 10% of the elms in the southern half of England had been killed, the severity of the disease has varied greatly. After 1937 there was a marked decline and the disease came to be regarded as an endemic problem of no great significance; although twig and branch infection was frequent the trees usually recovered. (Peace, 1960).

Recently the position has changed again. Since 1967 killing attacks have continued unabated in two main regions; alongside the Thames Estuary in Essex and Kent and in the counties of Gloucester, Worcester and Hereford in the Vale of Severn. There are also severe attacks in parts of Suffolk, Surrey, Hampshire and Sussex. This year the Forestry Commission has carried out a sample survey of the important elm areas of Southern Britain and the results of this will enable a better estimate to be made of the number of trees at risk and the present level of infection. It is certain that hundreds of thousands of elms of all sizes from young suckers to mature trees have died and that in some localities the loss has been total.

The disease also causes great losses in America and in the United States it is estimated that 500,000 trees die annually. Many of these are shade trees of high amenity value and this has resulted in a great deal of research being directed over many years towards chemicals means of control. In Britain we are hoping to benefit from this research since although it is not considered that a chemical control is likely to be found which could be applied to the millions of elms growing in woodland and hedgerow there is a great need for a treatment for trees of high ornamental or sentimental value.

The nature of the problem imposes certain constraints. Despite the timber value of large elm the main loss caused by Dutch elm disease in Britain today is a loss of amenity and the effects of any chemical control measures on factors such as wildlife have also to be considered. Until quite recently protective sprays of D.D.T. were applied in many American cities each spring. Applications were made before leaf burst to give complete cover of the one or two year old shoots in order to prevent feeding by the beetle and the associated introduction of the fungus.

Today such sprays are unacceptable and attention is being directed towards the provision of an effective but ecologically safe insecticide. Because of the problems of applying sprays to the twigs after leaf emergence such an insecticide must remain active for several months. One compound which has received much attention is methoxychlor, but its use for this purpose is still in the experimental stage.

Considerable interest has also been directed to systemic chemicals, both insecticides and fungicides. A systemic fungicide would seem particularly attractive as C. ulmi is a typical vascular wilt pathogen confined to the xylem until the tree is moribund. A fungicide moving up in the wood would thus be well placed to prevent the downward movement of the fungus from the initial infection points in the young twigs. Benomyl is a fungicide which has recently been investigated in this connection but the results so far reported have not been very encouraging (Biehn & Dimond, 1971; Hock & Schreiber, 1971). Some success has been achieved with soil application but only at very high rates and this has prompted increased research in techniques of stem injection. Good, although variable, uptake of a benomyl suspension in water was obtained in injection experiments on 10 m English elms this year but no benefits in disease control could be detected.

In addition to conventional approaches there is considerable research interest in naturally occurring chemicals. The United States Forest Service has reported the existence of a pheromone produced by virgin females of S. multistriatus which might be used to trap the male beetles of this species in the way already employed for the Gipsy Moth. D.L. Norris and his associates at the University of Wisconsin have been investigating the chemical basis of the attraction of Scolytus beetles to the bark of their host trees. It appears that both stimulants and repellants are involved, and it is hoped that some of these chemicals could eventually be used in beetle control.

One last suggestion has been made following a recent paper in which resistance to the disease in various elm clones has been correlated with vascular anatomy (McNabb et al 1970). It is postulated that the use of chemicals such as T.C.P.A. (1, 2, 6, trichlorophenyl acetate) might result in the modification of the xylem structure and lead to increased resistance.

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CHEMICAL CONTROL OF HONEY FUNGUS (ARMILLARIA MELLEA)

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Summary A phenolic emulsion marketed as a fungicide under the name Armillatox was tested to assess both its phytotoxicity and its ability to kill Armillaria mellea. When applied direct to bark a solution containing 8% a.i. did not injure roots (1.0-4.0 cm diameter) of sycamore trees (Acer pseudoplatanus) but even small quantities of a 4% a.i. solution killed roots of sycamore seedlings. When Armillatox was applied to a forest soil and an agricultural soil at rates of 10,000, 1,000 and 500 p.p.m. all three concentrations significantly reduced the total length of rhizomorphs produced by A. mellea in the forest soil but only the highest concentration did so in the agricultural soil. Ten thousand p.p.m. Armillatox also caused significant mortality of A. mellea mycelium under the bark and in the wood of sycamore stem segments buried in both soils.

INTRODUCTION

A. mellea is a specialised root-infecting fungus (sensu Garrett, 1970) which kills or rots roots of many tree species throughout the world. It causes disease in forests, as well as orchards, parks and gardens. Methods of chemical control have been investigated for over 40 years and quite a wide variety of chemicals have been tested in vitro (Guyot, 1928; Reitsma, 1932; Ritter, 1967), including 20 systemic chemicals (Cheo, 1968) but only a few have been tested in vivo: an arsenic compound was tested by (Prihoda 1957) and formalin was tested by (Sokolov 1964). However, neither compound proved effective. Work on control methods of all kinds has been reviewed by the latter author and by Twarowski and Twarowska (1959).

Fumigation with CS_2 has been the only method used successfully on a commercial scale. It has been used in Californian orchards between crops of citrus trees for many years. The biological effects of this method have been investigated by Bliss (1951) and Garrett (1957 and 1958).

Clearly, soil to be fumigated must be devoid of live roots so that under most circumstances this method is not applicable in parks and gardens where A. mellea can be a serious problem. Recently, however, a treatment involving use of a phenolic emulsion, which is marketed as a fungicide under the name Armillatox, has been advocated as a control method for use in this situation. It is recommended that a solution containing 4% a.i. (i.e. an 8% solution of Armillatox which is stated to contain 48% a.i.) should be applied to infected trees and stumps and to infested soil. It is claimed to "kill the fungus without being toxic to woody material""but contact with young fibrous roots .... should be avoided" (Anon., 1971). This paper reports the results of preliminary in vivo tests carried out with this substance.

METHOD AND MATERIALS

Phytotoxicity tests

In Experiment 1, 20 cm portions of two roots (1.0–4.0 cm diameter) on each of six trees 5–7 m tall were exposed carefully to avoid wounding. An Armillatox solution containing 4% a.i. was brushed onto both roots on three trees and the remainder were similarly treated with an 8% solution. Treatment was applied at the end of April, 1971.

In Experiment 2, 40 Scots pine (Pinus sylvestris) and 40 sycamore (Acer pseudoplatanus), 15–30 cm tall, were planted in 5 in (12.5 cm) pots containing soil A (an agricultural soil) and the same number of both species were planted in pots of soil F (a forest soil) on April 2, 1971. Each pot contained approximately 900 cc soil having a mean dry weight of 1,200 g. Pots were plunged in soil and watered as necessary. On June 22, 1971 a solution of Armillatox containing 4% a.i. was poured onto the soil around 10 plants of each species at rates of 314 ml, 31.5 ml and 15.5 ml per pot, equivalent to 10,000, 1,000, and 500 p.p.m. respectively based on the air dry weight of soil in each pot. Control plants received 314 ml water per pot.

Fungitoxicity test

Freshly cut segments of sycamore stem, 3 cm long x 1.5 cm diameter were autoclaved in flasks and allowed to become colonised by A. mellea in the manner described by Garrett (1956). After incubation at 25°C for $2\frac{1}{2}$ months one segment was buried in the centre of each of 200 1 lb (mean volume 350 ml) jam jars, half of which contained 317 g soil A at 30% of its moisture holding capacity (m.h.c.) and the other half contained 309 g soil F also at 30% m.h.c.. The moisture holding capacities of the two soils were 41 and 39 g water per 100 g air dry soil respectively. Jars were capped with polythene sheet and incubated at room temperature.

Rhizomorph growth was evident in almost all jars after $2\frac{1}{2}$ months incubation. Armillatox solution was then added to produce concentrations in the jars of 10,000, 1,000, 500, and 0 p.p.m. (based on the weight of air dry soil per jar) and to raise the moisture content to 75% m.h.c. Jars were incubated for a further three months.

RESULTS

Phytotoxicity tests

In Experiment 1, roots brushed with either 4% or 8% a.i. Armillatox showed no signs of injury five months after treatment. It was interesting to observe that young rhizomorphs, which were not present at the time of treatment, were growing epiphytically on one root treated with 4% Armillatox.

In Experiment 2, plants were assessed three months after treatment. All seedlings were killed in soils to which the largest volume (314 ml, a quantity sufficient to saturate the soil) of 4% a.i. Armillatox was applied, whereas roots

and shoots of control trees remained healthy. Application of 31.5 ml and 15.5 ml of 4% a.i. Armillatox caused moderate to severe leaf scorch in sycamore but only three plants were defoliated. Scots pine showed no definite needle symptoms. Nevertheless, root examination revealed that on most trees of both species 1.0-4.0 cm of the root collar just below soil level was either dead or exhibited severe bark necrosis (Table 1). In most cases roots below this point were still alive.

Table 1

Effect of 4% a.i. Armillatox on roots of sycamore and Scots pine seedlings

Vol. Armillatox (ml)	Soil	Tree Species	No. Treated	Condition of root collar		
				Dead	Necrotic	Healthy
31.5	A	Syc.	10	3(2)*	6(4)	1
		S.P.	10	0	8(0)	2
	F	Syc.	10	10(9)	0	0
		S.P.	10	6(0)	4(0)	0
15.5	A	Syc.	10	6(5)	4(1)	0
		S.P.	10	0	7(0)	3
	F	Syc.	10	9(7)	1(1)	0
		S.P.	10	3(0)	7(0)	0

* Figures in parenthesis represent numbers of plants which produced new roots between soil level and the damaged zone on the root collar.

Fungitoxicity test

Inocula and rhizomorph systems were washed from jars three months after treatment. The number of live rhizomorph growing tips was counted and rhizomorph length (both dead and live) was measured. Results are shown in Table 2.

Table 2

Survival and growth of A. mellea in soils treated with four concentrations Armillatox

Soil	Armillatox conc'n (p.p.m.)	Rhizomorphs Mean No. live tips	Rhizomorphs Mean length (cm)	No. inocula* with 50% or more mycelium dead under bark
	0	2.6	$166.8^{c} \pm 14.2$	0
A	500	3.6	$194.0^{c} \pm 8.6$	0
	1,000	4.8	$175.5^{c} \pm 8.7$	1
	10,000	2.0	$86.1^{d} \pm 11.1$	21
	0	4.4	$232.1^{b} \pm 12.6$	0
F	500	5.1	$170.6^{a} \pm 7.9$	0
	1,000	2.5	$178.3^{a} \pm 9.4$	0
	10,000	4.4	$151.8^{a} \pm 14.7$	9

* out of 25

Analysis revealed an interaction between soil and chemical concentration and therefore results for the two soils were analysed separately. Duncan's Multiple range test was used to compare the effect of concentration on rhizomorph length in each soil. In Table 2 mean rhizomorph lengths, indicated by dissimilar letters are significantly different at the 1% level.

In soil A only 10,000 p.p.m. Armillatox significantly reduced rhizomorph growth and survival of A. mellea in inocula. In soil F all concentrations of Armillatox reduced rhizomorph growth but only the highest concentration caused even partial death of A. mellea in inocula. A. mellea was alive under the bark and in the wood of all inocula in the control and 500 p.p.m. treatments of soil A, and in the control, 500 and 1,000 p.p.m. treatments of soil F.

DISCUSSION

For all practical purposes the life cycle of A. mellea is completed below ground. It spreads by means of rhizomorphs which grow out from a food base into the soil where they branch and anastomose to form a network. Rhizomorphs are not free living and growing tips remain connected to a food source through the network. On infested sites they run epiphytically over the root systems of most trees; many roots may be partly infected or have localised lesions (Redfern, 1968). Rhizomorphs also exhibit apical dominance and respond to mechanical severing or death of a growing tip by forming many branches at the severed end or behind the dead tip, each of which is able to cause infection (Redfern, 1966).

These features of the life history and growth habit of A. mellea must be taken into account when judging the effectiveness of a control method. A successful method must either kill the fungus in its food base (usually a tree root) or kill the rhizomorph network and prevent further growth from the food base. Since A. mellea may live for decades in tree roots the latter objective would require regular application of a fungicide or use of a persistent one. Furthermore, although most rhizomorphs are concentrated between 2.5 and 15.0 cm below the soil surface (Redfern, 1966), any fungicide would need to penetrate at least 0.5 m even to reach all the rhizomorph network.

In the experiments described above, inocula were placed vertically in the centre of each jar and it was noticable that in those inocula in which A. mellea had been partly killed, the dead portion was nearest the soil surface. Any dead rhizomorphs occurred in the upper $\frac{2}{3}$ of the jars and live rhizomorph growing tips, which were observed even in the highest concentration, mainly occurred in the lower $\frac{1}{3}$ of jars. Growing tips were evenly distributed in control jars. This suggests that a concentration gradient of Armillatox was probably established in each jar, which, in the case of the highest concentrations in both soils, varied from fungitoxic at the top to less than fungistatic at the bottom. The Armillatox may have been retained by the upper layers of soil. In a freely drained system use of a greater volume of Armillatox might have increased its depth of penetration, but clearly any further work with this substance must determine its distribution and fate in different soils.

In several jars rhizomorph regeneration had occurred behind dead growing tips. In each case 5-10 new rhizomorphs had formed a few centimetres behind the dead tip. Thus death of some rhizomorph tips in a network caused by a toxic concentration of a chemical may result merely in the formation of new tips in a part of the network unaffected by the chemical, or where the concentration is at a non-toxic level.

The phytotoxicity test shows that although an 8% a.i. Armillatox solution is apparently not toxic to the relatively thick bark of older sycamore roots, even quite small quantities of a solution half that strength will kill roots of sycamore seedlings. This substance cannot therefore be used as a soil sterilant at this strength in the proximity of young roots. Further work might determine a concentration of Armillatox <4% a.i. (and a suitable rate of application) which will kill A. mellea rhizomorphs while not injuring plant roots, but even so, in light of the above account of the growth habit of A. mellea the chances of successful control of this disease in this manner must probably be very small indeed.

Armillatox could perhaps be applied direct to the bark of older trees infected by A. mellea up to a concentration of 8% a.i., but if it is the thick bark of such trees that prevents injury to live tissue it might well similarly protect A. mellea mycelium beneath it. Moreover, the only practicable way of detecting infection (short of frequent root examination!) is by crown symptoms, and by the time such symptoms become evident the tree will be very close to death and probably beyond help anyway.

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POSSIBILITIES OF CONTROL OF A BRITISH OUTBREAK OF SPRUCE SAWFLY BY A VIRUS DISEASE

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Summary For the first time Spruce sawfly (Gilpinia hercyniae) has appeared as a serious pest in Britain where currently it is established in three adjacent Welsh forests and in two has reached epidemic numbers. Experience in North America indicates adequate natural population control can occur and persist when the sawfly is infected with a nuclear polyhedrosis virus (N.P.V.), though optimal depression of numbers probably occurs when parasites are present. Some relevant characteristics of the N.P.V. are described. In the Welsh population the pressure of natural enemies seems slight: predation is probably at a low level, no parasites exist but N.P.V. is present in some areas. The possibilities of obtaining general control by use of N.P.V., either of local or other origin are considered. Some safety problems arising from the use of viruses, and other microbiological agents for insect control are briefly mentioned.

European Spruce sawfly, Gilpinia hercyniae has only recently emerged as a pest in Britain. Currently epidemic levels of population exist in certain spruce forests in mid-Wales where there is evidence of at least four years defoliation.

For two reasons the problem is viewed with some concern. Firstly because in post war years greater emphasis has been placed on the planting of spruce than on any other tree and currently there may be a million acres in this country. The second reason arises from the pest potential of the sawfly which its career in North America has dramatically illustrated. The date of its introduction into the Nearctic is unknown, but when first discovered in 1930, in the Gaspe Peninsula, Canada, a large outbreak had already developed. Spread was very rapid and by 1938 12,000 square miles were estimated as being heavily infested. The affected area spread west of the Gaspe in Quebec throughout New Brunswick and northern Maine and into parts of Vermont and New Hampshire with lighter infestations extending from Nova Scotia to the northern shore of the St. Lawrence River and west to Ontario. It also appeared in Newfoundland.

From 1938 to 1942 the infestation declined and by 1943 no important defoliation was being caused. This decline coincided with the establishment of exotic parasites and the fortuitous appearance of a nuclear polyhedrosis virus (N.P.V.) disease which killed high percentages of larvae. The virus disease appeared to spread from south to north and was not evident, for instance, in the Gaspe until 1940 where it did not cause any striking reduction in population until 1942. By 1942 it was known to be distributed throughout the greater part of the range of the sawfly, though Newfoundland and areas in Ontario were still apparently free.

The origin of the disease is obscure. Prior to 1938 only rare individuals which might have been diseased were observed in the forest, though in the laboratory in Canada in 1936 small percentages of larvae began to die and mortality increased steadily until by 1939 it had become impossible to rear a single larva other than under sterile conditions. It was considered not improbable that the disease was introduced with parasite material from Europe where it is certain a similar if not identical condition of the more widely occurring Gilpinia polytoma existed (Balch et al 1944).

Parasite introduction resulted in the effective establishment of at least two species and on experimental plots in New Brunswick, where population studies were conducted continuously from 1938 to 1963, the changing incidence of these and of the virus disease was followed. Neilson and Morris (1964) analysed the accrued data using Morris's (1963) key factor approach and the results showed that disease and parasites constitute an almost ideal regulatory complex. The parasites are effective at very low sawfly density and are very sensitive to minor host density fluctuations at this level. On the other hand disease responds to density changes of greater magnitude and to minor increases of a long term nature. The two are considered complementary and compensatory. But it has also been shown (Bird et al 1961) in Ontario that in the absence of parasites disease is able to maintain Spruce sawfly densities at an endemic level, though one which is several times higher than that in New Brunswick where both parasites and disease are present. There is also unpublished evidence (Neilson et al) that parasites can control sawfly in the absence of the virus disease (Neilson et al 1964) but no details are available.

There is no evidence in the 50 generations reported on by Neilson and Morris of development of resistance to either the virus or the parasites and for this reason their controlling effect has continued undiminished for over 30 years in North America.

Characteristics of the virus disease

The disease is restricted to gut tissues and is contracted by feeding on contaminated foliage. Virus particles develop in cell nuclei and there groups of virions become occluded in small crystals of protein (0.8-1.5 μ diameter). Incubation varies with temperature, instar and initial dose but it is in the region of 5-10 days. The behaviour of infected larvae may be affected as early as day three after infection and is manifest in decreasing feeding activity which may cease altogether by day 4-6. Very large quantities of infectious polyhedra are produced. At death a 5th instar larva, for instance, may contain 2×10^8 polyhedra and preliminary observations indicate that in addition it may have already emitted a further 10^8 in a period of 2-4 days before death, in states readily available for the infection of further larvae, e.g. in unusually wet, adhesive frass and exuded fluids from the deranged gut. All larval instars are susceptible to infection and the LD_{50} varies between 50 and 500 polyhedra. Thus before death a 5th instar larva may emit between 400,000 and 4 million times the LD_{50} dose. After death the majority of the cadavers remain on the tree where they gradually break down releasing a further large quantity of polyhedra. By this massive release of polyhedra virus is spread through a population, a process which is aided by rain splash, and the movements of predators and parasites. Under Canadian conditions N.P.V. appears not to persist on the trees from one year to the next but, as the eonymph in the cocoon has a certain resistance to the effects of infection, 5th instar larvae infected late may, in the following generation, develop into virus-charged adults. Such adults appear to transmit virus by contamination of the foliage in and around which oviposition takes place (Neilson

et al 1968).

The effectiveness of virus control in part of the infested area in N. America seems to be enhanced by the presence of more than one generation of sawflies each year and the data analysed by Neilson and Morris (1964) relates to such a double generation area. A massive development of inoculum towards the end of the first generation leads to very high mortality in the second, and in addition highly motile adults, a proportion inevitably carrying virus, serve to spread the disease even more widely. Nevertheless because of the diapause pattern, depression of sawfly populations is delayed longer than might be expected. Sawflies of the first generation may hibernate until at least the following year, and perhaps longer (they are able to spend up to six years in diapause (Balch, 1939)), and so escape the greater infection risks to which the last generation of the year is exposed. On the other hand population regulation by virus seems possible, but is probably slower, in areas such as the Gaspe where there is only one generation annually.

The situation in Wales

Our limited experience of the sawfly population in mid-Wales suggests there is only one generation a year, with the possibility of a partial second under exceptionally favourable climatic conditions. For instance, this year (1971) the main adult flight did not take place until the 30th June and the constitution of the larval population in September indicated no second generation.

A careful scrutiny of the population revealed no parasites whatever and suggested predation to be low; the incidence of small mammals, which would account for cocoon loss, seemed not unduly high and bird predation of larvae was at a low level. Though rich in species the arthropod complex of potential predators was generally very low in numbers. Only two species were at all common, Aphidecta obliterata (Insecta, Coleoptera, Coccinelidae) and Mitopus morio (Arachnoidea, Phalangida), but their precise relationships to spruce sawfly in the field have yet to be clearly established. Thus in the absence of parasites and the weak effects of predation the mid-Wales population of sawfly parallels the situation which originally existed in North America. However, it differs in that a naturally occuring N.P.V. which is not the result of intervention by man is present in the Welsh population.

Early surveys, conducted first in the overwintering cocooned population and, after the beginning of July, in the larval populations failed to detect N.P.V. until about half way through August. It was then identified in the two main epidemic centres in Hafren Forest. By the beginning of September infection was heavy in one of these two areas but was apparently very slight in, or absent from, less heavily infested parts of the same forest and also Tarenig and Myherin Forests. Moreover by this time many larvae had already completed their development and had descended to the forest floor litter to cocoon and so had escaped the possibility of infection.

Possibilities of Control by Virus

There is no good reason to suppose the appearance of virus in Wales to be peculiar to 1971 and it seems very probable that virus epizootics were present in previous years during the development of the sawfly epidemic. If this is a true assessment of the situation we must question if this particular virus will, under natural circumstances, ever bring about a decline in sawfly numbers especially in

view of the apparent low level of action of other mortality factors.

Control may be achievable by one of three methods:-

1. Introduction of a more virulent N.P.V., if such is available. The presence of one N.P.V. in the field may be no barrier to the establishment of one more effective. In addition to the Canadian virus at least two other N.P.V's which may or may not prove to be different, are available; N.P.V. of Gilpinia pindrowi in Pakistan, which is definitely known to be cross infective to G. hercyniae, and N.P.V. of the closely allied European Gilpinia polytoma.

2. Introduction of parasites as additional mortality factors; these would possibly also enhance the horizontal transmission of N.P.V. by acting as passive vectors.

3. Spraying suspensions of polyhedra in, say, July when the larval population is young, so bringing forward artificially the period of greatest effect of the virus. This course of action would be strictly analagous to the system of control by virus currently practiced against Pine sawfly (Neodiprion sertifer) in this country and widely throughout the Holarctic.

Whichever virus is eventually selected must be carefully screened for a variety of possible undesirable properties. For convenience these may be grouped as human pathogenicity and ecological effects, the latter concerning the effects on both vertebrates and invertebrates in the same ecosystem, not forgetting the passage of virus through food chains. Finally the quality of any virus preparation dispersed in the forest must be controlled in order to be reasonably certain no batch contains additional and possibly dangerous micro-organisms. These problems, of course, are ones which are common to the use of all microbiological agents considered for insect pest control and as such indicate that the development of a common approach is in the interests of the protection of the community and the integrity of the 'on site' ecosystem. Much can be learnt from the concentrated experience accrued in the U.S.A. on similar viruses of Heliothis zea and Trichoplusia ni the former of which has after stringent tests been given provisional approval for use against this pest on cotton.

Spruce sawfly poses a threat to our largest natural timber resource and the possibilities of outright control in Britain with nuclear polyhedrosis virus or, at least, of the development of an integrated system of control in which virus is incorporated as a major mortality factor merits careful analysis. Some of the resources of the Unit of Invertebrate Virology (Natural Environment Research Council) are currently directed towards this problem and during the coming year we hope to work towards characterising the virus, or viruses, concerned to examine in detail the effects of N.P.V. in the field and to formulate the most appropriate control system for British conditions.

This note has been produced in co-operation with the Entomology Section of the Forestry Commission Research Station, Alice Holt.

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TETRACHLORVINPHOS AS AN ALTERNATIVE TO D.D.T. IN PINE LOOPER MOTH CONTROL

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Summary Screening trials were carried out during 1970 to find an alternative to the standard D.D.T. treatment for controlling an outbreak of the Pine looper moth (Bupalus piniarius). Seven insecticides at their highest rates gave similar mortalities to D.D.T. after 60 hours exposure, with the best in order of merit being phosalone, fenitrothion, N.R.D.C. 104 (a synthetic pyrethroid), and tetrachlorvinphos. After exposure to weathering, only phosalone and tetrachlorvinphos were comparable in effect to D.D.T. Tetrachlorvinphos was selected in preference to phosalone, despite its rather poorer performance because of its very low toxicity and lower cost. It proved satisfactory in a field trial and gave about 90% mortality in the control operation.

INTRODUCTION

The first outbreaks of Pine looper moth in Britain occurred in 1953 at Cannock Chase, Staffordshire, and at Culbin forest, Morayshire. Aerial spraying with D.D.T. at 1.12 kg a.i/ha (1 lb/ac) in water gave good control, Crooke (1959). D.D.T. at the same rate was used successfully in the only two subsequent outbreaks in 1957 and 1963. In the 1957 infestation at Tentsmuir, Bevan (1961) has described control by fogging with D.D.T. in a light oil. Attempts made at the time to find a suitable alternative to D.D.T. had been disappointing.

Since 1954 annual pupal surveys have been carried out in all forests with large areas of pine in the susceptible pole stage, Bevan (1954). High pupal counts at Wykeham forest, Yorkshire, in the winter of 1969 gave warning of a possible infestation with complete defoliation over a large area Bevan et al (1970). To cater for this eventuality a number of insecticides were tested in a fresh attempt to find a suitable alternative to D.D.T.

METHOD AND MATERIALS

Laboratory tests

Potted Scots pine transplants about 12 inches in height were sprayed with the insecticides under test at four rates equivalent to 0.28, 0.56, 1.12 and 2.24 kg a.i./ha ($\frac{1}{4}$, $\frac{1}{2}$, 1 and 2 lb a.i./ac) in 22.5 l water/ha, (2 gal/ac). Aerial spraying was simulated using a pendulum sprayer developed by Fisons Limited. In the case of B. thuringiensis, the rate was 2.24, 3.36, 4.48 and 5.60 kg actual/ha, (2, 3, 4, 5 lb/ac) in 56 l water/ha, (5 gal/ac). The treated plants were laid out in five

randomised blocks. Ten larvae were caged on each tree and the mortality was recorded at intervals over a 60 hour period. After a lapse of five days during which the trees were exposed out of doors to weathering, a fresh population of larvae was caged upon them and the mortality recorded as before.

Field Trials

Immediately prior to the main aerial control spraying operation at Wykeham, a field trial was carried out to compare the effectiveness of Tetrachlorvinphos with three other insecticides which had shown promise in the screening trials. Spraying was carried out from the ground using a mistblower. A randomised block design with two replicates was used. Larvae falling from the trees were collected in sampling trays over a period of three days. The trees were then overdosed with D.D.T. to bring down any surviving larvae.

RESULTS

Laboratory Screening

The percentage mortality was transformed to angles and treated by analysis of variance. Treatment means were tested against D.D.T. 0.56 kg/ha ($\frac{1}{2}$ lb/ac) rate at the 5% level. It was clear that the lowest rate ($\frac{1}{4}$ lb/ac) did not in most cases properly reflect the potential performance of the chemical. The best overall indication was obtained when the three highest rates were pooled and meaned, Table 1.

Table 1

Control of Pine looper moth on pine transplants
Mean % mortality for the 3 highest rates

Chemical	Formulation	Percentage Mortality after 60 hours	
		Before Weathering	After Weathering
D.D.T.	25% e.c.	100	81
Phosalone	33% e.c.	99	63
Fenitrothion	50% e.c.	99	10*
N.R.D.C. 104 ≠	25% e.c.	98	2*
Tetrachlorvinphos	24% e.c.	93*	54*
Trichlorphon	80% s.p.	89*	13*
Bromophos Ethyl	80% e.c.	76*	2*
Gamma B.H.C.	20% e.c.	70*	33*
B. thuringiensis (Midox B.T.B. 183)	25 billion Spores/g w.p.	5*	
Control	Nil	1*	3*

≠ An experimental synthetic pyrethroid.

* Significantly different to D.D.T. (mean of 3 highest rates).

The very low mortality recorded for B. thuringiensis was rather surprising, so instead of removing the original test larvae they were left undisturbed until the prepupal stage. All surviving larvae were healthy and microscopic examination showed no damage to the gut lining.

The mortality given by the different rates is shown in Table 2. It is probable that these are under estimates since the larvae were introduced approximately 6 hours after spraying and did not receive direct contact by spray droplets.

Table 2

Control of Pine looper moth or pine transplants
at 3 different rates of chemical treatment

* Significantly different to D.D.T. at 1.12 kg a.i./ha (1 lb/ac) at the 5% level

Chemical	Treatment Rate kg/ha	% Mortality	
		Before Weathering	After Weathering
D.D.T.	2.24	100	90
	1.12	100	84
	0.56	100	69
Phosalone	2.24	100	96
	1.12	100	60
	0.56	98	35*
Fenitrothion	2.24	100	11*
	1.12	98	4*
	0.56	98	16*
Tetrachlorvinphos	2.24	95	93
	1.12	100	60
	0.56	84*	10*
N.R.D.C.	2.24	100	3*
	1.12	98	0*
	0.56	98	3*
Gamma B.H.C.	2.24	98	76
	1.12	70*	18*
	0.56	40*	4*
Bromophos Ethyl	2.24	87	2*
	1.12	76*	2*
	0.56	63*	0*
Trichlorphon	2.24	94	9*
	1.12	93	15*
	0.56	84*	14*

Field trials

The population in Block 2 was much higher than in Block 1 making it impossible to combine the data. No significant difference was found between the treatments

probably because the very low number of degrees of freedom for error (3) gave an insensitive 'F' test. Generally however, Tetrachlorvinphos appeared to be satisfactory and as good or better than the other chemicals tested.

Table 3

Control of Pine looper moth in Field trials of Tetrachlorvinphos and other insecticides

Chemical	Treatment rate kg/ha	lb/ac	% kill Block I	Block II
Tetrachlorvinphos	0.56	½ lb	75	36
	1.12	1 lb	89	70
Fenitrothion	0.56	½ lb	11	28
	1.12	1 lb	37	86
Trichlorphon	0.56	½ lb	3	6
	1.12	1 lb	9	15
N.R.D.C.	1.12	1 lb	47	73
Control	Nil		0	0

DISCUSSION

The effectiveness of D.D.T. was fully confirmed in the screening trials. The logical choice for an alternative appeared to be phosalone, but since cheaper and much less toxic materials gave satisfactory control it was decided to select one of these. Tetrachlorvinphos was chosen, principally because of its very low toxicity to mammals and birds and its moderate to low toxicity to fish. Its rather longer short term persistence was reckoned to be an additional factor in its favour since it would allow greater latitude than its competitors in timing the control operation. The field trial indicated that Tetrachlorvinphos would provide adequate control at 0.56 kg a.i./ha (½ lb a.i. per acre) the rate actually used in the control operation. It also provided incidental confirmation that Fenitrothion would provide a further alternative. Spraying was carried out when 90% egg hatch has occurred and was forecast using as basis the date of pupal emergence and confirmed later by egg counts. Results from the control spraying operation carried out on August 18th and 19th showed about a 90% kill within 8 days, despite the heavy and prolonged rainfall immediately following treatment. Pupal counts in 1971 have been very low and confirmed the efficacy of the treatment.

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