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THE EFFECT OF SOIL STERILIZATION ON EFFICIENCY OF SOIL-APPLIED BENOMYL AND CARBENDAZIM FOR THE CONTROL OF SOME TOMATO DISEASES

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Summary Uptake of fungitoxicant into tomato foliage was greater from soil drenches of benomyl (100 mg/plant) when applied every 4 rather than every 8 weeks. In 1972 both frequencies of drenching controlled *Botrytis cinerea* infection (except fruit ghost-spotting) and increased tomato yields. Some *B. cinerea* isolates from lesions on benomyl-treated plants tolerated 28 mg MBC/l. *in vitro*. Tomato brown root rot (*Pyrenochaeta lycopersici*) was controlled by soil sterilization with steam or methyl bromide better than by benomyl or carbendazim* soil drenches.

Fungicide loss was less rapid in soil sterilized by steam or methyl bromide than in unsterilized soil, and this effect was greater with benomyl than with carbendazim. The cause of more rapid loss of soil fungicide residues from benomyl drenches applied in 1973 than in 1972 has not yet been determined.

INTRODUCTION

Evaluation of systemic fungicides for the control of tomato grey mould (*Botrytis cinerea*) showed that compounds forming methyl benzimidazol-2-ylcarbamate (MBC) or the corresponding ethyl ester effectively decreased leaf and stem infections and fruit rotting when applied as foliar sprays or soil drenches (Smith & Spencer, 1971). In 1971 substantial MBC residues were detected in soil 3 months after applying benomyl drenches to a tomato crop (Smith & Worthing, in press). In 1972, therefore, an experiment was commenced to study the long-term effects of repeated benomyl soil drenches (100 mg a.i./plant) on fungicide residues in plant and soil, incidence of air and soil-borne pathogens, and crop yields and values. In addition, these effects were assessed in relation to soil sterilization treatment and drenches of benomyl and carbendazim.

METHODS AND MATERIALS

Benomyl and carbendazim were used as the commercial wettable powders (50% a.i.). Fungicide treatments were applied to the soil surface around stems commencing when the tomato crop was at about the second truss flowering stage. Drenches (500 ml/plant) were applied with equipment designed to deliver a predetermined volume.

Crops were grown in glasshouse beds (brick earth soil with organic matter content 1 - 2% C). Soil was sterilized by steaming for 10 min at 100°C or methyl bromide fumigation (49 g/m^2) and the soil pH adjusted to about 6.5.

* Proposed BSI common name for methyl benzimidazol-2-ylcarbamate.

Leaf samples from similar positions on the stems of at least eight plants per treatment were bulked for chemical analysis. Eight to sixteen soil cores (25.4mm diam) were taken from each treatment at depths of 50, 100, 200 and 300 mm within the drenched area, and cores from the same depths were bulked before analysis. Amount of MBC was expressed as mg/m^2 of soil for the total depth sampled.

1-Carbamoylbenzimidazoles, such as benomyl, decompose rapidly in solution to form the corresponding benzimidazoles (Staab & Benz, 1961) and benomyl can be converted *in vivo* into MBC. The analytical method estimated the total fungitoxicant by converting any benomyl to MBC (Worthing, in press).

In vitro bioassay of leaf sap (Smith & Spencer, 1971) assessed fungitoxicity as the width of the 'annulus' of inhibited fungal growth surrounding the 0.1 ml drop of sap on an agar plate 'seeded' with *Fulvia fulva*. Using *B. cinerea* as the test organism a similar method tested for tolerance of isolates to MBC.

Tomato laminae were artificially inoculated *in vivo* with agar discs of mycelium (Smith & Spencer, 1971) but lesions were measured and leaves removed before infection had spread into stems. Incidence of tomato grey mould developing from natural inoculum was assessed as numbers of stem lesions per plant and weight of rotted fruit. The percentage of root system infected by brown root rot (including corky root) was estimated at intervals after planting.

RESULTS

Persistence and uptake of fungitoxicant from benomyl soil drenches

Tomato plants var. GCR 355 were transplanted on 7 April 1972 into steamed or unsteamed soil which was either left untreated or, commencing on 27 April, drenched with benomyl at 4 or 8 week intervals. Each treatment was replicated in four randomised blocks, and of the twenty-two plants per plot eight were used for recording *B. cinerea* infection and crop yields.

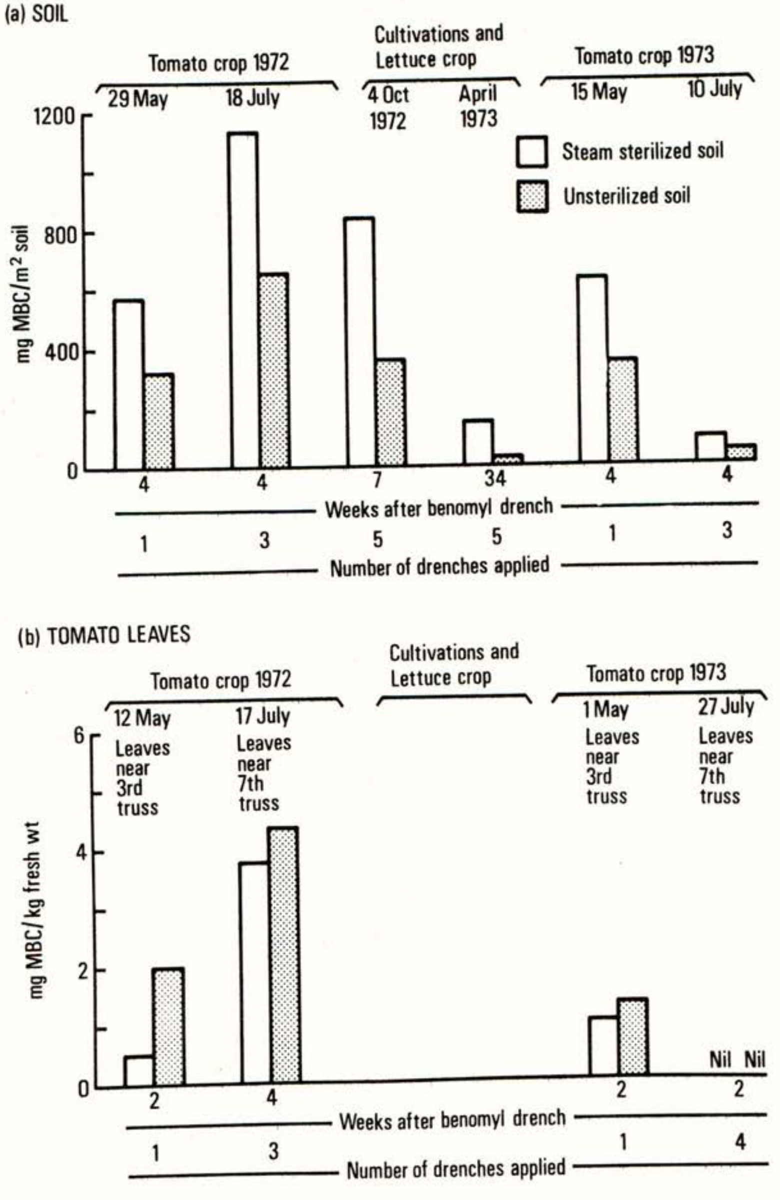
The experiment was continued in 1973, but with different combinations of steaming and benomyl treatments applied to some of the plots and with two replicates for any given treatment.

1972 During the first eight weeks of treatment fungitoxicant (expressed as MBC) disappeared less rapidly from steamed than unsteamed soil, and residues for the 4-weekly drenches are shown in Fig. 1a. Drenching every 8 weeks decreased soil residues but did not affect the proportion of MBC lost compared with 4-weekly applications. At the end of the crop, 23 weeks after the first drench, most MBC was detected in steamed soil treated every 4 weeks (835 mg MBC/m^2 soil) and least in unsteamed soil drenched every 8 weeks (224 mg MBC/m^2). Irrespective of treatment only traces of MBC were detected at a depth greater than 200 mm.

MBC content in foliage during the first 8 weeks of treatment indicated greater uptake from benomyl applied to unsteamed than steamed soil (foliage near third truss, Fig. 1b). Later on this effect was less apparent (foliage near seventh truss, Fig. 1b) but foliar concentration of MBC increased markedly in crops drenched at 4 rather than 8 week intervals. Fungicide concentrations in leaves at any given stem position increased when drenches were reapplied until the onset of senescence when, as in commercial practice, the old foliage was removed.

1973 In 1973 smaller differences in plant uptake of fungicide from drenches applied to steamed and unsteamed soil were detected (Fig. 1b). Increasing the frequency of drenching from 8 to 4 weeks again increased fungicide in soil and plant but the residues of both were much less persistent than in the previous season (Figs. 1a, 1b).

Fig. 1. Effect of benomyl drenches every 4 weeks to steamed and unsteamed soil on fungicide concentration in (a) soil and (b) tomato leaves in 1972 and 1973



Disease control and crop yield

In 1972 there was a low incidence of brown root rot when assessed 9 weeks after planting but after 26 weeks steam sterilization had controlled build-up of disease better than benomyl (Table 1).

Table 1

The effect of steam sterilization and benomyl soil drenches on incidence of tomato brown root rot incidence in variety GCR 355 in 1972 and 1973

Soil sterilization	Frequency benomyl drenches weeks	% brown root rot 1972			1973	
		weeks after planting				
		9	19	26	8	20
Steam	0	5.6	8.8	16.5	11.7	13.5
	4	7.0	9.5	11.6	10.5	10.2
	8	9.5	9.0	14.1	10.5	11.0
None	0	7.0	20.9	49.9	14.8	36.2
	4	9.2	15.5	28.5	16.0	40.0
	8	7.7	23.7	37.5	-	-

More *B. cinerea* stem lesions and fruit rotting occurred in untreated crops grown in steamed than unsteamed soil. Benomyl, applied every 8 weeks to steamed soil was the only fungicide treatment which never gave more than 1 mg MBC/kg fresh foliage near the third truss, and this was associated with more lesion development on this part of stems than with the other benomyl treatments. Moreover, when non-senescent, undamaged leaves of the same age were artifically inoculated with *B. cinerea* mycelium (MBC-susceptible strain) macroscopic lesions did not develop when the MBC content of similar leaves was greater than 1.5 mg/kg fresh weight.

Benomyl drenches whether applied at 4 or 8 week intervals significantly decreased the incidence of naturally occurring stem lesions and fruit rotting and increased the weight and value of the crop (Table 2). Yield increases were greater when drenches were applied to crops grown on steamed than unsteamed soil, because untreated crops yielded potentially more fruit but were more susceptible to loss by *B. cinerea* rotting when grown on sterilized soil.

Towards the end of cropping, stem lesions, mainly associated with *B. cinerea* colonisation of old peduncles, developed on some benomyl-drenched as well as on untreated plants. Multispore isolations from lesions on plants drenched every 4 weeks tolerated 28 mg MBC/l. when fungal growth was assayed *in vitro*, whereas isolates from untreated plants were susceptible to 0.5 mg MBC/l.

The lower concentration of fungitoxicant in the plants in 1973 was associated with the development of more *B. cinerea* stem lesions (Table 2). Irrespective of treatment no MBC-tolerant strains of *B. cinerea* were isolated from leaf lesions 6 weeks after drenching was started; monitoring for MBC-tolerant *B. cinerea* is continuing. Estimates of brown root rot 20 weeks after planting showed that only steam sterilization was effectively controlling the disease (Table 1).

Effects of soil sterilization methods on persistence of benomyl and carbendazim

The effect of soil sterilization on fungicide uptake into foliage and persistence in soil was further tested in 1973, and the study was extended to

Table 2

Effect of soil sterilization and benomyl soil drenches on crop yields and values (1972) and incidence of *B. cinerea* (1972 & 1973) in variety GCR 355

Soil sterilization	Frequency benomyl drenches (weeks)	1972 Fruit yield (kg/plot)≁	1972 Crop value (£/plot)	1972 Botrytis rotted fruit (kg/plot)	1972 No. Botrytis* stem lesions	1973 No. Botrytis** stem lesions
Steam	0	33.6	5.5	1.9	4.5	5.8
	4	38.2	6.5	0.6	0.6	3.2
	8	37.4	6.6	0.7	0.9	5.6
Means of benomyl treatments		37.8	6.6	0.6	0.7	4.4
None	0	33.4	5.9	1.8	3.5	3.0
	4	35.6	6.3	0.7	0.9	2.8
	8	35.3	6.1	0.7	0.7	-
Means of benomyl treatments		35.4	6.2	0.7	0.8	-
L.S.D.'s (P=0.05) for comparisons between mean values of benomyl treatments and untreated.		1.63	0.38	0.47	0.63	-

No. lesions/plant recorded* 19 weeks and ** 20 weeks after planting. ≁ 8 plants per plot.

compare unsterilized soil with two methods of sterilization when combined with benomyl or carbendazim ('Bavistin') drenches at equivalent rates. Each of the combined treatments was replicated in four blocks. B. cinerea infection and yield data were recorded on four of the eight plants per plot, the remaining plants being lifted at intervals for determinations of fresh weight, fungicide content and incidence of brown root rot. Variety GCR 93 was transplanted on 21 February into polythene troughs of maiden loam, left unsterilized or previously steamed or fumigated with methyl bromide. Fungicide drenches were applied on 8 March and repeated every 4 weeks until 1 June.

Initially there was a greater loss of MBC from carbendazim- than from benomyl-treated soils (Table 3), but subsequently residues from both compounds declined rapidly and no MBC was detected 6 weeks after the fourth application. Irrespective of fungicide used, there was a greater loss of MBC from unsterilized than from sterilized soil.

Table 3

The effect of soil sterilization and benomyl and carbendazim drenches on fungicide persistence in soil, control of brown root rot and crop yield in variety GCR 93

No. soil drenches	1	1	3	Brown root rot ≠≠ %		Yield kg/plot
Weeks after previous drench.	2	4	4			
Soil treatment	mg MBC/m^2 soil ≠					
Unsterilized						
None	-	-	-	57		17.80
Benomyl*	492	243	31	44		21.92
Carbendazim**	204	106	25	55		22.80
					Mean	20.80
Steamed						
None	-	-	-	6		25.49
Benomyl	780	305	37	2		26.63
Carbendazim	297	282	26	7		24.70
					Mean	25.60
Methyl bromide						
None	-	-	-	7		24.34
Benomyl	749	466	54	11		24.68
Carbendazim	284	263	44	12		23.95
					Mean	24.32
L.S.D. ($P=0.05$) for comparisons between yield means:						1.297

≠ Sampled to 150mm depth
* benomyl applied at 100mg a.i./plant ** carbendazim at 67mg a.i./plant
≠≠ sampled 22 weeks after planting.

The distribution of MBC in the plant showed that most fungitoxicant accumulated in leaves less in stems and least in fruit when plants were sampled 14 days after the first drench of benomyl and carbendazim to steamed and unsterilized soils respectively (Table 4).

Table 4

Distribution of fungicide in tomato plants after soil drenches of benomyl and carbendazim

Sample °	Benomyl*			Carbendazim ≠		
	Fresh wt g/plant	MBC mg/kg	MBC µg/plant	Fresh wt g/plant	MBC mg/kg	MBC µg/plant
Below truss 3						
Foliage	328	2.1	690	273	1.1	303
Stem	93	0.5	43	93	0.2	18
Fruit (green)	204	0.09	18	222	0.17	38
Above truss 3						
Foliage	80	1.7	137	113	0.9	99
Stem	35	0.1	3	40	0.1	4
Fruit (green)	None	-	-	None	-	-
Total/plant	740	-	891	741	-	462

Two weeks after drenching ° samples taken from 4 plants/treatment and bulked.
* 100mg a.i./plant to steamed soil and ≠ 67 mg a.i./plant to unsterilized soil.

Tomato grey mould did not develop, but assessments of brown root rot showed that soil sterilization by steaming or methyl bromide fumigation were more effective than benomyl or carbendazim in controlling the disease and increasing crop yields (Table 3).

DISCUSSION

Spencer (1972) has reviewed the activity of MBC-related fungicides for the control of several tomato diseases in addition to grey mould viz. tomato wilts (Fusarium oxysporum f. lycopersici, and Verticillium albo-atrum), and leaf mould (F. fulva). Benomyl has been investigated for the control of tomato stem rot (Didymella lycopersici) (Channon, 1972), Fusarium wilt and grey mould (Channon & Thomson, 1973). Commercial tomato growers have quickly appreciated the possibility of controlling some important tomato diseases with soil-applied systemic fungicides, especially as drenching decreases labour costs compared with spraying. Moreover, if biological methods are developed to control tomato pests some fungicides may be less toxic to predators and parasites when applied as drenches than as sprays, as Parr & Binns (1971) demonstrated on cucumbers.

Effective disease control depends on a fungitoxic but non-phytotoxic concentration at the infection site. MBC-type compounds can be absorbed by roots and translocated to stems, leaves and sepals, preventing or decreasing B. cinerea infection of these structures and also reducing fruit rotting initiated by mycelial spread from other infected parts of the plant. But relatively small amounts of fungitoxicant were detected in fruit, in agreement with earlier reports (Peterson & Edgington, 1971; Locke & Green, 1971), and in stems (Biehn & Dimond, 1970), which probably explains the failure of these materials to control ghost-spotting or prevent colonisation of moribund peduncles.

Schreiber et al. (1971) associated increased plant uptake of fungicide with high pH and low organic content of the soil. Thus, soil sorption may be a factor in regulating the amount of fungitoxicant available to the plant. The initial decrease in fungicide uptake associated in this study with steamed as compared with unsterilized soil may be attributable to effects not only on the fungicide residues but also on the plant. For example steaming the soil increased the potential fruiting capacity of the plants and also their susceptibility to B. cinerea infection. Smith (1972) reported similar effects on fungicide uptake, crop yields and incidence of tomato grey mould when crops were transplanted at an early (buds first visible) compared with a late (first anthesis) stage of growth.

However, in our experiments only 1-2% of the fungicide applied to the soil was detected in the plant, and negligible fungicide loss could be attributed to leaching. Since chemical analysis determined the total fungicide in the soil, what then is the fate of the remaining 98% of the applied fungicide dose? Helweg (1972) has demonstrated bacterial degradation of benomyl in vitro, and the present series of experiments showed greater fungicide loss when drenches were applied to unsterilized rather than sterilized soil. But in 1973, irrespective of treatment, there was a very marked decline in persistence of benomyl soil residues and increased incidence of B. cinerea during the period of drenching compared with the two previous years. Investigations are continuing to determine the cause of fungicide loss, because conditions regulating the persistence and uptake of fungicides from soil must be more clearly defined if economic disease control is to be consistently achieved.

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NOTES

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THE INTEGRATED USE OF TWO SYSTEMIC FUNGICIDES FOR THE CONTROL OF CUCUMBER POWDERY MILDEW

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Summary Damaging infections by strains of powdery mildew (*Sphaerotheca fuliginea*), tolerant to benomyl or dimethirimol, on a glasshouse cucumber crop were controlled by applying the two fungicides either alternately, depending on the tolerance of the primary infection, or together. The tolerance of the strains used experimentally did not change and no strain tolerant to both fungicides appeared. A quantative method for the bioassay of benomyl in cucumber leaves is described.

INTRODUCTION

Dimethirimol was introduced commercially for the control of cucumber powdery mildew (*Sphaerotheca fuliginea*) in 1968 but by 1969, strains of the pathogen able to attack treated plants had begun to appear, first in England and later in Europe (Bent *et al*. 1971). Likewise, strains of the organism tolerant to benomyl also appeared early in the commercial life of this compound (Schroeder and Provvidenti, 1969).

According to Kaars Sijpesteijn (1972) dimethirimol appears to depend for its activity on its interference with tetrahydrofolic acid - directed C-1 transfer reactions whilst the fungitoxicity of benomyl depends on its interference with nucleic acid metabolism.

Wolfe (1971) suggested that in order to minimise the emergence of tolerant strains of pathogenic fungi, fungicides with different modes of action should be applied either combined or alternately. As it is improbable that tolerance mechanisms to two fungicides would be present in a single strain of the pathogen, it should be possible to control cucumber powdery mildew using the two compounds even in the presence of strains of the pathogen tolerant to either of the fungicides.

Accordingly, dimethirimol and benomyl either alternately or together were applied throughout the season to cucumber plants inoculated with strains of the organism tolerant to each of the fungicides. Established infections were routinely examined throughout the season to determine the level of tolerance of the causal organism to either or both of the fungicides.

For convenience it would have been preferable to have used all fungicides as drenches, but in earlier work we had failed to achieve consistent uptake from benomyl drenches on cropping plants. However, in a separate experiment consistent uptake of fungicide was achieved thus enabling benomyl drenches to be used later in this experiment.

MATERIALS AND METHODS

Fungicide treatments

Standard commercial formulations of dimethirimol (20 ml "Milcurb"/plant as a drench) and benomyl (500 μg/ml "Benlate" sprayed to run off), as well as an experimental compound which had shown promise as a drench in pot tests, were used to treat cropping cucumber plants (cv. Cupra) grown as cordons on peat beds.

Treatments were as follows;

B + D - monthly applications of the two systemic fungicides at the same time,

D/B - dimethirimol, followed by benomyl when dimethirimol tolerant mildew established,

B/D - benomyl, followed by dimethirimol when benomyl tolerant mildew established,

EC - monthly drenches of the experimental compound in 0.2% aqueous acetone.

Each treatment was replicated in four plots each of four experimental plants.

Mildew strains and inoculation

A strain of the pathogen tolerant to approximately 5 ppm dimethirimol was kindly provided by ICI Ltd., (Mr. M. Woolmer, Jealott's Hill). A strain tolerant to >10 ppm methyl benzimidazole carbamate (MBC) the fungitoxic derivative of benomyl appeared at GCRI in 1972. These strains were maintained in isolation on pot plants treated with the corresponding chemical.

Leaves were shaken 24 hours before inoculum was required, to remove old conidia. Leaf discs 1 cm diam bearing young conidia were cut out, gently placed on the upper surface of leaves of experimental plants and held in place for 24 hours using a spring clip.

Plants in Treatments B + D and EC were inoculated each week with mildew strains tolerant to dimethirimol, to benomyl and to neither.

Plants in Treatment D/B, first drenched with dimethirimol were inoculated at weekly intervals with the dimethirimol-tolerant strain. When mildew infection was seen to be established these plants were sprayed with benomyl. Subsequent weekly inoculations were with benomyl-tolerant mildew until this strain was seen to have become established. Dimethirimol was then used again as a drench and the cycle repeated.

Plants in Treatment B/D also received the two fungicides alternately, starting with benomyl and were inoculated first with the benomyl tolerant organism.

Assessment of Tolerance

Throughout the growing season any newly established mildew was tested in the laboratory for tolerance to both fungicides either alone or in a mixture.

Petri dishes (6 cm diam) each received 10 ml of water or of fungicide solution from a range of concentrations from 0.1 - 20 μg/ml, plus benzimidazole at 10 μg/ml.

Apparently healthy leaves from untreated plants were dusted with young conidia of the infection to be examined. Discs (1 cm diam) were then cut from the leaf with a sharp cork borer and five discs were floated on the solution in each dish. The dishes were kept on the laboratory bench and the development of mildew was recorded on an arbitary scale 0-5.

Measurement of uptake of fungicide from benomyl drenches

In early experiments to demonstrate the presence of fungitoxicant in cucumber plants, tissues were deep frozen and thawed before being extracted in chloroform. This method was later modified to use only the sap expressed through a hypodermic syringe from deep frozen and thawed tissue. Aliquots ($25 \mu l$) were pipetted on to 3 MM chromatography paper which was then sprayed with a suspension of conidia of *Fulvia fulva* (250,000/ml) in Oxoid Czapek-Dox liquid medium allowing 0.05 ml/cm^2 paper sprayed from a de-Vilbiss Atomist sprayer at 1 kg/cm^2 pressure. After incubation at laboratory temperature for 72 hours the size of the zone of inhibited fungal growth, could, by means of a simple calibration curve, be directly related to the amount of toxicant present, measured as micrograms of MBC. It was found that the size of the zone of inhibition was not modified by the stimulants of fungal growth in the cucumber juice.

Yield

Fruits were harvested on Monday and Thursday of each week when the number and weight of all fruits from the four treated plants in each plot was recorded.

RESULTS

Powdery mildew infection

The aim in assessing powdery mildew infection was to determine when the actively sporulating pathogen was so established as to require treatment. The grades used (Figs 1-4) were therefore as follows:-

1 = infection established at primary inoculation
2 = infection spreading from primary inoculation
3 = infection (< 10%) on median uninoculated leaf
5 = infection 10-20% on median uninoculated leaf
10 = " 20-30% " " "
15 = infection becoming severe on all leaves
20 = plants moribund.

Treatment B + D During the season powdery mildew infection occasionally became established very lightly (Fig 1a) but after the monthly treatment with the two fungicides careful examination of the plants demonstrated that the visible mildew was no longer viable.

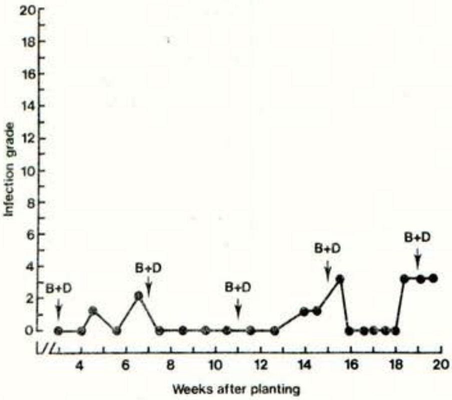
Treatment D/B The alternate use of dimethirimol and benomyl throughout the season successfully controlled the powdery mildew infection which developed from inoculations with strains of the organism tolerant to each of the fungicides. (Fig. 1b).

Treatment B/D Dimethirimol was slow to control the infection which developed after inoculation with benomyl-tolerant mildew. It was even slower to control the infection later in the season (Fig 1c, weeks 13-16), and at this time a second application of the fungicide was made. The organism here was found, in laboratory tests, to be dimethirimol-tolerant and the disease was controlled by a single benomyl drench (Fig 1c, week 16).

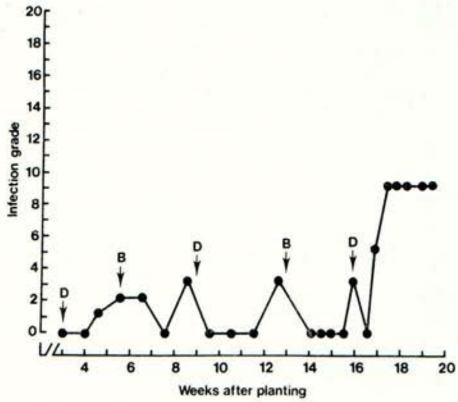
Fig. 1 Development of cucumber powdery mildew on cropping plants inoculated weekly with different strains of the organism and treated with various fungicides.

➡ application of fungicides.

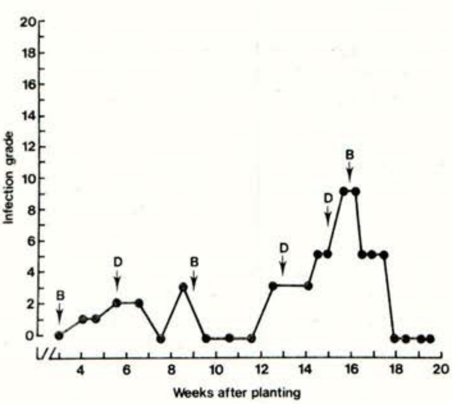
a) Treatment B + D. Benomyl and dimethirimol applied together monthly.



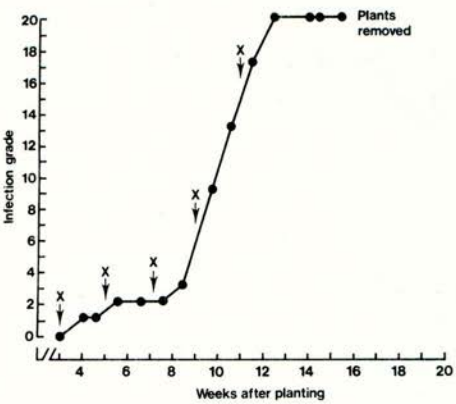
b) Treatment D/B. Dimethirimol applied first and alternated with benomyl, as needed.



c) Treatment B/D. Benomyl applied first and alternated with dimethirimol, as needed.



d) Treatment EC. An experimental compound, applied monthly.



Whereas actively growing powdery mildew is normally present on leaves as a clean white raised efflorescence, that killed by a systemically distributed fungicide has a dull cream flat appearance. The mildew present in Treatment B/D towards the end of the season and which in vitro was tolerant to 5 μg/ml dimethirimol can be described as 'cobweb'. Under low-power magnification there was much more mycelium than in normal strains while conidia were very sparse.

Treatment EC Powdery mildew was not controlled by the experimental compound after the first few weeks. (Fig 1d). As a result $3\frac{1}{2}$ months after planting, the plants had become moribund and constituted such an enormous source of inoculum that it was decided to remove them.

Measurement of uptake

The bioassay of extracts from mature cropping plants showed that following a root drench with 500 ml benomyl suspension at 250 μg/l the level of MBC in the median leaves was approximately 0.5 ppm after 2 days and 1.5 ppm after 7 days. It was therefore decided to apply the benomyl as a drench in Treatment B/D towards the end of the season (week 16 Fig 1c). Two weeks after the plants were drenched with 500 ml benomyl (250 μg/ai/ml), they were free from viable mildew.

Yield

Plants in Treatment EC were removed after 8 weeks cropping and yielded 33.4 kg/plot of 4 plants. Plants in Treatments B + D, D/B and B/D were cropped for 12 weeks and gave respectively 55.4, 65.1 and 63.0 kg/plot, yields which were not in fact significantly different. The lower yield from Treatment B + D was attributed to the known adverse effects of monthly sprays of benomyl on the predator Phytoseiulus persimilis (Parr and Binns, 1970) which was used throughout the experiment to control red spider mite (Tetranychus urticae). In Treatment B + D red spider mite caused considerable damage to leaves, resulting in a measurable loss of crop.

DISCUSSION

The purpose of this investigation was to determine whether the cucumber grower could use two different systemic fungicides either alternately or in combination, to control powdery mildew when tolerant strains appeared.

The progress of infection was assessed to determine when fungicide needed to be re-applied and therefore in practice it was only important to note when mildew was sufficiently established to pose a threat to the crop. A distinction was made between infection established at the inoculation point, and that which occurred elsewhere on the plants, possibly from inoculum elsewhere in the house. Thus in Treatment B + D (Fig 1a) infection was established at the point of inoculation twice and later in the season mildew appeared elsewhere on the plant. On each occasion the monthly simultaneous application of dimethirimol and benomyl quickly controlled the infection which at no time appeared likely to become unmanageable. In Treatment B/D the mildew developing after an application of dimethirimol to plants inoculated with benomyl tolerant mildew was found to be the dimethirimol-tolerant strain. Presumably this infection had arisen from strains present elsewhere in the house and it is probably significant that the plants on which it occurred had been sprayed with benomyl as long as four weeks previously.

At no time during the season was the tolerance of either strain found to have changed. Neither was there any indication that any strain had developed tolerance to both systemic fungicides.

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INSECTICIDE RESISTANCE TESTING AND CHEMICAL CONTROL OF GLASSHOUSE WHITEFLY

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Summary Fourteen out of 17 glasshouse whitefly populations were resistant to one or more of the insecticides, malathion, DDT and resmethrin. None of the populations tested was resistant to methomyl.

Azinphos-methyl was more effective against a susceptible population than malathion or DDT; some chemicals not normally used for whitefly control were partially effective.

Simulated HV sprays of oxamyl, mecarphon, methomyl, pyrethrin/resmethrin, pyrethrum/rotenone and WL 29319 were effective against a whitefly population resistant to both malathion and DDT.

Granules of aldicarb, dimethoate and oxamyl, and drenches of bendiocarb, oxamyl, mecarphon and methomyl applied to pot plants, were effective against larvae of the resistant population.

Sodium cyanide fumigation gave variable control and pirimiphos-methyl 'fog' gave good control of susceptible and resistant whitefly larvae.

Resumé Quant aux populations des Mouches blanches des serres, quatorze sur dix-sept populations ont résisté à un ou plusieurs des insecticides, malathion, DDT ou resmethrin. Pas une seule a résisté à methomyl.

Azinphos-methyl était plus effectif contre une population susceptible que malathion ou DDT.

Des pulvérisations d'oxamyl, mecarphon, methomyl, pyrèthre/resmethrin, pyrèthre/rotenone et WL 29319 étaient effectives contre une population résistante à tous les deux malathion et DDT.

Les insecticides systemics aldicarb, bendiocarb, dimethoate, oxamyl, mecarphon et methomyl, appliqués aux plantes dans les pots, ont été effectifs contre les larves de la population résistante.

Le cyanure de sodium a donné un résultat variable, et pirimiphos-methyl a donné un bon résultat contre des larves susceptibles et résistantes.

INTRODUCTION

Insecticide resistance in glasshouse whitefly (Trialeurodes vaporariorum) to malathion (6 of the 7 populations tested) and DDT (1 of the 6 populations tested) was recorded during 1972 in S.E. England (Wardlow et al., 1972). French et al. (1973) screened many chemical treatments against a malathion-susceptible and a resistant population, and found that bioresmethrin, DDT, DDT/BHC and pyrethrum were the most promising alternative materials for the resistant strain.

Testing of whitefly populations for insecticide resistance continued during 1972/73 to gain more information on the extent of resistance, to provide advice on control measures, and to monitor any development of resistance to the alternative chemicals.

Further insecticide screening included materials not normally used for whitefly control, and systemic insecticides suitable for use in pot-plant production.

INSECTICIDE RESISTANCE TESTING

French beans (Phaseolus vulgaris) were infested with eggs from about 1,000 adult whiteflies from each of 17 sites situated mainly in S.E. England.

Tests were carried out on first instar larvae using the method described by Wardlow et al. LC_{50}s for larval populations were derived from lines of best-fit to the data on log-probit graphs. These will be published elsewhere after statistical analysis together with data from tests on adults. Resistance factors based on the original unanalysed data are given in Table 1.

Table 1

Insecticide resistance testing of glasshouse whitefly populations 1972/73

S = susceptible; x4 = 4-fold resistance; N.T. = not tested

Whitefly population site*	Pesticide tested: Malathion e.c.	DDT e.c.	Resmethrin e.c.	Methomyl w.p.
A	S	S	S	S
B	S	S	S	S
C	S	S	S	S
D	S	S	x4	S
E	x2	S	S	S
F	x4	S	S	S
G	x4	S	N.T.	N.T.
H	x7	S	S	S
J	x22	S	N.T.	N.T.
K	x2	S	x5	S
L	x9	S	x5	S

Table 1 (continued)

Whitefly population site*	Pesticide tested Malathion ec.	DDT e.c.	Resmethrin e.c.	Methomyl w.p.
M	x4	x13	S	S
N	x12	x9	N.T.	N.T.
O	x12	x8	S	S
P	x12	x36	S	S
R	x4	x25	x6	S
S	x10	x38	x6	N.T.

* Population D was from Middlesex and F from Guernsey; the remainder were from Kent, Sussex and Surrey.

Only three populations out of 17 were susceptible to all four pesticides; only four were susceptible to malathion. Six out of 17 populations were resistant to both malathion and DDT and five out of 14 populations were resistant to resmethrin. There was no resistance to methomyl.

INSECTICIDE SCREENING

The techniques, leaf-dips and Potter tower treatments, simulating HV sprays, were described by French et al. Pesticides not normally used for whitefly control (including some acaricides/aphicides used in glasshouses) and a number of alternatives to malathion and DDT were first tested against a susceptible population from site A (Table 1). The best of the alternative pesticides were tested against a population which was 12-fold resistant to malathion and 8-fold resistant to DDT (site O); DDT and malathion were included for comparison.

Data from the untreated controls and the treated plots were analysed separately; mortality of untreated controls was low and did not significantly affect results. High-kill treatments generally gave heterogeneous errors and were omitted from analyses of treated data. Standard errors refer only to data marked by asterisk in Tables 2, 3 and 5.

Excepting methomyl (0.02%) which had a slight effect, no treatment seemed to affect egg viability.

Table 2

Effects of simulated HV sprays on a whitefly population susceptible to malathion and DDT

Chemical	Rate of use (a.i.)	Percentage kill				
		First instar	Second instar	Third instar	Pupae	Adults
Azinphos-methyl 22% e.c.	0.02%	100.0	100.0	98.5	62.4*	99.5
DDT 25% e.c.	0.09%	100.0	98.0	74.2*	30.8*	98.7
Demeton-S-methyl 58% e.c.	0.02%	95.2	36.2*	16.0*	0.5ø	21.1ø
Diazinon 20% e.c.	0.02%	100.0	100.0	69.5*	3.4ø	13.1ø
Dicofol 20% e.c.	0.02%	8.7*	38.8*	12.0*	5.2ø	10.2ø
Dioxathion 47% e.c.	0.06%	60.8*	41.7*	2.4*	1.7ø	52.1*
Endosulfan 20% e.c.	0.05%	99.0	98.7	86.6*	3.5ø	97.4
Formothion 43% e.c.	0.04%	100.0	99.4	87.6*	3.4ø	40.2*
Malathion 60% e.c.	0.11%	100.0	98.2	93.8*	42.6*	95.6
Mevinphos 99% e.c.	0.01%	97.0	62.8*	9.3*	1.4ø	63.6*
Quinomethionate 25% w.p.	0.02%	67.2*	11.6*	17.7*	11.4ø	17.9ø
Tetradifon 8% e.c.	0.02%	10.1*	9.7*	6.5*	2.7ø	5.9ø
Untreated control		1.5	1.1	1.2	0.8	4.8
Standard error		±11.8	±15.6	±10.1	±15.9* ± 3.8ø	±20.2* ± 4.8ø

* and ø Data to which S.E.'s refer

Azinphos-methyl was the best treatment; diazinon, endosulfan and formothion were generally as effective as malathion and DDT against larvae, although diazinon and formothion were less effective against adults. All other treatments were partially effective against larvae and adults.

Table 3

Effects of simulated HV sprays on a whitefly population resistant to malathion and DDT

Chemical	Rate of use (a.i.)	Percentage kill First instar	Second instar	Third instar	Pupae	Adults
Bendiocarb 80% w.p.	0.20%	85.6*	67.5*	42.7*	5.5*	68.4*
DDT 25% e.c.	0.09%	60.3*	29.2*	22.2*	7.7*	27.2*
Malathion 60% e.c.	0.11%	88.0*	56.3*	20.9*	1.1*	16.9*
Mecarphon 50% e.c.	0.03%	98.0	99.9	89.1	28.0*	87.2*
Methomyl 25% w.p.	0.02%	100.0	96.4	78.0*	39.5*	93.8
Oxamyl 25% e.c.	0.03%	99.0	86.9*	73.3*	14.4*	66.6*
Pyrethrin/resmethrin e.c.	-	98.5	99.7	96.3	17.2*	100.0
Pyrethrum/rotenone 1% and 1.5% e.c.	0.01%	95.4	93.3	68.7*	14.6*	98.3
W.L. 29319** 40% e.c.	0.08%	100.0	98.1	56.6*	55.5*	99.5
Untreated control		2.3	0.2	1.2	1.2	2.0
Standard error		±9.2	±8.2	±7.6	±10.4	±8.9

* Data to which S.E.'s refer
** Shell experimental insecticide

All treatments gave better control of all stages than malathion or DDT, and were fully effective (excepting bendiocarb) against first and second instar larvae.

FUMIGANTS

Two batches of bean plants were infested with eggs, larvae and pupae of susceptible and resistant whitefly populations (a single pot of 5 plants for each stage) and were treated with sodium cyanide (40% a.i.) on 3 February and 18 June in an 850 ft^3 glasshouse maintained at 70°F. A tray of damp soil was placed on the floor of the glasshouse and 28.4 g of powder was sprinkled over the surface. Plants were removed after 24 h and whitefly mortality was assessed after five days for larvae and 16 days for eggs.

Other batches of beans infested with susceptible and resistant whiteflies were placed in a large commercial glasshouse of tomatoes during a thermal fog treatment with 10% pirimiphos-methyl at 4 pints/ac on 18 June. Plants were removed after 24 h and mortality assessed as above.

Table 4

Effects of sodium cyanide and pirimiphos-methyl fumigation on glasshouse whitefly

S = malathion and DDT susceptible population;
R = malathion and DDT resistant population

Chemical	Percentage kill							
	First instar		Second instar		Third instar		Pupae	
	S	R	S	R	S	R	S	R
Sodium cyanide 2 February	98.1	79.7	98.5	69.8	94.2	67.9	6.8	1.4
Sodium cyanide 18 June	62.7	87.7	90.1	96.8	79.9	98.9	11.8	7.2
Pirimiphos-methyl 18 June	100.0	98.7	100.0	93.9	99.9	69.6	50.5	0.4

Effects of both chemicals on adults were difficult to determine because they flew off the plants; however, no live adults were seen after sodium cyanide and a high proportion of the glasshouse population of adults (7-fold resistant to malathion, susceptible to DDT) were killed after pirimiphos-methyl fumigation.

Against larvae, sodium cyanide gave variable results which differed on both occasions; pupae were only slightly affected. Pirimiphos-methyl gave good control of both populations but was ineffective against pupae of the resistant population.

POT TREATMENTS

Various rates of systemic chemicals were first tested against a susceptible population and the most efficient were then tested against a resistant population.

Five inch diameter plastic pots each containing five bean plants were exposed to adults for egg-laying for 48 h and treated with drenches or granules; each treatment was replicated three times. Drenches were applied at 100 ml per pot.

Granules at the rates shown in Table 5 were distributed on the soil surface by hand; and pots were watered with 100 ml daily until mortality of eggs and larvae was assessed on one leaf from each of three plants 16 days after treatment.

Table 5

Effects on glasshouse whitefly of systemic chemicals applied to bean plants in pots

Chemical	Rate of use (a.i.)	Percentage kill			
		Eggs		First instar larvae	
		Susceptible	Resistant	Susceptible	Resistant
Drenches					
Bendiocarb 80% w.p.	0.1%	1.2	1.1	93.1*	90.0*
Bendiocarb 80% w.p.	0.2%	2.6	2.4	99.0*	99.6*
Mecarphon 50% e.c.	0.013%	2.7	1.4	99.4*	100.0*
Mecarphon 50% e.c.	0.025%	9.1*	4.4*	100.0*	100.0*
Methomyl 25% w.p.	0.01%	30.6*	18.5*	99.4*	80.5*
Methomyl 25% w.p.	0.02%	53.8*	33.1*	100.0*	99.9*
Oxamyl 25% e.c.	0.013%	0	0	99.7*	88.6*
Oxamyl 25% e.c.	0.026%	0	0.8	100.0*	99.7*
Granules	g a.i./pot				
Aldicarb 10%	0.005	0	N.T.	93.2	N.T.
Aldicarb 10%	0.01	0.8	N.T.	96.5	N.T.
Aldicarb 10%	0.02	0	1.3	99.6*	100.0*
Dimethoate 5%	0.025	17.3*	5.1*	99.2*	80.2*
Dimethoate 5%	0.05	37.1*	22.2*	99.8*	99.8*

Table 5 (continued)

Chemical	Rate of use (a.i.)	Percentage kill			
		Eggs		First instar larvae	
		Susceptible	Resistant	Susceptible	Resistant
Oxamyl 10%	0.01	0	N.T.	83.3	N.T.
Oxamyl 10%	0.02	0	0	99.1*	96.5*
Untreated control		0	0	1.43*	0.72*
Standard error		±13.5		±7.2	

* Data to which S.E.'s refer
N.T. = not tested

Dimethoate and methomyl prevented some eggs from hatching; they were slightly less effective at both rates of use against the resistant population. At the higher rates all the pesticides gave excellent control of resistant larvae; at the lower rates control was generally less efficient than against the susceptible population.

Dimethoate and bendiocarb caused some foliar scorch.

DISCUSSION

Tests confirmed that malathion resistance is widespread in South-East England, and cases are occurring elsewhere. However, the degree of resistance of all these populations was lower than that in the worst cases previously tested by Wardlow et al. Resistance to DDT was more widespread than in previous tests and particularly noticeable at site S where DDT/BHC smoke had been used as an alternative against a population shown to be malathion resistant during 1971/72 winter. Resmethrin resistance was recorded for the first time at site D where pyrethrum HV spray had been used for many years and at site R where a bioresmethrin/resmethrin programme was initiated in early 1973 against a malathion/DDT resistant population. It would seem that once a population has built up resistance to one chemical, it may take less than a year of regular use to become resistant to another.

The results in Table 2 suggest that chemicals normally used on glasshouse crops for the control of other pests may also affect whitefly, and it is possible that some of these have contributed to the development of resistance.

Most of the alternatives to malathion and DDT in Table 3 show promise against resistant whiteflies. Pyrethrum/rotenone is already proving useful in the field as a short-persistence HV spray; pyrethrin/resmethrin would also prove a good alternative. The other treatments are either experimental or not cleared for glasshouse use under the Pesticides Safety Precautions Scheme in the United Kingdom, and further work is required on effects on plant growth.

Pirimiphos-methyl (an organophosphorus chemical) was very effective against susceptible whiteflies and almost equally effective against the malathion resistant population. Populations with higher resistance levels may prove more difficult to kill, but it seems that the thermal fog system is suitable for whitefly control.

The systemic materials gave promising results for routine preventative control on plants grown in pots, but further work is required on their effects and persistence on commercially grown crops. These treatments would not be suitable for the control of established infestations, as many larvae reach the resistant pupal stage before the pesticides are taken into the plant.

Most of the whitefly populations tested for resistance were from nurseries where the pest was out of control; resistance was often responsible but other causes were late initiation of a control programme, too few treatments or excessive intervals between applications, or inadequate spray cover of foliage.

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NOTES

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PROGRESS TOWARDS INTEGRATED PEST CONTROL ON YEAR ROUND CHRYSANTHEMUMS

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Summary The pest complex of chrysanthemums has been studied over a period of three years. The pest complex consisted of aphids, red spider mite, leaf miner and caterpillars. An integrated system of pest management is proposed. The peach-potato aphid was controlled by treating boxes of rooted cuttings with this aphid, previously exposed to the parasite for four days, at a rate of one aphid to ten plants. Red spider mite and its predator were introduced into the boxes at rates of one per plant and one per fifty plants respectively. The use of trichlorphon/pirimicarb sprays misted onto the apical parts of the plants gave complete control of other pests except caterpillars, which were controlled with high volume sprays of Bacillus thuringiensis. Complete management of the two major pests has so far resulted.

INTRODUCTION

Chrysanthemums may be grown sequentially throught the year in beds and pots. The continual presence of all growth stages associated with this system encourages pests especially at flowering time when risk of damage to blooms makes pesticide applications impractical.

By 1963 a number of growers were failing to control the peach-potato aphid (Myzus persicae) with organophosphorus insecticides and differences in susceptibility to parathion were shown (Wyatt 1963, 1964). The rapidity with which resistant strains could be distributed by the commercial trade in cuttings demanded a rapid reduction in the selection pressure. The success of an integrated pest management system for the pest and disease complex on cucumbers (Anon 1972) stimulated the development of similar systems for other crops especially tomatoes and chrysanthemums.

The first step towards biological control of those pests already creating resistance problems (the peach-potato aphid and red spider mite (Tetranychus urticae) on year round chrysanthemums has been successfully demonstrated on two commercial nurseries (Scopes, 1969) but the trials had to be terminated because of the build-up of hitherto minor pests.

METHODS AND MATERIALS

Four beds of chrysanthemums, 400 plants per bed, were sub divided into four equal plots, each planted alternately with two chrysanthemum cultivars, Tuneful and Fandango. The experiments were continued for three years. The former cultivar is susceptible to attack by the peach-potato aphid (Wyatt, 1965), while the latter is reputed to be prone to red spider mites.

Regular leaf samples were taken from both top and bottom parts of plants (50 leaves per 100 plants), while whole plant samples were taken when the plants were in flower (5 plants per 100 plants). The undersides of the leaves on the main axis only were examined and the numbers of pests and their enemies were recorded. Visual assessments were made of flower quality as affected by pest numbers. Crops of chrysanthemums were grown with or without biological or chemical treatments to determine their effects on other pests and natural enemies.

RESULTS AND DISCUSSION

Biological control

The peach-potato aphid. This aphid showed itself to be a perpetual pest on the chrysanthemums in these experiments, and the cultivar Tuneful appeared to favour the aphid compared with the cultivar Fandango. Vertical aphid movement on Tuneful followed the patterns demonstrated by Wyatt (1970) but this trend was not detected on Fandango. (Table 1).

Table 1

Proportion (%) of peach-potato aphid populations on the apical leaves during the vegetative growth of chrysanthemum plants

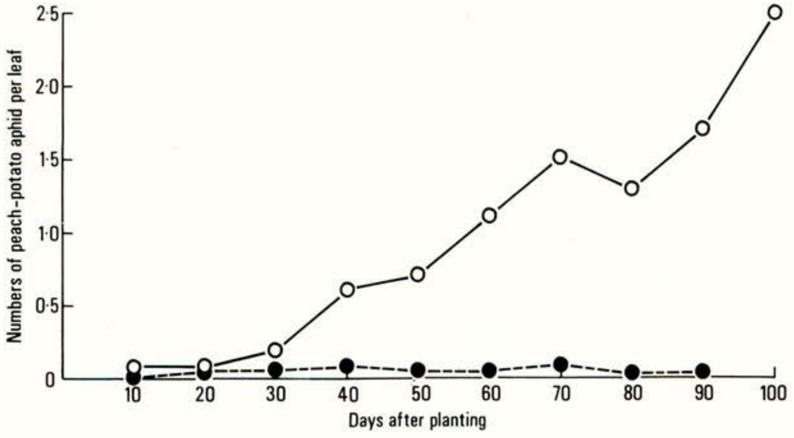
Tuneful				Fandango			
Crop 1		Crop 2		Crop 1		Crop 2	
Days after planting	%	Days after planting	%	Days after planting	%	Days after planting	%
0	-	0	-	0	-	0	-
15	-	15	-	15	-	15	-
27	33	30	75*	27	-	29	52
41	43	51	19	41	96	51	73
55	42	78	13	55	72	78	89
76	58	86	55	76	60	86	65
87	84	93	66	87	67	93	63
111	89			111	43		

* Represents four aphids.

Following many observations of flower damage, the unsightly presence of aphids in the blooms was found to occur when aphid numbers had reached an average of more than 3.0 per leaf. This contrasts with the large populations which the plant can tolerate in the vegetative state without harm (Gurney 1969, Scopes 1970).

In order to obtain a uniform introduction of the pest and its parasite Aphidius matricariae the boxes of cuttings were treated with aphids, that had previously been exposed to parasites for four days. The introduction rate was one aphid per ten cuttings (Scopes 1970) and the effect of such introductions is illustrated in Figure 1. Twenty seven consecutive crops were treated in this way, and no damage to either leaves or flowers has resulted. Only on eleven occasions did the numbers rise above 0.5 aphids per leaf. Such small numbers of this aphid occurred in the samples that it has been impossible to detect any vertical migration tendencies on the plants since biological control began.

Fig. 1. Numbers of peach-potato aphids developing on chrysanthemums (c.v. Tuneful) o—o and after treating boxes with parasitized aphids •---•. (Each line is the mean of three consecutive crops)



Red spider mite. A common and serious pest of chrysanthemums, and with severe infestations dense webbing makes flowers completely unmarketable. Damage estimation was difficult because it proved impossible to keep the predacious mite *Phytoseiulus persimilis* out of the trial glasshouse. However, mites were found in the flowers and webbing was formed when mite numbers averaged 8-10 per leaf, while webbing completely ringed flowers when numbers rose above about twenty-five per leaf. Treating boxes prior to planting with both red spider mites and predators at rates of one per plant and one per 50 plants respectively gave excellent control on 20 crops, and on no occasion did the numbers of red spider mites average more than 3.6 per leaf (Figure 2). Where the plants were invaded by chance migration of *Phytoseiulus* red spider mite was controlled on growing crops in about 60 days (Table 2).

Fig. 2. Numbers of red spider mite developing on chrysanthemums (c.v. Fandango) o—o and after treating boxes with mites and predators prior to planting •---•. (Each line is the mean of three consecutive crops.)

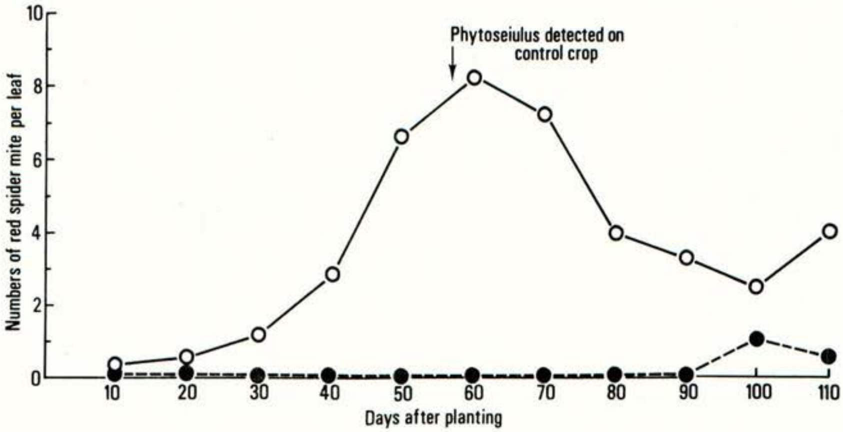


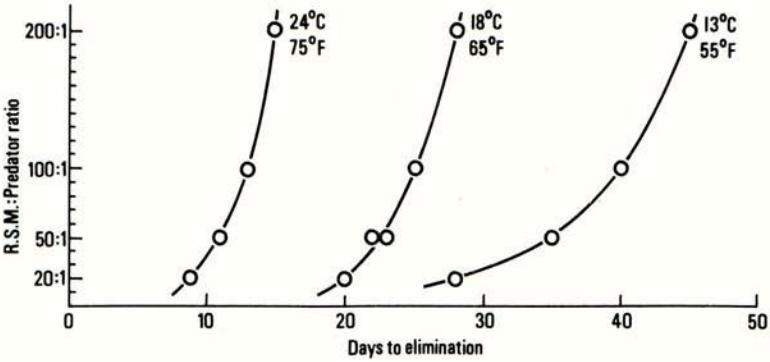
Table 2

Control of red spider mite with predators on chrysanthemums

Days after planting	Red spider mite : predator ratio Tuneful	Fandango
15		
27		
41		
55	961 : 1	730 : 1
62	221 : 1	125 : 1
76	132 : 1	21 : 1
87	15 : 1	11 : 1
111	3 : 1	2 : 1

A series of tests was then made to determine the effect of temperature on the speed of predatory control of red spider mite. Known numbers of mites were confined with a single female predator on french bean (*Phaseolus vulgaris*) leaves, floating on water, at either 13, 18 or 24°C. The results are shown in Figure 3.

Fig. 3. The effect of temperature on the elimination of red spider mites by *Phytoseiulus*.



Assuming that chrysanthemum temperatures are 16°C and above, it would appear that the box seeding treatment should be effective for about four weeks, plus a further three week period while the predator population declines with a decreasing food supply. However, in practice (Table 2) control took longer, perhaps because of the increases searching activity required on a bed of plants, and reinfestation by red spider mites.

Minor pests. The leaf curling plum aphid (*Brachycaudus helichrysi*), an aphid normally of occasional occurrence, can rapidly assume pest proportions when spray programmes are relaxed. This species inhabits the crown of the plant causing deformation and mottling of leaves. Migration into the flowers occurs as soon as buds expand and show colour. Migration to lower leaves occurs only when numbers in the crown became excessive. The honeydew excreted by this aphid crystallizes into characteristic sugary plate like crystals. Flower damage occurred when aphid numbers averaged more than three per leaf, though as many as 90 have been recorded on a single leaf.

Unidentified parasites have been developing on this aphid but their control potential has been negligible both in the laboratory and in the glasshouse. Entomophagous fungi have given natural control in the glasshouse on a number of occasions, but only after extensive damage had been caused. The chrysanthemum aphid (*Macrosiphoniella sanborni*), a dark brown glossy aphid, feeds primarily on the main stem of chrysanthemums especially at the top. It creates an unsightly appearance when large populations aggregate on flowering stems. Excellent control of this aphid has been achieved in the laboratory with the wasp parasite *Aphelinus asychis* but three attempts to establish it in the greenhouse have unaccountably failed.

The use of predators (Larvae of the green lacewing (Chrysopa carnea) and the tropical ladybird (Cycloneda sangiunea) against both this aphid and the leaf curling plum aphid (B. helichrysi) failed. In the former case the predators were unable to reach their prey living in the confined spaces between leaves, while the chrysanthemum aphid (M. sanborni) were not liked, or were toxic to the predators. Phytomyza syngenesiae is a common pest on chrysanthemums which is difficult to control with contract insecticides during most of its lifecycle, as from eggs to pupa it is protected within leaf tissues. The lifecycle was completed in laboratory tests in 17, 24, and 38 days at 23, 17 and 13°C respectively. Damage is caused by the larvae tunnelling within the leaves.

Excellent control has been achieved with the eulophid parasite Diglyphus isaea. The adult parasite kills leaf miner larvae when they are 2.0-2.5 mm long and lays its eggs beside the dead host. This occurs when the larvae are 7, 12 and 18 days old if developing at 23, 17 or 13°C respectively. During tests made in 1971 complete control of large populations of leaf miner was obtained within six weeks but, in 1972 the parasite failed to establish. It was concluded that this parasite was of little value for commercial exploitation on account of its unpredictable behaviour. Current tests suggest that either low light intensities and/or short days account for its failure to establish.
Caterpillars About twelve species of caterpillars are known to damage crops under glass but three species predominated in these experiments, the angle shades moth (Phlogophora meticulosa), silver Y moth (Autographa gamma) and the carnation tortrix (Cacoecimorpha pronuba). Damage occurs primarily during the early summer and early autumn and is spasmodic, however infestations have been successfully controlled by a drenching spray of 0.5% Bacillus thuringiensis (serotype 3b). The spray must be applied at about 400/450 gallons per acre and occasionally a second spray one to two weeks later may be necessary to kill survivors. The problem with such spray treatments is that Bacillus is a stomach poison which may be washed off the foliage by water where overhead spray-lines are used.

Other pests recorded on the crop have included onion thrips (Thrips tabaci) glasshouse potato aphid (Aulocorthum solani), black bean aphid (Aphis fabae) and two other unidentified aphids which did not increase or cause serious damage.

Use and integration of sprays

The complex of pests that may attack chrysanthemums makes a biological pest control system difficult to achieve, especially as many minor pests may occur only spasmodically, making such developments uneconomic. A combination of biological and chemical methods must therefore be employed. The recent introduction of aldicarb has simplified pest control on year-round chrysanthemums because of its persistent action against a wide spectrum of insects. It is therefore hoped that the programme proposed here will be the basis of a future integrated control programme should resistance to aldicarb occur in one or other pest. With the exception of aldicarb and pirimicarb other pesticides are toxic to Phytoseiulus so that integration would have to rely on careful placement or timing of sprays. The programme must take into account two pests, leaf miner and minor aphids both of which live primarily in the apical foliage of the plants. Red spider mite and the peach-potato aphid tend to live on the undersides of lower leaves especially when numbers are small, and may be protected by the dense canopy of foliage from pesticides applied above.

Successful integration largely depended on obtaining compatible chemical control of leaf miner. Leaf miners feed and lay eggs on the upper surfaces of young leaves on the chrysanthemum while the larvae develop within the leaf. Both control and protection from attack were obtained with several insecticides including pirimicarb, trichlorphon, bromophos, dimethoate, carbenothion and dioxathion applied to the upper surfaces of the apical leaves (Gurney in preparation). These compounds not only kill eggs and larvae for periods up to fourteen days but also protected the plant from attack for this period. Trichlorphon, although controlling leaf miner, appeared to exert a fumigant effect killing predators below and enabling spider mite outbreaks to occur.

Tests made during the summer of 1973 integrated the biological control of the peach-potato aphid and red spider mite with one of two different pesticide regimes. In one series bromophos was applied at 0.1% while in the second a mixture of dioxathion (0.1%) and pirimicarb (0.025%) was used. Sprays were applied every fpurteen days at the rate of eighty to one hundred gal/ac. Pests were re-introduced every three weeks throughout the crops. Both spray programmes provided excellent control of leaf miner but while bromophos gave fair control of caterpillars, only about 70% control of aphids was achieved. The dioxathion/pirimicarb mixture gave excellent aphid control but was not effective against caterpillars. The proposed integrated system is being tested during 1973 (Table 3) and indications are that both the peach-potato aphid and red spider mite are being maintained at very low levels while the other pests are being successfully controlled with sprays.

Table 3

Proposed system for integrated pest control for year round chrysanthemums

Pest	Biological programme	Spray programme
Peach-potato aphid.	Treat boxes prior to planting with aphids exposed to parasites at the rate of one aphid per 10 plants.	-
Red spider mite.	Treat boxes prior to planting with spiders and predators at rates of 1.0 and 0.02 per cutting respectively.	-
Leaf miner.	-	Dioxathion/pirimicarb spray every two weeks (80-100 gal/ac).
Minor aphids.	-	"
Caterpillars.	-	*Bacillus thuringiensis* spray at first symptoms (450 gal/ac).

It is envisaged that such an integrated programme will be viable from March to November, supplemented in the winter months by a complete spray programme. This would be necessary because parasite efficiency falls off during short days and in periods of low light intensities (Letton 1973, McDevitt 1973). However this break would also permit any *Aphidius* hyperparasites which became established to be destroyed.

Acknowledgements

The authors wish to thank Miss S.J. Brown for performing the laboratory studies.

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THE ACTIVITY OF MORPHOLIN DERIVATIVES IN MIXTURES WITH CARBENDAZIM FOR THE CONTROL OF POWDERY MILDEW OF ROSES

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Summary Tests were conducted, over a period of two years, comparing dodemorph and carbendazim, alone and in mixture, for control of powdery mildew (*Sphaerotheca pannosa*) on roses. All tests were carried out under conditions of severe infection. The mixture gave excellent disease control, proving superior to either material used alone. No phytotoxicity was noted over a wide range of cultivars. Comparison of two formulations of carbendazim indicated that a formulation in oil showed comparable activity at a lower rate of active ingredient. Tests with a mixture of tridemorph and carbendazim showed less advantage than did the dodemorph mixture. In addition some phytotoxicity was recorded with tridemorph.

Resume Des essais comparant le dodemorph et le carbendazim seul et en melange pour lutter contre l'oidium (*Sphaerotheca pannosa*) sur rosier ont ete conduits durant une periode de deux ans. Tous les essais ont ete conduits dans des conditions d'infestation severe. Le melange a donne un excellent controle de la maladie, se revelant superieur a chaque produit utilise seul. Aucune phytotoxicite n'a ete notee sur un grand nombre de varieties. La comparison de deux formulations de carbendazim montre que la formulation huileuse presente une activite comparable a une dose faible de m.a. Les essais avec un melange de tridemorph et de carbendazim ont montre moins d'interet que le melange avec le dodemorph. De plus une certaine phytotoxicite a ete enregistree avec le tridemorph.

INTRODUCTION

The activity of dodemorph against powdery mildew of roses (*Sphaerotheca pannosa*) has been previously reported (Frost & Pattisson 1971). It was shown that dodemorph is very effective under glass, but that outdoors control was less effective - unless the spray intervals were reduced or the concentration increased. The effect of the morpholin derivatives is believed to be mainly curative and it was considered likely that improved control may result by using a mixture including a fungicide with a mainly protectant activity.

Carbendazim - the proposed common chemical name for methyl benzimidazole carbamate - was tested in mixtures. Initially, tests were conducted with a mixture of carbendazim and dodemorph, while later tests were also carried out with a mixture of carbendazim and tridemorph.

METHOD AND MATERIALS

All the trials were laid down in a randomised block design, with a minimum of three replicates. Materials were applied using either a pressurised sprayer or a motorised knapsack mistblower. In each case application was to 'run-off'.

Assessment was made by either dividing the leaflet into quarters and recording the presence or absence of mildew in each quarter, or by counting the number of leaflets infected.

In each case assessment was carried out on the abaxial surface of the leaf, with only young growth being examined.

All assessment figures were converted to percentage for presentation, and then to angles for analysis.

Formulations used:

Dodemorph - an emulsifiable concentrate containing 40% w/v a.i.
Tridemorph - an emulsifiable concentrate containing 75% w/v a.i.
BAS 3460F - a wettable powder containing 50% w/w carbendazim
BAS 3465F - an oil formulation containing 25% w/v carbendazim

RESULTS

Tables 1 and 2 show the effect of a mixture of dodemorph and carbendazim as BAS 3460F at two sites. In each case mildew was severe at commencement of the trial.

Table 1

The comparitive effect of application of dodemorph, carbendazim and a mixture of the two materials to the cv. Papa Meilland on levels of mildew infection

Treatment	conc. a.i. %	% infection of mildew (converted to angles)	Actual % infection
Dodemorph	0.2	29.3	24.0
BAS 3460F	0.05	38.2	38.0
Dodemorph + BAS 3460F	0.1 + 0.025	23.4	15.7
Untreated		69.2	87.5
LSD - p = 0.05		13.3	

Site - Colchester, Essex

Assessment was made 7 days after the third application. The interval between applications was 14 days.

Table 2

The comparitive effect of application of dodemorph, carbendazim and a mixture of the two materials to the cv. Papa Meilland on levels of mildew infection

Treatment	conc. a.i. %	% infection of mildew (converted to angles)	Actual % infection
Dodemorph	0.2	61.2	76
BAS 3460F	0.05	55.2	67
Dodemorph + BAS 3460F	0.1 + 0.025	31.7	28
Untreated		76.5	94
LSD - p = 0.05		10.2	

Site - Oxford

Assessment was made 13 days after the third application. Interval between application was 14 days.

Table 3 illustrates the effect of varying the formulation of carbendazim in mixture with dodemorph. Mildew levels were severe at commencement of the trial.

Table 3

The comparitive effect of application of dodemorph, two formulations of carbendazim and mixtures of dodemorph and carbendazim, to two cvs. on levels of mildew infection

Treatment	conc. a.i. %	% infection of mildew (converted to angles)		Actual infection	
		A	B	A	B
BAS 3460F	0.05	49.3	44.2	57.5	48.5
BAS 3465F	0.0125	44.0	48.7	48.2	56.5
Dodemorph	0.2	35.5	33.1	33.5	30.0
Dodemorph + BAS 3460F	0.2 + 0.025	25.0	17.3	18.0	8.8
Dodemorph + BAS 3465F	0.2 + 0.006	27.2	23.9	21.0	16.5
Untreated		67.0	65.4	84.7	82.5
LSD - p = 0.05		11.5	9.6		

Site A - Colchester, Essex - cv. Papa Meilland. Assessment was made 8 days after the fifth application. The interval between applications was 14 days.

Site B - Oxford - cv. Super Star. Assessment was made 10 days after the third application. The interval between applications was 15 days.

Table 4 illustrates the effect of a mixture of tridemorph and carbendazim. Mildew levels were severe at commencement of the trial.

Table 4

The comparitive effect of application of tridemorph, carbendazim, a mixture of the two materials and dodemorph, to two cvs. on levels of mildew infection

Treatment	conc. a.i. %	% infection of mildew (converted to angles)		Actual % infection	
		A	B	A	B
Dodemorph	0.2	31.3	45.6	27.3	51.0
Tridemorph	0.0375	35.3	43.5	33.5	47.5
Tridemorph	0.075	30.0	36.3	25.0	35.0
Tridemorph + BAS 3460F	0.0375 + 0.025	28.2	31.5	22.5	27.5
BAS 3460F	0.05	42.7	52.5	46.0	63.0
Untreated		55.5	90.0	67.9	100.0
LSD - p = 0.05		6.2	15.9		

Site A - Colchester, Essex - cv. Duke of Windsor. Assessment was made 7 days after the third application. The interval between applications was 14 days.

Site B - Oxford - cv. Super Star. Assessment was made 10 days after the third application. The interval between applications was 15 days.

In trial A, tridemorph at 0.075% a.i. caused slight scorch on the foliage of the cultivar Duke of Windsor.

In all trials BAS 3460F at 0.05% a.i. left a visible deposit on the foliage - this was not apparent with any other treatment. Phytotoxicity was only seen with tridemorph at 0.075% a.i. The mixture of dodemorph and BAS 3460F has been applied across a wide range of cultivars with no signs of phytotoxicity. The information on the mixture of tridemorph and BAS 3460F is limited to the cultivars included in the trials.

DISCUSSION

The results presented in Tables 1 and 2 are from trials carried out in 1972. In each case the mixture of half rates of dodemorph and carbendazim gave superior control of mildew to the full rate of either material used alone. In Table 2 the difference between the mixture and either ingredient was significant at p = 0.05. The levels of mildew at assessment in Table 2 were considerably higher than in Table 1, probably because assessment was carried out at a longer interval after the final application.

Further work was carried out in 1973, and as in 1972, a mixture of half rates of dodemorph and carbendazim gave superior control of mildew to the full rate of either used alone (Table 3). Comparison of two formulations of carbendazim indicated that a formulation in oil (BAS 3465F) showed comparable activity to the wettable powder formulation (BAS 3460F) at a lower rate of active ingredient.

Tests with mixtures of tridemorph and carbendazim (Table 4) showed little advantage in disease control compared with tridemorph alone. Tridemorph applied at 0.75% a.i. caused some leaf scorch on the cultivar Duke of Windsor.

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NOTES

Proceedings 7th British Insecticide and Fungicide Conference (1973)

RESULTS OF 1972 U.K. TRIALS WITH ALDICARB ON GLASSHOUSE ORNAMENTALS

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Summary Trials to compare the efficacy of two 10% granular formulations of aldicarb (Temik 10 G aldicarb pesticide) with commercial spray programmes were conducted on roses and chrysanthemums. Assessments were made for aphid, red spider mite and chrysanthemum leaf miner. The results show that a new coal based formulation is equal in pesticidal activity and persistence to one based on corn cob and that a single application of either provided equal or superior control to that obtained from commercial spray programmes. Aldicarb at 4 and 5 lb/ac, applied to chrysanthemums shortly after planting, provided effective control of aphid, leaf miner and red spider up to marketing. Five and 10 lb/ac on roses controlled aphid and red spider for 9 - 14 weeks. There were no symptoms of phytotoxicity on any of the many cultivars treated.

Résumé Les essais réalisés avaient pour but de comparer deux formulations granulées contenant 10% d'aldicarbe (Témik 10 G) aux programmes classiques de pulvérisation en cultures de roses et de chrysanthèmes. L'appréciation d'efficacité a concerné les pucerons (*Myzus persicae*), les acariens (*Tetranychus urticae*) et les mineuses des feuilles de chrysanthème (*Phytomyza syngenesiae*). Les résultats obtenus montrent d'une part qu'une formulation nouvelle sur support de charbon minéral est équivalente à celle à base de rafle de maïs en ce qui concerne l'activité et la rémanence, d'autre part qu'une seule application de l'une ou l'autre formulation d'aldicarbe donne des résultats égaux ou supérieurs à ceux obtenus avec les programmes conventionnels. L'aldicarbe à 4 et 5 lb/ac, appliqué peu après plantation, a assuré un contrôle efficace des pucerons, des mineuses, et des acariens sur chrysanthèmes jusqu'à la commercialisation des fleurs. Sur roses des doses de 5 et 10 lb/ac ont contrôlé les pucerons et les acariens pendant 9 à 14 semaines. Aucun symptôme de phytotoxicité n'a été visible sur les variétés qui ont été traitées.

INTRODUCTION

During the years 1967 - 1971 researchers, including French and Ludlam (1972), Gould (1968, 1969), Osborne (1970a, 1970b) and Worthing (1967, 1968) investigated the efficacy of aldicarb, formulated as a 10% granule on corn cob, for the control of pests on glasshouse ornamental crops. Remarkably good and persistent control of many pests including peach potato aphid (*Myzus persicae*), red spider mites (*Tetranychus urticae*), chrysanthemum leaf miner (*Phytomyza syngenesiae*), white fly (*Trialeurodes vaporariorum*) and nematodes was invariably achieved without crop phytotoxicity.

A new formulation based on coal was developed for use in the U.K. in 1972 for reasons outside the scope of this paper. The trials reported here were laid down in 1972 with two main objectives, firstly to compare the efficacy of this new formulation with that of the original corn cob and secondly to confirm the many results of other researchers on glasshouse ornamentals.

Fourteen trials, seven each on roses and chrysanthemums were located in Kent, Sussex, Essex and Hertfordshire.

METHOD AND MATERIALS

All trials were of randomised block design with two to eight replicates. Plot size varied between 130 and 800 sq. ft. according to bed size. Owing to the danger of a rapid build-up of pests in high value commercial glasshouse crops, it was not practicable to include untreated controls, nor because of the problem of drift was it feasible to include spray treatments in the block design. However, assessments were made on comparable areas receiving commercial spray programmes which varied at the different sites.

Details of the treatments used at each site are shown in Table 1.

Table 1

Aldicarb in lb a.i./ac

Trial	Crop	Aldicarb coal			Aldicarb corn cob		
1,5,6,7	Roses		5	10			10
2,3,4	"		5	10		5	10
8,9	Chrysanthemums	4	5		4		
10-13	"	4	5			5	
14	"	4	5		4	5	

Granules were applied 1 - 3 weeks after the chrysanthemums were planted, and, when possible, during active vegetative growth on the roses. On the chrysanthemums the application was made overhead either by hand using a 'pepper-pot' or with the Midget Kyoritsu applicator. A 'pepper-pot' or a Horstine Farmery motorised knapsack granule applicator was used on the roses. The motorised applicator was used to blow the granules onto the beds from the side at ground level. A thorough overhead watering was given immediately after each application to ensure soil incorporation.

Assessments were made at approximately 4 week intervals. Fifty leaves per plot or 100 leaves per treatment, sampled at random from the lower half of the plants, were assessed for red spider mites and leaf miners using the scale given below:-

0 =	nil infestation or leaf damage		
1 =	1 - 4 mites per leaf or	5%	leaf mined
2 =	5 - 10 " " " "	15%	" "
3 =	11 - 20 " " " "	30%	" "
4 =	21 - 40 " " " "	60%	" "
5 =	41 or more" " " "	100%	" "

The presence or absence of aphids was also recorded during these observations.

On several trials the incidence of pests after treatment with aldicarb was so isolated and patchy that assessments had to be qualitative rather than quantitative.

RESULTS

I. Effect of Formulation

a) Red spider on roses

Table 2

Control of red spider (mites per leaf)

Trial	6 weeks 10 lb coal	6 weeks 10 lb corn cob	13/14 weeks 10 lb coal	13/14 weeks 10 lb corn cob
1a	17.7	18.0	NR	NR
1b	4.5	0.7	NR	NR
2	0.0	0.0	0.0	0.1
3	0.0	0.1	6.9	7.2
4	0.0	0.0	3.4	2.0

1a - var. Baccara
1b - var. Pink Sensation
NR - Not recorded

b) Red spider on chrysanthemums

Table 3

Control of red spider (mites per leaf)

Trial	7/8½ weeks 5 lb coal	7/8½ weeks 5 lb corn cob	9/10 weeks 5 lb coal	9/10 weeks 5 lb corn cob	15 weeks 5 lb coal	15 weeks 5 lb corn cob
10	0.0	0.0	NR	NR	NR	NR
11	0.1	0.0	0.5	0.2	NR	NR
13	NR	NR	0.0	0.0	0.0	0.0

Results (Tables 2 and 3) show equal efficacy for both formulations in the control of red spider mite. Other data not presented here showed a similar effect on aphid and leaf miner.

II. Comparison of Aldicarb with Grower's Spray Programmes

a) Red spider on roses

Table 4

Control of red spider (mites per leaf)

Trial	Weeks after treatment	5 lb coal	10 lb coal	Growers' spray	Grower's spray programme
1a	6	18.5	17.7	29.7	Formetanate every 14 days
1b	6	7.2	4.5	14.7	" " " "
2	10	0.1	0	0.1	'Plictran'* applied in sixth week
4	10	5.8	1.5	14.9	'Pentac'** applied in fourth week
5	9	0	0	0†	3 x 'Pentac', 3 x dimethoate
6	14	0†	0	0†	4 x 'Pentac', 4 x nicotine
7	13	0†	0	0	2 x 'Plictran'/dimethoate, 1 x 'Pentac'

† Small isolated patches of spider mites.
* 'Plictran' - Tricyclohexyltin hydroxide.
** 'Pentac' - Bis pentachlorocyclopentadienyl.

b) Aphid on roses

Table 5

Control of aphid (% shoots infested)

Trial	Weeks after treatment	5 lb coal	10 lb coal	Growers' spray	Grower's spray programme
3	9	3	1	83	Pirimicarb applied at 8½ weeks
3	11	29	21	91	" " " " "

c) Red spider on chrysanthemums

Table 6

Control of red spider (mites per leaf)

Trial	Weeks after treatment	Aldicarb*	Growers' spray	Grower's spray programme
10	8½	0.0	1.2	Quinomethionate, diazinon, and schradan at 7 weeks
11	9	1.0	16.8	Dichlorvos/pirimicarb at 2 weeks, dicofol/tetradifon at 5 weeks, dicofol/diazinon at 8 weeks
12	10	0.0	0.0	Pirimicarb every 14 days, formetanate or dioxathion every 10 days
13	15	0.0	10.2	Dicofol/diazinon at 6 weeks
14	10	0.0	0.0	DDT/formetanate, quinomethionate/nicotine, 4 x quinomethionate/pirimicarb, 2 x formetanate/pirimicarb, 2 x quinomethionate/DDT

*Mean of aldicarb at 4 and 5 lbs on coal and corn cob.
Isolated patches of aphid, and/or red spider were recorded on trials 12 and 14.
No red spider was found on the aldicarb plots.

Results (Tables 4, 5 and 6) show control of red spider and aphid given by a single application of aldicarb was superior to that provided by commercial spray programmes.

III. Effect of Dose

a) Red spider on roses

Table 7

Control of red spider (mites per leaf)

Trial	6 weeks Grower's spray programme	 5 lb coal	 10 lb coal	10 weeks Grower's spray programme	 5 lb coal	 10 lb coal
1a	29.7	18.5	17.7	NR	NR	NR
1b	14.7	7.2	4.5	NR	NR	NR
2	0.1	0.0	0.0	0.1	0.1	0.0
3	2.1*	0.4	0.0	21.1*	3.1	3.0
4	5.1	0.0	0.0	14.9	5.8	1.5
Mean	10.34	5.22	4.45	12.03	3.00	1.50

*Untreated when assessed.

b) Red spider on chrysanthemums

Table 8

Control of red spider (mites per leaf)

Trial	7/8½ weeks Grower's spray programme	 4 lb coal	 5 lb coal	9/10 weeks Grower's spray programme	 4 lb coal	 5 lb coal	15 weeks Grower's spray programme	 4 lb coal	 5 lb coal
10	1.2	0.0	0.0	NR	NR	NR	NR	NR	NR
11	1.9	0.1	0.1	16.8	2.2	0.5	NR	NR	NR
13	NR	NR	NR	2.5	0.0	0.0	10.2	0.0	0.0

Results (Tables 7 and 8) show only small differences in the control of red spider mite with the range of doses used. The effect on aphid (data not presented) and leaf miner (see Table 9) followed a similar pattern.

IV. Effect of Aldicarb on Chrysanthemum Leaf Miner

Table 9

Control of leaf miner (% mined leaves)

	Trial 8			Trial 9		
Weeks after treatment	4	8	10	4	8	10
Grower's spray programme*	0	20(16)	38(21)	0	28(6)	46(13)
4 lb corn cob	0	0(4)	0(7)	0	0(2)	1(5)
4 lb coal	0	0(4)	0(6)	0	0(4)	3(7)
5 lb coal	0	0	0(2)	0	0(2)	0(3)

Figures in brackets show damage from feeding punctures when no mining occurred.
*Untreated when assessed.

Results show that both formulations of aldicarb at 4 lb a.i./ac prevented all mining for up to 8 weeks and at 5 lb until marketing.

DISCUSSION

The results presented in this paper show that the new coal formulation of aldicarb is as biologically effective as the corn cob formulation in terms of pesticidal activity and persistence. Both formulations gave a similar control of red spider mite, chrysanthemum leaf miner and peach potato aphid. Aphid control on chrysanthemums and carnations (data not presented) followed a similar pattern with both formulations.

Our results also show that all aldicarb treatments gave equal and usually superior control to that obtained by the commercial spray programmes used on the trial sites. These programmes varied from one to four sprays on roses and up to ten sprays on chrysanthemums applied during the period of these trials (Tables 4, 5 and 6).

With one exception, control of red spider on roses was maintained for 9 - 14 weeks by a single application of aldicarb and confirms the results of French and Ludlam (1972).

Initially there was little effect from dose. Control of red spider mite on roses was comparable at 6 weeks from 5 and 10 lb aldicarb. On chrysanthemums, the control of red spider and leaf miner with 4 and 5 lb aldicarb was comparable at 7 and 8 weeks. Likewise dose had little effect on the degree of aphid control. Persistence was however improved on some trials at the higher rates.

The results of the trials on chrysanthemums reflect those of Gould (1968, 1969), Worthing (1967, 1968) and Osborne (1970a, 1970b) who have shown that aldicarb at 2.4 to 5.0 lb a.i./ac provided excellent control of red spider, aphid and leaf miner on chrysanthemums, which usually lasted up to marketing when applied shortly after planting.

No symptoms of phytotoxicity were observed on any of the many rose and chrysanthemum cultivars treated, indicating the high degree of crop safety associated with the use of aldicarb on glasshouse ornamental crops.

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CONTROL OF DISEASES OF GLASSHOUSE CROPS AND ORNAMENTALS WITH THIOPHANATE-METHYL

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Summary Thiophanate-methyl (NF.44) has been evaluated as a fungicide for use in glasshouses for the control of powdery mildew of cucumbers (*Sphaerotheca fuliginea*), roses (*S.pannosa*), and ornamentals, and *Botrytis cinerea* on tomatoes, cucumbers, lettuce and ornamentals.

Replicated and grower trials in heated and coldhouse tomato crops, using foliar sprays of thiophanate-methyl at 16 oz a.i./100 gal + wetter, or 8 oz a.i./100 gal as a soil drench, confirmed good control of stem lesions caused by *Botrytis*, although ghost spotting of fruit was not controlled as effectively.

Against *Botrytis* and powdery mildew of cucumbers, good control was demonstrated using a rate of 8 oz a.i./100 gal as a spray or drench at monthly intervals, although in one case benzimidazole resistance was encountered. Control of glasshouse red spider mite (*Tetranychus urticae*) was noted at one site. Although levels of *Botrytis* on lettuce were low, grower trials demonstrated control with thiophanate-methyl at 8 oz a.i./ 100 gal, and this dose rate was also effective against powdery mildew and *Botrytis* on a wide range of glasshouse-grown ornamentals, and powdery mildew on roses.

Sommaire On a evalué en serre le thiophanate-methyl comme fongicide pour contrôler l'oïdium de concombre (*Sphaerotheca fuliginea*), l'oidium du rosier (*S.pannosa*) et des cultures florales, et pour contrôler le *Botrytis cinerea* des tomates, des concombres, des laitues et cultures florales.

On a affirmé le contrôle efficace des lesions de la tige produit par *Botrytis* avec les essais répétés et du cultivateur en serre chauffée et non-chauffée, utilisant le thiophanate-methyl (100g/hl + le mouillant comme pulverisation foliaire ou 50g/hl comme traitement du sol) bien qu'on n'ait pas contrôlé assez efficacement les tâches sur les fruits.

On a aussi montré un contrôle efficace contre le *Botrytis* et l'oïdium des concombres avec une dose d'emploi de 50g/hl; soit par pulverisation soit par traitement du sol, une fois par mois. Dans un cas, on a trouvé une résistance characteristique du 'benzimidazole'. Dans un essais on a noté un contrôle efficace d'araignée rouge (*Tetranychus urticae*). Bien que les niveaux de *Botrytis* sur les laitues etaient basses, les essais des cultivateurs ont montré un contrôle avec thiophanate-methyl. Aussi, la dose d'emploi etait efficace contre l'oïdium et le *Botrytis*, sur une gamme des plantes cultivées en serre, et aussi contre l'oïdium des rosiers.

INTRODUCTION

Thiophanate-methyl (50% w/w formulation - trade name 'Mildothane*') has previously been shown to control a number of diseases in top fruit (Cole et al 1971) and soft fruit (Gilchrist and Cole 1971). Following successful trials in lettuce, tomatoes and cucumbers at various research centres, Smith & Spencer (1971), Fairfield E.H.S. (Annual Report 1971), Stockbridge House E.H.S. (Annual Report 1971), it was decided to evaluate further the material in glasshouse-grown ornamentals, roses and carnations.

The data contained in this paper concern replicated trials carried out by staff of May & Baker Ltd and Murphy Chemical Ltd., together with user trials where growers applied the material to their own crops.

METHODS AND MATERIALS

(i) Tomatoes The main disease problems of tomato-growing are the conditions caused by infection of *Botrytis cinerea*. This can produce stem lesions, usually following de-leafing, or 'ghost spots' on the fruit which can lead to downgrading. Replicated and user trials were set up during 1972-73 to evaluate spray and drench applications of thiophanate-methyl for *Botrytis* control.

Four replicated trials were set up in 1972. Programmes were based on routine monthly intervals for soil drenches, and 14-day intervals for sprays, commencing on or before disease onset. Drench applications were carried out by pouring one pint of thiophanate-methyl at various dose rates around the base of each plant whilst sprays were applied by means of a knapsack sprayer at a volume rate of about 200 gal/ac. The plots consisted of 10 plants with 4 replications of each treatment at each site.

Seventeen user trials were set up in 1973 to evaluate further the material in tomatoes at a rate of 16 oz a.i./100 gal plus non-ionic wetter as a foliar spray, or 8 oz a.i./100 gal as a soil drench. Sites were selected on both heated and cold crops in the major English tomato-growing areas including a wide range of cultivars. High volume sprays were applied at over 200 gal/ac with hand lances or, in one case, through an overhead sprinkler system, whilst low volume sprays were applied with motorised knapsack equipment. Soil drenches were applied to the base of the plant either through a hand-held hose or through various types of dilutor. One pint of fungicide suspension was applied to each plant during drenching. The treatments were carried out by the growers themselves, and seven growers agreed to leave untreated controls in the same house.

Planting took place from February until June - later plantings being unheated crops. All trials were on crops growing directly into sterilised soil. The growers were encouraged to adhere to a 14 day programme if spraying was their normal practice, or a monthly programme in the case of soil drenches. Treatments commenced either at onset of disease or, where regular spraying was the usual practice, the initial application was made at about the two-truss stage.

Following queries from growers about the possibility of applying thiophanate-methyl through dilutors, normally used for liquid feeds, an exercise was mounted to examine such a feasibility. The system was first calibrated using water to determine the variation in flow rate at various points in the house. 0.5 lb a.i. thiophanate-methyl, was made up in a suspension of water before being poured into the 2 gallon Cameron dilutor tank. The coarsest dilution rate (1:50) was used which allowed the fungicide to be applied as quickly as possible. The 2 gallon of

* Registered trade mark of May & Baker Ltd.

suspension was thus distributed in 100 gallons of water and as the throughput of each nozzle was 1 pint every 20 minutes, the system was allowed to operate for that time when samples were taken from various positions in the house for chemical analyses.

(ii) Cucumbers Three replicated trials were carried out in 1972 to compare soil drenches and H.V. sprays for the control of powdery mildew (*Sphaerotheca fuliginea*) and grey mould (*Botrytis cinerea*) on cucumbers. For the soil drenches 1 pint of suspension of various rates (4 - 12 oz a.i./100 gal) was poured around the base of each plant whilst sprays (6 - 10 oz a.i./100 gal) were applied by a knapsack sprayer at the rate of 200 gal/ac. Each treatment consisted of seven plants replicated four times at all sites. An additional nine user trials were carried out in 1973 to confirm the 1972 results. These trials were located in various regions and included a wide range of varieties and growing conditions. High and low-volume foliar sprays and soil drenches were included, the latter being favoured by those growers employing biological control methods. In most cases untreated areas were left, and, in addition, thiophanate-methyl was compared with benomyl. Thiophanate-methyl was recommended to be used at 8 oz a.i./100 gal as a spray or drench to be carried out at monthly intervals.

(iii) Lettuce The incidence of grey mould on lettuce can be a serious problem, especially on those crops grown under glass under conditions of poor ventilation. Five trials, involving a range of cultivars, were carried out by lettuce growers in various parts of the country. The area sprayed with thiophanate-methyl was approximately one-tenth of an acre at each site. The fungicide was used at 8 oz a.i./100 gal. In three trials the fungicide was compared with benomyl - the growers standard - and untreated plants, and in two other trials with benomyl alone. Two trials were sprayed using a Cheshunt sprayer, two with a knapsack sprayer, and one through overhead irrigation. Spraying at 14 day intervals was recommended, but participating growers tended to revert to the previous practice of applying 2 sprays only in a 6-8 week season, paying particular attention to protection of the newly planted lettuce. Zineb was often added to thiophanate-methyl for the control of downy mildew, which can be as great, or more of a problem then grey mould.

(iv) Ornamentals Thiophanate-methyl was treated on a wide variety of ornamental pot plants for disease control (mainly powdery mildew and grey mould) and species tolerance. Rates used were 4, 8 and 12 oz a.i./100 gal applied through an ASL Killaspray. H.V. foliar application or soil drenches were used and comparisons made with benomyl and untreated controls. Treatments were replicated 3 or 4 times and assessments varied depending on crop and disease (see Table 4).

(v) Roses Four replicated trials are reported - three under glass and one field crop where thiophanate-methyl was applied for the control of powdery mildew. (Tables 4 & 6)

RESULTS AND DISCUSSION

Both the replicated and user trial results from 1971 and 1972 are presented in the following tables, together with discussion.

Table 1

Control of tomato *Botrytis* - 1972 replicated trials

Treatment	Dose rate oz a.i. 100 gal	Mean No. main stem lesions/plant*				
		Site 1		Site 2	Site 3	Site 4
SPRAYS						
thiophanate-methyl	8 oz	-	-	0.44	1.00 b	0.25 bc
" "	12 oz	-	-	1.31	0.35 a	0.28 bc
" "	16 oz	-	-	0.12	0.25 a	0.48 c
thiophanate-methyl + wetter ∅	16 oz	-	-	0.94	0.10 a	0.18 ab
benomyl + wetter ∅	8 oz	-	-	0.44	0.30 a	0.20 ab
DRENCHES						
thiophanate-methyl	4 oz	0.87	1.50 b	0.44	0.30 a	0.09 ab
" "	6 oz	0.50	0.87 ab	1.25	0.25 a	0.11 ab
" "	8 oz	0.44	0.75 ab	0.31	0.10 a	0.04 a
" "	12 oz	0.31	0.19 a	-	-	-
benomyl	4 oz	0.94	0.94 ab	0.31	0.10 a	0.06 ab
Control	-	1.12	7.06 c	2.06	1.60 c	0.85 d
Date assessed		3/8	12/10	25/8	10/11	12/12
Cultivars		Moneycross		Eurocross BB	Moneycross	Stacos

* Most lesions resulted from fungal infection of leaf scars after trimming.
∅ Alkyl aryl polyoxyethylene glycol - non ionic, used at manufacturer's recommended rate for tomatoes.

Duncan's Multiple Range Test: (Duncan 1955) figures suffixed by the same letter are not significantly different at the 5% level. Comparisons valid within sites only.

Assessments on 3/8, 25/8 not significant.

(i) Tomatoes The results of the replicated work (Tables 1 and 2) indicate little difference in *Botrytis* control between fortnightly sprays and monthly drenches, although in trials at Fairfield E.H.S. (Annual Report 1971), monthly sprays were found to be more effective than monthly drenches. The same workers found foliar sprays of 10 oz a.i. thiophanate-methyl/100 gal gave good control of stem *Botrytis*, whilst in our own work, best control wasobtained with the highest rates tested, viz. 16 oz a.i./100 gal. The addition of a non-ionic wetter improved disease control, especially ghost spotting. A rate of 8 oz a.i. thiophanate-methyl/100 gal applied at 1 pint per plant gave the most satisfactory control of grey mould when used as a soil drench.

No phytotoxicity was noted to any of the 24 cultivars checked. In the user trials a grower who used the material through overhead irrigation equipment complained of chemical deposit on the fruit; this was hardly surprising in view of the large volume of suspension - 1000 gal/ac - used in the trial.

Disease levels were low throughout the user trials, and comparisons between the treatments were therefore difficult. There appeared to be little difference in the numbers of stem lesions between treated and untreated plants. Both drenches and sprays produced this effect although, unlike the previous replicated work, it was not possible to compare both application methods at the same site. No significant effect was noted against ghost spotting in the user trials - a fact previously shown by workers at Fairfield E.H.S. (Annual Report 1971) and Smith & Spencer (1971).

Chemical analyses of samples from the various points in the house in which thiophanate-methyl was applied by dilutor, confirmed the ability of this equipment to apply a fairly uniform dose of fungicide over the treated area. Care would be required to ensure that such a system was initially clean, since admixture in the lines with previously applied nutrients could lead to incompatibility which in turn could lead to line blockage. Four participating growers used thiophanate-methyl through similar systems to that described above, and, whilst infection was low, they appeared satisfied with this method of application.

Table 2

Tomatoes - Control of ghost-spot (*Botrytis cinerea*) - 1972 replicated trials

Treatment	Dose rate oz a.i./100 gal	% fruit ghost-spotted* Site 2	% fruit ghost-spotted* Site 3
SPRAYS			
thiophanate-methyl	8 oz	14.0	13.5
" "	12 oz	21.5	16.7
" "	16 oz	15.4	5.2
thiophanate-methyl + wetter ø	16 oz	10.0	1.7
benomyl	8 oz	21.6	6.0
DRENCHES			
thiophanate-methyl	4 oz	23.0	0.0
" "	6 oz	25.2	8.8
" "	8 oz	27.7	6.5
benomyl	4 oz	19.4	4.8
Control	-	26.1	25.0
Date assessed		26/7	27/11

ø alkyl aryl polyoxyethylene glycol - non ionic, used at manufacturer's recommended rate for tomatoes.

* two trusses on three plants per plot assessed.

(ii) Cucumbers Replicated trial results (Table 3) indicate the effectiveness of both sprays and drenches against powdery mildew. At Site 1 mildew spread rapidly after the third drench, and, whilst untreated plants were severely weakened, 8 oz a.i./100 gal thiophanate-methyl gave good protection against the disease, confirming previous work carried out at Stockbridge House E.H.S. (Annual Report 1971) and at the Glasshouse Crops Research Institute (Smith & Spencer 1971). The curative property of thiophanate-methyl at this dose was also demonstrated when the infected controls were treated to prevent spread of the disease. At Site 2, mildew levels were low, although spray and drench treatments of thiophanate-methyl provided control. At a third site, a benzimidazole-resistant strain of mildew was encountered which was not controlled by either thiophanate-methyl or benomyl.

Table 3

Control of cucumber diseases - 1972 replicated trials

Treatment	Dose rate oz a.i./ 100 gal	Mean mildew index per leaf (0-5 scale) + Site 1			Site 2	Mean No. Botrytis lesions per plant Site 1
SPRAYS						
thiophanate-methyl	6 oz	-	-	-	0.19 a	-
" "	8 oz	-	-	-	0.08 a	-
" "	10 oz	-	-	-	0.04 a	-
thiophanate-methyl + wetter	8 oz	-	-	-	0.04 a	-
benomyl	4 oz	-	-	-	0.01 a	-
DRENCHES						
thiophanate-methyl	4 oz	0.46 c	2.57 c	1.18 abc	0.12 a	2.96 bc
" "	6 oz	0.19 b	1.06 b	0.86 abc	0.13 a	1.96 ab
" "	8 oz	0.03 a	0.35 a	0.43 ab	0.09 a	2.04 ab
" "	12 oz	0.02 a	0.26 a	0.27 a	-	1.25 a
benomyl	3 oz	0.58 c	2.40 c	2.06 c	0.09 a	2.92 bc
Control	-	3.02 d	4.86 c	1.65* bc	1.20 b	3.80 c
Date assessed		3/8	14/8	13/10	2/10	3/8
Cultivar			Sporu		Femdam	
Growing medium			Straw bales		Soil	

* All control plots at Site 1 were drenched with thiophanate-methyl 8 oz a.i./100 gal on 14.8.72 and 12.9.72 to save them from dying off due to crippling mildew infection.

+ 0 = no infection 5 >50 mildew lesions/leaf.

Duncan's Multiple Range Test: figures suffixed by the same letter are not significantly different at the 5.0% level. Comparisons valid within sites only.

Grey mould infection occurred only at Site 1, and 8 oz a.i./100 gal thiophanate-methyl gave improved control over the recommended rate of benomyl where these materials were used as soil drenches.

In the user trials little disease was seen, although in cases where powdery mildew started to build up, thiophanate-methyl appeared to reduce the infection confirming previous trial work. At one site, a response to glasshouse red spider mite was recorded and a 95% reduction of active mites noted. Acaricidal activity of thiophanate-methyl had previously been noted by Cranham et al (1971) and this activity would appear to be a useful bonus in a glasshouse situation. At another site improved yield was obtained, probably due to control of unspecified root diseases.

(iii) Lettuce Disease levels were generally low, but at one site, 60% of untreated lettuce were infected with grey mould compared with 10% on thiophanate-methyl areas. At a second site, 14 and 12 plants respectively were infected from thiophanate-methyl and benomyl plots out of a treatment total of 1250.

Table 4

Control of diseases of glasshouse-grown ornamentals

Crop	Disease	No. of sprays	Percent disease control: thiophanate-methyl a.i./100 gal 12 oz	8 oz	4 oz	benomyl a.i./100 gal 4 oz	Control Infection Level
Begonia (leaf)	Mildew	3	100	100	69	87	1.6 *
" (flower)	"	3	72	50	31	22	36.0 ⊕
Cineraria	"	2	-	100	-	92	12.5 +
Cyclamen	Botrytis	1	-	98	-	88	64.0 ⊕
Hedera	Mildew	3	100	100	99	100	23.7 *
"	Botrytis	3	100	92	100	93	1.3 x
Hydrangea	Mildew	3	99	84	97	81	12.5 ≁
Kalanchoe	"	2	100	100	100	100	0.9 *
Mini-rose	"	3	100	95	39	80	2.1 x
Pelargonium	Botrytis	2	100	75	87	62	2.0 x
Rose - Lovita canina	Mildew	3	68	60	66	48	26.0 *
" - Pink Sensation	"	4ø	60	50	26	49	86.0 *
Saintpaulia	Botrytis	3	82	100	100	100	0.7 *
"	"	3	94	89	74	82	2.0 x

Assessments reported are those made within two weeks of final spray application.

* Percent leaf cover by mildew
⊕ Percent infection
\+ No. leaves infected
x Disease severity grade 0-3 scale
≁ No. mildew spots/leaf
ø Final two sprays with added wetter

Other species (including above) treated without damage to 8 oz a.i./100 gal:-

Abutilon, Aphelandra, Azalea, Calceolaria, Capsicum (ornamental), Carnation, Chlorophytum, Chrysanthemum, Coleus, Freesia, Fuchsia, Gloxinia, Impatiens, Peperomia, Pilea, Poinsettia, Primula, Rhoicissus, Schizanthus, Sideritus, Tradescantia, Zygocactus.

(iv) Glasshouse-grown ornamentals A wide range of glasshouse-grown ornamentals (flowering pot plants, house plants, etc.) were treated and results against diseases such as grey mould and powdery mildew (Table 4) have shown thiophanate-methyl at 8 oz a.i./100 gal to be as effective as commercial standards such as benomyl. Disease tolerance of grey mould to both these materials has been encountered at one or two locations, and this has usually been associated with previous regular use of benomyl (e.g. in one case six applications in 11 weeks). In this sort of situation, cross-tolerance to thiophanate-methyl is inevitable.

Table 5

Control of grey mould on cyclamen

Sprayed: 16.4.73 (one application only - foliar spray)

Treatment	a.i./100 gal	Percent Botrytis infected leaves (days after spraying) 15	22	39
thiophanate-methyl	8 oz	1.6	3.0	22.2
benomyl	4 oz	8.0	10.6	49.2
water control	-	64.0	74.5	85.0

A persistence trial on cyclamen (Table 5) showed that efficacy of thiophanate-methyl against grey mould started to decline after about 3 weeks. Up to this time excellent control was obtained compared with that from benomyl.

Table 6

Control of Rose powdery mildew (Sphaerotheca pannosa)

Treatment	% a.i. HV	Under glass * Percent mildew leaves	buds	Field crop ≁ Percent mildew leaves	buds
thiophanate-methyl + wetter	0.05	2.7	10.8	5.5	0.0
chloraniformethan	0.025	0.4	9.6	1.5	3.0
wetter alone	0.025	27.5	88.0	24.4	51.0
water control	-	52.0	94.0	26.5	73.0

* Glass:- 4 sprays at weekly intervals - assessed 1 week after last application
≁ Field:- 5 " " fortnightly " " " " "

(v) Roses Initial experimental work in 1972 was continued in 1973 and two typical results - one under glass and one field situation - are shown in Table 6.

In both cases, thiophanate-methyl with the addition of non-ionic wetter significantly reduced the level of powdery mildew infection, though the level of control on the leaves is slightly inferior to that given by chloraniformethan. On the flower buds, however, control matches that given by chloraniformethan. Results from two additional replicated trials, are included in Table 4 and indicate good control with 8 oz a.i./100 gal thiophanate-methyl compared with benomyl at 4 oz a.i./ 100 gal.

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CONTROL OF VARIOUS DISEASES IN GREENHOUSE GROWN HORTICULTURAL CROPS WITH THERMALLY INDUCED DUSTS OF CHLORTHALONIL IN SICILY

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Summary Trials were carried out in greenhouses in Sicily, to test the activity of a sublimable formulation of chlorthalonil against the main pathogens of certain horticultural crops.

The results obtained have provided evidence of the product's efficacy against the micro-organisms considered except those agents of powdery mildew and they have allowed us to distinguish, for each parasite, in an environment particolarly favourable to diseases, the safety interval between treatments. The advantages gained by a smoke-bomb treatment of easy and speedy execution are also considered.

Résumé Sont considerées des essais de la lotte fongicide réalisées en serres en Sicile, per veréfier l'activité contemporainne de la formulation sublimable de chlorthalonil contre les pathogènes principales d'alcunes coltures maraichères.

Des résultats obtenus ont mis en évidence l'efficacité du produit contre les microrganismes considerés sauf ces agents di Oidium et ils nous ont permis de distinguer, per chacun parasite, dans un ambiant particolièrement favorable aux maladies, l'intervalle de sécurité entre traitements. En outre, les avantages gagnés d'un traitement fumogène de facile et rapide exécution sont contemplés.

INTRODUCTION

The wide spectrum of chlorthalonil's activity against pathogens present in vegetable crops (Turner et al. 1964; Turner et al. 1965) , and its inherent property of self-sublimation, if it is heated to more than 100°C and of auto-condensation upon cooling its vapour to form a very fine dust, (Lockhart 1963), has led us to consider a special formulation of 20% active ingredient chlorthalonil which holds particular interest for the control of greenhouse diseases. At the beginning of 1969, (Ruggeri 1970), we undertook a series of experiments in the provinces of Ragusa and Syracuse (Sicily), where there are approximately 8000 hectares of greenhouses and where, because of the climatic and agricultural conditions, the results of fungicidal control by means of the customary products in wettable powder form do not always prove satisfactory.

In this island, in fact, the greenhouses made of wood, each of which is covered by plastic and covers a surface area of 1000m^2, for the most part, are not heated artificially. Therefore, it follows that the crops, submitted to great changes in temperature from the usually warm day to the fresh night, are quite sensitive to infection on account of this unbalance. Moreover, the sandy ground, the high temperatures and the increased production necessitate frequent irrigation which stimulates a high humidity, thus, encouraging epidemics. The rise of diseases is also facilitated by the lavish applications of nitrogenous fertiliser, by the continual reintroduction of the same crop for years upon the same plot, by the co-existence of diverse species in the same greenhouse and, finally, by the density of planting. The crops, cultivated either alone or together, are: tomato, egg-plant, peppers, French bean, vegetable marrow, cucumber and water-melon.

Keeping in mind the properties of sublimable chlorthalonil, we have made a series of trials on these crops in order to:

- test the fungicidal action of only one active ingredient against the majority of pathogens in these crops, hoping to avoid the frequent errors made by farmers who are not very experienced in preparing mixtures or in recognising diseases
- reduce the total of treatments, given the high persistence of the active ingredient
- to avoid the distribution of water upon the plants during the treatment so as not to augment the humidity in the environment
- to reduce the visible residues on the crops
- to put at disposal a product of speedy and easy use, to be used within a very short period after the occurence of the conditions favourable to infections.

METHOD AND MATERIALS

The experiments were carried out in standard greenhouses of $1000m^2$, of average height 2m, and were arranged in series, all, as far as was possible, under identical conditions.

Each greenhouse was considered a unit and then the crop contained therein was treated, from the beginning to the end of the cycle, with the formulation indicated in Tables I to 5. In every greenhouse, the fungicidal action against only one pathogen of each product was estimated at a fixed interval between one application and another. In order to ascertain the presence of the pathogen, artificial inoculation was performed, withdrawing infected material from other greenhouses and preparing acqueous suspensions of conidia with them. For each crop and for each parasite an inoculated greenhouse was used as an untreated control. No replicates were used because the methodology employed and the large number of plants under observation seemed to us adaguate to guarantee results; the total number on $1000m^2$ was as follows: 3500 tomato-plants, 2500 peppers, 1500 egg-plants, 1000 vegetable-marrows, 2300 cucumbers. The transplantation of tomato, pepper and egg-plant occurred in October while that of vegetable-marrow and cucumber in November. On all these crops the treatments were carried out at fixed intervals of 6-8-10-12 days from the date of transplantation to the end of the diseases' appearance, using as aforementioned, a greenhouse for each product and for each interval in the cases of the campaign against Phythopthora, Pseudoperenospora, Alternaria, Botrytis, Cercospora, Colletotrichum and Cladosporium . As to zineb and maneb, the treatments are usually made every 5-6 days. Against Leveillula and Erysiphe, besides their respective basic treatment, dinocap was added 7 times (Tables 3-4-5); against Botrytis specific products were added (benomyl, folpet) at the start and at the end of blossoming and were applied at fruit-setting and during maturation for a total of 5 treatments.

In all, 15 treatments were applied to tomato and egg-plant and 10 to pepper, cucumber and vegetable-marrow, using 0.5 l./1000m^2 of mixture each time according to the stage of vegetation for all the products except chlorthalonil. The application of the latter was made for every 250 m^3 of environmental volume by placing an open can which contained 20g. of active ingredient upon the central corridor of the greenhouse and activating the sublimation by means of a paper match. The dosages of the other products were, in terms of active ingredient per litre water: zineb, maneb and mancozeb 2g., dinocap 0.25g., zineb & benomyl 2g. & 0.3g., maneb & folpet 2g. & 1.5g. The treatments were always carried out in the afternoon when the internal temperature of the greenhouse did not pass the 25°C mark.

The results were assessed in three different schedules, removing every time for each assessment 2000 leaves and 2000 fruits and surveying the recent infections upon them. We did not assess the infected surface area but, rather, the number of organs affected, measuring the success of the results by the complete healthiness of the leaves and fruits and not by the extent of their lesions. The results obtained, expressed in percentage infection are reported in Tables I to 5.

RESULTS

Valid conclusions can be drawn from the results. All the products have shown a varying effectiveness according to the interval between treatments but chlorthalonil, on the whole, proved itself the most persistent as is indicated below.

Chlorthalonil applied regularly every 6-8 days gave good control of *Phythopthora*, *Alternaria*, *Septoria*, *Cladosporium*, *Cercospora*, *Pseudoperenospora* and *Colletotrichum*. Using the same intervals, very satisfactory results were also obtained against *Botrytis*. Used every 10 days, the degree of infection found was still acceptable with the aforementioned pathogens while at every 12 days it was effective only against *Cercospora* and *Septoria*. As to its efficacy against *Leivellula* and *Erysiphe*, in both cases it was poor. Against *Phythopthora*, this active ingredient has shown some capability in blocking the predeveloped infections. Zineb and maneb gave results inferior to those already described against *Phythopthora*, *Cladosporium* and *Alternaria* at all different intervals. Their results were good, on the contrary, against *Septoria* and *Cercospora* up to 10 days. Dinocap gave an excellent and satisfactory control over *Leivellula* and *Erysiphe* in all the trials. Zineb and benomyl in their anti-grey mould capacity behaved similiarly to chlorthalonil used alone while the mixture of maneb and folpet employed for the same purpose proved inadaquate in all the applications.

In conclusion, we have had confirmed the wide spectrum of chlorthalonil's activity and its ability to protect greenhouse vegetable crops, even in its sublimable formulation, from the most common and serious pathogens except powdery mildew. As a side observation to the tests, we calculated the average time necessary to treat a standard greenhouse. With smoke-bomb chlorthalonil, the treatment took 10 minutes while the products in wettable powder form needed one and a half hours, using a nozzled tube atomizer. No phytotoxicity was observed in the trials with chlorthalonil, even when the treatment co-incided with the blossoming period. What is more, the vegetation did not show any residues visibly from the formulation.

DISCUSSION

Substantially the tests gave the expected results; in the first place, the possibility to control different parasites with a single product including Botrytis against which the results obtained are not essentially different from those gained by 5 specific treatments of benomil. It is advisable to resort to mixtures of chlorthalonil and other active ingredients in the Sicilian environment, only for crops prone to powdery mildew, for which we prescribe the addition of chinocap or sulphur.

Referring to the intervals between treatments, we consider, for the sake of brevity, only tomato as the crop most affected by diseases of all the crops mentioned; we can, therefore, suggest 8 days as the safety interval for chlorthalonil, whose schedule ranges from the middle of December to the middle of March, when normally in the case of zineb and maneb, one must make applications every 5-6 days. After transplantation and towards the end of the cycle, one can treat every I2-I5 days. This greater persistence allows one to omit 4-7 treatments, a factor which taken together with the fact of not having to add other active ingredients and of less time required for the application, gives a greater profitability to the crop. One could argue in the same way for the other crops as well. We find particularly interesting the possibility of not being compelled to wet the vegetation during treatment, in an environment where for many days and for many hours a day the humidity passes the 90% mark, and of being able to disinfect the wooden structure of the greenhouses by means of the smoke clouds.

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Table I

The effect of applications of thermally induced dusts of chlorthalonil on tomato

Treatment	every 6 days fruits	every 6 days leaves	every 8 days fruits	every 8 days leaves	every IO days fruits	every IO days leaves	every I2 days fruits	every I2 days leaves	fruits	leaves
	% Phythophthora infestans infection									
Chlorthalonil	0.0	0.0	0.0	0.0	0.5	0.9	II.3	I7.4	-	-
Zineb	4.I	7.3	6.5	IO.2	27.4	39.5	32.8	59.2	-	-
Maneb	3.9	8.7	3.8	I2.6	25.2	43.8	40.3	62.4	-	-
Untreated	-	-	-	-	-	-	-	-	54.4	87.2
	% Alternaria solani infection									
Chlorthalonil	0.0	0.0	0.0	0.0	0.0	0.7	I.9	2.5	-	-
Zineb	2.I	4.0	3.5	5.7	4.6	5.8	7.2	IO.4	-	-
Maneb	3.0	3.2	2.9	2.5	3.5	4.7	8.0	II.3	-	-
Untreated	-	-	-	-	-	-	-	-	I4.3	43.I
	% Botrytis cinerea infection									
Chlorthalonil	0.6	0.0	I.3	0.2	I.9	I.5	8.I	7.0	-	-
Zineb&benomyl	0.4	0.0	I.0	0.4	2.3	2.0	3.5	2.8	-	-
Maneb&folpet	3.5	I.2	4.5	3.0	7.4	5.2	IO.3	9.5	-	-
Untreated	-	-	-	-	-	-	-	-	55.7	26.4

Treatment	leaves	leaves	leaves	leaves	leaves
	% Septoria lycopersici infection				
Chlorthalonil	0.0	0.0	0.0	I.3	-
Zineb	0.0	0.0	I.3	7.4	-
Maneb	0.0	0.0	I.7	5.2	-
Untreated	-	-	-	-	33.4
	% Cladosporium fulvum infection				
Chlorthalonil	0.0	0.0	I.9	22.6	-
Zineb	3.5	7.8	I6.5	44.5	-
Maneb	4.0	8.0	I3.4	40.I	-
Untreated	-	-	-	-	7I.9

Table 2

The effect of applications of thermally induced dusts of chlorthalonil on egg-plant

Treatment	every 6 days fruits	every 6 days leaves	every 8 days fruits	every 8 days leaves	every IO days fruits	every IO days leaves	every I2 days fruits	every I2 days leaves	fruits	leaves
	% Phythophthora infestans infection									
Chlorthalonil	0.0	0.0	0.0	0.0	0.3	0.6	IO.6	I5.2	-	-
Maneb	I.8	5.6	4.3	8.2	22.5	33.6	39.4	46.2	-	-
Zineb	2.0	5.0	4.0	IO.4	20.0	26.I	40.6	40.3	-	-
Untreated	-	-	-	-	-	-	-	-	47.4	56.9
	% Botrytis cinerea infection									
Chlorthalonil	0.5	0.0	2.0	0.6	2.5	I.4	7.0	5.2	-	-
Maneb&folpet	5.4	3.0	II.2	8.6	I9.7	IO.8	24.I	I2.5	-	-
Zineb&benomyl	0.0	0.0	0.0	0.0	2.0	I.0	4.5	3.0	-	-
Untreated	-	-	-	-	-	-	-	-	44.3	20.6

Table 3

The effect of applications of thermally induced dusts of chlorthalonil on pepper

Treatment	every 6 days fruits	every 6 days leaves	every 8 days fruits	every 8 days leaves	every 10 days fruits	every 10 days leaves	every 12 days fruits	every 12 days leaves	fruits	leaves
% Phythophthora capsici infection										
Chlorthalonil	0.0	0.0	0.0	0.0	0.0	0.2	I.6	5.3	-	-
Zineb	0.0	2.3	2.0	7.8	5.6	I2.0	I0.5	27.2	-	-
Maneb	0.0	2.5	4.I	9.6	5.8	I7.0	8.3	3I.9	-	-
Untreated	-	-	-	-	-	-	-	-	40.2	55.4
% Botrytis cinerea infection										
Chlothalonil	0.0	0.0	0.0	0.0	I.0	0.2	5.3	4.0	-	-
Zineb&benomyl	0.0	0.0	0.0	0.0	I.0	0.0	4.0	4.0	-	-
Maneb&folpet	I.4	0.0	5.6	I.2	I0.4	5.6	I9.3	5.6	-	-
Untreated	-	-	-	-	-	-	-	-	47.5	I8.3

Treatment	every 6 days leaves	every 8 days leaves	every 10 days leaves	every 12 days leaves	leaves
% Leveillula taurica infection					
Chlorthalonil	I0.3	I5.5	I9.5	33.2	-
Dinocap	0.0	0.0	0.0	2.0	-
Untreated	-	-	-	-	56.2
% Cercospora unamunoi infection					
Chlorthalonil	0.0	0.0	0.0	I.4	-
Zineb	0.0	0.0	0.5	5.2	-
Maneb	0.0	0.0	0.7	5.0	-
Untreated	-	-	-	-	3I.4

Table 4

The effect of applications of thermally induced dusts of chlorthalonil on cucumber

Treatment	every 6 days leaves	every 8 days leaves	every 10 days leaves	every 12 days leaves	leaves
% Erysiphae chicoriacearum infection					
Chlorthalonil	7.2	II.3	I7.5	29.I	-
Dinocap	0.0	0.0	0.0	3.0	-
Untreated	-	-	-	-	7I.2

Treatment	fruits	leaves	fruits	leaves	fruits	leaves	fruits	leaves	fruits	leaves
% Cladosporium cucumerinum infection										
Chlorthalonil	0.0	0.0	0.0	0.0	0.0	I.3	I0.2	8.5	-	-
Mancozeb	0.0	0.0	0.0	I.3	0.0	I.8	I3.5	I0.2	-	- -
Untreated	-	-	-	-	-	-	-	-	5I.4	40.2
% Colletotrichum lagenarium infection										
Chlorthalonil	0.0	0.0	0.0	0.0	0.0	0.0	7.2	5.4	-	-
Mancozeb	0.0	0.0	0.0	0.0	0.0	0.0	6.3	6.0	-	- -
Untreated	-	-	-	-	-	-	-	-	43.5	2I.4

Table 5

The effect of applications of thermally induced dusts of chlorthalonil on vegetable marrow

Treatment	% Erysiphae chicoriacearum infection				
	every 6 days	every 8 days	every IO days	every I2 days	
	leaves	leaves	leaves	leaves	leaves
Chlorthalonil	5.4	IO.6	I8.2	32.5	-
Dinocap	0.0	0.0	0.0	I.6	-
Untreated	-	-	-	-	68.4
	% Pseudoperenospora urbensis				
	leaves	leaves	leaves	leaves	leaves
Chlorthalonil	0.0	0.0	0.0	IO.4	-
Zineb	0.0	I.5	7.2	25.2	-
Untreated	-	-	-	-	73.8

NOTES

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RECENT WORK ON THE BIOLOGY OF THE SHEEP HEADFLY IN BRITAIN

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Summary The muscid fly *Hydrotaea irritans* is a non-biting species which occurs throughout Britain and can cause serious irritation by swarming around the heads of livestock. In the north of England and southern Scotland the species is associated with self-inflicted lesions on the heads of sheep, especially those of a horned breed or those without woolled heads. Larvae do not appear to feed on manure in the farm environment but to breed in leaf litter in thickets and woodland. The fly appears mainly to pass through a single generation in the year: males appear first but almost entirely disappear before the majority of female flies are found. *H. irritans* avoids low humidity and is inactive in strong winds. The present problem may be the result of short-term population change, but the introduction of non-vulnerable flocks into affected areas may be the most effective long-term measure to reduce headfly losses.

Résumé *Hydrotaea irritans* est une espèce non-piqueuse qui se rencontre partout dans la Grande Bretagne et qui peut occasioner une irritation sévère a force de pulluler autours des têtes des bestiaux. Au nord de l'Angleterre et au sud de l'Écosse, l'espèce s'associe a des lésions infligées par les moutons sur leurs propres têtes surtout chez ceux d'une race à cornes ou à têtes dépourvues de laine. Les larves, à ce qu'il paraît, ne se nourrissent pas sur le fumier aux alentours de la ferme, mais multiplient dans la litière de feuilles des bois. La mouche complète, pour la plupart, une génération pendant l'année: les mâles apparaissent les premiers, mais disparaissent à peu près tous avant que l'on retrouve la plupart des femelles. *H. irritans* évite une basse humidité, et se montre inactive dans les vents forts. Le problème actuel peut découler d'une variation éphemère de la population, mais l'introduction de troupeaux non-vulnérables dans les régions affectées pourrait être, a long terme, la mesure la plus efficace pour réduire les pertes.

INTRODUCTION

The muscid fly *Hydrotaea irritans* is a non-biting species not unlike the common housefly, and is familiar throughout Britain through its habit of swarming around woodland in the summer and feeding avidly on perspiration. It causes serious irritation to livestock by swarming round their heads to feed on eye and other secretions, and in certain areas in the north has, over the last three years, led to a high proportion of head lesions in sheep. These injuries are self-inflicted, and caused by rubbing against trees or scratching with a foot in attempts to alleviate the irritation. Once the head skin is broken the resulting sore is even more attractive to the flies. It becomes more severe and often secondarily infected, and the animal lies under a hedge and may scarcely feed.

The problem is not new, and a variety of traditional remedies are in use which were widespread several decades ago. However, it is now assumed by many farmers that the newer organophosphorus sheep-dips marketed for protection against the sheep blowfly will also give protection against headfly worry. The result has been bitter complaints about 'non-effectiveness' of treatments which make virtually no contact with a fly which scar cely settles on the fleece at all.

It soon became apparent that there was no ready answer to the call for effective control measures especially in the absence of the most basic information on the biology and behaviour of the fly, and a project was begun at Weybridge at the end of 1971 to learn more about this species. Observations were made on fly populations from the beginning of the 1972 fly season and this has been extended in 1973. The investigation also involved maintenance of flies in the laboratory and observations on developmental stages in the laboratory and in the field.

FIELD WORK ON H. IRRITANS POPULATIONS

METHODS

Although headfly damage is rarely seen in the south of England, H. irritans is numerous in many places within reach of the laboratory at Weybridge, and the fly population studies were based on standardised catching in two areas. These were of different types: a hill site with extensive open spaces, high on the South Downs near Dorking, and a lower area in thick woodland at Ottershaw, near Woking.

At a number of marked catching stations in the Downs area timed catches were made weekly throughout the summers of 1972 and 1973. At Ottershaw, however, daily catching for longer periods was carried out as this was the main source of flies for laboratory colonies. On their return to the laboratory the sexes and reproductive stages of the flies were recorded.

RESULTS

Despite the apparent differences between the two areas the results were generally in agreement. Totalled weekly results for the Downs catches during the two seasons are shown in Fig. 1. A remarkable but entirely consistent feature is the early appearance of many male flies and their early disappearance just as the number of females began to rise. A similar result was obtained this year in Northumberland (N. French, pers. comm.). Curiously, the appearance of spermatozoa in the spermathecae followed about a week after the drop in numbers of males was evident. There is no indication that the population make-up differed from one type of habitat to another, but it was clear that larger numbers of flies were associated with the margins of woodland, and that they swarmed in the open for short periods only.

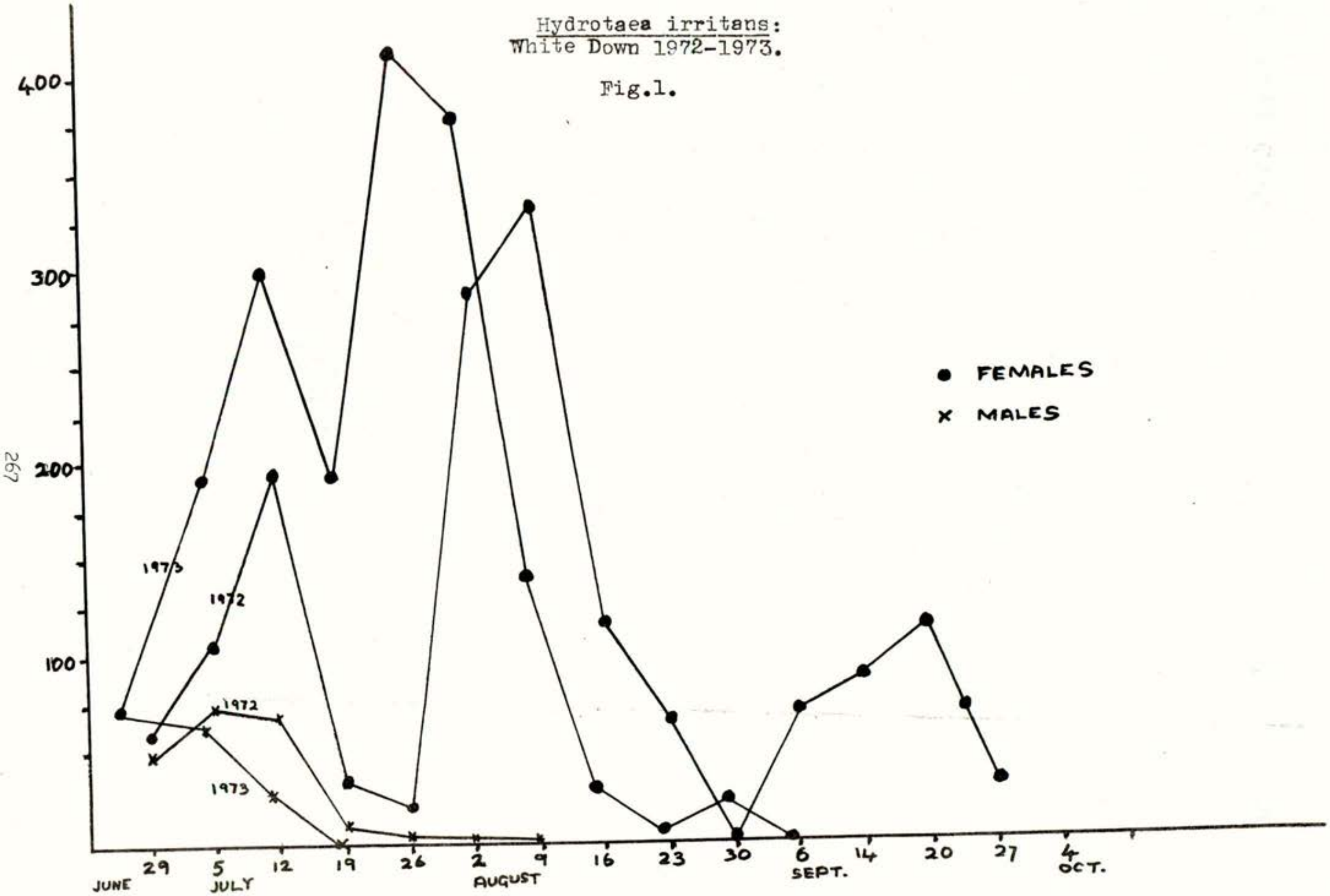
An attempt was made to relate fly numbers broadly to temperature and humidity, on the basis that numbers caught were an index of fly activity. No exact relationship emerges (Fig.2) but there appears to be a level of humidity and temperature below which activity is negligible. On occasions when high temperature and humidity did not support a high level of activity in the fly season, a strong wind was almost always noted. It has been pointed out by Nielsen et al (1971) that H. irritans will not fly when wind speeds are greater than 6m/s.

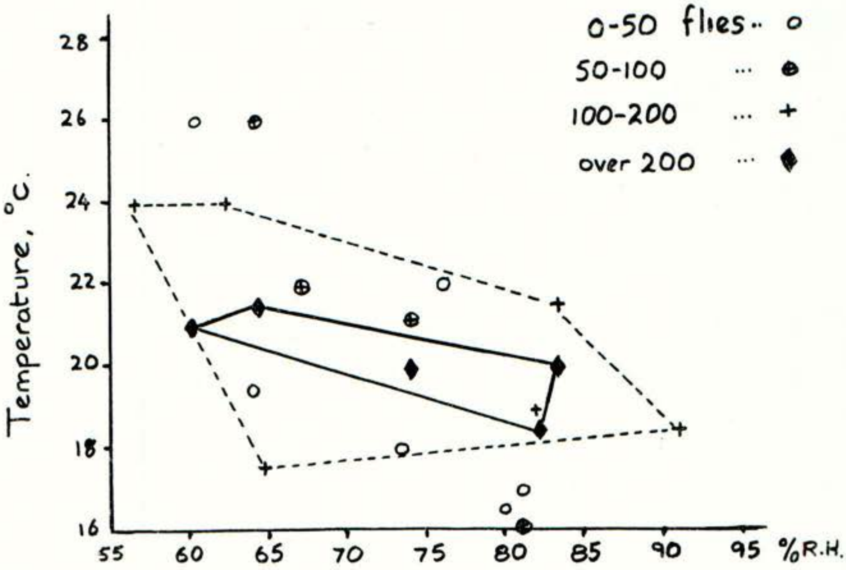
A striking difference between the result for 1972 and that for 1973 is the sudden population fall in late July of this year. The significance of this will be discussed after the other results have been given.

Observations made in the epidemic areas of Northumberland showed that the field activity of H. irritans did not differ in any distinct way from that in Surrey,

Hydrotaea irritans:
White Down 1972-1973.

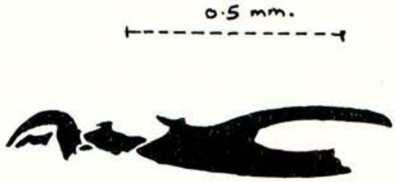
Fig.1.





H.irritans caught on various days during July 1973.

Fig.2.



H.irritans: Cephalopharyngeal skeleton,3rd instar.

Fig.3.

300 miles further south, except that the first emergence of flies in any number was delayed by 2-3 weeks in this cooler climate. Swarms were most frequently associated with woodland patches in the sheep-grazing areas, but moderate numbers of flies could be found swarming on open hillsides with a deep heather mat, which appeared to provide resting places as an alternative to the shade of trees. The range of resting sites may thus be wider than was at first anticipated. Surveys across moorland, and even in forest reserves covering considerable areas, indicated that the 'reservoir' population of breeding H. irritans, whether or not sheep happen to be present, is very great.

Affected sheep were examined on many farms with a history of headfly injury. Despite much time and trouble on the part of farmers in attempts to combat the condition, a number of sheep showed severe lesions and were in a distressed state. Hunter (1972) recently reported that over 50% of farms in the endemic area had suffered some loss, as shown by an extensive postal survey.

Frequently, horned breeds of sheep with non-wooled heads appeared to be the most seriously affected, many farmers mentioning particularly the Blackface type.

Mastitis was found to be frequent in several flocks; this is in agreement with findings in Denmark of Bahr (1955) where a study was made of the involvement of this fly in spreading this type of infection in cattle.

LABORATORY OBSERVATIONS

METHODS AND MATERIALS

Flies were maintained after collection in 45 cm washable net cages in sheltered outdoor conditions, and were provided with water, sugar, honey pads and fresh blood protein. The cages were suspended over trays of moistened peat as an oviposition medium. A variety of further oviposition media was offered in petri dishes: these included faeces of sheep, chicken, rabbit and cattle, and also damp moss as described by Nielsen et. al. (1971); also peat, moist bark, turf and decayed vegetation. Cages were established in sequence, new flies being put in for 1-2 weeks only to permit reasonable dating of biological changes.

RESULTS

Only the early cages contained many males, as later on es were begun when the wild populations were almost exclusively of females. The males mostly died within 4-5 weeks, but many females were remarkably long-lived, and despite handling and the artificial conditions 20% of these survived for over 10 weeks in the first cage in 1972: these would not have been newly emerged when they were collected. Oviposition was observed in the cages from the beginning of the third week in July of both years. No gravid females were caught in the field until September, but the histology of the ovaries of a number of specimens in August suggested that eggs had been laid. It is possible that gravid females take part less readily in the typical swarming behaviour and are thus less likely to be collected. Females in laboratory colonies at this time regularly showed maturation of eggs at about the 7th day after being put into cages with a supply of blood protein. The alternative interpretation would be that through the most serious months of 'fly worry' no eggs at all were being laid.

The shining white eggs were about 3 mm long with an indentation along one side, and were scattered on the tray surface or laid in crevices in the leaf litter. If no substrate was provided they were eventually dropped to the cage bottom where they soon dried up. At outdoor temperatures they hatched by the fifth day. Several hundred eggs per week were obtained from some cages by early September.

Larvae emerged when the litter had been kept in a moist atmosphere: trays were changed every 3-4 days. The larvae were of a typical phaonid type, and were white at first becoming parchment yellow as they grew. The buccal skeleton is characteristic and is the most useful guide to positive idientification (Fig.3). Many larvae were left in the trays for observation of development, but this appeared to stop after about the third week in these conditions. Other larvae, removed from the litter mainly by a flotation technique, were reared individually in petri dishes with a wide variety of natural media, but leaf litter again was the most suitable; development was still halted after a little growth had taken place.

At about the third week it was found necessary to provide small larvae of other species as food: cannibalism would otherwise occur. This carnivorous habit of H. irritans is not a great surprise as it is known in other Hydrotaea species (Keilin, 1917). When feeding, an abrupt initial penetration is followed by rhythmic sucking of the prey contents for up to an hour. Larvae appeared to go into diapause in November, becoming active again the following March. They began to pupate in mid-May of this year, but the few survivors did not emerge. The sequence is, however, in agreement with the expected emergence of most adults towards the end of June.

From the field a very large number of soil samples was taken from suitable habitats where swarming was known to have occurred, and these were maintained under natural conditions for observation but no adult flies emerged from them. With other samples, especially a number taken later in the season, sieving and flotation techniques were used to search for larvae but in only two samples were larvae found which appeared to be of a Hydrotaea type; identification beyond genus is in any case not possible. These samples came from the edge of a Downs beechwood, and from a clearing in the birch woodland at Ottershaw. Despite extensive sampling through the winter period not a single overwintering larva could be found.

DISCUSSION

The changes that have been described in the sex ratios and reproductive stages of the population suggested that only a single main generation occurs each year. The late date of insemination shown by the spermathecae, and the late onset of oviposition support this suggestion. Although Hammer (1941) did not specify such a limited cycle, he pointed out that H. irritans differs markedly in its biology from the other species of the genus.

A carnivorous diet, as is shown to occur in H. irritans is not unusual amongst muscid fly larvae and many phaonid and other species found in the woodland habitat form a complex and interrelated ecosystem which may be delicately balanced. Main predators of H. irritans may include Polietes lardaria (Keilin, 1917) and Morellia spp.

The unexpected collapse in H. irritans population size observed in late July of this year can most probably be accounted for in such a way. At a time when the main increase in population numbers was anticipated on the basis of previous results few flies appear to have succeeded in emerging; the reduction could very well have occurred in the larval stage. At the critical time of year, moreover, Polietes and Morellia, amongst the commonest predacious species, were much in evidence. It has for some time seemed probable that the complaints of excessive headfly numbers were attributable to a non-permanent population imbalance, and this evidence supports the suggestion. Excessive numbers of any species are often reduced abruptly by disease or predator build-up. In the case of H. irritans it would be premature to speculate at this point about whether a reasonable equilibrium level will eventually be reached.

ECONOMIC IMPORTANCE OF HEADFLY AND ITS CONTROL

The problem of headfly injury causes losses to a number of farmers in the area approximately between Newcastle-on-Tyne and Edinburgh, but could be considered not to be a problem on a national scale as, at most, $2\frac{1}{4}$ million sheep are maintained in the area of risk, and almost half may be on farms which are not affected. For those concerned, however, it is a most serious problem especially as it can not yet be prevented with any confidence, and may lead to secondary infection or blowfly attack of the open wounds.

The fly itself moreover is of extremely widespread occurrence, and may be assumed to have a significant role in transmitting various infective agents of cattle and sheep in the summer months (Bahr, 1955; Kirkwood and Tarry, 1973). Even their part in the disturbance of livestock should not be under-estimated. It has, for examples, been shown that the health of cattle can be seriously affected by another non-biting muscid fly, Musca autumnalis (U.S.D.A. 1959).

CONTROL OF HEADFLY INJURY

It would be out of place to discuss control measures in any detail at this stage in the progress of the work. Three approaches however appear to be available:

i) Protection by repellants against insect attack has been investigated in many parts of the world for very many years, but advance is slow and they still seem to be of value for short periods only. It is claimed that useful protection may be obtained from some insecticidal preparations recently marketed but evidence about their efficacy is not yet available and may not be easy to establish in the field situation. Treatment is generally confined to the livestock as breeding areas are very diffuse and could not readily be treated; hill forests for example often extend for many miles.

ii) A number of farmers have been obliged to avoid the most severely affected pastures during July and August. Several were found to keep sheep housed during the daytime and let them out for grazing only in the evenings. This measure appeared to be effective but is not possible on a large proportion of hill farms.

iii) Relative immunity from attack has been claimed for breeds which are hornless and have woolled heads. Such breeds do not always thrive under hill farm conditions, but the experimental introduction of suitable crosses into the worst-affected areas is to be encouraged.

Clearly there is at present no simple answer to the problem of headfly worry which, at best, must be expected to recur spasmodically. A compromise including the methods mentioned may be all that can be offered for some time to come.

Acknowledgements

For the success of the work described I am indebted to my colleague A. C. Kirkwood who has shared the effort both in the field and the laboratory.

My thanks are also due to Dr. S.B. Kendall for his support and interest.

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Proceedings 7th British Insecticide and Fungicide Conference (1973)

1,1-BIS(p-ETHOXYPHENYL)-2-NITROPROPANE, A NEW INSECTICIDE FOR THE CONTROL OF FLIES ON DOMESTIC ANIMALS

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Summary A new insecticide, 1,1-bis(p-ethoxyphenyl)-2-nitropropane, has shown good activity against flies on domestic animals. When applied to the coats of animals as a spray, or by immersion dipping, it affects flies sufficiently rapidly to prevent skin puncture by biting species, or oviposition by sheep blowflies (*Lucilia* spp.). This activity is believed to be caused by rapid toxic effect after contact, rather than by true repellent action. The compound is active for several days on animal hair, and for a much longer period on the wool of sheep. The report contains data on mammalian toxicity of this insecticide, and on its activity against other ecto-parasites of animals.

Résumé Un nouvel insecticide, 1,1-bis(p-ethoxyphenyl)-2-nitropropane, s'est démontré actif contre les mouches sur les animaux domestiques. Quand on l'applique au poil des animaux en forme de pulvérisation ou par immersion dans un bain, il affecte les mouches assez rapidement pour empêcher la piqûre de la peau par les espèces piquantes, ou la porte des oeufs par *Lucilia* spp. On croit que cette activité se doit à un effet toxique rapide après le contact, plutôt qu'à une vraie action répulsive. Le composé est actif pendant plusieurs jours sur le poil, et pendant une période beaucoup plus longue sur la laine des moutons. Ce rapport contient des données de la toxicité mammifère de cet insecticide et de son activité contre d'autres parasites externes des animaux.

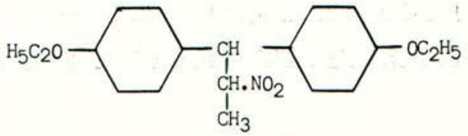
INTRODUCTION

An efficient insecticide for the control of flies on domestic animals should produce a rapid toxic effect on insects so that they are prevented from biting or worrying the host. It should also be sufficiently persistent on animal hair to render unnecessary very frequent treatments. Few, if any, of the insecticides at present available exhibit this combination of activity.

1,1-bis(p-ethoxyphenyl)-2-nitropropane was discovered at the Division of Applied Chemistry, Commonwealth Scientific & Industrial Research Organization, Melbourne, Australia, (Holan, 1971), where it was given the code number "GH74". The compound has been examined for activity against ectoparasites of animals at the Wellcome Research Laboratories, Berkhamsted, and in field trials in Britain and overseas. It has useful activity against several insects of economic importance, but is of particular interest because, in our experience, it exhibits unique properties of speed of action against flies on animals, combined with persistence on hair and wool.

* Formerly the Cooper Technical Bureau.

PHYSICAL AND CHEMICAL PROPERTIES



Molecular formula and weight:	$C_{19}H_{23}NO_4$ 329.4
Wiswesser line notation:	WNY & YR DO2 & R DO2
Chemical and physical properties:	GH74 is an off-white crystalline solid, m.p. 56.5^{o}, b.p. 160^{o}/10^{-3} Torr Although sparingly soluble in methanol, polyethylene glycol 200, and kerosene, it is readily soluble in xylene and olive oil.
Residue analysis:	Residues in tissues of animals may be determined by gas-liquid chromatography using 2% OV101 at 240^{o} and an electron capture detector.

MODE OF ACTION

The mode of action of GH74 has not been established. It has a very rapid toxic effect on flies coming into contact with deposits on animal hair or wool. This is described in more detail later.

The compound has no observable vapour toxicity against sheep blowflies (Lucilia sericata) or stable flies (Stomoxys calcitrans), when these are exposed to concentrations of up to 2%.

TOXICOLOGY

GH74 is of moderate acute oral toxicity to small mammals and of low dermal toxicity. The following data were obtained in toxicological studies, using a sample of GH74 that had been synthesised on a laboratory scale.

The acute oral LD50 in female rats and mice was 264 mg/kg and 336 mg/kg respectively, with 50% w/v GH74 in vegetable oil.

The acute intraperitoneal LD50 in female mice was 200 mg/kg, with 50% w/v GH74 in vegetable oil, whilst the acute dermal LD50 in female rats was $>$ 6,400 mg/kg, 50% w/v GH74 in xylene.

Ocular irritancy; a 20% w/v solution of GH74 in corn oil caused no visible effects of irritancy when using the Draize test with rabbits.

A 21 day feeding study in male and female rats, with a control group and groups of rats receiving 200, 600 and 2,000 ppm GH74 in the diet, has shown no significant change in blood chemistry, haematology, or organ weights.

Calves weighing approximately 100 kg have been sprayed with up to 45 g GH74 in the form of an aqueous emulsion. No clinical signs of toxicity were seen and there were no significant changes in the haemogram, blood chemistry, bodyweight, or electrocardiogram. This dose represents approximately ten times the envisaged therapeutic dose.

BIOLOGICAL ACTIVITY

In laboratory screening tests GH74 showed activity against a wide range of insect species, but was selected for further investigation on account of its rapid effect on flies on animals. This activity was first observed when mice, which had been sprayed with an emulsion of GH74, were exposed to artificially reared *S. calcitrans*. Calves were used in subsequent tests.

Myiasis, caused by larvae of the sheep blowfly (*Lucilia spp.*), is one of the most important diseases of sheep in several of the largest sheep rearing countries. GH74 has been tested for activity against *L. sericata* larvae, and also against gravid *L. sericata* females.

METHOD AND MATERIALS

Biting flies

Four calves, weighing 90-160 kg, were sprayed with 1.136 litres (two pints) each of an emulsion of GH74 at the following concentrations: 1.0%, 0.5%, 0.25% and 0.1%. The same four calves were used in each test to assess the effect of each concentration.

The treated calves were exposed in pairs at daily intervals after spraying to about 100 starved adult *S. calcitrans* in a fly chamber. Counts of flies present on the calves were made at minute intervals between three and seven minutes inclusive after release. After each day's exposure as many flies as possible were collected, and the percentage mortality determined 24 hours later.

Blowflies

A modified du Toit test was used to assess larvicidal activity *in vitro* (du Toit and Fiedler, 1953). This was followed by larval implantation tests on sheep that had been dipped in 0.25% GH74 emulsion.

Activity against adults was assessed by immersing a group of seven sheep, for 30 seconds, in 0.25% GH74 emulsion. These animals, and a further seven untreated controls, were exposed at weekly intervals to approximately 1,000 gravid female *L. sericata*, for six hours, in a fly chamber. An attractant was applied to the back of each sheep before exposure, to encourage oviposition on the fleece. At the end of each exposure period, deposits of eggs on each sheep were recorded and removed.

RESULTS

S. calcitrans

The average number of *S. calcitrans* landing on each calf at minute intervals is given in Table 1. The mortality of captured unengorged flies, 24 hours after exposure, is shown in Table 2, and of engorged flies in Table 3.

Table 1

Numbers of S. calcitrans on treated calves

3-7 minutes after release of flies

Average number of flies per animal at minute intervals

Days after treatment	Concentration of GH74 1.0%	0.5%	0.25%	0.1%	Untreated
1	2.3	2.4	4.7*	8.7	(31.6)
2	4.2	4.1	6.1	10.7	(28.6)
3	5.6	3.5	5.0*	13.4	(31.6)
4	7.1	4.3	6.8	-	(29.0)
7	8.1	7.8	14.4	-	(26.4)
10	18.0	18.1	29.9	-	(28.4)
14	19.6	-	-	-	

* Average of two calves only.

Table 2

Percentage mortality of captured unengorged S. calcitrans

24 hours after exposure

Days after treatment	Concentration of GH74 1.0%	0.5%	0.25%	0.1%
1	100	95	99*	92
2	100	97	87	66
3	99	91*	82*	77
4	95	63	46	-
7	95	80	74	-
10	52	67	45	-
14	66	-	-	-

* Mortality of flies from two calves only.

Table 3

Percentage mortality of captured engorged S. calcitrans

24 hours after exposure

Days after treatment	Concentration of GH74 1.0%	0.5%	0.25%	0.1%
1	-	-	-	• 100
2	-	-	-	0
3	-	-	-	36
4	-	-	-	-
7	-	0	14	-
10	0	46	28	-
14	0	-	-	-

Shortly after treatment, flies alighted on the calves, but stayed for not more than 3-5 seconds. Knock-down was seen after 45-60 seconds. The majority of these flies did not recover. After a period of several days, depending on the treatment concentration, flies were able to settle on the coat and begin probing. This was soon interrupted by a withdrawal reaction, characterised by the fly extending its front legs and subsequently falling off and flying away.

This toxic effect prevented engorgement for some 10 days on animals treated with 1.0% GH74, for 7 days after treatment with 0.5%, and 3 days with 0.25%. Insecticidal activity was also well maintained, in that 7 days after treatment there were mortality rates of 95%, 80% and 74% respectively in unengorged flies collected after exposure to the three highest concentrations. Ten days after treatment the mortality in groups treated with 1.0% and 0.5% was 52% and 67% respectively.

Blowflies

In the du Toit test GH74 exhibited poor larvicidal activity, the minimum lethal concentration being 20 ppm. This was confirmed in the larval implantation trial, in which larvae were able to establish themselves in the fleece of sheep four weeks after treatment with 0.25% GH74.

In the groups exposed to adult blowflies, eggs were deposited on untreated sheep during the first week's exposure and on each subsequent occasion.

On sheep dipped in 0.25% GH74 the treatment prevented oviposition for 39 weeks on clean wool to which attractant had been applied. In an earlier experiment eggs were deposited on naturally soiled wool in the perineal area, 6 weeks after sheep had been dipped in 0.25% GH74.

DISCUSSION

The repellent and lethal effects of GH74 on a population of visiting biting flies, such as S. calcitrans, would be to reduce fly worry by affecting flies before they are able to bite the host, and also to reduce the total fly population. Against a resident fly population such as the horn fly (Lyperosia irritans), or the buffalo fly (L. exiqua), the lethal effect would be the more significant. This has been confirmed in field trials reported later.

The poor larvicidal activity of GH74 reduces its value for protecting sheep against blowfly myiasis. Although the repellent and lethal activity of the compound prevent oviposition by gravid female flies which come into contact with treated wool, the masking effect of faeces etc, permits eggs to be deposited in soiled fleece from which larvae are able to invade clean treated wool, and cause normal myiasis.

It is possible that the use of GH74 as a sheep dip, for several seasons, could effect a reduction in the population of blowflies, and consequently the incidence of myiasis, but it is unlikely that such a practice would be of interest to farmers so long as effective persistent larvicides are available.

Field trials

A herd of approximately 500 dairy cattle in Rhodesia was sprayed to saturation on three occasions with 0.1% GH74 wash, to give an estimated deposit of 2.73-3.57 g of insecticide per animal. Excellent control of S. calcitrans and Musca domestica was obtained for 7-14 days, depending on weather conditions.

In South Africa, saturation spraying with 0.1% GH74 gave good control of S. calcitrans for 7 and 14 days respectively in two dairy herds.

Against horn fly (L. irritans) 0.5 g GH74, applied as a 0.5% spray to the back-line of cattle in Colombia, gave more than 90% control for 13 days, reducing to 50% after 15 days. In Australia effective control of buffalo fly (L. exiqua) was provided for 15-19 days after the application of approximately 2.8 g GH74 per animal as a back-line spray.

A small scale trial was carried out in the New Forest area of Hampshire, England, when there was a large mixed population of flies. Treatment of very large cattle with 1.5 litres each 1% GH74, as a light mist spray, gave 7 days' protection against a fly infestation, of which the highest incidence occurred on the animals' faces. Later in the summer, spraying the same animals with 0.5% gave 10 days' protection against an infestation that contained a larger proportion of flies normally resident on the bodies of cattle.

During this trial the following genera were collected and identified on or near the treated cattle: Tabanus, Haematopota, Chrysops, Stomoxys, Lyperosia, Hippobosca, Musca, Hydrotoea, Lucilia, and Calliphora.

In field trials in different countries GH74 has also been found effective against Glossina, Simulium and Culicoides, on animals.

Other parasites of cattle

GH74 has low ixodicidal activity against larvae of normal and organo-phosphorus resistant cattle ticks, Boophilus microplus, but is resisted by larvae of an arsenic/organo-chlorine resistant strain of B. decoloratus. It has no significant activity against engorged female ticks.

No systemic activity has been demonstrated against migrating warble larvae (Hypoderma spp.) in cattle.

Other parasites of sheep

The sheep head fly (Hydrotoea irritans) causes lesions on the heads of sheep in localised areas of northern England and in Scotland. Preliminary evidence from field trials suggests that dipping sheep in 0.125% GH74 gives good control of the fly for 4-6 weeks.

Dipping in 0.01% GH74 eradicated infestations of the sheep ked (Melophagus ovinus). Sheep dipped in 0.05% were protected from reinfestation for 15 weeks.

Infestations of the body louse of sheep (Damalinia ovis) were eradicated by dipping in 0.01% GH74. Sheep dipped in 0.05% were protected from reinfestation for 23 weeks. There was evidence of cross resistance in a dieldrin-resistant strain of D. ovis, and dipping in 0.125% was necessary to eradicate infestations of this parasite.

Infestations of the face louse of sheep (Linognathus ovillus) were not eradicated by dipping in 0.05% GH74, the highest concentration tested.

GH74 had no effect on sheep scab mites, Psoroptes ovis, at a concentration of 0.01%.

It is intended that the observations mentioned above will be reported in detail in subsequent publications.

Acknowledgements

I wish to acknowledge the work of many of my colleagues at the Wellcome Research Laboratories, Berkhamsted, and with associated Wellcome research units overseas, whose results and information have been largely used in compiling this paper.

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NOTES

THE DEVELOPMENT OF A NEW COMPOUND ACTIVE AGAINST RESISTANT TICKS

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Summary Results of experimental studies with clenpyrin on ticks are reported. As a handspray at concentrations of 2000-3000 ppm and in a plunge-dip at concentrations of 1500 ppm clenpyrin is effective against resistant cattle ticks (Boophilus microplus). The mode of action of clenpyrin is different from that of organophosphorus esters. It paralyses the ticks and inhibits maturation of eggs in the ovary Treated ticks are unable to break down protein. It does not inhibit acetylcholinesterase.

Résumé Un rapport est établi concernant les résultats des études experimentelles avec la clenpyrine sur des tiques résistantes. La clenpyrine agit donc en des concentrations de 2000 à 3000 ppm sous forme des spray et de 1500 ppm sous forme de dip. La clenpyrine a un mecanisme d'action différent de l'esters d'acide phosphorique, elle paralyse les tiques, inhibe le développement des oeufs dans les ovaires et la dégradation des proteins chez les tiques. Elle n'inhibe pas l'esterase d'acetylcholine.

INTRODUCTION

For some years an ever increasing number of strains of one-host cattle ticks (Boophilus spp.) have been found that are resistant to organic phosphorus compounds and carbamates. Wharton and Roulston (1970), O'Sullivan and Green (1971), Stendel (1969) and Hart and Batham (1969).

These strains (for example the Ridgelands-, Biarra-, Mackay-, Mt.Alford-strain of Boophilus microplus in Australia and the Berlin-strain of Boophilus decoloratus in South Africa) differ in behaviour towards individual phosphorusesters and in degree and mechanism of resistance. Numerous cross resistances amongst the phosphorus compounds and carbamates indicate however, that the acetylcholinesterase system is involved in both types of acaricides.

It would seem therefore unlikely that a new promising compound with lasting effect will be found within the group of cholineesterase inhibitors. It is rather to be expected that preparations with other mechamisms of action will in future be more successfully employed against such strains, Wharton (1967).

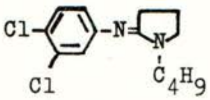
Whilst searching for such substances, we found in the cyclic amidine series, numerous compounds active against ticks; these were equally effective against both sensitive and resistant strains, Enders et al (in press).

Chemical and biological screening of this group finally led to the choice of clenpyrin.

This paper presents the results of experimental laboratory and animal studies with clenpyrin.

METHOD AND MATERIALS

Clenpyrin is 2-(3,4-dichlorophenyl-imino)-1-N-butylpyrrolidone, its structural formula is as follows:



(I)

Tolerance data for clenpyrin:

Rat, acute, oral:	LD_{50}	2586 mg/kg
Mouse, acute, oral:	LD_{50}	745 mg/kg
Rat, acute, dermal:	LD_{50}	>2000 mg/kg
Calf, acute, oral:	100 mg/kg without toxic symptoms 250 mg/kg mild toxic symptoms 500 mg/kg moderate toxic symptoms	
Cattle, acute, dermal:	up to 20000 ppm no toxic symptoms 50000 mild toxic symptoms.	

Ticks For these investigations we used ticks of a sensitive strain (Yeerongpilly) and of various resistant strains (Ridgelands, Biarra, Mackay and Mt.Alford) of B.microplus. The ticks were bred on female Friesian cattle weighing 400 kg, aged 2.5-3 yr). During the test the animals were housed in climatised stalls at a temperature of 25°C ± 1°C and 60% relative humidity ± 10%.

In vitro-test on adults
From the 21st day after infection the fully engorged female adult ticks were carefully collected by hand shortly before dropping off. Prior to the test and during the post-observation period the ticks were kept in a climatised room at 27°C ± 1°C and 80% relative humidity ± 10%.

The formulation for the test was prepared by mixing 3 parts of active substance with 3.5 parts ethylglycolmonomethyl ether and 3.5 parts nonylphenolpolyglycol ether (quantities in weight %) and diluting the resultant e.c. to the desired concentration with WHO Standard Hard-water (342 ppm soluble components with reference to $CaCO_3$).

Groups of 25 ticks per concentration were placed in test-tubes each containing 36 ml test-solution and shaken for 1 min at 96 rev/min. The ticks were then transferred to plastic beakers, the bases of which were covered with a round filter. In experiments with fixed times of exposure the ticks were carefully rinsed with tap water at the end of the exposure period.

The criterion of efficacy was inhibition of voluntary movements of the body and limbs, and prevention of laying of fertile eggs.

The activity of the substance is expressed as a percentage. A 100% means that no voluntary movement was demonstrable and egg laying was completely prevented. 0% means that normal movement was demonstrable and that fertile eggs were layed in normal amounts. The paralysing action was assessed 24 hours after application. Egg laying was assessed 14 days after application (quantitative) and fertility at 28-35 days.

In vivo spray and dip test The in-vivo tests were carried out as follows: Over a period of 28 days cattle were infected three times a week, on each occasion with 2000-4000 unfed larvae of B.microplus which were 21-28 days old. Under the conditions chosen for the test, development of B.microplus on the host animal is complete after 20-21 days. On account of the multiple infection one finds from the 21th day of infection all stages of development of the ticks (larvae, metalarvae, nymphs, metanymphs and adults) in large numbers on the animals.

On days 21, 22 and 23 after commencement of the test, the number of fully engorged ticks on the animals was counted. These figures served as a measure of the severity of the infection in the individual animal.

On the 23rd day after infection the cattle were dipped in the desired concentration of active substance for 1 min or sprayed carefully with 10 l of the concentration under test.

The number of developed, engorged female ticks were counted daily on the treated and control animals in the three weeks after treatment. The ticks that were collected were fixed to cardboard with adhesive tape. The quantity of eggs layed per female was determined after 14 days and the percentage of fertile eggs after 28-35 days. The number of developed ticks on the treated and untreated control animals was compared. Taking into consideration the length of the development period of the individual stages of the ticks, it was thus possible to calculate the percentage efficacy of the test concentration against the various stages.

RESULTS

In vitro dip tests on adult ticks   The comparative dip tests on the engorged female adult ticks of the various strains of B.microplus showed that clenpyrin paralyses and inhibits eggs laying in all the strains tested. There was no difference in action of clenpyrin on either the sensitive Yeerongpilly strain or the resistant Ridgelands-, Biarra- and Mt.Alford strains of B.microplus. Only in the case of the Mackay strain was the action of clenpyrin somewhat less (Table 1). The Mackay strain is a resistant strain known to detoxify organophosphorus esters. This capacity may be the cause for the slightly reduced efficacy of clenpyrin.

Table 1

B.microplus / fully engorged female adults in vitro
Effect of clenpyrin on inhibition of egg production on various strain

Concn of a.i. in ppm	% inhibition of deposition of fertile egg batches Yeerongpilly-strain(s)	Ridgelands-strain(r)	Biarra-strain(r)	Mackay-strain(r)	Mt.Alford-strain(r)
8192	100	100	100	100	100
4096	100	100	100	100	100
2048	100	100	100	95	100
1024	85	95	90	90	70
512	50	60	40	0	10
256	0	20	0	0	0
contr.	0	0	0	0	0

Onset of action   In order to determine the speed of action of clenpyrin, the paralysing effect was determined at fixed times after application. The results show that depending on concentration, the paralysing action of clenpyrin in B.microplus begins after only 1 hour and is complete after 8 hours (Table 2).

Table 2

B.microplus (resistant Biarra strain) / fully engorged female adults in vitro.
Paralysing effect of clenpyrin at different times after application

Concn of a.i. in ppm	Paralysing effect in %, h after application: 1	2	4	8	12	24	48	72
10000	<50	<50	>50	100	100	100	100	100
3000	0	0	<50	100	100	100	100	100
1000	0	0	0	100	100	100	100	100
300	0	0	0	<50	<50	<50	<50	>50
contr.	0	0	0	0	0	0	0	0

Dependence of in vitro activity upon duration of exposure

Ticks were exposed to clenpyrin for different lengths of time. It was shown that concentrations of 1000 ppm exerted a definite paralysing effect and inhibited egg laying after only a 1/4 minute exposure. Concentrations of 3000 ppm and 1000 ppm were fully effective after 1 and 2 minutes (Table 3).

Table 3

B.microplus (resistant Biarra strain) / fully engorged female adults in vitro

Inhibitory effect on egg production and paralysing effect of clenpyrin in relation to time of exposure

Concn of a.i. in ppm	Efficacy in % / Time of exposure in min 1/4	1/2	1	2	4	8
10000	100 / 100	100 / 100	100 / 100	100 / 100	100 / 100	100 / 100
3000	60 / 100	90 / 100	100 / 100	100 / 100	100 / 100	100 / 100
1000	0 / 40	70 / 70	50 / 60	100 / 100	100 / 100	100 / 100

upper left number = limited exposure time = washing off ticks with tap-water after exposure time

lower right number = unlimited exposure time = ticks not washed after exposure time

Influence of water hardness on in-vitro activity Tests were carried out with normal tap water, WHO Standard Hard-Water and extremely hard water (Amberley water) of various degrees of pollution. It was found that neither the degree of hardness nor the extent of pollution in any way influenced the activity of clenpyrin (Table 4).

Table 4

B.microplus (resistant Biarra strain) / fully engorged female adults in vitro

Inhibitory effect of clenpyrin on egg production in artificially polluted dip fluid and different types of water

Concn of a.i. in ppm	type of water	pollution with	Efficacy in %				
		% faeces	0,5	1,0	2,0	4,0	8,0
		% clay	0,5	1,0	2,0	4,0	8,0
	tap-water		100	100	100	100	100
10000	WHO-water		100	100	100	100	100
	Amb.water		100	100	100	100	100
	tap-water		100	100	100	100	100
3000	WHO-water		100	100	100	100	100
	Amb.water		100	100	100	100	100

Dip stability in vitro Appropriate tests were made to discover whether clenpyrin is stable in polluted aqueous solution at concentrations intended for treatment. These studies showed that clenpyrin maintains its biological activity for more than 128 weeks when stored at 28° and 40°C in clean water. In strongly polluted dips clenpyrin was stable at 28°C for more than 128 weeks and at 40°C remained biologically active for more than 32 weeks (Table 5).

Table 5

B.microplus (resistant Biarra strain) / fully engorged female adults in vitro
Inhibiting effect on egg production of clenpyrin in %, after storage for different periods

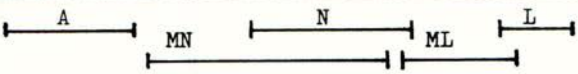
Concn of a.i. in ppm	Dip fluid	Storage temp.	weeks of storage 4	8	16	32	64	128
3000	WHO	28°	100	100	100	100	100	100
3000	water	40°	100	100	100	100	100	100
3000	artific. polluted dip fluid 10% faeces + 10% clay	28°	100	100	100	100	100	100
3000		40°	100	100	100	100	90	40

In vivo spray test As a handspray at concentrations of 2000 ppm and higher, clenpyrin is at least 99% effective against the parasitic stages of B.microplus (Biarra strain). The paralysing action of clenpyrin was a 100% against ticks, which at the time of application existed on the host as larvae, metalarvae, nymphs and adults. Some ticks which at the time of application were metanymphs, survived treatment and also layed eggs (Table 6).

Table 6

B.microplus (resistant Biarra strain) / all developmental stages in vivo (cattle) / Number of engorged ticks with fertile egg batches before and 1-21 days after hand-spraying with clenpyrin

Conc of a.i. in ppm	Days before treatment -2-±0	Days after treatment +1-3	4-6	7-9	10-12	13-15	16-18	19-21	1-21
	Number of engorged ticks with fertile egg batches								
1000	613	290	146	215	264	213	63	48	1239
1500	1356	29	38	144	49	6	0	0	266
2000	634	3	0	12	5	2	0	0	22
2500	394	2	0	0	32	29	0	0	63
3000	231	3	1	0	11	1	2	0	18
contr.	1016	644	731	1313	1360	535	389	216	5188



Approximate stage of development at time of treatment

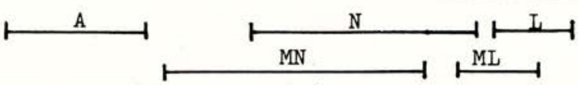
A = Adults, MN = Metanymphs, N = Nymphs, ML = Metalarvae, L = Larvae

In vivo dip tests In plunge-dips (freshly prepared unpolluted dip) at concentrations of 1500 ppm, clenpyrin was almost a 100% effective. Since it was discovered in the in vitro tests that the Mackay strain of B.microplus was somewhat less sensitive than other strains, this strain was used in the dip tests. It was found, that at concentrations of 2000 and 2500 ppm, the number of ticks on the animals were reduced by more than 99%. Also here, some ticks survived, which at the time of treatment were metanymphs (Table 7).

Table 7

B.microplus (resistant Biarra- and Mackay strains) / all developmental stages in vivo (cattle).
Number of engorged ticks with fertile egg batches before and 1-21 days after plunge-dipping with clenpyrin

conc of a.i. in ppm	Number of engorged ticks with fertile egg batches Days before treatment -2 - +0	+1-3	4-6	7-9	10-12	13-15	16-18	19-21	1-21
250*	450	454	73	600	504	320	131	39	2121
500*	1276	732	81	187	533	347	100	64	2044
1000*	1076	121	0	1	7	7	6	2	144
1500*	463	0	0	0	1	0	1	0	2
2000**	368	0	0	4	6	4	0	0	14
2500**	445	0	0	0	2	0	0	0	2
contr.1*	989	1032	656	1868	2826	3178	1158	585	11303
contr.2*	1114	1005	338	450	198	317	199	174	2681
contr.3*	654	1093	970	1058	288	327	359	41	4136
contr.4**	642	681	390	759	460	1014	522	61	3887



Approximate stage of development at time of treatment

A = Adults, MN = Metanymphs, N = Nymphs, ML = Metalarvae, L = Larvae

* = Biarra strain, ** = Mackay strain

Mode of activity The behaviour and appearance of the ticks after application of clenpyrin is substantially different from that seen after treatment with the organophosphorus compounds and formamidines. The phosphorus compounds cause a slow shrinking of the ticks over several days resulting finally in mummification with a brownish-yellow colouration; the formamidines cause a slight discolouration, but at first no macroscopic changes. By contrast, clenpyrin causes increasing paralysis of the treated ticks. They change their form to a bloated appearance and are coloured at first light grey and later towards black before slowly dying.

In order to demonstrate inhibition of motility, the movement of the ticks was measured as follows: The ticks were placed on blotting paper, and as they moved they produced scraping noises. Since the noise level is so low, the measuring equipment has to be enclosed in a lead box in order to exclude external sounds. The lead cases were in turn wrapped in synthetic foam material and placed in an incubator. The noise produced by the ticks was detected by a microphone, amplified, separated from interference and converted to impulses. Every tenth signal so produced was applied to a counter. By means of a timer the stored impulses are printed out and the counter reset to zero as required.

The motility measurement shows that movement of the extremities of the ticks is completely inhibited only a few hours after application of clenpyrin. A comparative test with an organophosphorus compound showed that the paralysing action was slower in onset and incomplete. A formamidine showed an increase in motility typical for this class of compounds.

The mode of action of clenpyrin is fundamentally different from that of other acaricides and insecticides, which act by inhibition of cholinesterases. After treatment with clenpyrin, the musculature of the tick completely loses its ability to contract. A further consequence of treatment is a rapid fall in oxygen consumption of the ticks.

The effects apparent after only 1-2 days are inhibition of egg maturation in the ovary, a fall in the quantity of glycogen and glucose demonstrable in the ticks and a rise in RQ to values over 1.0. After two days it is clear that the protein taken up by the treated ticks has not been digested. The metabolic processes observed in the controls namely the degradation of protein, the formation of guanine and haematin, and the synthesis of glycogen and lipid associated with egg production and also the secretion of wax onto the body surface, are prevented by treatment. Clenpyrin has no demonstrable effect on the activity of the acetylcholinesterase of tick larvae and engorged females. To date, it has not been possible to explain why treated ticks are unable to break down protein.

DISCUSSION

If a preparation is to be successfully employed against resistant and sensitive ticks of a given species, then it must be effective against both. Such activity is most likely to be discovered among substances belonging to classes of compounds not previously employed to combat ticks.

Both as regards <u>in vitro</u> and <u>in vivo</u> mechanism of action, clenpyrin differs completely from known acaricides such as organophosphorus compounds, carbamates, chlorinated hydrocarbons and also from formamidines. The paralysing action on all parasitic stages takes place so rapidly, that sucking ticks have no time to withdraw the hypostome from the skin of the host and thus although dead remain for a certain time hanging on the animal. Also the various physico-chemical and toxicological properties of clenpyrin are very favourable for application under practical conditions. The extremely good stability even in polluted dips and in very hard water obviates the addition of dip stabilisers. The sedative action observed with some other formamidines does not occur with clenpyrin, so that higher concentrations are tolerated without symptoms.

Acknowledgements

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A NEW TRIAZAPENTA-DIENE COMPOUND ACTIVE AGAINST CATTLE TICKS OF MAJOR IMPORTANCE IN SOUTH AFRICA.

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Summary In field trials 1,5-di(2,4 dimethylphenyl)-3-methyl-1,3,5-triazapenta-1,4-diene, a new triazapenta-diene compound, was shown to be highly effective against both susceptible and resistant strains of important cattle ticks. The compound rapidly expelled attached ticks, gave long residual protection against reinfestation and indirectly controlled some stages by an apparent systemic effect. It showed activity against most species over a wide concentration range and had a significant ovicidal effect when gravid females were treated.

INTRODUCTION

The control of ticks on cattle in South Africa has long been a matter of prime importance to stock owners over much of the more intensive natural grazing areas of the country, both from the point of disease control and the physical debilitation and mechanical tissue damage that often result from field infestations. Since the introduction, in the late 19th century, of arsenic for tick control these parasites have progressively developed resistance to this and other ixodicides introduced for their control. The present indications are that both the degree and diversity of tick resistance in South Africa is increasing and it is thus vital that new chemicals are sought of different type and activity.

Attention has already been drawn to the high activity of a new compound, 1,5-di-(2,4-dimethylphenyl)-3-methyl-1,3,5-triazapenta-1,4-diene* (proposed common name, azaform), against some Australian cattle tick strains by Palmer et al (1971), and against ticks of major importance in South Africa by Harrison et al (1972).

This paper describes the effects of applying azaform to naturally infested cattle by handspraying at weekly intervals.

METHODS AND MATERIALS

Experimental concentrates of 50% and 60% by wt of a w.p. and 12.5% by wt of an e.c. were used. For purposes of biological comparison in vivo, a commercial dioxathion/chlorfenvinphos composite dip concentrate was used at the recommended dilution. Field trials were carried out on three farms in different parts of the Eastern Cape littoral of South Africa. On each farm tick-infested cattle were divided into treatment groups of 4 animals.

*The Boots Company Limited, Nottingham.

Procedures for treatments, tick counts and assessments described by Baker and Thompson (1966) were used. The animals were hand sprayed at weekly intervals, using lances supplied by a mechanical pump at a pressure of 17.5 - 21.09 kPa. About 12 l. of wash were applied to each animal. Adult two and three-host ticks were counted but their immature stages and the adult and immature stages of blue ticks (Boophilus decoloratus) were assessed by the techniques mentioned earlier. Where investigations were aimed at establishing a possible systemic effect by azaform the animals' ears and much of the head were left untreated at each spraying and observations were made of the inner ear grooves, the predilection site of the immature stages of the red-legged tick, (Rhipicephalus evertsi). These sites were initially cleansed of tick life.

Expellency was assessed by stationing of a treated animal over a canvas sheet laid on the floor of a cattle crush, following a preliminary draining period. Two containers were fastened to the animal to collect urine and faeces. Continuous collections of expelled ticks were made from the surface of the canvas for eight hours after treatment. At prescribed intervals collected ticks were placed in separate containers in an incubator at 80% r.h. and 26°C to assess their survival rate. Other procedures included the placing of recently expelled flat ticks in well secured ear and tail bags on untreated animals, to observe their reaction.

RESULTS

Blue ticks (B. decoloratus)

Against Berlin type resistant strains, azaform established an effective control at a concentration of 0.001% (Fig. 1). At this level only light infestations of larvae were found at each weekly inspection and engorgement was minimal. Nymphs were absent after the second treatment and adults after three weeks. 0.025% dioxathion/0.025% chlorfenvinphos did not control blue ticks as effectively as did 0.001% azaform.

Red-Legged Ticks (Rhipicephalus evertsi)

Azaform at all concentrations effectively controlled larval reinfestations of this two-host tick after two weekly treatments (Fig. 2). Flat larval ticks were frequently encountered on the 0.025% dioxathion/0.025% chlorfenvinphos treated groups.

Observations on immature stages (Fig.3) indicated a possible systemic action by azaform when sprayed at 0.025% and 0.05%. Only flat larvae were seen in the ears of the 0.025% group on three occasions over a 17 week period, despite deliberately imperfect treatments. 0.0125% azaform was not as good but gave acceptable results. 0.025% dioxathion/0.025% chlorfenvinphos did not prevent the free emergence and engorgement of nymphal stages.

Reinfesting adult ticks were markedly reduced by treatment with 0.006% azaform. (Fig.2). No engorged females were found on this group after the second treatment. 0.025% dioxathion/0.025% chlorfenvinphos did not prevent the full engorgement of females between treatments.

Brown ear-ticks (Rhipicephalus appendiculatus)

Against larvae of this three-host tick, azaform proved highly effective in maintaining control over reinfestation at concentrations of 0.0035% and above. (Fig.4). Nymphal attachment was significantly reduced by 0.005% azaform, at which

Fig. 1. Results of treatments against blue ticks

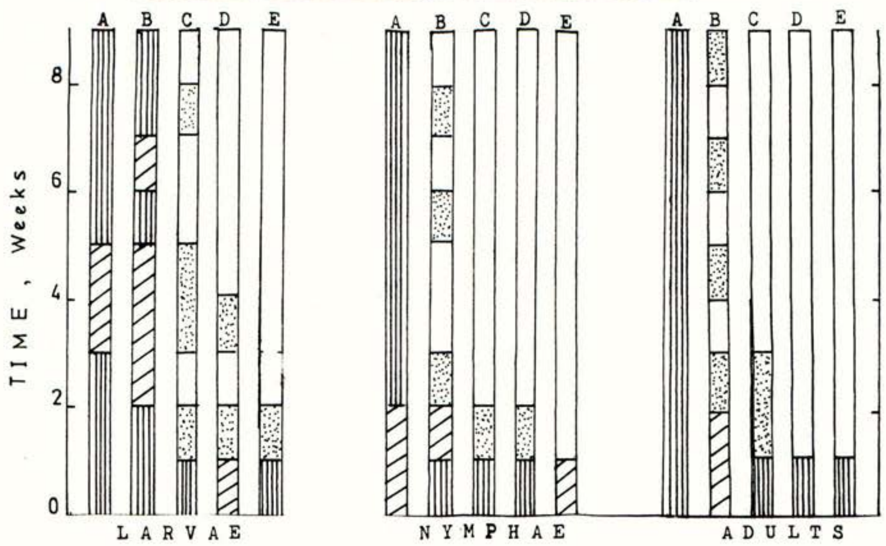
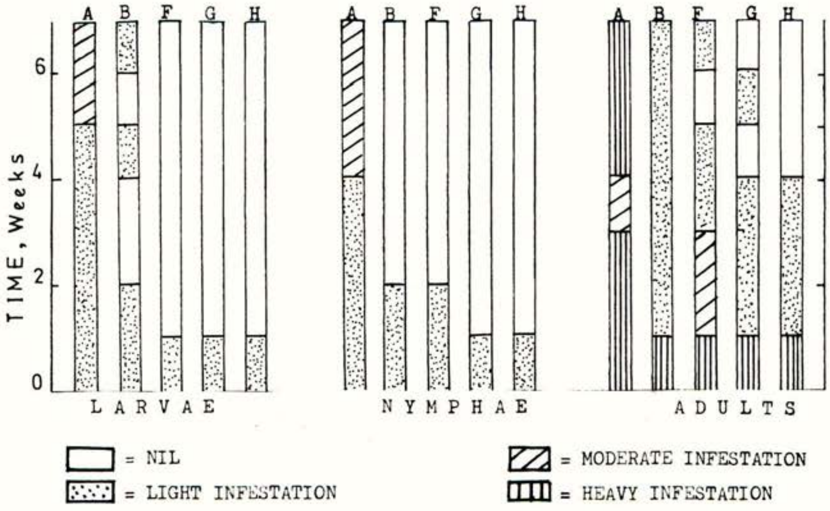


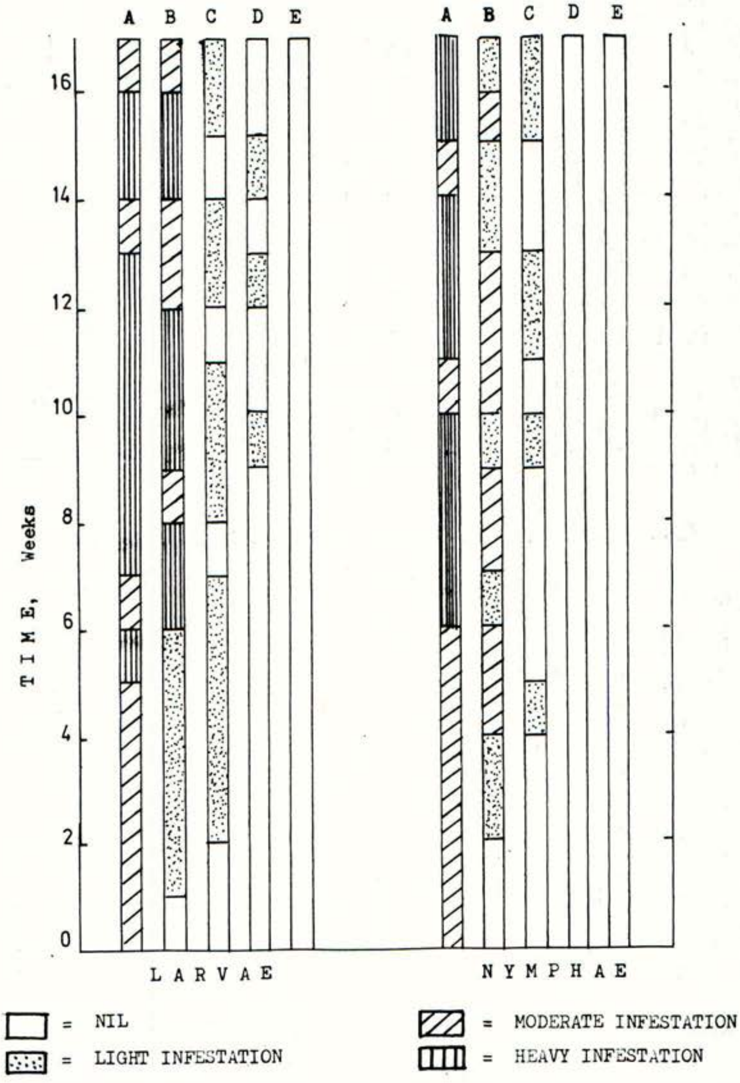
Fig. 2. Results of treatments against red-legged ticks



Average from groups of four animals,
Weekly intervals of treatment

A untreated controls; B 0.025% dioxathion/ 0.025% chlorfenvinphos; C 0.001% azaform; D 0.002% azaform; E 0.006% azaform; F 0.002% azaform; G 0.004% azaform; H 0.006% azaform

Fig. 3. Results of treatments against red-legged ticks

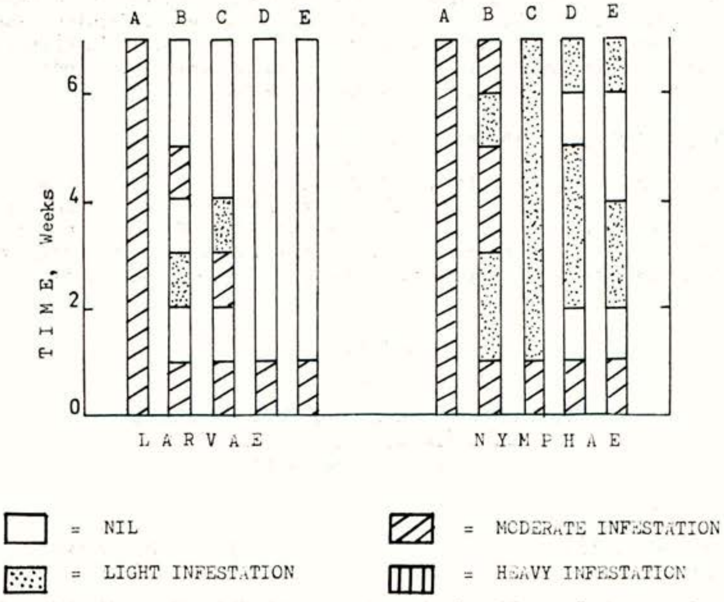


Average from groups of four animals,

Weekly intervals of treatments

A untreated controls; B 0.025% dioxathion/ 0.025% chlorfenvinphos; C 0.0125% azaform; D 0.025% azaform; E 0.05% azaform

Fig. 4. Results of treatments against brown ear-ticks



Average from groups of four animals

Weekly intervals of treatments

A untreated controls; B 0.025% dioxathion/ 0.025% chlorfenvinphos; C 0.0025% azaform; D 0.0035% azaform; E 0.005% azaform.

level minimal engorgement was recorded. Little or no nymphal activity was observed at 0.0125%. 0.025% dioxathion/0.025% chlorfenvinphos permitted regular attachment and frequent engorgement of both larval and nymphal stages.

Against heavy adult infestations, 0.025% azaform almost completely inhibited feeding and markedly reduced numbers of re-infesting female ticks (Fig.5.). Highly satisfactory levels of control, superior to that of 0.025% dioxathion/0.025% chlorfenvinphos, were recorded by the 0.0125% and 0.00625% azaform concentrations.

Bont ticks (*Amblyomma hebraeum*)

Within the concentration range 0.00625% - 0.025%, azaform gave excellent control of adult bont ticks, the reduction in the number of feeding females being noteworthy (Fig.6). Short interval counts indicated that re-infesting female ticks did not commence feeding within a six day period following treatment as compared to four days for 0.025% dioxathion/0.025% chlorfenvinphos.

Expellency Observations (Tables 1 and 2)

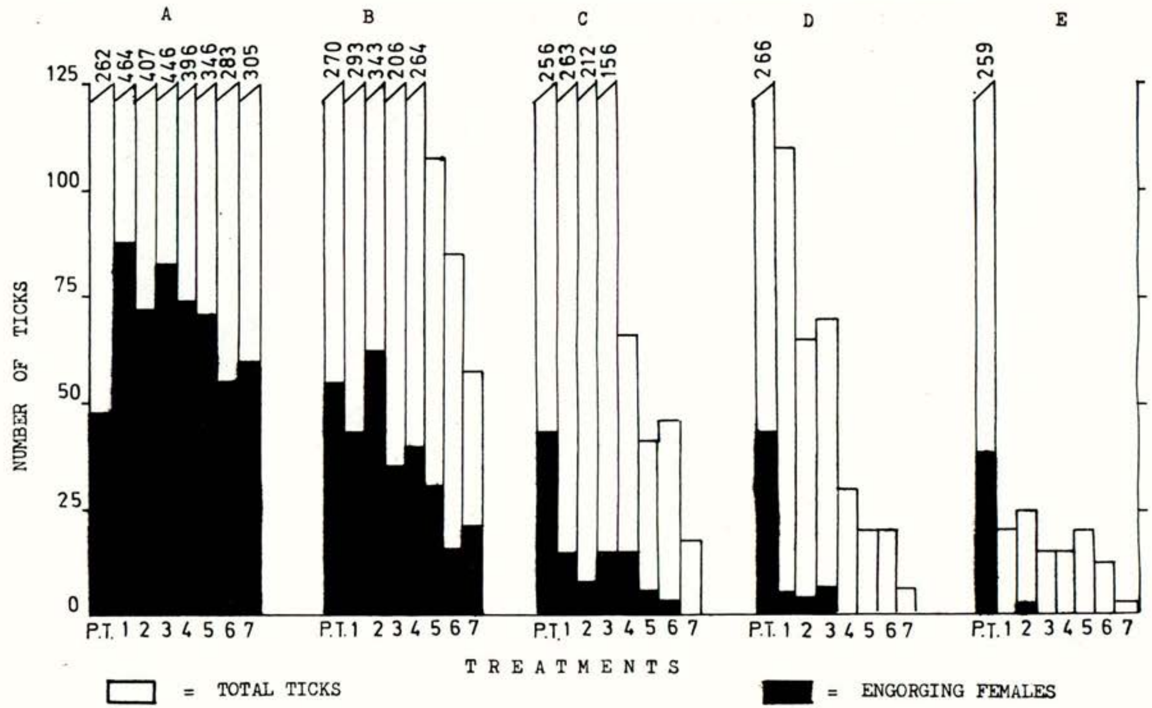
A marked expellency of attached ticks was observed within one hour after treatment. Flat ticks failed to survive longer than 48 hours after collection either under optimum incubator conditions or when transferred to a new, untreated host. No attempt was made to re-attach to the new host. Only small numbers of expelled gravid female blue ticks survived to lay viable egg batches.

Table 1

Expellency effect on male and unfed female *Amblyomma hebraeum* treated *in vivo* with 0.025% azaform e.c.

Time elapsed between treatment and expulsion	Number of ticks	% Mortality after 48 hours
Pretreatment	832 (*in situ*)	-
0 - 1 hours	112 (expelled)	100
1 - 2 hours	279 "	100
2 - 3 hours	244 "	100
3 - 4 hours	111 "	100
4 - 5 hours	13 "	100
5 - 8 hours	9 "	100
Manually removed		
8 hours	64	100

<u>Fig. 5. Results of treatments against adult brown ear-ticks</u>

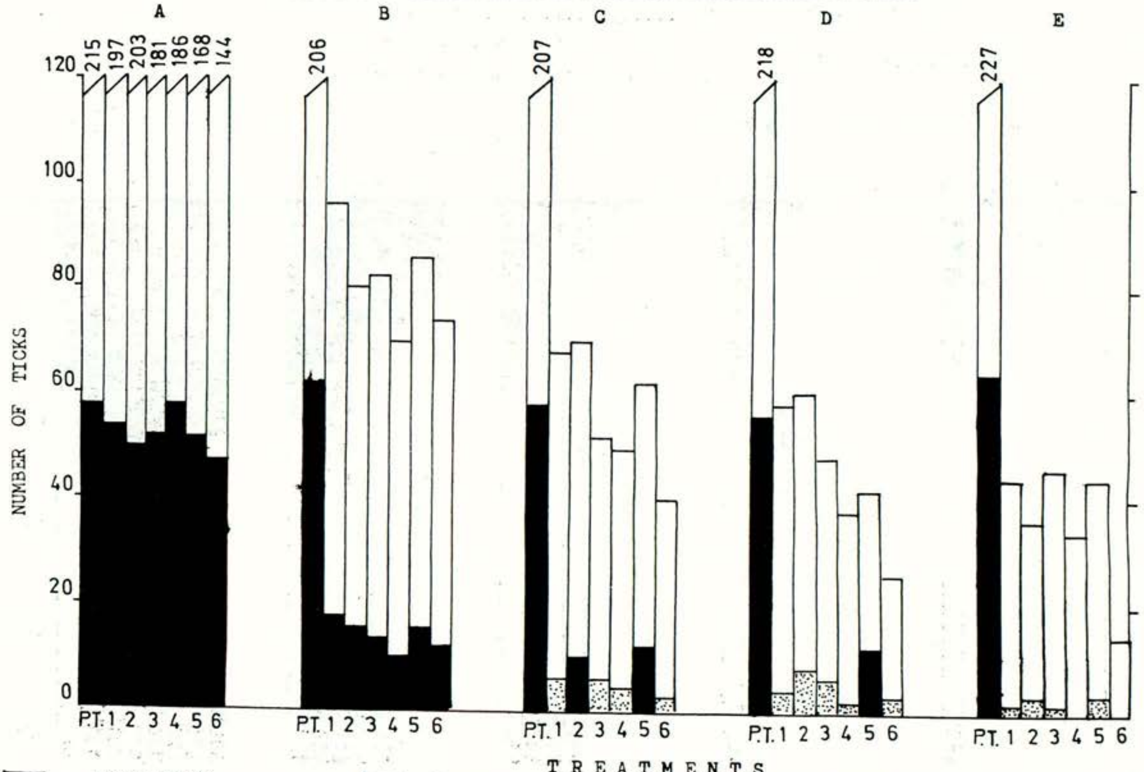


Average from groups of four animals.
Weekly intervals of treatments

PT = PRE-TREATMENT

A untreated controls; B 0.025% dioxathion/0.025% chlorfenvinphos; C 0.00625% azaform; D 0.0125% azaform; E 0.025% azaform

Fig. 6. Results of treatments against adult bont ticks



= TOTAL TICKS = INITIAL FEMALE ENGORGEMENT = ENGORGING FEMALES

Average from groups of four animals. Weekly interval of treatment. PT = PRE-TREATMENT

A untreated controls; B 0.025% dioxathion/0.025% chlorfenvinphos; C 0.00625% azaform;
D 0.0125% azaform; E 0.025% azaform.

Table 2

Expellency effect of azaform on gravid female *Boophilus decoloratus* treated *in vivo*

Formulation and wash concentration	Time elapsed between treatment and expulsion	Number of ticks	Percent ticks laying viable eggs
A. Wettable Powder			
0.0125%	0 - 1 hours	54	2
	1 - 2 hours	36	8
0.025%	0 - 1 hours	60	6.6
	1 - 2 hours	38	0
Control	-	10	100
B. Emulsifiable Concentrate			
0.025%	1 - 5 hours	247	0.5
Control	-	20	100

DISCUSSION

Spectacular results were achieved with azaform in these trials. Comparison with dioxathion/chlorfenvinphos clearly demonstrates the significant margin of difference existing between azaform and an established commercial acaricide.

Azaform was shown to possess a number of highly desirable ixodicidal features:

(a) An ability to expel established tick infestations rapidly. This promotes quicker healing of tick damage and reduces the chances of disease transmission.

(b) An extended period of protection against all reinfesting tick stages. This is of particular significance with the brown ear-tick which, by virtue of its disease potential, can be classed as the most important of the African Continent. In its adult stage it is also the most difficult species to counter by established treatment regimens.

(c) An apparent systemic effect whereby indirect tick control is achieved. Poor control of tick life within the ears of cattle is an inherent problem of present spraying and dipping procedures.

(d) A significant ovicidal effect on gravid females of value in reducing the tick infestation on farms.

(e) A wide spectrum of ixodicidal activity in the field within the concentration range 0.001% - 0.05%.

(f) A similar level of activity against both resistant and susceptible strains of different tick species.

Further field trials aimed at establishing the use of this compound in both sprays and plunge dips are in progress.

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