THE USE OF VISIBLE MUTANT MARKERS IN THE STUDY OF RESISTANCE OF HOUSE FLIES TO INSECTICIDES

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Summary Recessive visible mutants on known linkage groups can be used to locate and isolate single resistance factors in strains of house flies in which resistance to one or more insecticides is controlled by more than one gene.

This is explained by an example where the resistance factors to a number of insecticides were located and isolated from the diazinon-selected SKA strain of house flies by using visible markers.

INTRODUCTION

Resistance to chlorinated insecticides celebrates this year its 21st anniversary. First detected in the house fly in Sweden it has become now a world-wide problem, and over 180 species of insect pests and mites are known to have developed resistance to one or more insecticides (Georghiou 1965).

Resistance can be confined to a single group of closely related insecticides or extend to insecticides of unrelated chemical groups. In the house fly we can usually distinguish the following types of resistance spectra:

Group I: DDT and its related compounds, e.g. methoxychlor, TDE, o-chloro DDT, etc,
Group II: γ-BHC and the cyclodienes, e.g. dieldrin, aldrin, heptachlor, etc,
Group III: organophosphates and insecticides of Group I,
Group IV: carbamates and often insecticides of Group I,
Group V: pyrethroids and insecticides of Group I,
Group VI: miscellaneous.

House flies selected with an insecticide of group I or II tend to develop strong resistance only to insecticides of the group with which they have been selected, but those selected with group III and sometimes with group IV almost always develop resistance to DDT and its analogues, and often to the cyclodienes as well. More often than not such insects are considerable more resistant to the chlorinated insecticides with which they have not been in contact than to the organophosphates with which they were selected.

It is generally thought that the same mechanism is responsible when resistance is confined to a single group of chemicals, but where the resistance spectrum covers a range of insecticides of unrelated chemical groups the reasons for resistance are more difficult to explain, and this is where genetics offer the greatest help.

The possible mechanisms that result in the broad resistance spectra known in the house fly include:

a/ a series of factors each specific to its own group of insecticides
b/ factors that protect the insects against one or more insecticides of unrelated chemical groups
c/ a mixture of a and b.

Unfortunately the terminology to describe the different types of resistance mechanisms and their combinations is confused, because it is partly based on what is

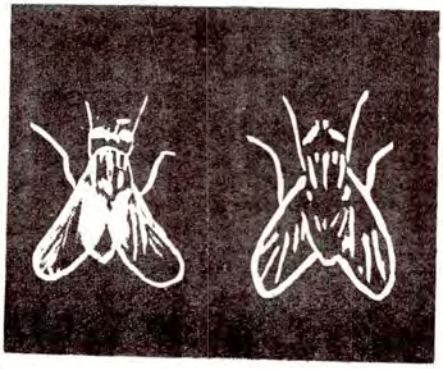
known about individual resistance mechanisms, and resistance spectra for which mechanisms are unknown. It is proposed therefore to use the term specific cross-resistance for a single property ensuring cross-protection against various toxicants of the same group, non-specific cross-resistance for a single property ensuring cross-resistance to various toxicants of different groups, and multiple resistance for the coexistence of specific or non-specific cross-resistance mechanisms in the same strain. When more than one factor contributes to resistance to one compound or a group of compounds resistance is apposite.

The analysis of crosses between normal susceptible and resistant flies by genetical and biochemical methods has led to the discovery of DDT-dehydrochlorinase (DDT-ase) and gene a, the low ali-esterase factor linked with resistance to organophosphates, and to the elucidation of some of the simpler cross-resistance spectra. With the genetical techniques then available, it was difficult to analyse complex resistance spectra and isolate single resistance factors in multiple-resistant strains. The discovery of house fly mutants and the allocation of these mutants to the known linkage groups has enabled rapid progress in the genetics of resistance. The techniques used and some of the results obtained are presented below.

House fly genetics Mutants are individuals that deviate from the normal or wild type, and whose deviations are heritable. The mutant is dominant when the character reappears unchanged in the hybrids, i.e. first generation or F1, is recessive when the hybrid resembles the normal parent and the mutant reappears only in F2 the second generation, and is intermediate when the hybrid resembles both the normal and mutant parents. Mutations can affect either visible characters, such as colour of eyes, form of wings etc. (fig. 1), or biochemical or physiological factors such as resistance to toxins, response to light etc.

Figure 1

Photograph of a multi-marker mutant fly (left) and a normal fly (right). The mutant factors of the multi-marker fly are: brown body (II linkage group), ocra eyes (III linkage group), aristapedia (modified arista on antena, V linkage group) and curved wings (VI linkage group).



The term phenotype refers to the way the individual looks, whereas its genotype describes its genic formula and the way it breeds. When the two members of a given pair of genes in an individual are alike, this individual is homozygous for the given factor; when the two members differ in expression, the individual is heterozygous for the given factor.

The house fly has six pairs of chromosomes of which five pairs, the autosomes, are not sex-determining. All the known visible mutants are on the five autosomal linkage groups numbered from II to VI, which correspond to the five pairs of autosomes. All genes on the same chromosome are linked and so belong to the same linkage group. Most of the mutants have poor phenotypic properties which often affect survival, but a few good mutants for each of the five autosomes not only have the right genetic properties, but also differ enough from the normal type to be recognised easily, and can therefore be used as markers for their linkage groups. These marker mutants are all recessive and are used to locate and isolate the resistance factors in unmarked resistant strains.

Location of the resistance factors To locate the linkage group of the resistance factor(s), each pair of autosomes of the resistant fly is substituted after suitable crosses by the corresponding mutant pair from the susceptible flies. Flies in which substitution has occurred are distinguished by inspection because they are marked by the mutant, and in thoseflies the marked chromosome cannot carry resistance factors because the chromosome pair has been inherited only from the susceptible strain. Thus if the marked fly differs in resistance from the unmarked flies it means that a resistance factor has been removed through substitution. Hence in the resistant parent a resistance factor is present on this chromosome (Table 1a). Where resistance is on a pair of chromosomes other than the pair substituted both mutant and normal flies have the same range of tolerances, because the resistance factor and the mutant are not linked and therefore segregate independantly (Table 1b).

Table 1a

Segregation of the mutant and resistance factor when the factors are on the same linkage group

a - recessive mutant R - dominant resistant
+a - dominant normal +R - recessive susceptible

Parental cross: $\frac{a}{a}\ \frac{+R}{+R}$ x $\frac{+a}{+a}\ \frac{R}{R}$

mutant susceptible normal resistant

F1: $\frac{a}{+a}\ \frac{+R}{R}$

normal resistant

Test-cross: ♀♀ $\frac{a}{a}\ \frac{+R}{+R}$ x ♂♂ $\frac{a}{+a}\ \frac{+R}{R}$

mutant susceptible normal resistant

Progeny: $\frac{a}{a}\ \frac{+R}{+R}$ mutant susceptible

$\frac{a}{+a}\ \frac{+R}{R}$ normal resistant

Table 1b

Segregation of the mutant and resistance factor when the factors are on different linkage groups. (The symbols are the same as in Table 1a).

Parental cross:	$\frac{a}{a}$;	$\frac{+R}{+R}$	x	$\frac{+a}{+a}$;	$\frac{R}{R}$		
	mutant	susceptible		normal	resistant		
F1:			$\frac{a}{+a}$;	$\frac{+R}{R}$			
			normal	resistant			
Test-cross: ♀♀	$\frac{a}{a}$;	$\frac{+R}{+R}$	x	♂♂	$\frac{a}{+a}$;	$\frac{+R}{R}$	
	mutant	susceptible			normal	resistant	
Progeny:	$\frac{a}{a}$;	$\frac{+R}{+R}$	mutant susceptible				
	$\frac{a}{a}$;	$\frac{+R}{R}$	mutant resistant				
	$\frac{a}{+a}$;	$\frac{+R}{+R}$	normal susceptible				
	$\frac{a}{+a}$;	$\frac{+R}{R}$	normal resistant				

The technique used us as follows: The two parents, i.e. the marker-carrying susceptible strain and the unmarked resistant strain both homozygous for their respective factors are crossed, and the F1 progeny, which is normal in appearance is tested for resistance to determine the expression of the resistance factor(s). F1 males are then crossed with susceptible mutant females; this is the test-cross. The direction of this cross is important in the test-cross because crossing-over, that is an exchange of parts between the chromosome members of the same pair, occurs almost exclusively in females. Thus by using F1 males, where crossing-over does not occur, the identity of the chromosome member of the resistant parent is preserved. The mutants that reappear in the test-cross progeny and the normal flies are tested to determine the tolerance range of the phenotypes and thus locate the resistance factor.

The factors of resistance can be located either by substituting each linkage group of the resistant parent in turn with a single marker by crossing the resistant flies with single markers, or all the factors of resistance can be located simultaneously by crossing the resistant strain with a multi marker strain, where all the autosomes are marked. Thus in this test-cross progeny five of the phenotypes will retain only one of the autosomes from the resistant parent as shown.

Resistant parent		Susceptible parent
$\frac{+^{a}}{a}\ ;\ \frac{+^{b}}{b}\ ;\ \frac{+^{c}}{c}\ ;\ \frac{+^{d}}{d}\ ;\ \frac{+^{e}}{e}$	x	$\frac{a}{a}\ ;\ \frac{b}{b}\ ;\ \frac{c}{c}\ ;\ \frac{d}{d}\ ;\ \frac{e}{e}$

Quintuple markers in test-cross progeny

$\frac{+^{a}}{a}\ ;\ \frac{b}{b}\ ;\ \frac{c}{c}\ ;\ \frac{d}{d}\ ;\ \frac{e}{e}$	normal for a
$\frac{a}{a}\ ;\ \frac{+^{b}}{b}\ ;\ \frac{c}{c}\ ;\ \frac{d}{d}\ ;\ \frac{e}{e}$	normal for b
$\frac{a}{a}\ ;\ \frac{b}{b}\ ;\ \frac{+^{c}}{c}\ ;\ \frac{d}{d}\ ;\ \frac{e}{e}$	normal for c
$\frac{a}{a}\ ;\ \frac{b}{b}\ ;\ \frac{c}{c}\ ;\ \frac{+^{d}}{d}\ ;\ \frac{e}{e}$	normal for d
$\frac{a}{a}\ ;\ \frac{b}{b}\ ;\ \frac{c}{c}\ ;\ \frac{d}{d}\ ;\ \frac{+^{e}}{e}$	normal for e

Bio-assays will indicate which of the unsubstituted linkage groups carry the dominant resistance factor(s).

Isolation of resistance factors in the homozygous conditions Tests on the test-cross progeny show where the dominant or intermediate factor is present. Even then they only indicate the resistance conferred in the heterozygous condition. To study one particular resistance factor it is preferable to isolate and retain this factor by inbreeding, rather than repeat the test-cross each time the resistance factor is required.

The technique of isolating the resistance factor(s) of single linkage groups is an expansion of the technique to locate the resistance factors.

If we assume that there is only one incompletely dominant factor of resistance (R) in the strain investigated which segregates in opposition to the marker a of the same linkage group, all the marked individuals in the test-cross progeny lack the resistance factor and have the $\frac{a\ ;\ +^{R}}{a\ ;\ +^{R}}$ genotype, whereas all the normal individuals are heterozygous for the marker and the resistance factor and are represented by $\frac{a\ ;\ +^{R}}{+^{a}\ ;\ R}$, where the upper symbols represent the genotype of the chromosome member of the susceptible marker strain and the lower symbols the genotype of the chromosome member of the resistant parent (Table 1a). The progeny of these apparently normal flies will fall into three different classes:

a/ $\frac{a\ ;\ +^{R}}{a\ ;\ +^{R}}$ homozygous for the marker and fully susceptible

b/ $\frac{a\ ;\ +^{R}}{+^{a}\ ;\ R}$ normal in appearance, incompletely resistant

c/ $\frac{+^{a}\ ;\ R}{+^{a}\ ;\ R}$ normal in appearance, fully resistant

The marked individuals in class a/ carry no resistance factor provided cross-over is small and these flies are discarded, but it is impossible to distinguish between the heterozygotes for resistance (b) and the homozygotes (c) by visual inspection because both classes are of the normal phenotype. For this reason the flies of the normal phenotype are bred in single pairs in the hope that both single pair parents will be fully homozygous for resistance. Such a pair will give in its F1 and all subsequent progenies only flies of the normal type without the marker. The two other types of crosses will give marked individuals either in F1 or F2. Thus the cross :

$\frac{a\ ;\ +^R}{+^a\ ;\ R}$ x $\frac{a\ ;\ +^R}{+^a\ ;\ R}$ gives marked individuals in F1,

$\frac{a\ ;\ +^R}{+^a\ ;\ R}$ x $\frac{+^a\ ;\ R}{+^a\ ;\ R}$ gives marked individuals in F2, and

$\frac{+^a\ ;\ R}{+^a\ ;\ R}$ x $\frac{+^a\ ;\ R}{+^a\ ;\ R}$ gives normal flies only.

The absence of the marker in F2 et al. indicates that the chromosome pair has probably been inherited from only the resistant parent. However, the progeny of such a pair must be tested thoroughly over several generations to determine its purity, because cross-over or the heterozygosity of the resistant parent prevents the homozygosity of the resistance factor in single pair progenies.

Application of the genetical techniques to SKA flies

Cross tolerance of SKA flies For about 10 years we have bred a strain of flies, the SKA strain, which was derived from two field strains resistant to diazinon, viz. the Latina strain from Italy, and the 203a strain from Denmark. The SKA strain has been selected with diazinon at each generation and is now very resistant to this compound (x500>). Toxicological and biochemical work showed that this strain carried several resistance factors, and that the strain had a wide resistance spectrum. The SKA flies are also very resistant to ethyl chlorthion (x450), moderately resistant to parathion (x65), ethyl chlorthion (x80), ethyl fenchlorphos (x30) and ethyl malathion (x22) and are almost susceptible to malathion (x4), fenchlorphos (x2) and Dichlorvos (x2). The SKA flies are extremely resistant to dieldrin.

Location of the factors of resistance in SKA flies We first located two major resistance factors to diazinon by crosses between single marker strains and SKA flies followed by test-crosses and bio-assays on the test-cross progenies. The two factors are linked with ocra (III linkage group) and ar (V linkage group). There were no dominant factors on the 2nd and 6th linkage groups, because the log-dose-probit lines of the markers and wild types were identical, but the differences in the tolerances of the mutants and wild flies in the ocra and ar test-crosses showed the presence of resistance factors segregating in opposition to the two markers.

The two major resistance factors were then isolated in opposition to the corresponding markers by crossing the susceptible double marker ocra;ar with SKA flies, using the technique described above. Of the four classes segregating in the test-cross progeny, viz. wild, ocra, ar, and ocra;ar, we retained only the ocra and ar mutants. In ocra flies the factor on the III linkage group was missing, because it was substituted by the chromosome pair carrying the ocra mutant which came from the susceptible double-mutant parent. Therefore ocra flies only had the resistance factor on the V linkage group. Similarly ar flies where the V linkage group was inherited completely from the susceptible parent carried only the resistance factor on the III linkage group. Each resistance factor was then isolated in a homozygous condition by single-pair mating, and flies with resistance on the V linkage group had the ocra marker on the III linkage group, whereas those with resistance on the III linkage group had the ar marker on the V linkage group.

By means of this technique we showed that the two factors are incompletely dominant and that neither factor confers by itself great resistance to diazinon even when homozygous (Table 2). This experiment also indicated the possible presence of

Table 2

Degree of tolerance to diazinon of the three factors of diazinon-resistance in SKA flies

Resistance factor(s)	Linkage group	Degree of resistance
R2	II	c. x 2
R3	III	c. x 14
gene a	V	c. x 14
R2; R3; a	II, III, V	c. x 500

a third factor because the ocra;ar flies of the test-cross progeny, in which the III and V resistance factors were absent were 1.5 times more resistant to diazinon than the ocra;ar susceptible parent, and there was a 2-3 fold difference in resistance between the strains carrying the single dominant factor. The third factor has now been located and isolated, and is on the II linkage group. When alone it confers very weak resistance to diazinon c. x 2 but increases the resistance of the two major factors and probably acts as a modifier.

The two major factors of resistance to diazinon in SKA flies are: gene a for low ali-esterase on the V linkage group, and a sesamex susceptible factor, R3, on the III linkage group. Flies carrying R3 lose their resistance to diazinon after pretreatment with sesamex, and resemble flies of the Fc strain (Oppenoorth, 1965).

Although the two major factors of resistance give almost identical results with diazinon (Table 2) they definitely differ. Gene a, the gene for low ali-esterase on the V linkage group shows specific cross-resistance confined to OPs such as parathion, chlorthion, "ethyl" malathion, and diazinon. R3 the factor on the III linkage group shows no specific cross-resistance. It protects the flies against diazinon and DDT but not the OPs mentioned above. Also the two factors differ in their response to diazinon after pre-treatment with sesamex (Table 3). Resistance in flies carrying R3 disappears completely after pre-treatment with sesamex, but increases in flies carrying gene a. Thus synergism and antagonism to the same compound can co-exist within the same strain as it does in the SKA strain.

Table 3

Response to R3 and gene a to diazinon after pre-treatment with 2 µg/fly of sesamex

Resistance factor	Diazinon alone LD50 µg/fly	Diazinon after sesamex LD50 µg/fly	Activation by sesamex
R3	0.79	0.048	x 11
gene a	0.76	1.35	x 0.6

We have recently located and isolated the factors of resistance to diazinon, DDT and dieldrin, by crossing the SKA flies with the quadruple marker susceptible marker strain bwb, (brown body II); ac (curved wings, VI); ar (aristapedia, V); ocra (yellow eyed, III) (fig. 1) using the same techniques as before, and found that resistance to diazinon is on the 2nd, 3rd and 5th linkage groups, to DDT on the 2nd, 3rd and 5th linkage groups and to dieldrin on the 4th linkage group (Table 4). The

Table 4

Distribution of resistance factors in linkage groups of SKA house flies

2	3	4	5	6
DDT x 2.5 diazinon x 2 parathion x 2 zectran	DDT x 15 diazinon x 15 (sesamex sensitive factor)	dieldrin x 100> γ-BHC	DDT-(DDT-ase) x 100> gene a diazinon x 15 chlorthion x 10 parathion x 15	
brown body bwb	ocra-eye ocra	-	aristapedia ar	curved wing ac

factors for resistance to diazinon (gene a) and DDT (DDT-ase) although on the same linkage group (V) are each of the specific cross-resistance type, the factor of resistance to these two compounds on the 3rd linkage group is probably of the non-specific cross-resistance type, because both compounds are synergised by sesamex. The factor on the 2nd linkage group is probably also of the non-specific cross-resistance type and almost certainly acts as a modifier to the major factors of resistance to DDT, diazinon, parathion and dieldrin.

The factor of resistance to dieldrin is of the specific cross-resistance type, because it affects only the cyclodienes and γ-BHC.

DISCUSSION

It seems that in the SKA strain great resistance to diazinon is brought about by the interaction of two weak and one very weak factor of resistance.

Multiple resistance in flies selected with diazinon can arise for various reasons. The automatic development of resistance to DDT in house flies selected with diazinon can develop either through the selection of individuals in which the factors on the II and III linkage groups are common to both insecticides or the selection of flies where DDT-ase and gene a are linked. With either, resistance to DDT will considerably exceed resistance to diazinon because DDT-ase by itself or the combination of the factors on the II and III linkage groups give strong resistance to DDT, whereas gene a or the factors on the II and III linkage groups give weak resistance to OPs.

Cross resistance to cyclodienes is probably unconnected with selection by diazinon. In the SKA flies dieldrin resistance is located on a linkage group which has no known cross-resistance to diazinon. It was probably inherited from the 203a strain which had developed resistance to chlordane in the field. Resistance to dieldrin has decreased over the years and now less than 5% of the SKA flies are

resistant to dieldrin. Also resistance to dieldrin may not necessarily develop through selection with an insecticide. It has developed in a susceptible population propagated from early emerging adults (Georghiou et al., 1963).

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THE EFFECTS OF PLANT GROWTH-RETARDING COMPOUNDS ON THE BEHAVIOUR OF SOME PESTS AND DISEASES OF BLACKCURRANT

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Summary

Chlormequat applied as a foliar spray at 5000 ppm to young shoots of Malvern Cross did not protect these from infestation by blackcurrant gall mite as well as it had done on the less vigorous variety Wellington XXX. The more powerful retardant, N-dimethylamino succinamic acid at 1000 ppm was more effective. Good control of blackcurrant leaf spot was obtained on fruiting bushes of Baldwin with chlormequat, but by the end of the season this treatment resulted in a higher level of American gooseberry mildew than on untreated shoots. N-dimethylamino succinamic acid at 1000 ppm did not appear to affect either leaf spot or mildew. Whilst growth retardants may act against pests and diseases in several ways, it is suggested that an important factor may be the availability of suitable habitats, which are reduced in number during mite migration, allowing the plant to 'escape'; the plant may also be made unfavourable for the development of leaf spot, but favourable for mildew, by a high rate of post check growth, thus changing the plant's degree of resistance to these diseases.

INTRODUCTION

Experiments at Long Ashton on the effects of plant growth retardants on pests and diseases followed earlier attempts to control a gall-forming eriophyid mite (Cecidophyopsis ribis) by interfering with the host/parasite relationship, using quaternary ammonium compounds, systemically acting. Growth retardants have been defined as chemicals which limit elongation of the stem without malformations of leaf, stem or flower (Cathey 1964). They are known to increase plant resistance to stress from high and low temperatures, drought, air pollutants and high salt concentrations, and on woody plants to promote the initiation and development of flower buds. There have been reports of growth regulators giving some control of pests and diseases; van Emden (1964) found that Chlormequat (2-chloroethyltrimethyl ammonium chloride) reduced the reproduction of the cabbage aphid on brussels sprouts and Tahori et al , (1965a) found that the same compound had an anti-feeding effect on the cotton leafworm; also that it reduced the reproduction of the oleander aphid (1965b). Tahori et al also found (1965c) some reduction of wheat stem rust and southern stem rot fungus on bean seedlings whilst Sinha and Wood (1964) observed that chlormequat gave some protection against verticillium wilt on tomatoes. Smith and Corke (1966) found that blackcurrant bushes (var. Wellington XXX) given 3 foliar applications of chlormequat at 5000 ppm, had 73% fewer mite-infested buds than the untreated bushes. At harvest time on fruiting bushes, var. Baldwin, there was 43% less leaf spot infection and 66% fewer Botrytis-infected berries than on the unsprayed bushes. The treatment had little effect on American gooseberry mildew on fruiting bushes but by the end of the year this was more severe on the chlormequat treated shoots of Wellington XXX, previously cut back, than on the unsprayed bushes.

Field experiments were continued in 1966 to compare the effectiveness of chlormequat at different concentrations and times against blackcurrant pests and diseases and to compare its effects with that of a more powerful growth retardant, N-dimethylamino succinamic acid.

METHOD AND MATERIALS

To test the ability of the growth retardants to protect healthy bushes from infestation with blackcurrant gall mite, heavily infected plants were placed at intervals along rows of healthy 1 year old Malvern Cross bushes and left unsprayed so that each

plot consisted of 5 bushes on either side of an infection source. There were 4 replicates per treatment set out in a randomised block design. All chemicals were applied to the foliage at H.V. by hand lance and the treatments were; (1) Chlormequat 5000 ppm on three occasions; (a) at the 'grape' stage (April 1st); (b) at full blossom (April 26); (c) on May 12th; (2) Chlormequat 2500 ppm at a + b + c; (3) Chlormequat 1250 ppm at a + b + c; (4) Chlormequat 5000 ppm at a; (5) Chlormequat 5000 ppm at b; (6) Chlormequat 5000 ppm at c; (7) N-dimethylamino succinamic acid 1000 ppm at a + b + c; (8) Unsprayed control; (9) Endosulfan 0.05% at a + c.

In another part of the same plantation treatments (1), (2), (3), (7) and (8) were each repeated on 60 fruiting bushes of the variety Baldwin, arranged in randomised plots, to assess the effect of the growth retardants on disease incidence and yield. Zineb (0.15%) applied 4 times was also included in this experiment. On the Baldwin bushes assessment of infection by leaf spot was made in July by using the system of Clarke and Corke (1956). The numbers of mite infested buds on the Malvern Cross were counted in December. American gooseberry mildew was assessed on both varieties in July by classifying leaves in a series of infection grades (1-10), and again in November by classifying shoot infection according to Corke and Jordan (1965). Crop weights were taken on the Baldwin bushes in 1966 and on the Malvern Cross in 1967. All buds were counted and dissected in December 1966 on a sample of shoots from each treatment on the Malvern Cross, to ascertain effects on numbers of buds and on the flower/vegetative bud ratio. Labelled shoots were measured weekly on each Malvern Cross treatment to determine the intensity and duration of the growth check.

Buds were also dissected on bushes which were sprayed with chlormequat at 5000 ppm, after mites had invaded them, to determine if the retardant had had any eradicant effect.

In the laboratory mites were brought into contact with chlormequat to find if there was any direct toxicity.

RESULTS

Table 1 (Part 1)

Growth retardants - effects on blackcurrant gall mite, American gooseberry mildew

Treatment	Spraying dates 1966 a = April 1st, b = " 26th, c = May 12th	Mite infested buds/40 bushes	American gooseberry mildew July (Leaf grades 1-10)	American gooseberry mildew Nov. Mean No. in grade 5 (10 bushes) ‡	Mean final shoot length (in)
Chlormequat (5000 ppm)	a + b + c	113	3.2	69.0*	17.8
Chlormequat (2500 ppm)	a + b + c	114	3.1	43.0	17.5
Chlormequat (1250 ppm)	a + b + c	127	2.1	28.2	16.8
Chlormequat (5000 ppm)	a	135	3.2	51.0	19.1
Chlormequat (5000 ppm)	b	101	3.2	27.2	17.6
Chlormequat (5000 ppm)	c	146	3.5	45.5	19.1
N-dimethylamino succinamic acid (1000 ppm)	a + b + c	63*	3.1	45.7	14.5
Unsprayed control		118	3.2	40.2	16.7
Endosulfan	a + c	52*			

‡ Grade 5 is the most heavily infected category
* Significantly different from control at 5.0%

Table 1 (Part 2)

Growth retardants - effects on blackcurrant gall mite, American gooseberry mildew, mean total shoot increment, mean total No. buds per shoot, % flower buds, and yield var. Malvern Cross

Treatment	Spraying dates 1966 a = April 1st b = " 26th c = May 12th	Mean total increment/ shoot (in)	Mean total No. buds/ shoot	% flower buds	Total crop wt in 1967 (lb)
Chlormequat (5000 ppm)	a + b + c	12.8	32.4***	48.7*	10.0
Chlormequat (2500 ppm)	a + b + c	11.5	-	-	7.0
Chlormequat (1250 ppm)	a + b + c	9.7	-	-	4.7
Chlormequat (5000 ppm)	a	12.8	29.0	35.0	6.5
Chlormequat (5000 ppm)	b	11.7	28.8	36.4	5.0
Chlormequat (5000 ppm)	c	13.0	30.0*	40.8	7.2
N-dimethylamino succinamic acid (1000 ppm)	a + b + c	11.8	27.1	9.4‡	2.2
Unsprayed control		10.3	26.7	35.8	5.7
Endosulfan	a + c				

* Significantly different from control at 5.0%
*** " " " " 0.1%
‡ Significantly different from all other treatments at 0.1%

Uneven growing conditions on this plot resulted in large variations between blocks which reduced significance between treatments.

Table 2

Growth retardants - effects on leaf spot, American gooseberry mildew and yield var. Baldwin

Treatment	Spraying dates a = April 1st b = April 26th c = May 12th	Leaf spot (% total leaf area affected)	American gooseberry mildew (leaf grades 1-10)(July)	Crop wt 1966 lbs/plot
Chlormequat (5000 ppm)	a + b + c	8.9***	4.29*	9.2
Chlormequat (2500 ppm)	a + b + c	5.1***	4.79**	7.9
Chlormequat (1250 ppm)	a + b + c	5.0***	2.85	10.6
N-dimethylamino succinamic acid (1000 ppm)	a + b + c	25.2	3.87	8.8
Unsprayed control		22.9	3.48	9.2
Control with zineb	a + b + c + May 26	5.5***	4.68**	8.6

* Significantly different from control at 5.0%
** " " " " 1.0%
*** " " " " 0.1%

DISCUSSION

Gall mite (Table 1) In contrast to the 1965 result on Wellington XXX, where three applications of chlormequat at 5000 ppm reduced the numbers of mite infested buds by 73%, there was no significant reduction of infested buds on Malvern Cross in 1966, and no clear indication of a relationship between mite infestation and concentration of chlormequat. However, N-dimethylamino succinamic acid, used at 1000 ppm reduced the number of mite infested buds by approximately 50% - a similar reduction to that obtained with endosulfan, a standard control measure. This retardant is more powerful than chlormequat. Whilst the mean total increment and final shoot length measurements do not indicate much difference between these compounds, shoot measurements made at weekly intervals throughout the growing season showed that N-dimethylamino succinamic acid treated shoots took much longer to recover from the growth check than those receiving chlormequat, but before the end of the season they had a faster growth rate and caught up. The period during which mites migrate from galls to new axillary buds is from March to June but it has been shown (Smith 1962) that the majority of mites migrate during the blossom period, especially at the full blossom stage which corresponded with spray b in 1966. Mites can only penetrate buds at a certain stage of development; those in the axils of the youngest leaves near the shoot apex are protected by overlapping tissue and a dense mass of hairs whilst older buds are also impenetrable. If the numbers of buds in susceptible growth stages are limited by reduction in stem elongation rate at the time when the majority of mites are searching for them, appreciable reductions in infestation might be expected. The extent and duration of the growth check appears to be related to concentration of retardant, as is also the rate and amount of subsequent growth and, for example, with chlormequat at 2500 ppm and above, this exceeds that of the untreated. However since only a few mites had not migrated by this time the plant escaped severe attack. There is an indication that the application of chlormequat at full blossom (spray b) was more effective against mite invasion than pre or post blossom applications. Malvern Cross has a different growth pattern to Wellington XXX (Wilson and Adam 1967); its shoots grow faster in May when many mites are seeking buds and such a difference may be at least partly responsible for the better control on Wellington XXX in 1965. When buds of Wellington XXX which had became infested with mites and which had subsequently been treated with chlormequat, were dissected, there was no indication of increased mortality so that this growth retardant did not have mite eradicant properties. Mites which were deliberately brought into contact with chlormequat under laboratory conditions did not show any increased mortality and whilst there are a number of ways in which growth retarding compounds may increase resistance to the establishment and development of this mite it is suggested that the reduction in numbers of suitable available habitats may be an important factor.

Leaf spot (Table 2). Reduction in infection on fruiting bushes of Baldwin was similar to that obtained with 5000 ppm in 1965 and similar also to that obtained from 4 sprays of zineb. Concentrations of chlormequat down to 1250 ppm were equally effective but no reduction was found with N-dimethylamino succinamic acid at 1000 ppm. The incidence of blackcurrant leaf spot is correlated with the vigour of host plant growth; it succeeds best on older leaves, (Marsh and Maynard 1930) and it is likely, therefore, that the rapid production of young tissue as the growth check disappears presents an unfavourable habitat, and the earlier beneficial effects of the retardant are prolonged.

Mildew (Tables 1 and 2). As was the case with Wellington XXX in 1965, by the end of the season there was more mildew on those plants of Malvern Cross which received 5000 ppm chlormequat than on the untreated controls. The amount of mildew on leaves in July on both Malvern Cross and Baldwin, and on shoots in November, varied with concentration of chlormequat as also did the total increment and final shoot measurements. The smallest amount of mildew was found where the least amount of post-check growth had taken place, and since it has been shown that, in contrast to leaf spot, mildew succeeds best on young rapidly growing tissue (Jordan V. W. L. 1967), it is possible that a more suitable habitat for the disease has been provided. With chlormequat at 1250 ppm there is an indication of some control, but this was not found with 1000 ppm N-dimethylamino succinamic acid on Baldwin or Malvern Cross.

Effect on yield and potential yield. (Tables 1 and 2). Chlormequat at 5000 ppm increased both the total numbers of buds and the proportion of flower buds on Malvern Cross, and this resulted in a yield increase. However, dissections revealed that there were more mildew infected buds on the treated shoots, so that the potential yield was not achieved. N-dimethylamino succinamic acid, however, greatly reduced the proportion of flower buds and this is probably the main reason for the low yield of this treatment. On the fruiting bushes of Baldwin, where retardants were applied and yields recorded in the same season, the proportion of flower trusses on treated and untreated plants was similar and there were no significant increases in yield. The amount of leaf spot in this experiment did not appear to affect yield, but the increase in mildew on the shoots treated with the higher concentrations of chlormequat may have adversely affected yields.

Experiments are in progress to determine other mechanisms which may be involved and to assess the potential role of plant growth regulators in pest and disease control.

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THE BIOLOGICAL EFFICIENCY OF A PRILL FORMULATION OF DAZOMET WITH PARTICULAR REFERENCE TO OUTDOOR SOIL STERILISATION

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Summary The performance of a prill formulation of dazomet as a soil steriliser particularly for outdoor use is described. Information is given relating to the control of nematodes, a number of fungal diseases and a range of weeds in a number of crop situations. The activity of the prill is compared with that of the powder formulation and shown to be similar at equal active ingredient rates.

In supporting laboratory and greenhouse studies the release of methyl isothiocyanate from prill and powder formulations of dazomet is compared, and the sensitivity of a range of soil-borne fungal pathogens is evaluated.

INTRODUCTION

At the 3rd British Insecticide and Fungicide Conference in 1963, Hams and Collyer[2] reported on the biological spectrum of dazomet, described the vertical and lateral spread of the evolved gas in the soil, and discussed the methods of incorporation necessary to achieve maximum control. The sterilisation of glasshouse soils using the powder formulation of dazomet is now well established practice. There are also many outdoor situations where use of the product is justified and where it has been used successfully but the nature of the present commercial powder formulation renders general outdoor usage impractical except under conditions of minimum air movement.

Since 1962, collaborative work has been in progress with B.A.S.F. on a number of granular types of formulation, most of which proved unsuccessful because release of methyl isothiocyanate in the soil was insufficiently rapid. More recently in the course of this work there emerged a satisfactory prill type formulation which has been subjected to gas release studies and to a programme of work designed to investigate spectrum of activity and field performance.

MATERIALS

Throughout the work described in this paper two formulations of dazomet were used, a) the standard 85% powder product and b) the prill formulation containing virtually 100% active ingredient.

Laboratory Investigations

a) M.I.T.C. Release Studies

Methods

The method used in the chemical estimations of methyl isothiocyanate (M.I.T.C.) evolved from dazomet formulations in contact with the soil is a modification of that described by Hughes, Read and Smith (1960)[3]. The apparatus used was as follows; 4 glass cylinders, each of 25 cm long and 5 cm diameter, were filled with 170 g. soil, treated with 42.3 mg a.i. dazomet. Air was drawn through each column of soil, flushing the M.I.T.C. into a series of 4 wash bottles filled with ammonia (0.880 specific gravity, diluted 1 to 2). The soil was flushed through daily, the air current being maintained for at least 1 hour.

After the flushing procedure, the ammonia samples were bulked together, made up to a known volume and the absorption measured at 235 mμ, using a Hilger 'Uvispek'

Spectrophotometer. Any M.I.T.C. in the original soil sample is converted by the ammonia to methylthiourea, which absorbs strongly at 235 mμ. Comparison of the optical density with known standards gave quantitative results.

In preliminary experiments, it was shown that the recovery of a known amount of M.I.T.C. from a solution in acetone placed in the soil tube was 100% ± 5%.

Laboratory results and discussion

Chemical determination of the M.I.T.C. released from the powder and prill formulations of dazomet indicate that the prill tends to give more variation in laboratory experiments. This is seen in the case of the following parameters.

Soil moisture content

Whereas changes in moisture content have some small effect on the evolution of M.I.T.C. from the powder, larger effects can be shown with the prill, (see Table 1.)

Table 1.

Release of M.I.T.C. by both prill and powder from Channel Island soil (C.I.L.)

Water content	Prill - M.I.T.C. released (% theory)	Powder - M.I.T.C. released (% theory)
% loss on drying at 100°C		
15	73.0	81.5
13	37.0	65.9

15% water is nearly field capacity for this soil, but, as is usually the case with sandy soils, even the small drop in moisture content to 13% results in an obvious drying of the soil, with a marked change in its appearance and texture. At the higher soil moisture level (15%) the yields of M.I.T.C. are comparable, but at 13% the prill apparently suffers by comparison with the powder. It is, however, clear that the soil in the tube is very far from being an accurate representation of the soil in the natural state. In particular, the soil in the tube is subject to gradual drying out as air flows through it, whereas in natural conditions, the soil below surface is usually moist, especially during the recommended treatment period. Nevertheless, should soil dry out immediately after the treatment in the field, biological activity is unlikely to be impaired since in a subsequent experiment it was shown that water added to dry soil several days after treatment could lead to the evolution of M.I.T.C.

Variation of soil type

In a limited survey of soil types, no obvious correlation was found between M.I.T.C. release and soil type. The powder was consistently good in all types tested and although the prill tended to give rather more variation, no difference was detectable between prill and powder in terms of nematode and fungus control at equivalent rates of use. We conclude, therefore, that the M.I.T.C. release experiments do not give an accurate measure of the field performance, but rather act as a very sensitive indicator of changes induced by variables affecting M.I.T.C. release.

Effect of soil temperature

The laboratory experiments carried out to determine the effect of soil

temperature on the rate of M.I.T.C. release by prills and powder indicated that, as might be expected, the release rate is reduced at lower temperature in both cases. Temperatures at which these measurements were made ranged from 4°C (39°F) to 17°C (62°F). Total yields measured after 9 days, however, were unaffected with one exception, this being where a prill was subjected to low soil moisture. Under these circumstances the overall yield fell. It is however, unlikely in the field that low soil moisture would occur at times of the year when soil temperatures as low as 4°C are experienced.

Effect of prill size

The prill particle size varies broadly from 100 μ to 400 μ in diameter. In chemical and biological tests the rate of release of gas from different sized particles was compared and it was found possible to arrive at an optimum particle size spectrum for consistent biological effect.

b) Biological Spectrum

Method

The laboratory tests described were intended to supplement the information obtained in both glasshouse and field experiments, by establishing the sensitivity of dazomet to a range of fungi known to be pathogenic to certain commercial crops.

The paper cup method previously described in detail (Hams and Collyer 1963)[2] was employed with certain minor modifications. These involve the substitution of waxed paper cups for the plastic containers, a modification in the starting concentration of dazomet employed viz. 95.2 mg of powder in 160 ml of water, and the use of a different assay method for Plasmodiophora brassicae. With P. brassicae, soil known to be naturally infected was treated with dazomet in an identical manner to the other soils in the test, but instead of incubating, kale seeds were sown in the pots and allowed to grow at ordinary greenhouse temperatures. After 10 days, visual assessment of the seedlings was made, after which they were removed from the soil, washed well and the roots stained in aceto-carmine (Samuel and Garrett 1945)[4]. Microscopic examination of the root hairs enabled an assessment of the infection to be made. The results obtained are shown in Table 2.

The dazomet powder was applied as an aqueous suspension to overcome the difficulty of evenly incorporating such small amounts of test chemical into the soil, and to simplify the process of providing the lower rates of application. The depth of soil in the containers rarely exceeded 4 cm, and by reference to results reported by Hams and Collyer[2] it will be seen that penetration of soil by M.I.T.C. vapour gives a control of fungal growth up to 1.5 in. from its source. It can be safely assumed that the soil in the containers in the current tests was evenly affected by the vapour produced by the various dose levels. This procedure thus satisfactorily simulates the effect obtained in the field where various rates of dazomet powder product (viz. 400, 200 etc. lb/acre) are incorporated in the soil to a depth of 9 in.

Results and discussion

The results show that all fungi tested except Fusarium oxysporum, f. sp. phaseoli are satisfactorily controlled by dazomet 85% powder used in this test at a concentration equivalent to 200 lb/acre. Several fungi were controlled at half that rate.

Although these are in vitro results which might, at first, be considered to flatter the performance of the product, it must be realised that the fungi were present in the test soil to a much greater extent than would normally occur in the field. On balance, therefore, it is to be expected that in soils where the above fungi are known to affect crops adversely, treatment of the growing area with 200 lb 85% dazomet powder per acre incorporated to a depth of 9" would produce a marked

improvement in crop health.

Table 2.

Determination of relative sensitivity of a range of fungi to 3 rates of dazomet

Fungus	lbs per acre 85% dazomet powder			
	0	100	200	400
Pythium ultimum	9	0	0	0
Sclerotium cepivorum	9	6	0	0
Stemphylium radicinum	10	10	0	0
Rhizoctonia solani	10	9	1	0
Sclerotinia sclerotiorum	9	9	3	0
Phoma lingam	9	8	0	0
Ophiobolus graminis	9	0	0	0
Fusarium oxysporum f. sp. phaseoli	9	9	8	6
Helicobasidium purpureum	10	0	0	0
Phytophthora citricola	9	0	0	0
Sclerotium tuliparum	10	2	0	0
Botrytis allii	10	9	0	0
Didymella lycopersici	10	10	0	0
Colletotrichum atramentarium	9	0	0	0
Plasmodiophora brassicae	Many plasmodia in root hairs and cortex	Some plasmodia in root hairs and cortex	No plasmodia	No plasmodia
	Plants small; root systems restricted	Plant size and root development moderate	Plants healthy and thriving	Plants healthy and thriving

Key to assessment scale

0 = absence of fungus

10 = complete surface coverage by fungus

Field Investigations

Methods

Trials were of two broad types, detailed trials involving replicated layouts, and user trials involving unreplicated plots of ½ acre in Jersey and 100 to 300 yd^2 in the United Kingdom.

Dose rates of 100 to 340 lb/ac of the prill were used in comparison with the equivalent rates of the 85% powder formulation.

Application was either by perforated hand shaker or by one or other of a number of proprietary applicators the most consistently accurate and convenient of which were the various Sisis models.

Incorporation, essential to the functioning of dazomet, was almost always by rotary cultivator fitted with 'L' shaped blades (Hams and Collyer)[2] and more rarely by hand forking. Different depths of incorporation were compared in certain circumstances where it appeared that deep incorporation (8 to 9 in) might not be necessary. Various sealing procedures were compared in an endeavour to determine the most efficient technique for outdoor use.

The question of time lapse between application and planting has been considered in order to arrive at the most practical commercial recommendation.

The benefits occurring from the use of the dazomet prill have usually been measured in terms of improved crop growth, yield (where possible) and weed control. Quantitative assessment of eelworm or of fungus diseases is often not practical in this type of work but the history of the site gives a useful background against which to measure results.

The first field trials in the series were carried out in Jersey where the prill was compared with the powder formulation and also with metham sodium and D - D against potato root eelworm (Heterodera rostochiensis), Verticillium spp. and Didymella spp. in outdoor tomatoes. More recently in Jersey, trials have been conducted prior to planting cauliflowers with a view to suppressing Plasmodiophora brassicae in that crop and Heterodera rostochiensis in the potato crop due to follow later in the year.

About 130 trials have been initiated this year in the United Kingdom on a range of commercially important crops in situations where various nematode species and/or soil-borne fungus diseases were known to have a serious limiting effect on crop growth. Not all the trials are complete at the time of writing this paper and in other cases where trials are complete it has not yet been possible to collate all relevant data. It is, however, possible to report progress on many of the trials and to indicate the potential of the prill form of dazomet for use in outdoor crops.

Results

Trials in Jersey C.I.

The five trials carried out in 1966 in specially selected fields of high nematode count were the culmination of a 4-year programme of work in which it became known that dazomet at rates of 100 to 200 lb/ac of the 85% powder incorporated into the soil to a depth of 8 to 9 in gave adequate suppression of potato root eelworm sufficient to ensure commercially acceptable yields of tomatoes. In the 1966 trials in which the prill and powder were compared, the equality of performance of the two formulations was very clearly demonstrated in terms of crop height and vigour, foliage colour, truss and fruit size and maturity. The results indicated a very significant suppression of potato root eelworm and of Didymella spp. and Verticillium spp. which were present in the untreated plots. In all cases, dazomet was equal in effect to metham sodium and D-D at recommended rates and quite often was found to be superior to these compounds.

The technique of using dazomet in Jersey is streamlined for rapid execution. Application by a 6 in tractor mounted Sisis spreader is followed by rotary cultivation at the same width using 'L' shaped blades. Sealing is by rolling - the only practical method for large scale usage. Trial experience over several years has shown that the optimum time intervals between application, opening up, and planting are 2 weeks in each case. During the second 2-week period a second opening up cultivation should be made to ensure liberation of all M.I.T.C. from the soil.

Trials in the United Kingdom

For convenience these are reported under the specific crops on which most information is so far available. Weed control is considered separately as are various aspects of procedure.

Tomatoes

A series of trials was laid down in autumn 1966 to compare the efficacy of prill and powder formulations of dazomet in glasshouse crops. In detailed trials 340 and 170 lb/ac of the prill were compared with the powder product at 400 and 200 lb/ac. In user trials only the full rates were used, (normal glasshouse rate of the powder product is 400 lb/ac).

In all trials the full rates of both formulations gave equally excellent results against potato root eelworm, wilts and corky root. The intensity of the infestation can be appreciated from the fact that plants in the control plots were frequently killed by nematodes and fungi very soon after planting. Table 3 gives the results of two typical detailed trials.

Table 3.

Detailed trials at two tomato sites to compare different rates of dazomet prill and powder by plant vigour at both sites and tomato yield at one

Treatments	Weston-on Trent (medium loam)			Lenton (medium loam)				Tomato yields from Lenton trial in lbs and ozs Plots			
	1	2	3	1	2	3	4	1	2	3	4
Prill 340 lb/ac	5	5	5	5	5	5	5	22.12	22.6	22.0	22.5
Prill 170 lb/ac	5	4	5	4	3-4	3	3-4	22.6	22.1	19.12	20.4
Powder 400 lb/ac	5	5	5	5	5	5	5	22.3	22.0	22.5	21.0
Powder 200 lb/ac	5	5	5	4	4	4	4	21.3	21.0	20.14	21.5
Untreated	1	1	1	1	0	1	1	11.4	5.0	10.6	10.14
Plot size	10 x 17 ft			5 x 13 ft				Yield taken from four plants/plot			

Key to assessment scale

5 represents the healthiest and most vigorous plants
0 represents the poorest plants

Celery

A number of very successful trials in self-blanching, earthed-up and glasshouse celery were conducted.

One trial was carried out at Methwold on a deep light peat. The prill at 300 lb/ac was applied on April 16th by standard technique and Dwarf White celery was

planted on May 26th. Growth of celery was better than that in control and in nearby chloropicrin plots. Weed control was outstandingly good and still virtually complete on September 22nd. The plot is on a known Aratis mosaic area populated by Xiphinema sp. and it is hoped that information will be available later on the effect of the prill on the levels of these nematodes.

At a site in Essex, successful control of Phoma spp. and of subsequent bacterial soft rot and other basal rots by 300 lb prill/ac led to a 7-day advancement in maturity compared with control. At this site, the untreated crop contained depressed patches deemed to be due to Paratylenchus spp. Such patches were absent in the adjacent dazomet-treated area. Investigation is in progress to confirm presence of this nematode in these patches.

Five successful celery user trials carried out in the Newent area demonstrated 7 to 10 days advancement in maturity, with increased yield, brought about by the control of basal rots.

Lettuce

In most of the glasshouse trials on tomatoes reported earlier in this paper, the tomatoes were preceded by lettuce, the growth of which was improved equally by powder and prill forms of dazomet.

An interesting feature of the work with dazomet on lettuce has been the fact that, in addition to generally better growth and more even maturity, heart formation has been improved in the dazomet plots as compared with control. Table 4. giving figures from a trial on deep light peat at Methwold, illustrates the point.

Table 4.

Percentage of lettuce plants forming hearts

Replicates	Dazomet prill 300 lb/ac	Control	% Improvement in dazomet plots
1	94	39	140
2	88	50	76
3	83	50	66
4	100	90	11
5	91	64	42

NB. In the control plots from which the above figures were taken the seedbed had been rotary cultivated as in the dazomet-treated plots.

Upon examination, the non-hearting lettuce plants were found to have poorer root formation than those which had hearted normally. The causal organism involved is still in process of being identified.

Potatoes

A number of trials has been carried out on potato root eelworm in potatoes (as distinct from the trials in tomatoes) but at the time of writing the details of only one trial as recorded in Table 5 are available. (Data presented with the kind permission of Dr. C. E. Taylor, Scottish Horticultural Research Institute).

Table 5.

Control of Heterodera rostochiensis by application of dazomet prill

YIELDS

	Weight (lb oz)	Ware	Seed	Chats
A. Untreated				
1	10 lb 15 oz	18	32	57
2	12 lb 14 oz	14	41	44
3	10 lb 13 oz	20	36	71
Total	34 lb 10 oz	52	109	172
B. Treated				
1	12 lb 12 oz	34	23	15
2	17 lb 3 oz	36	22	16
3	13 lb 15 oz	46	22	11
Total	43 lb 14 oz	116	67	42

In the trial recorded in Table 5, dazomet prill was applied by hand shaker at the rate of 400 lb/ac on April 15th, 1967 and incorporated by forking. The ground was turned over on May 7th and potatoes planted on May 13th. It became obvious at crop emergence that all M.I.T.C. had not been released by planting time for the treated plants were slower in emergence and initial growth. By lifting on September 7th all haulms of the control plants were dead or dying back while those of the treated plants were still upright, dark green and vigorous. The results in the table indicate the marked increase in yield, number of ware tubers and decrease in number of chats in the dazomet-treated plots.

Reports of other trials have been received where use of dazomet prill at 300 lb/ac has led to marked increases in yield of potatoes but full details of these trials are not yet available.

Brassica Crops

A number of extremely interesting results has been observed in trials in cabbage and cauliflower crops. Full details are not yet available but in these trials control of clubroot by dazomet at 300 lb/ac and sometimes at 150 lb/ac, has resulted consistently in markedly increased vigour, increases of 3 to 4 in. in height, and a 2 week advancement in maturity and harvesting.

Beans, runner

Results are available of one trial in "bean-sick" soil where plants grown after use of dazomet prill (300 lb/ac), although planted 10 days later than the adjacent control plots, caught up with the latter and were recorded as yielding 30 lb of beans per 100 yd row in excess of the control plots at each picking.

Flower crops

There is considerable experience of the use of dazomet powder formulation in

chrysanthemums. In the current series of trials the prill was used with good effect before the planting of outdoor chrysanthemums in soil known to have been infested with nematodes and Verticillium spp. In all trials, suppression of these organisms resulted in more vigorous, taller plants (up to 10 in. increase in height). Trials in stocks also showed healthier and more rapid growth than in control plots. A number of trials have been arranged in bulb crops against nematodes and bulb rots. Indications to date are of good control of both types of organism.

Other crops

Trials are still in progress on the following crops, onions (white rot), carrots (nematodes), hops (fluctuating wilt), roses, top fruit (replant diseases), strawberries (nematodes and red core), raspberries (nematodes) and forestry. It is not possible at this stage to report on any of these.

Range of action of dazomet

It is appropriate to record here the names of the main organisms so far known from the literature and from the combined work of B.A.S.F. and Boots to be susceptible to dazomet at the rates considered in this paper.

Plant parasitic nematodes

Free-living root nematodes (eg., Pratylenchus spp., Paratylenchus spp., Rotylenchus spp., Hoplolaimus spp.), and root gall eelworms (Meloidogyne spp.); considerable activity is also shown against cyst-forming nematodes of the genus Heterodera (eg., potato or beet eelworms) and stem eelworms (Ditylenchus dipsaci) occurring in the soil.

Soil pests

Wireworms, cockchafer larvae, leather-jackets and other soil-living stages of insect pests.

Soil fungi

eg., the causative agents of damping-off, wilting and root-rot; Aphanomyces spp., Pythium de Baryanum and other Pythium spp., Bremia lactucae, Phytophthora infestans, P. cactorum, P. nicotianae, Peronospora spp., Thielaviopsis basicola, Verticillium alboatrum, Didymella lycopersici, Fusarium spp., Phoma apiicola, Alternaria tenuis, Sclerotium cepivorum, Stemphylium radicinum, Rhizoctonia solani, Sclerotinia sclerotiorum, Phoma lingam, Ophiobolus graminis, Helicobasidium purpureum, Phytophthora citricola, Sclerotium tuliparum, Botrytis allii, Colletotrichum atramentarium, and the clubroot organism Plasmodiophora brassicae.

Weeds

Over the course of years with the dazomet powder, and during the trial series with the prill formulation, a good deal of evidence on the weed control properties of the product has been amassed. Weed control has frequently been excellent when powder or prill have been used to control nematodes, fungi, etc. Results do not appear to be affected by soil type, in fact some of the best results have occurred on peat soils. Control of perennial weeds, particularly couch grass Agropyron repens, has been consistently good as has been that of perennial broad-leaved weeds such as dock and thistle. With such weeds however, any particles of root below the treated zone can eventually recolonise the sterile area.

Recolonising of sterilised areas from weed seeds introduced from outside is a very common occurrence. Tears in polythene sheeting used to seal the ground have demonstrated the ease with which sterilised areas can rapidly become reinfested. Weed seeds can be carried in on boots and equipment, from the air both by wind and by birds and also of course by over-deep cultivations.

There is no doubt that seeds germinating, and rhizomes lying, in the treated zone are very effectively killed but the extent to which weed seeds are killed is not exactly known and this is the subject of further investigation. It is certain that growers who use dazomet powder or prill for soil sterilisation can expect to obtain some degree of weed control, but the extent and duration of this will be very closely related to the care exercised by the grower in avoiding the reintroduction of weed material in to what has become a perfect growing medium.

Procedure for field use of dazomet prill

The trials have shown that application of the prill outdoors can be made extraordinarily accurately by use of simple perforated hand shakers. There is no drift nor dust hazard with this material which has excellent flow properties. For the larger areas normally envisaged in outdoor usage a proprietary applicator will be necessary. The prill is ideally suited for such application and although all types of machine are not sufficiently accurate to be recommended the Sisis 'Truspred' range has been found to be satisfactory.

The same rules of incorporation apply as with dazomet powder, the principles having been laid down quite clearly by Hams and Collyer[2].

In the trials reported here, good results, particularly of weed control, were often associated with shallower incorporation but this aspect requires more consideration before a recommendation can be made.

The question of soil temperature at the time of using dazomet prill has been considered. The laboratory investigations reported earlier in this paper suggested the possibility of slower M.I.T.C. release by prill than by powder at low temperatures when the soil was dry. It is not considered likely that such dry soil conditions will occur in the field when soil temperatures are around 40°F. In the field trial programme several comparisons of powder and prill were made at around 40°F and no difference could be detected between the two formulations. If there is ever any doubt of this in a particular case, a "cress test" to determine persistence of M.I.T.C., carried out on soil samples representative of the total depth of incorporation would soon resolve the matter.

For outdoor use, a period of 4 weeks between application and planting has proved to be satisfactory in all trials in Jersey and in the United Kingdom. Under the conditions usually prevailing 2 weeks has proved sufficient for release of gas and the second fortnight enables that gas to be removed from the soil by successive cultivations. At the lowest soil temperature under which use is envisaged viz. around 40°F it may well be preferable to leave the soil undisturbed for three weeks and then to carry out several cultivations in the fourth week to ensure the soil is free of gas before planting. Particularly under these conditions the "cress test" should be carried out to ensure that all gas has disappeared from the soil.

Some comparisons between different methods of sealing after rotary cultivation of the prill, have been made. In general, rolling was found to be the most practicable method but was slightly inferior to the use of polythene sheeting or flooding. Choice of method will usually depend on facilities available.

Conclusions

From this programme of work it is clear that the soil sterilising performance of dazomet prill equals that of the powder when used in the glasshouse at equivalent rates of active ingredient.

The prill has been shown to be easy of application and from the biological results it can be inferred that under glasshouse and field conditions, release of M.I.T.C. is as satisfactory as with the well established powder product.

The availability of the prill formulation makes possible the use of dazomet

in a wide range of outdoor situations. At a rate of 300 lbs/ac the prill has been shown to have a very valuable potential in crops such as celery, lettuce, potatoes, brassicas, beans and a range of flower crops. Other crops still under investigation will broaden even more the scope of the product.

Control of nematodes and soil fungi in the crop situations reported has resulted in significant improvement of crop vigour, maturity and yield. Good control has also frequently occurred of a range of annual weeds and such important perennial weeds as Agropyron repens (couch).

Acknowledgements

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Acknowledgement is also due to the collaboration of officers of the National Agricultural Advisory Service and other official bodies and to the many growers who have co-operated in the programme of work reported.

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PHOSALONE - A WIDE SPECTRUM ORGANOPHOSPHORUS INSECTICIDE

by D. L. Colinese and H. J. Terry

May and Baker Ltd., Dagenham, Essex

Summary

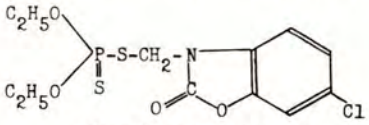
Phosalone is a non-systemic organophosphorus insecticide originally discovered in the research laboratories of Société des Usines Chimiques Rhône-Poulenc, Paris. It is active by direct or residual contact action against a wide range of species including aphids, active stages of mites and the larvae of Diptera, Lepidoptera and Coleoptera. It is of relatively low toxicity to mammals and is not scheduled under the United Kingdom Agriculture (Poisonous Substances) Regulations.

Field trials carried out by Rhône-Poulenc and May & Baker Ltd. since 1963 have established the commercial use of phosalone to control a wide range of pests of apples, pears and peaches, including codling moth, (Cydia pomonella) various leaf- and fruit-eating tortricid species, aphids and mites. It is also used on sugar beet, brassica crops, potatoes, vines and cotton.

The low toxicity of phosalone and its broad spectrum of activity make it suitable for use on a wide range of agricultural and horticultural crops in temperate and tropical conditions.

INTRODUCTION

Phosalone is the recommended common name for a new broad spectrum organophosphorus insecticide and acaricide. Phosalone has the structure:



Phosalone 11,974 R.P.

It is the most promising of a series of insecticidally active organophosphorus esters discovered in the laboratories of the Société des Usines Chimiques Rhône-Poulenc in France in 1961 and first reported by Desmoras et al (1963).

PHYSICAL AND CHEMICAL PROPERTIES

(a) Physical properties

Appearance:	White crystalline solid
Odour:	Slight alliaceous
Melting point:	43-45°C
Vapour pressure:	Virtually nil

Stability: Stable for at least one month at 50°C and at least 12 months at normal storage temperature.

Solubility: Soluble in acetone, chloroform, ethanol, methanol and most aromatic solvents.

Insoluble in water, cyclohexane and light petroleum

Phosalone has been approved for commercial use in France since 1964, in Australia in 1966 and it was cleared for use in the United Kingdom in 1967. Its widest use is on apples and pears but in addition to these crops, it is used in France on beet, potatoes, brassicas, beans and other vegetables, rape, lucerne, strawberries, vines and ornamentals. Experiments on cherries, plums, peaches, cotton and sunflowers are in progress elsewhere.

(b) Toxicology

Phosalone is a typical inhibitor of cholinesterase. The data given in Table 1. show that it is considerably less toxic than parathion, azinphos-ethyl or ethion and it is not scheduled under the United Kingdom Agriculture (Poisonous Substances) Regulations.

Table 1.

Acute Toxicity of Phosalone

(LD50 in mg/kg; aqueous suspension)

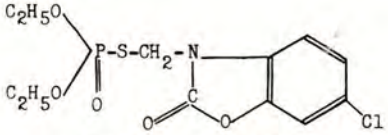
Species	Route	Phosalone	Parathion	Azinphos-ethyl	Ethion
Mouse	Oral	180-205	10	8.2	45
Rat ♀	Oral	135-170	2.6	6.2	36
Rat ♂	Oral	120	-	10.5	-
Guinea-pig	Oral	82-150	5	3.2	40
Rat ♀	percutaneous	390	10	-	-
Rabbit	percutaneous	LD20-1000	45	-	-

Daily oral doses of 7.5 and 15 mg/kg administered to rats for 5 weeks and to dogs for one month resulted in no abnormality in growth, behaviour or pathology and in feeding experiments with rats, phosalone was administered at concentrations up to 250 p.p.m. in the diet for over one year without any change being observed in growth, behaviour or haematology.

Phosalone is relatively non-toxic to game birds. Laboratory experiments with quail and mallard ducks showed that phosalone was considerably less toxic than DDT and that neither species would be at risk when subjected to normal applications of the compound. Pheasant chicks on an experimental orchard suffered no ill effects after being in contact with spray drift and direct applications of phosalone sprays.

Standard tests carried out on various species of fish showed phosalone to be considerably less toxic than DDT. Several practical field experiments have been carried out in this country and overseas on the toxicity to honeybees. The present evidence is that phosalone at a rate of 10 oz/acre can be used without hazard to bees, provided that worker bees are not actively foraging at the time of spraying.

In common with many other phosphorus esters, phosalone can undergo metabolism by oxidation or hydrolysis. A toxic oxidation product, phosalone-oxon, is formed in plants:



Phosalone-oxon 12,244 R.P.

but the concentration remains minimal because it is degraded more rapidly than the phosalone from which it is derived. The hydrolysis products are non-toxic. Among those that have been identified is a glycoside metabolite containing the benzoxazolone ring.

(c) Residue studies

Risks associated with a treated crop are of course related to the residue at harvest, if any. Three procedures have been developed for the determination of residues of phosalone in crops. Using gas liquid chromatography, the preferred method, it is possible to determine concentrations down to 0.02 p.p.m. The second method makes use of hydrolysis followed by colorimetric determination of the copper complex of liberated diethyl dithiophosphoric acid. A biological method using Daphnia as the test organism gave results in close agreement with the other methods (Desmoras, 1964). The sensitivity of the colorimetric and biological methods are 0.1 p.p.m. and 0.2 p.p.m. respectively with recoveries of the order of 80-95%.

Phosalone is fairly persistent in treated plants. It has been shown that phosalone, although not systemic, penetrates the plant tissue to some extent and is present in plant tissue up to 15 days after application.

Table 2.

Decline of residues in apples - France

Interval between treatment and spraying (days)	Residue concentration (ppm) Biological method	Chemical method
0	1.5 ± 0.2	1.3
7	0.9 ± 0.1	0.85
14	0.8 ± 0.1	0.75
28	0.5 ± 0.1	0.35

The results in Tables 3-5. obtained in the United Kingdom and the U.S.A. illustrate the residue levels found in fruit following practical farm application of phosalone.

Table 3.

Summary of crop residue data on apples and pears - United Kingdom 1965-1966

Variety	Application rate % w/v H/V	Application rate oz/acre L/V	No. of Applications	Phosalone residue ppm G.L.C.	Phosalone residue ppm Colorimetric	Interval between last application and residue analysis (days)
Cox's Orange Pippin Site 1966		6.6	3	0.2	-	70
		9.9	3	0.49	-	70
Cox's Orange Pippin Site II		6.6	2	0.05	-	59
		9.9	2	0.09	-	59
Jonathans 1966		6.6	3	0.28	-	70
		9.9	3	0.42	-	70
Conference 1965	0.075		2	0.07	0.1	21
	0.075		2	0.02	0.1	28
	0.075		2	1.16	1.27	35
	0.075		2	0.80	0.72	42
	0.075		2	0.27	0.25	54
Williams 1965	0.075		2	0.33	0.30	21
	0.075		2	0.12	0.1	28
	0.075		2	0.44	0.32	35
	0.075		2	0.29	0.30	42

N.B. The recommended application rate in the United Kingdom is 10.0 oz a.i./acre

Table 4.

Summary of residue data on plums - United Kingdom 1967

Variety	Application rate % w/v H/V	Application rate oz/acre L/V	No. of Applications	Phosalone ppm G.L.C.	Interval between last application and residue analysis (days)
Czar	0.03	-	1	0.41	26
Yellow egg	0.03	-	1	1.33	24
Victoria Site I	0.03	-	3	0.80	11
			3	0.56	20
Victoria Site II	0.03	-	2	0.98	20

Table 5.

Summary of residue data on apples - U.S.A. 1966

Variety	Application rate Conc. % H/V	No. of Applications	Residues obtained after the following interval (days)* after last application			
			14	21	28	28+
Northern Spy	0.03	6	0.6	-	0.58	-
	0.03	6	0.19	-	0.08	-
	0.03	8	0.12	0.1	-	0.1 (29)
Mackintosh	0.04	8	-	-	0.7(26)	1.4 (32)
	0.06	1	1.23	-	-	1.14(29)
Golden Delicious	0.06	5	3.03	-	2.25	-
	0.06	6	3.75	-	-	3.9 (29)
Northern Spy	0.06	7	0.18	-	-	0.15(29)
	0.06	8	0.18	0.1	-	0.1 (29)

* or the day approximating to this interval as shown in brackets.

In the soil phosalone breaks down much more rapidly than parathion. Five breakdown products, all of very low toxicity, have been identified. Residues from very heavy applications of 85 oz phosalone/acre were 3 p.p.m. after 14 days and only 0.5 p.p.m. after 28 days.

Experiments with simulated heavy rain have shown that it does not migrate in the soil.

BIOLOGICAL PROPERTIES

Laboratory and greenhouse experiments

The preliminary screening experiments with phosalone were carried out in the laboratories of the Société des Usines Rhône-Poulenc in Paris and have already been reported, (Desmoras et al 1964). The laboratory results, summarised in Table 6. show a contact activity against hemipterous and lepidopterous larvae of the same order as azinphos-methyl.

Table 6.

Phosalone - Summary of laboratory results

Method of Application	Technique	Insect	Unit of Dose*	Phosalone	Azinphos-methyl	Vamidothion
Contact	Residue on glass slide	Acanthoscolides obtectus	LC_{90} µg/ml	2	0.5	-
		Calandra granaria		0.5	1.0	-
		Tribolium confusum		5	1.0	300
		Musca domestica		30.0	20.0	500
	Direct to insect	Gryllus domesticus	LD_{50} µg per insect	15±3	3±1	33
		Mamestra brassicae		0.8±0.3	0.5±0.2	-
		Cirphis unipunctata		1.2±0.2	1.5±0.3	-
		Musca domestica		0.1±0.02	0.1±0.03	10
Systemic	Root uptake	Aphis rumicis	LC_{50} µg/ml insect	100	10	0.1
Ingestion	Addition to feed	Gryllus domesticus	LD_{50} µg per insect	10±2	1.5±0.3	100
		Mamestra brassicae		4±1	2±0.3	
Ingestion and Contact	Addition to culture	Ceratitis capitata	LD_{50} µg/g	0.2±0.05	0.1±0.02	5±1
		Cirphis unipunctata				
	Contact on leaves	Mamestra brassicae	LC_{50} µg/ml	20	20	200
		Cirphis unipunctata		30	50	200
		Plutella maculipennis		20	30	200
		Tetranychus urticae		25	40	20

* concentration (LC) or dose (LD) which gives 50 or 90% mortality.

Phosalone had virtually no systemic activity, but was an effective stomach poison. Greenhouse experiments showed that phosalone was effective by contact action against aphids, caterpillars and mites for periods up to 15 days after spraying with concentrations of from 0.02 to 0.04% a.i.

Table 7.

Persistence of phosalone in greenhouse experiments

Number of days after application at which phosalone gave 95% control

Plant	Parasite	Dose % a.i.	Phosalone	Azinphos-methyl	Vamidothion
Nasturtium	Aphis rumicis	0.01	4	4	6
		0.02	12	6	15
		0.04	15	15	15
Broad bean	Aphis fabae	0.01	12	5	12
		0.02	15	8	15
		0.04	15	15	15
Cabbage	Plutella maculipennis	0.01	8	10	inactive
		0.02	12	10	"
		0.04	15	15	"
French bean	Tetranychus urticae	0.01	10	10	15
		0.02	12	10	15
		0.04	20	12	15

FIELD USE

The broad spectrum of activity indicated by the laboratory work, suggested that phosalone had a potential use on a wide range of crops that were commonly attacked by a number of different insect species. Examples of use in orchard and field crops has already been reported by Cessac and Burgaud (1964). Trials have continued in the United Kingdom, U.S.A and many other countries.

Apples, pears and peaches

Deciduous fruits are amongst the more important crops attacked by a complex of insect pests. Phosalone is now commercially available in Gt. Britain, France and other European countries and Australia for the control of codling moth, tortrix caterpillars, aphids and red spider mite on apples, pears and peaches. Trials are also being continued in Canada, U.S.A, South Africa and New Zealand. The recommended dose varies with the particular insect species, the intensity of insect attack and local spraying conditions. Thus in the United Kingdom, where codling moth is seldom a serious problem and attacks by tortricid moths are often sporadic, three applications of a dose of 10 oz a.i./acre are sufficient for good control of caterpillars (Table 8.).

Table 8.

Control of codling and tortrix caterpillars in apples - United Kingdom 1966

Treatment	Dose L.V. a.i./ac.	Dose H.V. % a.i.	% damaged fruit Site 11 H.V. Tortrix	Site 12 H.V. Tortrix	Site 13 H.V. Tortrix	Site 13 H.V. Codling	Site 14 L.V. Tortrix	Site 14 H.V. Tortrix	Site 14 H.V. Codling
Phosalone 33% e.c.	6.6	0.021	7.8	8.8	0.3	2.9	5.1	1.5	0.4
	9.9	0.031	2.7	1.9	0.5	2.4	1.2	0	0
Azinphos-methyl 22% e.c.	6.6	0.021	8.9	4.3	0.6	5.1	4.3	4.3	0
	9.9	0.031	4.6	4.7	0.5	2.8	1.8	0	0.6
Control	-	-	12.6	15.1	2.6	15.9	27.7	22.7	1.2

Site details:	Site no.	11	12	13	14
	Variety	Bramley	Cox	W. Pearmain	Cox
	Dates sprayed	15/6	16/6	15/6	13/6
		29/5	30/6	29/6	28/6
		12/7	13/7	12/7	11/7

In France, codling moth is the more serious problem, there often being two generations in the season and trials have shown that eight applications of organophosphorus or chlorinated hydrocarbon insecticides are necessary (Table 9.).

Table 9.

Control of codling moth on apples, France 1963

Treatment Dose % active H.V.	Number of apples Total	Number of apples No. attacked	% attack
Phosalone 0.06 e.c.	688	10	1.7
Phosalone 0.04 w.p.	657	9	1.3
Azinphos-ethyl	658	16	2.4
Parathion + ethion 0.03 and 0.06	623	11	1.7
Control	1,125	251	22.5

Site details: Variety: Winter banana

Spray volume: 8 applications 170 gal/acre

In Australia, high volume spraying is widely practised and a dose of 0.05% phosalone applied at approximately 300 gal/acre, has given good control of codling moth and light brown apple moth (Austrotortrix postvittana). This application also controlled red spider mites. In the United Kingdom the normal three applications for caterpillar control of 10 oz a.i./acre will control mites throughout the growing season (Table 10).

Table 10.

Control of red spider mite on apples - United Kingdom 1966

Number of active mites per 30 leaves on given date

Treatment	Dose L.V. a.i./acre	Dose H.V. % a.i.	Site 13 H.V. 27/7	Site 16 H.V. 21/6	Site 16 H.V. 4/7	Site 16 L.V. 23/6	Site 16 L.V. 4/7
Phosalone 33% e.c.	6.6	0.021	2	489	5	375	0
	9.9	0.031	0	189	4	135	0
Azinphos-ethyl 22% e.c.	6.6	0.021	71	304	17	193	6
	9.9	0.031	31	524	30	401	5
Control			303	2,107	740	1,166	13

Site details:	Site no.	13	16
	Variety	W. Pearmain	W. Pearmain
	Dates sprayed	12/6	21/6
		15/6	23/6
		29/6	4/7

The current trials work in Canada shows that phosalone will give good control of European red mite (Panonychus ulmi) and also the two spotted mite (Tetranychus telarius) although the emulsifiable concentrate formulation appears to be more active than the wettable powder (Table 11).

Table 11.

Control of European red mite (Panonychus ulmi) on apples - Canada 1967

Number of live mites on 90 leaves on given date

Treatment no.	Compound and formulation	Dose % a.i. H.V.	Site 8 21/6	Site 8 10/7	Site 8 26/7	Site 8 16/8	Site 5 10/7	Site 5 27/7	Site 5 7/8
1	Phosalone e.c.	0.04	160	48	10	24	32	64	224
2	"	0.06	200	16	0	0	48	0	48
3	Phosalone w.p.	0.04	144	192	16	144	144	224	668
4	"	0.06	120	48	0	112	88	576	416
5	Azinphos-methyl and dicofol*	0.03	1620	4144	880	2048	1408	992	-
6	'Imidan' and dicofol*	0.06 0.04	1756	3120	432	112	784	976	192
7	Control		1104	2656	1184	2064	1272	1488	5136

* Dicofol ('Kelthane') to treatments 5 and 6 on Site 8 on 20th July, 5 and 6 on Site 5 on 28th July and to 5, 6 and 7 on 8th August.

Site details: (Table 11.)	Site no.	5	8
	Variety	Red Delicious	Northern Spy
	Location	Walsh, Ontario	Waterford, Ontario
	No. of sprays	7	7
	Volume	175 g.p.a.	H.V.

Phosalone also gave good initial and persistent activity against aphids as the results in Table 12. show.

Table 12.

Control of apple aphid (Aphis pomi) - Canada 1967

Treatment no.	Compound and formulation	Dose % a.i. H.V.	Mean % infestation on given date 27/6	5/7	8/7
1	Phosalone e.c.	0.04	2.4	8.7	2.7
2	"	0.06	0.3	5.9	1.7
3	Phosalone w.p.	0.04	1.1	7.4	2.0
4	"	0.06	0.1	3.5	0.9
5	Azinphos-methyl w.p.	0.03	14.5	37.1	32.6
6	'Imidan' w.p.	0.06	13.7	37.7	31.8
0	Control	-	50.0	80.3	90.6

Site details:	Variety	McIntosh
	Location	Aylmer, Ontario
	No. of sprays	7
	Volume	H.V.

Other pests of deciduous fruits that are controlled by phosalone are Oriental peach moth, (Cydia molesta) in France and Australia, plum curculio (Conotrachelus nenuphar) in Canada, and various winter moth caterpillars in the United Kingdom (Gould et al, 1967) and in France (Cessac and Burgaud, 1964).

Arable crops

Phosalone has given good control of both seed beetles (Meligethes aeneus) and weevils (Ceuthorrhynchus assimilis) in rape grown for oil seed production in Western Central Europe (Tables 13. and 14).

Table 13.

Number of live rape seed beetles captured at given intervals after spraying

Product	Dose active material g/ha	Number of live beetles 2 days	4 days	5 days
Phosalone e.c.	360	19.7	29.5	23.2
	540	9.7	6.7	11.7
Phosalone w.p.	500	8.7	8.5	13.7
Endosulfan e.c.	225	7.5	7.5	17.5
Toxaphene e.c.	2025	4.5	5.5	11.2
Control	-	51.5	70.0	62.7
LSD 5%		11.1	17.4	12.4

Table 14.

Number of live rape seed weevils captured at given intervals after spraying

Product	Dose active material g/ha	Number of live weevils 2 days	3 days
Phosalone e.c.	600	9	10
	1000	2	3
Phosalone w.p.	1000	2	4
Toxaphene e.c.	4000	17	11
Toxaphene w.p.	5000	15	5
Endosulfan e.c.	600	20	14
Control		47	37
LSD 5%		13.5	9.3

Phosalone is also effective against the Colorado beetle (Leptinotarsa decemlineata) and is used on potatoes in Switzerland and in countries of Northern Europe. In Australia trials have shown that a dose of 7 oz a.i./acre will control the potato tuber moth (Phthorimaea operculella). It is also used on asparagus, beet lucerne and vines.

Numerous trials have been carried out in many parts of the world including the U.S.A, U.S.S.R. and Central Africa on the use of phosalone for the control of pests of cotton. Its polyvalent properties make it suitable for the control of bollworms, mites and aphis although the addition of DDT is necessary if bollweevils are present. Trials are at present being carried out on the control of jassids in cotton in Pakistan.

Types of formulation

Phosalone is available as emulsifiable concentrates of various concentrations or a wettable powder (30%); an oil-based formulation is available in France and there are also experimental formulations of dusts and granules. As stated above, the emulsifiable concentrate formulation is more active than the wettable powder against red spider mite, but otherwise both formulations are commonly used on deciduous fruits. Both formulations have been well tolerated by all the main U.K. varieties.

The oil-based formulation of phosalone (oleo-phosalone) has been proved to be particularly effective against the winter eggs of European red mite (Panonychus ulmi) and San Jose scale. The emulsifiable concentrate and wettable powder formulations are compatible with formulations of commonly used fungicides including dinocap, captan and dodine acetate. Mixtures with strongly alkaline compounds such as lime sulphur or Bordeaux mixture should be avoided.

CONCLUSIONS

The broad spectrum of insecticidal activity of phosalone and its relatively low mammalian toxicity makes it suitable for use on a wide range of agricultural and horticultural crops.

It has rapid activity, with a persistence on plants of about two weeks. Under field conditions a dose of from 0.03% to 0.06% a.i. H.V. gives good results against adults and larvae of Coleoptera, Dipera and Lepidoptera on aerial parts of plants as well as against aphids and mites.

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DYFONATE ®, A PROMISING NEW PHOSPHONODITHIOATE SOIL PESTICIDE

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Stauffer Chemical Company

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SUMMARY

The soil insecticide Dyfonate (O-Ethyl S-phenyl ethylphosphonodithioate) was discovered and developed in the laboratories of the Stauffer Chemical Company. Chemically it is a phosphonodithioate insecticide in which a carbon alkyl bonds directly to the phosphorus atom. Introduction of this group in the molecule created an insecticide with a high order of activity against a wide variety of soil inhabiting insects many of which have developed resistance to chlorinated hydrocarbon insecticides. Coupled with high insecticidal activity, Dyfonate shows good persistence in soils and a high degree of hydrolytic stability.

In recent years increasing attention has been directed to the problem of developing soil insecticides having utility for control of soil inhabiting pests which have developed resistance to chlorinated hydrocarbon insecticides.

To this end we have synthesized and developed a member of a new class of phosphonodithioate insecticides which are represented by the generalized structural formula shown in Fig. 1. The synthesis, physical and chemical properties and insecticidal activity of this series of compounds were described by Menn and Szabo (1965).

Based on activity in the laboratory and greenhouse, Dyfonate, formerly coded as Stauffer N-2790, appeared to be the compound of choice among the series of related analogs which were tested.

The structure and chemical name of Dyfonate are shown in Fig. 2. The physical properties of Dyfonate are outlined in Fig. 3. The relatively high degree of hydrolytic stability is a useful attribute insuring adequate persistence in soils. McBain and Menn (unpublished data) reported that in several types of agricultural soils and at various levels of moisture, the time for 50% (T_{50}) degradation of Dyfonate ranged from 11 to 16 weeks. The T_{50} values for Dyfonate indicate that it is a moderately persistent soil insecticide, in comparison to Trithion (Menn et al., 1960) and Diazinon (Getzin and Rosefield, 1966).

The acute toxicological data are summarized in Table I. The 10% granular formulation (10G) appears to be moderately toxic based on toxicity evaluations in rats and the rabbit. The 5G formulation is considerably safer based on the rabbit dermal toxicity value.

The mammalian toxicity of technical Dyfonate indicates that it should be treated as a highly toxic organophosphorus compound. However, the granular formulations appear to be considerably safer to handle.

The bioactivity of Dyfonate in comparison to its dithioate analog is shown in Table II. Insecticidal bioassays were carried out by use of the following procedures: Toxicity tests to house flies, Musca domestica L., were conducted employing a dry film contact bioassay, LD_{50} values were determined after 48 hours from log concentration-probit plots. Fifth instar cockroach nymphs P. americana (L.) were sprayed with aqueous suspensions of the insecticides. Third instar larvae of the salt-marsh caterpillar, Estigmene acrea (Drury), were exposed to leaves of sour dock, Rumex crispus L., which were dipped in aqueous suspensions of the test compound. Mortality counts were made 72 hours post treatment. It is obvious from the data presented in Table II that Dyfonate is a more active insecticide than its analog (II). In laboratory tests Dyfonate showed a high order of activity against house fly larvae which were exposed to soil containing uniformly incorporated Dyfonate. In the soil bioassay Dyfonate had an LC_{50} of 0.13 ppm in comparison to its phosphorodithioate analog (II) (>10ppm) and Diazinon (0.5ppm). Another advantage favoring Dyfonate is the low cross tolerance (4X) to Parathion resistant and multiresistant (9.3X) house flies in comparison to a cross resistance factor of (29X) for both resistant strains with the phosphorodithioate analog (II). The cross resistance data suggest that compounds of the phosphonodithioate group may usefully serve where insect resistance has developed to currently used materials.

Field testing by State and Federal Experiment Stations has shown that Dyfonate is a highly promising soil insecticide as shown in Table III. It is applied as a broadcast or band treatment followed by incorporation into the soil. It has also shown promise as an in furrow treatment at time of seeding of certain crops. A variety of soil inhabiting pests have been controlled on numerous crops including corn, Irish potatoes, tobacco, peanuts, sugar beets, cole crops and sugar cane.

The tests shown (Table III) represent a typical cross section of the data that have been obtained. Highly favorable results have also been obtained on: (1) field corn (white grub and rootworm control); (2) sweet corn, bush beans, carrots, asparagus, table beets, tomatoes, field corn (symphylan control); (3) radish, cauliflower, rutabaga (cabbage maggot control); (4) peanuts (granulated cutworm); (5) numerous crops and numerous species of wireworms not shown in Table III.

Current recommendations in the U.S.A., for corn rootworm control, range from 1/2 to 1 pound active ingredient per acre in a 6-8" band over corn rows 38-40" apart. Other U.S.A. recommendations expressed as lbs. active ingredient per overall acre are: Asparagus 3-10; cole crops and radish, 2; sugar beets, 4; sweet potatoes, 2; peanuts, 4; field and sweet corn, for garden symphylan, 2; and tobacco, 2.

To date, very promising results have been obtained in Western Europe with Dyfonate for control of wheat stem bulb fly in winter wheat when applied in a seed treatment formulation.

Additional field trials are underway to evaluate the efficacy of Dyfonate against carrot rust fly (psila rosae), onion and

cabbage maggots, wireworm cockchafer (melolontha spp.) and other soil pests in Europe.

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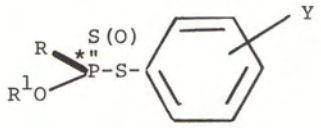

R, R^1 = CH_3 to C_3H_7

Y = H, CH_3 to C_4H_9, CH_3O, Cl

*Phosphonate bond

FIG. 1 Generalized Structural Formula for Aryl Phosphonodithioates

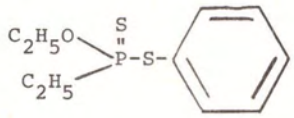

FIG. 2 Structural Formula of Dyfonate
O-Ethyl S-phenyl ethyl phosphonodithioate

Physical state:	Liquid
Specific gravity:	1.154 $\frac{20^{\circ}C}{20^{\circ}C}$
Refractive index:	$n_D^{30} = 1.585$
Vapor pressure:	0.21 microns at 25°C
Solubility:	Soluble in kerosene, xylene, acetone, ethanol
	slightly soluble in water (13 ppm at 22°C)
Hydrolytic 1/2 life at 40°C:	pH_7 = 74 days
	pH_{10} = 1.8 days

FIG. 3 Physical and Chemical Properties of Dyfonate

TABLE I. Acute Oral and Dermal Toxicity of Dyfonate

Species	Formulation	Application	LD_{50} in mg/kg
Rat ♂	Technical	Oral	8 - 17 (6) [1]
Rat ♀	Technical	Oral	4
Mouse ♂	Technical	Oral	15
Guinea Pig	Technical	Dermal	278
Rabbit	Technical	Dermal	147
Rabbit	5% Granular	Dermal	>5000
Rat ♂	10% Granular	Oral	79
Rat ♀	10% Granular	Oral	48
Mouse ♂	10% Granular	Oral	108
Rabbit	10% Granular	Dermal	349

[1] Range obtained from 6 determinations made with 6 separate technical and analytical grade samples.

TABLE II. Comparative Insecticidal Activity of Dyfonate and its Phosphorodithioate Analog

Test Insect	Insecticidal LD-50 Values	
	I C_2H_5 S P-S- C_2H_5O	II C_2H_5O S P-S- C_2H_5O
Musca domestica susceptible strain	4.3 µg/25 ♀	56 µg/25 ♀
Musca domestica [1] Parathion resistant	18 µg/25 ♀	1600 µg/25 ♀
Musca domestica [1] Multiresistant strain	40 µg/25 ♀	1600 µg/25 ♀
Periplaneta americana nymphs	0.025%	>0.10%
Estigmene acreae larvae	0.01%	>0.10%
Musca domestica larval soil assay	0.13ppm	>10ppm

[1] Data from Menn and Szabo (1965).

TABLE III. FIELD PERFORMANCE SUMMARY OF DYFONATE IN COMPARISON WITH OTHER INSECTICIDES FOR CONTROL OF SOME MAJOR ECONOMIC SOIL INSECTS IN THE U.S.A. AND CANADA.

SPECIES	CROP	APPLICATION RATE (LBS.A.I./A)	COMPOUND	RESULTS	
Tobacco wireworm (*Conoderus vespertinus*)	Tobacco (Transplants)	1.13	Dyfonate	3.8%	Injured plants after 20 days
		2.50	Aldrin	59.5%	
		0.83	Parathion	22.9%	
		0.86	Diazinon	16.3%	
		2.30	DiSyston	58.8%	
Southern corn rootworm (*Diabrotica undecimpunctata*)	Peanuts	1.5	Dyfonate	0.60%	Damaged peanuts
		2.5	Diazinon	0.60%	
		2.0	Phorate	5.48%	
Sugar beet wireworm (*Limonius californicus*)	Sugar beets	4.0	Dyfonate	100.0%	Control after 4 weeks
		4.0	Parathion	59.0%	
Sugar beet root maggot (*Tetanops myopaeformis*)	Sugar beets	1.0	Dyfonate	60.0%	Control at harvest
		1.0	Diazinon	40.0%	
Wireworms (*Melanotus communis*) (*Ctenicerca lobato*) (*Agriotes mancus*) (*Dolopius pallidus*)	Irish potatoes	5.6	Dyfonate	85.0%	Marketable tubers
		7.0	Diazinon	85.0%	
Pacific coast wireworm (*Limonius canus*) Western field wireworm (*Limonius infuscatus*)	Irish potatoes	5.0	Dyfonate	14.2%	Damaged tubers (1 or more punctures)
		4.0	Aldrin	20.5%	
		8.0	Diazinon	19.5%	
		8.0	Parathion	16.8%	

(continued)

TABLE III. (Continued)

SPECIES	CROP	APPLICATION RATE (LBS.A.I./A)	COMPOUND	RESULTS	
Onion maggot (Hylemya antiqua)	Onions	2.0	Dyfonate	0.5%	Damaged bulbs
		2.0	Diazinon	2.8%	
		2.0	Bay-37289	1.7%	
Garden Symphylan (Scutigerella immaculata)	Soil test	2.0	Dyfonate	5	Weeks of 85% or better control after soil exposure for 1 day
		5.0 and 10.0	Diazinon	0	
		2.0	Bay-37289	0	
	Pole Beans	2.0	Dyfonate	5.1	Yield (tons/A)
		2.0	Zinophos	4.0	
		4.0	Diazinon	3.9	
Cabbage maggot (Hylemya brassicae)	Cabbage	1.6	Dyfonate	96.3%	Healthy plants
		2.0	Diazinon	98.1%	
	Turnips	0.6 (oz.)	Dyfonate	94.2%	Control
		0.6 (oz.)	Thimet	84.0%	
		0.6 (oz.)	Guthion	70.3%	
		1.2 (oz.)	Diazinon	64.6%	
		1.2 (oz.)	Aldrin	5.2%	

N-4543, A PROMISING NEW PHOSPHONODITHIOATE FOLIAR INSECTICIDE-ACARICIDE

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SUMMARY

The insecticide acaricide N-4543 {O-Isobutyl S-(phthalimidomethyl)ethylphosphonodithioate} was synthesized and developed in the laboratories of the Stauffer Chemical Company. The direct bonding of isobutoxy and ethyl groups to phosphorus produced an active phosphonodithioate ester. This configuration imparts greater biological activity to this compound than that found in the corresponding phosphorodithioate analog. N-4543 is moderately toxic to warm blooded animals, and toxic to a number of economically important insect and mite pests attacking deciduous, citrus and selected field crops.

In the course of chemical synthesis on heterocyclic phosphonodithioate insecticides we discovered a highly active series of insecticides and acaricides represented by the empirical formula shown in Fig. 1. These compounds differed from the better known organophosphorus esters in that they have an alkyl group (R_1) which is directly bonded through carbon to the phosphorus atom. Extensive research work conducted in Europe and in the USA; (Schrader 1965), Fukuto et al. (1959), Razumov et al. (1957), and (Menn and Szabo, 1965) has shown that in many instances the phosphonate analogs of corresponding phosphate esters were more toxic to insects and mammals. The latter feature rendered many of these compounds useless for crop protection due to their high mammalian toxicity. The physical properties of N-4543 are summarized in Fig. 3.

Compound N-4543 was selected for development from a large number of analogs on the basis of a high order of insecticidal and acaricidal activity and a more favorable mammalian toxicity. These data are summarized in Table I. According to the classification of Hodge & Sterner (1943) N-4543 falls in the category of moderately toxic compounds. The degradation products, containing the phthaloyl moiety and/or the phosphonic acid residue, are only slightly or non toxic. The fate of the phthalimide residue has been studied extensively in conjunction with metabolic studies with the insecticide Imidan® {N-Mercaptomethyl phthalimide-S-(O,O-Dimethylphosphorodithioate)} by Menn and McBain (1965), Ford et al. (1966), Chamberlain (1965) and McBain and Menn (unpublished data). These investigations have shown that the heterocyclic ring is rapidly metabolized in vivo

and hydrolyzed in aqueous systems primarily to two innocuous metabolites; phthalamic and phthalic acids. Consequently, this type of compound represents a biodegradable insecticide which is not expected to accumulate in the environment.

In Table II, we have presented a summary of the insecticidal and acaricidal spectrum of activity as determined in laboratory experiments conducted at the Agricultural Research Center, Stauffer Chemical Co., Mountain View, California. Briefly, these tests were conducted as follows: House fly, Musca domestica L., bioassays were made employing a dry film contact method. LD_{50} values were derived from Log concentration-probit plots 48 hours later. Third instar larvae of the salt-marsh caterpillar, Estigmene acrea (Drury), were exposed to leaves of sour dock, Rumex crispus L., which were dipped in aqueous suspensions of the test compound. Mortality counts were made 72 hours post treatment. Toxicity to black bean aphids, Aphis fabae (Scop.), and Lygus bugs, Lygus hesperus (Knight), was obtained from contact spray tests of the insecticide suspension directly on the insect in the case of the lygus bug, and on nasturtium seedlings, Nasturtium sp. infested with aphids. Toxicity to two spotted mites, Tetranychus urticae (Koch), was determined by spraying test suspensions of the candidate acaricide on pinto bean plants, Phaseolus sp. infested with the mites. Mortality counts for aphids and Lygus bugs were made 72 hours after treatment and for mites one week after spraying. Data presented in Table II, indicate that N-4543 in comparison with malathion is an active insecticide with a high level of acaricidal activity. The corresponding phosphorodithioate analog was significantly less insecticidal than N-4543.

Insecticidal data from field trials are summarized in Table III and acaricidal data from field trials are summarized in Table IV for certain major pests attacking deciduous and citrus fruit crops. Table V shows preliminary insecticidal comparisons of the major economic pests on certain field crops.

An analysis of the insect and mite control data obtained to date reveals that N-4543 is a relatively broad spectrum phosphonodithioate insecticide with acaricidal properties.

The data presented here show that compound N-4543 has a favorable potential for insect and mite control in deciduous and citrus fruit crops and certain agronomic and vegetable crops.

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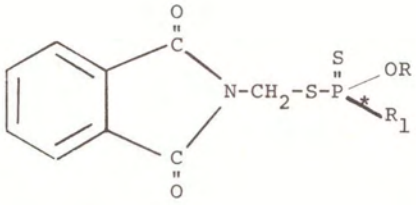
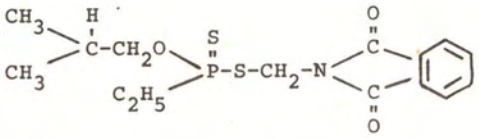


$R=CH_3$ to C_5H_{11}

$R_1=CH_3$ to C_4H_9

*Phosphonate bond

FIG. 1 Generalized Structural Formula of Phthalimido Phosphonodithioates



N-4543

FIG. 2 Structural Formula of N-4543 O-Isobutyl S-(phthalimidomethyl)ethylphosphonodithioate

Physical state:	White crystalline powder
Melting point:	58-60°C
Boiling point:	127°C at 1 micron pressure
Thermal stability:	16 hours at 100°C
Vapor pressure:	<0.01 microns at 25°C
Solubility:	Soluble in xylene, acetone
	Sparingly soluble in kerosene
	Slightly soluble in water (<15ppm)

FIG. 3. Physical Properties of N-4543

TABLE I. Acute Oral and Dermal Toxicity of Technical N-4543

Species	Application	LD_{50} in mg/kg
Rat ♂	Oral	75
Rat ♀	Oral	23
Mouse ♂	Oral	316
Mouse ♀	Oral	430
Rabbit	Dermal	121

TABLE II. The Efficacy of N-4543 as an Insecticide and Acaricide in Laboratory Tests

	LD_{50} Values Contact Bioassay	
Test Organism	Malathion	N-4543
Musca domestica, adults	11 µg/25 ♀	8 µg/25 ♀
Estigmene acrea, larvae	>0.1%	0.03%
Aphis fabae, adults, and nymphs	0.003%	0.005%
Lygus hesperus, adults, and nymphs	0.001%	0.005%
Tetranychus urticae, adults and larvae	0.03%	0.001%

TABLE III. FIELD PERFORMANCE SUMMARY OF N-4543 IN COMPARISON WITH OTHER INSECTICIDES FOR CONTROL OF SOME MAJOR ECONOMIC INSECTS IN DECIDUOUS AND CITRUS FRUIT.

SPECIES	CROP	COUNTRY	APPLICATION RATE	COMPOUND	RESULTS	
Codling moth (Carpocapsa pomonella)	Apples	Australia	.04%	N-4543	2.4%	(Infested fruit)
			.01%	Sevin	10.0%	
			.05%	Guthion	1.32%	
		Argentina	.12%	N-4543	1.16%	(Injured fruit)
			.12%	DDT	1.75%	
			.12%	Guthion	1.20%	
		U.S.A.	*1.0 lbs./ 100 gal.	N-4543	100.0%	
			*1.0 lbs./ 100 gal.	Guthion	57.8%	(Control)
Red-banded leaf roller (Argyrotaenia velutinana)	Apples	Canada	*1.0 lbs./ 100 Imp. gal.	N-4543	0.11%	(Fruit injury after 20 days)
			*0.5 lbs./ 100 Imp. gal.	Guthion	1.53%	
Apple aphid (Aphis pomi)	Apples	France	50 gm/100 L	N-4543	100.0%	(Control-31 days)
			25 gm/100 L	Methyl demeton		
Pear psylla (Psylla piri)	Pears	France	50 gm/100 L	N-4543	85.0%	(Control-21 days)
			40 gm/100 L	S103	72.0%	
(Psylla pyricola)	Pears	U.S.A.	*2.0 lbs./ 100 gal.	N-4543	17	(Number live nymphs after 5 weeks)
			*1.5 lbs./ 100 gal.	Guthion	51	

*Rates not expressed as a.i.

(continued)

TABLE III. (Continued)

SPECIES	CROP	COUNTRY	APPLICATION RATE	COMPOUND	RESULTS	
Oriental fruit moth (Grapholitha molesta)	Apples	Japan	.042%	N-4543	50%	(Killed
			.05%	Sumithion	25%	larvae)
	Peaches	U.S.A.	*1.0 lbs./ 100 gal.	N-4543	90%	(Reduction
			*0.5 lbs.+ 5 oz.	Guthion + Sevin	94%	after 27 days)
Green peach aphid (Myzus persicae)	Peaches	France	100 gm/100 L	N-4543	65.3%	(Control
			50 gm/100 L	Mevinphos	72.5%	after 11 days)
Diamondback moth (Plutella maculipennis)	Cabbage	Australia	.04% a.i.	N-4543	95.7%	(Control
			.05% a.i.	Diazinon	91.0%	after 14 days)
Banded cucumber beetle (Diabrotica balteata)	Bush beans	U.S.A.	1.0 pints/ 100 gal.	N-4543	0.5	(Number of beetles per
			1.0 pints/ 100 gal.	Azodrin	0.3	row, 3 days)
Cereal leaf beetle (Oulema melanopus)	Oats	U.S.A.	0.85 lbs. a.i./A	N-4543	98.8%	(Larvae control
			1.0 lbs. a.i./A	Malathion	79.2%	14 days)
Colorado potato beetle (Leptinotarsa decemlineata)	Potatoes	U.S.A.	0.425 lbs. a.i./A	N-4543	2	(Number live larvae after 9 days (ck=499).

*Rates not expressed as a.i.

TABLE IV. FIELD PERFORMANCE SUMMARY OF N-4543 IN COMPARISON WITH OTHER INSECTICIDES FOR CONTROL OF SOME MAJOR ECONOMIC MITES IN DECIDUOUS AND CITRUS FRUIT.

SPECIES	CROP	COUNTRY	APPLICATION RATE	COMPOUND	RESULTS	
European red mite (Panonychus ulmi)	Apples	Italy	0.2% a.i.	N-4543	10	(Number of mites/leaf after 17 days)
			0.2% a.i.	Acarthane	12	
	Apples	France	50 gm/100 L a.i.	N-4543	79.5%	(Control after 14 days)
			40 gm/100 L a.i.	Guthion	78.9%	
	Apples	Japan	.02% a.i.	N-4543	97.2%	(Control after 24 hours)
			.02% a.i.	Kelthane	97.5%	
Citrus rust mite (Phyllocoptruta oleivora)	Oranges	U.S.A.	5.1 oz. a.i./ 100 gal.	N-4543	0.4%	(Infested leaves after 63 days)
			2+2 oz. a.i./ 100 gal.	Tedion + Chlorobenzilate	0.4%	
Citrus red mite (Panonychus citri)	Oranges	U.S.A.	0.637 lbs. a.i./100 gal.	N-4543	100.0%	(Control after 15 days)
Kanzawa spider mite (Tetranychus kanzawai)	Kidney beans	Japan	100 ppm a.i.	N-4543	100%	(Control adult- 24 hrs.)
			100 ppm a.i.	CMP	100%	
					98.9%	(Control egg- 10 days)
					99.5%	

THE CONTROL OF CABBAGE ROOT FLY WITH THIONAZIN

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Summary Experimental work is described with the organophosphorus insecticide thionazin for control of cabbage root fly in transplanted brassica crops. Field experiments carried out in 1966 and 1967 showed that spot treatments of thionazin 10% granules at rates of 0.016 gm a.i. or more /plant gave effective control of cabbage root fly when applied either late in April to early-planted crops or within a week of transplanting to crops planted from late April onward. Foliar band treatments of thionazin applied at 1.72 and 2.33 oz. a.i./1000 yd of row in 6-inch wide bands reduced root fly damage but were not completely effective. Residues of thionazin in brassica crops treated with spot applications of 0.05 gm a.i./plant were less than 0.01 ppm at harvest.

INTRODUCTION

The use of granular organophosphorus insecticides for cabbage root fly control has become increasingly popular in recent years. The two principal reasons for this have been the discouragement by the Ministry of Agriculture and other bodies, of the use of the persistent organochlorine insecticides, following the "Review of the Persistent Organochlorine Pesticides" (1964); and the appearance and spread of organochlorine-resistant cabbage root fly populations. Resistance of cabbage root fly to dieldin dip and drench treatments is now widespread in the West Midlands and has been reported from other areas.

Results from trials carried out in 1964 and 1965 showed that the organophosphorus insecticide thionazin (O,O-diethyl O-2 pyrazinyl phosphorothioate) was promising for cabbage root fly control on transplanted brassica crops when applied as a drench (Coaker & Finch 1964) and as a band of granules into the planting furrow (Caldicott & Lindley 1965). On swedes a band of granules applied to the soil surface before drilling at 2.5 lb a.i./acre gave good root fly control (Wright 1965).

Subsequent work has been concentrated on spot applications of thionazin granules to transplanted brassica crops, in view of the low cost of spot treatments compared to granular band treatments and liquid drenches. This paper describes two years' trials in which spot and foliar band treatments were tested on transplanted brassicas.

METHOD AND MATERIALS

In all the trials described in this paper a 10% formulation of thionazin on Fuller's Earth granules (22/44 mesh) was used. In two trials phorate (O,O-diethyl S-(ethyl thiomethyl) phosphorodithioate) was also used, in the form of 10% granules on Fuller's Earth (22/44 mesh).

The materials used for comparison were diazinon 5% granules and chlorfenvinphos 9.2% granules.

Four trials were laid down in 1966 (Caldicott & Isherwood, 1967.) One was on Primo cabbage in Bedfordshire and another on summer cauliflower in Worcestershire. The two other trials were on Brussels sprouts. Neither of the latter suffered an appreciable root fly attack and they are not referred to any further.

In 1967 nine trials were laid down, three each on Primo cabbage, cauliflower (early summer and summer) and brussels sprouts. One of the cauliflower sites and two of the sprout sites were abandoned because no root fly attack materialised. Of the remainder, two of the Primo cabbage trials were in Worcestershire, and one in

Bedfordshire. One of the cauliflower trials was in Lincolnshire and one in Worcestershire and the Brussels sprout trial was in Bedfordshire.

All the trials were laid down as randomised block designs with four blocks. Plot size was 30 to 60 plants.

In the 1966 trials thionazin granules were applied at 0.05 and 0.025 gm a.i./plant. Phorate 10% granules were also used in these trials at the same rates. Diazinon 5% granules at 0.06 gm a.i./plant were included for comparison. Treatments were applied by hand using small volumetric measures.

In the 1967 trials thionazin spot treatments were applied at lower rates. It was intended to apply 0.025 and 0.015 gm a.i./plant but the rates actually delivered were slightly higher than these. Mean dosages delivered were 0.025 and 0.030 and 0.016 to 0.019 gm a.i./plant respectively. The treatments were applied with a hand applicator supplied by Horstine Farmery Limited.

Chlorfenvinphos 9.2% granules, applied at 0.016 gm ai/plant, were included for comparison. This treatment was applied with a "Birlane" hand applicator, as supplied by Shellstar Limited.

In view of the difficulty of applying spot treatments by hand to large acreages, foliar band treatments of thionazin were also tested in the 1967 trials. Two rates were used, 2.33 and 1.72 oz a.i./1000 yd of row. For a crop planted on 24-inch rows (e.g. cauliflower) these rates are equivalent to 1.06 and 0.78 lb a.i./acre. For a crop planted on 36-inch rows (e.g. Brussels sprouts) the equivalent rates are 0.70 and 0.52 lb a.i./acre. Treatments were applied as 6-inch wide bands over the foliage with a "Horstine Farmery Microband" applicator mounted on a wheelbarrow frame.

Crops planted before mid-April were treated in the third or fourth weeks in April (i.e. at or shortly before the commencement of root fly oviposition). Crops planted later than this date were treated within seven days of transplanting.

In the 1966 trials assessments were made of root damage on a 0 to 10 scale as used by Wright (1953, 1965). Twenty roots per plot were examined in the field and graded. The figures obtained were totalled and the total divided by two to give an index for each plot on a 0 to 100 scale. On the cauliflower trial an assessment of yield was made by measuring the diameter of the curds shortly before cutting. Twenty plants per plot were assessed and graded on a scale ranging from 0 (no curd formed) to 5 (> 6 inches in diameter). The figures were summed to give an index on a 0 to 100 scale. It was not possible to take yields on the cabbage trial.

In the 1967 trials root damage assessments were made in the same way. During the growing season periodic counts were made of the numbers of plants showing visible symptoms of cabbage root fly attack and of the numbers of plants which died. In the cabbage trials 20 heads per plot were weighed at cutting, and the mean head weight was calculated. In the cauliflower trials assessments were made of the percentage of plants forming curds. No assessments of yield were made in the Brussels sprout trials.

For statistical analysis, all percentage figures were transformed to angles. Other figures were analysed without transformation.

In the 1966 trials samples were taken from crops which had received spot treatments of thionazin and phorate at 0.05 gm a.i./plant, and residue analyses were carried out using a gas chromatography method.

RESULTS

The results of the 1966 trials are summarised in Table 1.

Table 1

Effects of spot treatments on cabbage root fly damage, 1966

Treatment	Primo cabbage mean root damage index (angles)	cauliflower mean root damage index (angles)	cauliflower yield index (angles)
Control	61.5	53.0	47.1
Thionazin (spot) 0.05 gm a.i./plant	25.3	32.2	61.2
Thionazin (spot) 0.025 gm a.i./plant	25.5	38.9	67.5
Phorate (spot) 0.05 gm a.i./plant	19.6	36.6	54.3
Phorate (spot) 0.025 gm a.i./plant	25.5	42.0	57.0
Diazinon (spot) 0.06 gm a.i./plant	19.7	37.8	45.7
L.S.D. 5%	10.9	11.2	8.3

Root damage to cabbage was greatly reduced by all treatments. In the cauliflower trial the level of root damage at cutting was reduced significantly by all treatments except phorate at 0.025 gm a.i./plant, but considerable damage had occurred in all cases. It is probable that much of this damage occurred late in the life of the crop, and had little or no effect on yield. Yields were significantly increased by both thionazin treatments and by phorate at 0.025 gm a.i./plant.

In 1967 the effects of the first generation of cabbage root fly were generally not severe. In the three trials carried out on Primo cabbage no plant losses occurred as a result of root fly attack, and there were no differences in yields between any of the treated groups and the controls. Some root damage, however, had occurred in each trial. Results of root damage assessments are shown in Table 2.

Table 2

Cabbage root fly damage in Primo cabbage trials, 1967

Treatment	Mean root damage index (angles)		
	A (Worcestershire)	B (Bedfordshire)	C (Worcestershire)
Control	22.4	31.3	28.0
Thionazin (spot) 0.025 gm a.i./plant	13.7	4.8	3.4
Thionazin (spot) 0.015 gm a.i./plant	17.4	6.2	5.3
Thionazin (band) 2.33 oz a.i./1000 yd row	19.9	14.0	19.8
Thionazin (band) 1.72 oz a.i./1000 yd row	16.2	19.4	21.7
Chlorfenvinphos (spot) 0.016 gm a.i./plant	13.5	6.2	4.9
L.S.D. 5%	NS	5.5	7.0

Excellent control of root damage was obtained in trials B and C with spot treatments of thionazin (both rates) and chlorfenvinphos. Significant reductions in root damage were obtained with band treatments of thionazin, at both rates in trial B and at the higher rate in trial C, but complete control was not obtained. In trial A root fly attack developed late and some damage occurred on all plots.

Table 3

Cabbage root fly damage and yield in summer cauliflower, 1967

Treatment	A (Lincolnshire)		B (Worcestershire)	
	Mean root damage index (angles)	Mean % heads formed (angles)	Mean root damage index (angles)	Mean % heads formed (angles)
Control	38.7	29.1	10.7	56.2
Thionazin (spot) 0.025 gm a.i./plant	11.9	60.5	10.2	56.2
Thionazin (spot) 0.015 gm a.i./plant	16.0	66.9	9.2	55.9
Thionazin (band) 2.33 oz a.i./1000 yd row	44.6	35.6	12.0	66.7
Thionazin (band) 1.72 oz a.i./1000 yd row	39.4	41.1	12.5	57.7
Chlorfenvinphos (spot) 0.016 gm a.i./plant	15.5	60.2	0.0	63.5
L.S.D. 5%	7.3	8.0	4.4	NS

The results of the two cauliflower trials carried out in 1967 are shown in Table 3. In trial A the root fly attack was very severe and many plants were lost on the untreated plots. Spot treatments of thionazin and chlorfenvinphos again gave excellent control and no plants were lost from root fly damage following these treatments. Root damage was reduced to a low level and yields were more than doubled in comparison with the untreated plots. Thionazin band treatments increased the yield slightly and reduced the numbers of plants lost from root fly attack, but adequate control was not attained.

In trial B the root fly attack was light and occurred very late in the life of the crop. Larvae were first found 10 weeks after treatment, at the time when the first cutting began. At this date the effect of thionazin spot treatments was no longer evident.

Table 4

Cabbage root fly damage on Brussels sprouts, 1967

Treatment	Mean % plants showing root fly symptoms 9 weeks after treatment	Mean root damage index 14 weeks after treatment (angles)
Control	15.8	30.5
Thionazin (spot) 0.025 gm a.i./plant	0.0	25.0
Thionazin (spot) 0.015 gm a.i./plant	0.0	-
Thionazin (band) 2.33 oz a.i./1000 yd row	4.2	-
Thionazin (band) 1.72 oz a.i./1000 yd row	7.5	-
Chlorfenvinphos (spot) 0.016 gm a.i./plant	0.8	25.5
L.S.D. 5%	6.7	-

The Brussels sprout trial (Table 4) suffered root fly attack over a long period. Severe symptoms of damage were first seen in early June, six weeks after treatment. The assessment of plants showing root fly symptoms was made nine weeks after treatment, and at this date spot treatments of thionazin and chlorfenvinphos were giving completely effective control of the attack. Band treatments of thionazin gave poorer results probably due to a less effective dose per plant; some plants were lost, though the losses were less than on the control plots. Root damage assessments were made at the end of July, 14 weeks after treatment, and at this date a later root fly attack was in progress which was not checked by spot treatments of either insecticide. This attack, however, had no effect upon the growth of the crop.

Residues

Following spot applications of 0.05 gm a.i./plant, no detectable residues (< 0.01 ppm) of thionazin or phorate were found in either the cauliflowers sampled 12 weeks after treatment or the Brussels sprouts sampled 26 weeks after treatment.

DISCUSSION

The trials described in this paper have shown that spot treatments of thionazin 10% granules at rates of 0.016 to 0.019 gm a.i./plant give effective control of cabbage root fly for a period of approximately two months. Root fly attack occurring two months or more after transplanting would not be expected to affect the crop. Up to the present, only hand applicators have been used to apply spot treatments on a large scale. It may be possible in the future to apply such treatments using tractor-mounted equipment, either combined with machine planting or as a separate operation. The necessity for the treatment to be applied very soon after transplanting and the variable growth habit of the crop at this stage present practical difficulties to such developments.

The main advantages of spot treatment are the small amount of insecticide used and consequently the low cost. Band treatment, however, despite its higher cost, is more acceptable to large-scale growers because large acreages can be treated quickly.

Band treatments 6 in wide of thionazin at a rate equivalent to 1.0 lb a.i. per acre for 24 in rows gave partial control of cabbage root fly in the 1967 trials. It is possible that this dose would be more effective if applied in a narrower, more concentrated band, provided that this could be applied accurately to the rows. Further trials are planned to investigate narrow-band applications.

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THE ACTIVITY OF THE DITHIOCARBAMATE COMPLEX IN CUFRAM Z AGAINST POWDERY MILDEWS AND OTHER FUNGI

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Summary Experiments in control of downy mildew in hops (Pseudoperonospora humuli) led to observations that a degree of control of powdery mildew (Sphaerotheca macularis) was also being exerted. This feature, unusual for a dithiocarbamate, has been examined further and experiments carried out to find levels of control exerted by 'Cufram Z' on powdery mildew in roses, blackcurrants, apples and vines. Results are reported from investigations on control of these and other diseases, and some ideas put forward on possible modes of action.

Success has been found in the use of 'Cufram Z' as a cereal seed dressing in Canada, and for control of a number of diseases in citrus in Southern States of America. Results are reported.

INTRODUCTION

The dithiocarbamate group of fungicides which are in current use are principally used for control of downy mildew types of disease, and other materials, notably dinocap and sulphur, are used for control of powdery mildews. In the light of this it was with some surprise that evidence of control by 'Cufram Z' of fungi in the powdery mildew group was found.

'Cufram Z' is the trade name which has been given to the dithiocarbamate complex formerly code-named Z.M.C.5. A paper describing this was read at this conference in 1965. (Giles, D., et al., 1965).

The material was developed as a result of early work with dithiocarbamic acid oxidation products, in particular monomeric ethylene thiuram monosulphide, which showed that traces of ferric and cupric ion were effective in increasing activity and spectra. It was also felt that the inclusion of copper into the dithiocarbamate might impart a longer protective life to the complex.

'Cufram Z' is an octadithiocarbamate corresponding to the empyrical formula

$$(C_4H_6N_2S_4)_4^{-}Zn_{1.55}Mn_{1.4}Cu_{0.942}Fe_{0.108}$$

and its physical properties are well suited to its formulation as an 80% wettable powder.

The standard rate of use is at 1½ lb/acre in 60 gallons of water, with higher volumes of similar concentration for situations where the foliage density is high.

The mammalian toxicity of 'Cufram Z' is low, acute oral L.D.50 for rats being 2,700 mg/kg, and it is not irritating to the skin.

No phytotoxicity has been observed at any level or cumulative level of application. The crop plants on which it has been used include potatoes, tomatoes, hops, vines, celery, roses, apples, citrus, coffee, rice, blackcurrants, strawberries, lettuce, and cereal seed.

Full residue data on hops, potatoes and blackcurrants was collected through the 1966 and 1967 seasons, and gives a complete picture of residue levels and disappearance curves of the material on the foliage. Comparisons were made with maneb and mancozeb on potato foliage, and a residue after spraying of approximately 2 p.p.m. of wet leaf weight was found. The original material disappeared to control

level (untreated) in 16-18 days in potato foliage. Fungitoxic activity from breakdown products extends a little beyond this period.

EXPERIMENTAL RESULTS

Experiments in hops in 1965 gave the first indication that some activity against hop powdery mildew (Sphaerotheca macularis) might be exerted. In 1966, trials were widened to examine powdery mildew control in blackcurrants and roses.

In a trial in hops at Frittenden, Kent, in 1966, no powdery mildew occurred on the 'Cufram Z' plot, though the level of this disease was low throughout the trial. The comparison treatments were metiram and zineb.

Plot size: 1 acre, one replication. Variety: O.T.48.
Applications: 8, starting 3rd June and finishing 28th August, made by standard commercial equipment.
Sampling: 50 lateral shoots averaging 110 cones each, at random from each treatment, assessed on 8th September, 1966.

Results

Table 1.

Control of hop diseases, Frittenden, 1966.

	'Cufram Z'	Metiram	Zineb
% sampled laterals showing downy mildew infection	78%	86%	88%
% sampled laterals showing powdery mildew infection	0%	6%	6%
% downy mildew infected cones	2.9%	2.8%	3.2%

In 1967, however, in a trial at Spelmonden, Kent, an infection of powdery mildew developed in hops which were given no fungicidal treatment other than 'Cufram Z'. Conditions for the disease were good, and on 9th August a disease level of 2.1% of cones was found, and an application of 2 lb/acre of sulphur had to be made. Control of downy mildew was again very good. The comparison treatment was treated with 'Cuprokylt' (Copper oxychloride W.P. 50% Cu.).

Plot size: 4 acres, one replication. Variety: Bullion.
Applications: 9, starting 10th June and finishing 23rd August, made by standard commercial equipment.
Sampling: 40 lateral shoots at random from each treatment, assessed 22/8/67.

Results

Table 2.

Control of hop diseases, Spelmonden, 1967.

	'Cufram Z'	Copper oxychloride
% sampled laterals showing downy mildew infection	48%	65%
% sampled laterals showing powdery mildew infection	60%	50%
% downy mildew infected cones	1.8%	2.5%
% powdery mildew infected cones	2.1% *	1.4%

*Received only one sulphur application, whereas comparison treatment received regular sulphur treatments through the season.

This contrary experience was also found in trials for control of blackcurrant

powdery mildew (Sphaerotheca mors-uvae). In 1966, 'Cufram Z' held mildew well despite being applied at the low rate of $\frac{3}{4}$ lb/100 gal, at 200 gallons per acre, in a trial which also examined control of blackcurrant leaf spot (Pseudopeziza ribis).

Site: Wrottesley, Staffordshire. Plot size: 240 yd of row.
Variety: 'Cufram Z' treatment - Westwick Choice. Others Wellington XXX.
Treatments: 1) 'Cufram Z' at $\frac{3}{4}$ lb/100 gal, 200 gallons applied.
2) Mancozeb with zineb " " "
3) Oxythioquinox, 11 lb/100 gal " " "
4) Control, no treatment.
Applications: 4 at fortnightly intervals from 26th April.
Assessments: On dates indicated by the Corke key for heavier levels of infection, and Preece key for lower infection levels.

Results.

Table 3.

Control of leaf spot, 1966.

Date	Control		Mancozeb + zineb		Oxythioquinox		'Cufram Z'	
	P.*	C.*	P.	C.	P.	C.	P.	C.
5.7.66	25.7	-	5.0	-	5.0	-	11.2	-
19.7.66	-	31.6	10.6	-	23.3	-	13.0	-
5.8.66	-	31.3	20.5	-	27.0	-	15.3	-
15.8.66	-	32.0	23.9	-	27.5	-	20.0	-
8.9.66	-	90.5	-	30.0	-	49.0	-	69.0

*P = Preece key, C. = Corke key. (Clark, G.M., et al., 1955).

Table 4.

Control of powdery mildew, 1966.

Date	Control	Mancozeb + zineb	Oxythioquinox	'Cufram Z'
5.7.66	-	-	-	-
19.7.66	3.5	1.8	0.6	1.9
5.8.66	1.45	2.55	0.95	1.2
15.8.66	1.6	2.9	1.25	1.05
8.9.66	62.0	53.5	42.5	29.5

Results looked encouraging, but the powdery mildew result was reversed in 1967 trials, when it was found that only a very slight inhibition of powdery mildew had occurred at Reading, though fair control was observed at Wrottesley where the level of powdery mildew infestation was low. The details of the trial at Reading are as follows:-

Plot size: 100 yd of row, one replication. Variety: Wellington XXX
Treatments: 1) 'Cufram Z' at $2\frac{1}{2}$ lb/acre in 100 gal.
2) Mancozeb at $2\frac{1}{2}$ lb/acre in 100 gal.
3) Control, not treated.
Applications: Six, starting 14th April and finishing 28th June.
Assessments: Leaf spot did not develop until the end of the season, and assessments were not made until that time.

Results

Table 5.

Control of blackcurrant leaf spot, Reading 1967.

Date	'Cufram Z'		Mancozeb		Control	
	P.*	C.*	P.	C.	P.	C.
14.9.67.	19.0	-	31.0	-	51.0	23.0

*P = Preece key, C. + Corke key.

Results

Table 6.

Control of blackcurrant powdery mildew, Reading 1967.

Date	'Cufram Z'		Mancozeb		Control	
	1*	2*	1	2	1	2
14.9.67	65.0%	5.0	69.0%	4.6	71.5%	5.8

*1. This assessment is the percentage of shoots showing some infection.
*2. This is an estimate on a 0 - 10 scale of leaf area destroyed on infected shoots.

Very hard frosts in May destroyed most of the developing fruit, and the crop was not picked. Effects on yield could therefore not be measured.

In a N.A.A.S. trial carried out at Wrottesley, Staffordshire, much less powdery mildew was encountered, but leaf spot was present at a much higher level. Details are as follows:

Plot size: 240 yd of row. Variety: Baldwin
Treatments: 1) 'Cufram Z' at 1½lb/100 gal, applied at 200 gal/acre.
2) Mancozeb at 1½ lb/100 gal, applied at 200 gal/acre.
3) Oxythioquinox at 22 lb in 200 gal/acre.
4) Control, not treated.
Applications: Seven, made starting 24th March, finishing 30th June.
Assessment: At 4th September showed the following:

Results

Table 7.

Control of blackcurrant leaf spot, Wrottesley 1967.

Date	'Cufram Z'		Mancozeb		Oxythioquinox		Control	
	P.*	C.*	P.	C.	P.	C.	P.	C.
4.9.67.	34.2	-	9.7	-	50.7	-	95.9	22.8

*P = Preece key, C. = Corke key.

Table 8.

Control of blackcurrant powdery mildew, Wrottesley 1967.

Date 4.9.67.	'Cufram Z'	Mancozeb	Oxythioquinox	Control
Slight mildew	7.0	10.0	5.6	10.6
Severe mildew	2.3	4.8	2.8	3.2

As with the 1966 results, the mildew figures suggest that a measure of control has been exerted where only a light infection occurred. In the event of heavy infection, 'Cufram Z' cannot be relied upon to check the attack. This experience parallels that observed in the hops, where in 1966 (when conditions were less favourable for powdery mildew development) no disease at all was found in the 'Cufram Z' treatment, whilst in 1967, (a season suited to powdery mildew development) the disease eventually overwhelmed the fungicide.

A 55% inhibition of apple powdery mildew (Podosphaera leucotricha) at 500 p.p.m. 'Cufram Z' was obtained in in-vivo greenhouse screening trials, at Long Ashton, - a degree of activity unusual for a compound of the dithiocarbamate class. Field trials carried out in the course of 1967 on young Miller trees gave no apparent mildew control however.

A degree of control was obtained of rose powdery mildew (Sphaerotheca pannosa) on Rosa canina in a trial carried out in 1966. Details were as follows:

Layout: Randomised block, 3 replications. Plot size: 10 bushes.

Treatments: 1) 'Cufram Z' at 2½ lb/100 gal.
2) Maneb at 2½ lb/100 gal.
3) Dinocap at 2½ lb/100 gal.
4) Control, not treated.

Applications: Two, on June 27th, and July 11th, 1966.

Assessments: These were made at intervals following application using the key given in appendix I. Figures given are means of three replicates.

Results

Table 9.

Control of rose powdery mildew, 1966.

Days after 1st application	'Cufram Z'	Maneb	Dinocap	Control
0	7	8	4	8
3	4	4	4	8
8	5	3	2	12
14	8	10	6	22
Days after 2nd application				
0	8	10	6	22
5	28	50	60	70
10	26	30	55	73
17	33	42	50	72
25	33	48	45	78
30	38	57	50	78

Results of 1967 trials are not to hand at the time of preparing this paper.

Full investigations of control of potato blight (Phytophthora infestans) in 1966 and 1967 led to approval of 'Cufram Z' for use in this crop. (It is also approved for downy mildew control in hops). The main features of a series of trials carried out in East Anglia and other areas were as follows:-

Results

Table 10.

Summary of potato blight control trials, 1965 and 1966.

	Control	'Cufram Z'	Mancozeb	Maneb	Fentin Hydroxide
1965 series	100	128	-	124	122
1966 series	100	127	127	120	-

Figures are yield indexes for blight free ware tubers, averaged from all trials, control = 100.

Table 11.

Tuber blight control in potatoes, 1965 and 1966.

	Control	'Cufram Z'	Mancozeb	Maneb
Mean % blights in ware size tubers	10.6	4.5	5.6	8.0

Two other aspects of experimental results obtained with 'Cufram Z' merit attention. In Canada, Co-operative seed dressing trials carried out in Winnipeg by H.A.H. Wallace of the Canadian Department of Agriculture have shown great promise for 'Cufram Z' as a seed dressing against bunt of wheat (Tilletia caries), and loose smut of barley (Ustilago nuda) and oats (Ustilago avenae).

Table 12.

Control of seed borne cereal diseases, Canada 1966.

Material	Dosage - oz/bushel		Disease rating		
	Cereals	Flax	Bunt	Oat smut	Barley smut
Control			20.13	11.40	4.3
Control			13.19	8.16	10.46
Panogen 15D	0.75	1.50	0.00	0.08	1.63
Ceresan M	0.50	1.00	0.25	0.00	1.69
'Cufram Z'	2.00	4.00	0.00	0.21	0.50
Least significant difference			2.97	2.22	4.25

Table 13.

% emergence of treated seed

Material	Crop	
	Flax	Rye
Control	69.5	58.3
Control	65.8	61.3
Panogen 15D	65.5	65.8
Ceresan M	64.9	59.5
'Cufram Z'	60.2	61.6
Least significant difference	7.0	8.0

These effects are being examined further. Certain Fusarium spp. however, notably F. nivale are not controlled by 'Cufram Z'.

A further sphere of activity in which 'Cufram Z' shows special promise is control of various diseases of citrus, including melanose, anthracnose tear stain (Colletotrichum gloeosporioides), and russeting. This last disfigurement is caused by the citrus russet mite (Phyllocoptes oleivorus), which is excellently controlled by 'Cufram Z'. With such a wide spectrum of activity in citrus disease control, simplification of elaborate spray programmes may become possible. Further, separate and independent observers have noticed improved colour and vigour in foliage of citrus and hops after treatment with 'Cufram Z', and there is a suggestion that the mineral content may be producing a mild foliar nutrient effect. The complete lack of phytotoxicity in the citrus crop is also of great significance.

MODE OF ACTION

Following accumulation of evidence of activity of 'Cufram Z' against powdery mildew fungi in 1966 as well as control of downy mildew diseases, efforts were made to ascertain the breakdown products of 'Cufram Z' in order to discover how contact between the fungicide and the pathogen was made. This had been assumed to be principally by breakdown of the parent material and redistribution of the derivatives in the leaf surface water film. The activity of the dithiocarbamates as inhibitors of a wide range of enzymes is tabulated by Thorn and Ludwig (Thorn G.D., et al., 1962), and interference with fungal enzyme systems by dithiocarbamates and derivatives is postulated by Chefurka (Chefurka, W., 1957). Further, several workers, notably Liebermeister (Liebermeister, K., 1950), have shown that the presence of copper can greatly increase the enzyme inhibitory powers of various dithiocarbamates. ('Cufram Z' contains 5% by weight of copper).

The method of activity and means of contact outlined above would

satisfactorilly explain the control of fungi whose spores germinate in a water film. Under dry conditions when powdery mildew spores are germinating this way would seem unlikely. Hislop (Hislop E.C., 1967), at Long Ashton has recently demonstrated the possibility of maneb and other dithiocarbamates acting in the vapour phase, or producing volatile or gaseous toxicants as breakdown products. This would explain activity which maneb and 'Cufram Z' (both of which are ethylene bis-dithiocarbamates) show to some powdery mildews under certain conditions.

The disappearance curves of maneb and 'Cufram Z' from hop foliage as calculated by combined disulphide, (Clarke method, Clarke, D.G., et al., 1951) give an approximately straight line to control level in 21 days. Hislop found that maneb deposits on bean leaves gave off vapour toxic to Botrytis fabae for up to 4 weeks, which allowing for the absence of weathering under laboratory conditions would seem to be in agreement. The metal ion concentration however tends to persist rather longer, which would support the suggestion that the sulphur component of the parent compound could be lost as a vapour.

Since it is unlikely that the gaseous toxicants identified from dithiocarbamates - methyl isothiocyanate (Munnecke, D.E., et al, 1962), hydrogen sulphide (Barratt, R.W., et al., 1947) carbon disulphide (Cox, C.E., et al., 1951), or carbonyl sulphide (Moje, W., et al., 1964) - are released directly from maneb, mancozeb, or 'Cufram Z', investigations to try to find the breakdown chain have been carried out. Using polarographic methods it has been established that the main breakdown product occurring in maneb and 'Cufram Z' is ethylene thiuram monosulphide. Polarographs from newly made and old maneb, and 'Cufram Z' were recorded. In addition to the ethylene bis-dithiocarbamate wave produced, a further wave, most marked in the old maneb sample, was observed. This wave was increased by additions of standard amounts of ethylene thiuram monosulphide (E.T.M.) and identified as being caused by E.T.M. Standard additions of ethylene thiuram disulphide (E.T.D.) showed a well defined cathodic wave as well as the conventional anodic wave, neither of which resembled the response obtained from maneb or 'Cufram Z'. E.T.D. was therefore presumed to be absent from newly prepared material, although minute amounts of E.T.D. have been detected on crops treated with these materials. Small amounts of ethylene thiourea (E.T.U.) have also been detected in residues on crops, but was found to be none-electroactive at the mercury electrode.

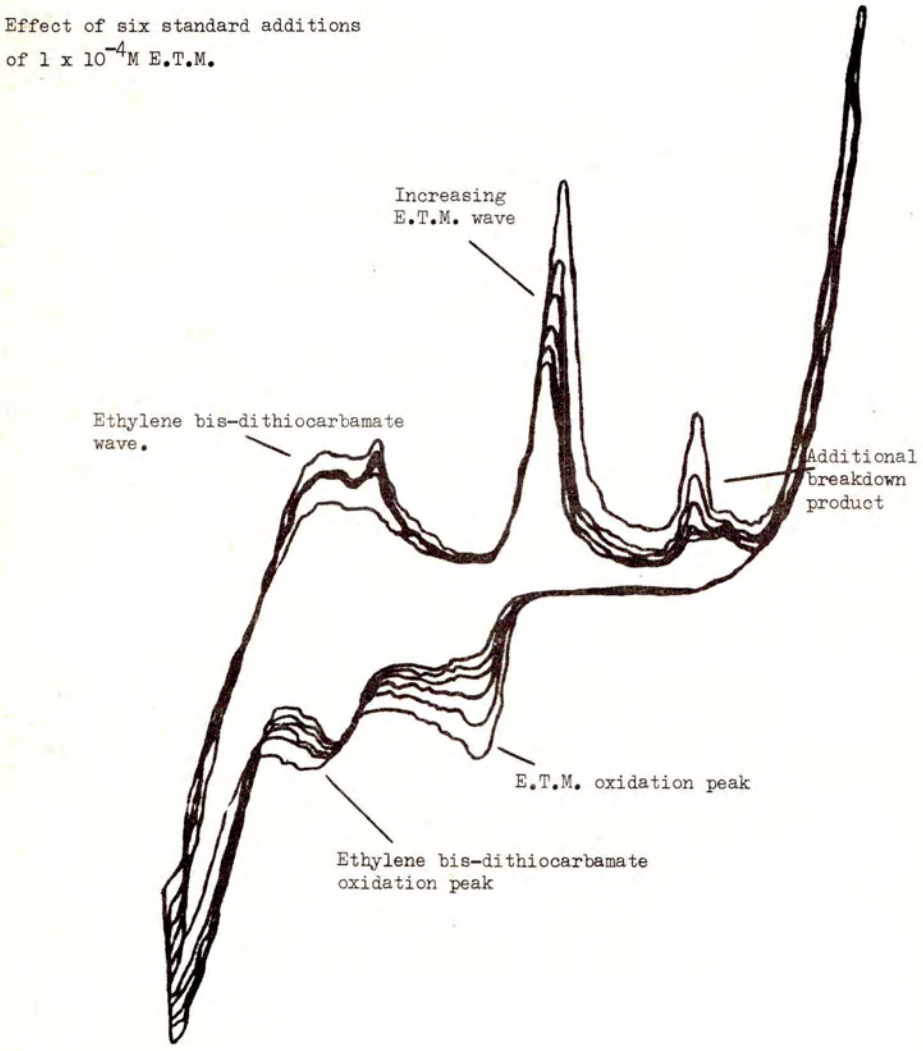
These observations were confirmed using a cyclic voltammetry technique. In this the dropping mercury electrode is replaced by a hanging (stationary) mercury drop electrode. The applied potential signal is made much faster than in the conventional polarographic technique, and in addition the wave form of the signal is changed from a simple regularly increasing voltage to a triangular wave function. Thus the applied potential signal increases cathodically over 0.5V and is then immediately reversed. This reversal in the direction of the potential sweep results in the products formed at the electrode during the cathodic sweep being re-oxidised during the reverse anodic sweep, which gives important information on the reversibility of the electrode process, and the nature of the products formed in the electrode reaction. Cyclic voltogrammes have also shown the presence of further breakdown products, but these have not yet been identified.

A further method used in investigations, 'Cathodic stripping', has again confirmed the presence of E.T.M. and further breakdown products. The tautomers and polymers of E.T.M. differ quite markedly in some respects, and the nature of the E.T.M. found has not yet been completely established. Figure 1 shows a cyclic voltogramme obtained from newly made maneb, to which six separate additions of E.T.M. were made.

In pilot plant production of monomeric E.T.M. by oxidation of nabam a quantity of elemental sulphur was found to be the main impurity in the manufactured product. In the formulation of E.T.M. on the leaf therefore, the possibility exists that elemental sulphur is formed, which could account for some of the activity against powdery mildews which has been observed. Thorn and Ludwig (Thorn G.D., et al., 1962) suggest that E.T.M. may arise via the intermediate ethylene thiuram

FIGURE I

Cyclic voltogramme of newly made maneb 5 x 10^{-4}M ethylene bis-dithiocarbamic ion



disulphide with loss of sulphur.

One possible course of breakdown of E.T.M. is the formulation of ethylene thiourea with the loss of a molecule of carbon disulphide. The presence of traces of E.T.U. on crops treated with 'Cufram Z' has been demonstrated.

The following points are concluded:

1) Ethylene thiuram monosulphide has been found and confirmed as being a major breakdown product of 'Cufram Z' and also of maneb.

2) In the presence of copper, and to some extent iron, the fungicidal activity of E.T.M. - itself a highly fungitoxic material - is greatly increased. In 'Cufram Z' these two factors occur in combination, and it is to this that the excellent activity against downy mildews is attributed.

3) E.T.M. can give rise to E.T.U. and a molecule of carbon disulphide, and this reaction could be responsible for vapour phase activity from dithiocarbamates as described by Hislop. It could also be a factor causing some inhibitory effects against powdery mildews in the case of 'Cufram Z'.

4) In the formation of E.T.M. elemental sulphur is produced. A measure of control of powdery mildews might therefore be due to formation of free sulphur on the leaf by oxidation of 'Cufram Z' to E.T.M.

APPENDIX I

Key for intensity of rose powdery mildew

Recordings on 5 bushes per plot using 5 branches of each bush as a unit for observation.

% Fungus Intensity

.01% Occasional leaves with partial infection.

.1% 5 leaves per bush with partial infection.

.5% 10 leaves per bush with partial infection.

1% 20 leaves per bush with partial infection.

1% 5 leaves per bush with partial infection + 5 with entire infection.

5% 10 leaves per bush with partial infection + 10 with entire infection.

10% 20 leaves per bush with partial infection + 20 with entire infection.

20% 40 leaves per bush with partial infection + 40 with entire infection.

50% 100 leaves per bush with entire infection.

100% All leaves with entire infection.

Acknowledgements

We gratefully acknowledge permission from the N.A.A.S. West Midland Region to reproduce results obtained in blackcurrant trials. Many thanks are also due to Dr. E.C. Hislop, for allowing us to read his paper prior to publication.

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NEW SYNTHETIC INSECTICIDAL COMPOUNDS RELATED TO THE PYRETHRINS

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Summary In the most potent insecticidal esters of chrysanthemic acid, the alcoholic component has a side chain with an activated methylene group held by a planar ring in a definite stereochemical relation to the acid part of the molecule.

Of the compounds that fulfil this condition, the most toxic have spatial arrangements very similar to those in the natural pyrethrins. This situation is found in 5-benzyl-3-furylmethyl (+)-trans-chrysanthemate, the best synthetic compound so far.

The toxicity and susceptibility to synergism of this compound and others related to it are compared with those of other pyrethroids and of insecticides of other classes.

INTRODUCTION

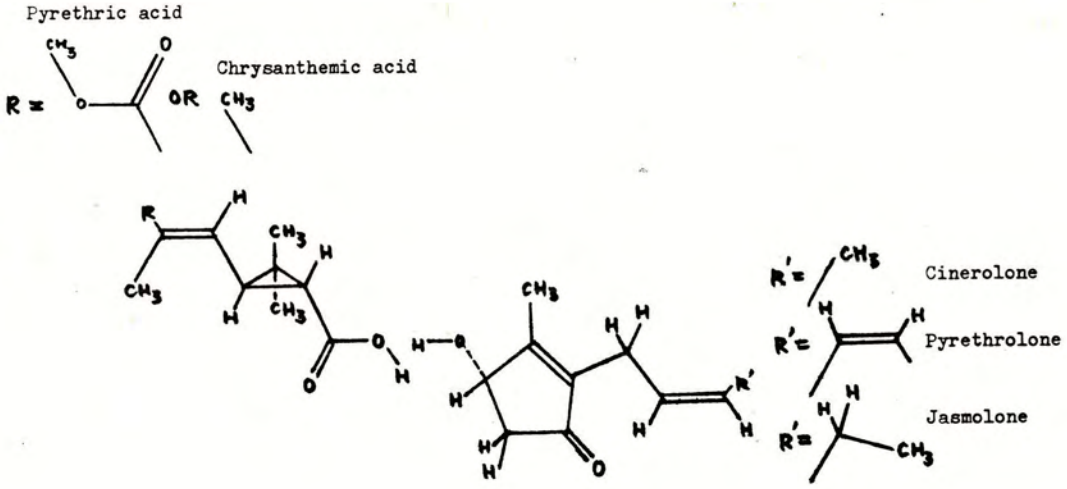
Very potent insecticides are found amongst the organic compounds of phosphorus, chlorine, or nitrogen. The purpose of this paper is to show that compounds containing only carbon, hydrogen and oxygen can equal and surpass in toxicity to insects the best compounds from these other classes.

RESULTS AND DISCUSSION

Of many acids investigated, (+)-trans-chrysanthemic acid present in the natural pyrethrins (Figure 1) gives the most toxic esters with the appropriate alcohol. But the alcohols of the natural esters and of allethrin, furethrin and cyclethrin, which are 2-alkenyl-3-methylcyclopentenolones, can be replaced by furylmethyl alcohols to give esters that maintain the stereochemical features essential for great toxicity while apparently resisting more successfully than the natural esters the detoxifying processes insects bring against them. Thus these furylmethyl chrysanthemates are more toxic than the natural pyrethrins.

For greatest toxicity in compounds related to the pyrethrins, an activated methylene group in the side chain of the alcoholic part of the molecule must be held in the appropriate steric relationship to chrysanthemic acid. Figure 2 shows that in the natural pyrethrins and in 5-benzyl-3-furylmethyl (+)-trans-chrysanthemate (the best compound in the present series), this relationship is very similar. To many species of insects these two classes of compounds at the top of Figure 2 are more toxic than the benzyl esters (including dimethrin, barthrin etc.,) and neopynamin, where it may be inferred that the ideal steric relationship is less precisely attained.

Fig. 1



Natural pyrethrins

Fig. 2

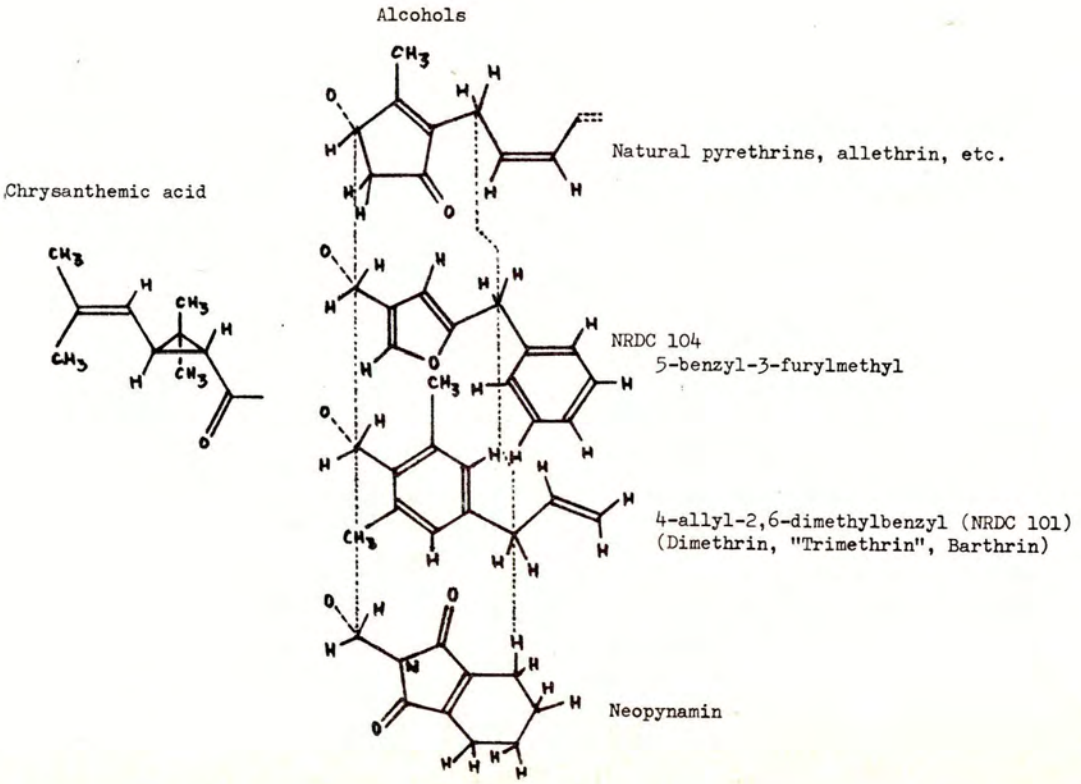

Alcohols
Chrysanthemic acid
Natural pyrethrins, allethrin, etc.
NRDC 104
5-benzyl-3-furylmethyl
4-allyl-2,6-dimethylbenzyl (NRDC 101)
(Dimethrin, "Trimethrin", Barthrin)
Neopynamin

Figure 3 shows the approximate relative toxicities of 5-benzyl-3-furylmethyl (+)-trans-chrysanthemate (NRDC 107), of 5-benzyl-3-furylmethyl (+)-trans-pyrethrate (NRDC 106) of the natural pyrethrins and of other known insecticides to houseflies (Musca domestica), mustard beetles (Phaedon cochleariae) and worker honeybees (Apis mellifera), determined by topical application techniques used in this laboratory. 5-Benzyl-3-furylmethyl (±)-cis-trans-chrysanthemate, m.p. 43-48°, (NRDC 104) is the most toxic compound tested so far, of any class of insecticide to 1-2 day old unfed females of Anopheles stephensi and Aedes aegypti (personal communication from Dr. A. B. Hadaway) and is about five times as toxic as the natural pyrethrins to the vetch aphid (Megoura viciae) (this work). NRDC 104 has been found as toxic or more toxic than the natural pyrethrins to representatives in the orders Orthoptera, Hemiptera, Coleoptera, Lepidoptera and Diptera, indicating a broad spectrum of activity.

An important property of the natural pyrethrins is their rapid knockdown action. 5-Benzyl-3-furylmethyl (±)-cis-trans-chrysanthemate (NRDC 104) knocks down houseflies slower than the natural pyrethrins but when knocked down very few insects recover. However, 5-benzyl-3-furylmethyl (+)-trans-pyrethrate (NRDC 106) had a somewhat better knockdown power 10 and 15 min after treatment and killed many more houseflies 24 h after treatment than the same dose of pyrethrum, when tested by a topical application technique (Sawicki, 1963a). Thus 15 min after treatment, 0.025 µg/fly of pyrethrum knocked down 66% of the flies but the same dose of NRDC 106 knocked down all the flies; 24 h later only 10% of the flies treated with pyrethrum were dead, against 60% with NRDC 106.

A comparison of concentrations required to knock down 50% of a housefly population at various times (Fig. 4) shows that the natural pyrethrins and neopynamin give more rapid initial knockdown than NRDC 104 which does not show maximum effect until 80 min. However, after knockdown a large proportion of the flies treated with the pyrethrins and neopynamin at 13 and 11 ppm respectively, recover, so that at the end of 21 h concentrations as large as 116 and 112 ppm are needed to kill 50% of the populations. In contrast, few insects recover from the initially slower knockdown with NRDC 104 and the LD50 (for kill) at 21 h is only 3.4 ppm. The KD50-time curves for pyrethrins and neopynamin are of the type found by Sawicki and Sawicki and Thain, (1963a,b,c) for pyrethrum and its separate constituents in the absence of a synergist, where paralysis is followed by recovery. The curve for NRDC 104, where few insects recover, resembles that obtained by Sawicki (1963c) for pyrethrum and its separate constituents in the presence of a synergist, where recovery from knockdown was suppressed.

This similarity between the behaviour of NRDC 104 and synergised pyrethrins implies that houseflies may find NRDC 104 more difficult to detoxify than the natural esters, for synergists probably inhibit the processes by which insects detoxify insecticides. Further evidence concerning this suggestion is given in Figure 5. Pyrethrins and NRDC 104 were compared in the absence of synergist in the usual way. Then houseflies were pretreated with a dose of sesamex (2 µg/fly) known from other experiments to be non-toxic to them but sufficient to inhibit all their detoxifying power. With sesamex pretreatment, NRDC 104 was synergised 12 fold but the natural pyrethrins were synergised to the more spectacular extent of 200 fold; the difference in toxicity between the unsynergised compounds was much diminished when detoxification of the pyrethrins was suppressed. However, the results in the lower part of Figure 5 indicate that mustard beetles find NRDC 104 and the pyrethrins either similarly easy or difficult to detoxify, for the greatest synergistic factor obtained even with a massive dose of synergist was 4 times.

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Fig. 3

Relative potencies of new compounds and other insecticides

	Mustard beetle	Housefly - susceptible strain	Worker honeybee
5-Benzyl-3-furylmethyl (+)-trans-chrysanthemate	1000[a]	1000[b]	1000[c]
5-Benzyl-3-furylmethyl (+)-trans-pyrethrate	630	110	370
Natural pyrethrins	160	18	48
Allethrin	11	17	1.8
Azinphos methyl			120
BHC			29
Carbaryl	23		5.7
Demeton methyl	5.4		13
DDT		39	1.5
Diazinon	13	180	29
Dichlorvos (DDVP)			
Dieldrin	100	330	42
Dimethoate	6.6		57
Malathion		11	29
Parathion	70	370	

[a] LD50 0.00071 µg/insect [b] LD50 0.0063 µg/♂ fly [c] LD50 0.0063 µg/insect

Fig. 4

Knockdown of houseflies (Musca domestica)

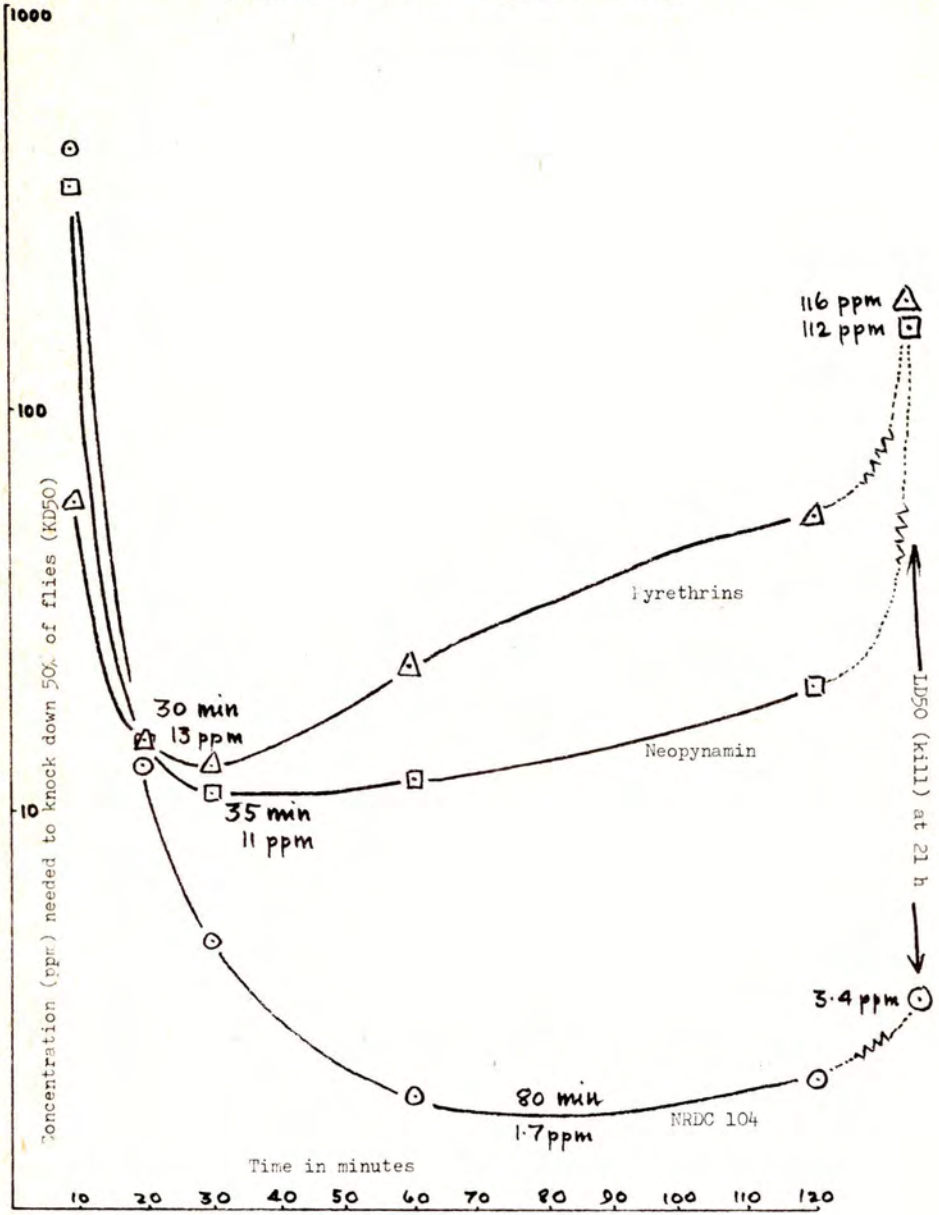

Fig. 5

Synergism

Houseflies : LD50 values (µg/♀ fly) - Topical application

	Compound alone	After pretreatment with 2 µg sesamex	Synergistic factor
Pyrethrins	0.35	0.0017	200
NRDC 104	0.016	0.0013	12

Mustard beetles : LD 50 values (µg/insect) - Topical application

	Compound alone	With piperonyl butoxide	
		1 µg/insect	10 µg/insect
Pyrethrins	0.023	0.010	
NRDC 104	0.025	0.020	
	0.041	0.040	
	0.0095		0.0020

COMPARATIVE STUDIES ON THE EFFECTIVENESS OF DIFFERENT DNBP ESTERS ON SPIDER MITES

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Summary Since dinitrophenol derivatives are of great interest for mite control, the effectiveness of three DNBP esters, viz. 2-sec-butyl-4,6-dinitrophenyl-3,3-dimethyl-acrylate (binapacryl), 2-sec-butyl-4,6-dinitro-phenyl-isopropylcarbonate (dinobuton), 2-sec-butyl-4,6-dinitro-phenylstearate, DNBP itself and 2,4-dinitro-capryl-phenyl-crotonate (dinocap) was studied by means of the slide-dip method on four laboratory strains of spider mites: Tetranychus urticae (susceptible Bad Soden strain), Tetranychus urticae (resistant Baardse strain), Tetranychus cinnabarinus and Metatetranychus ulmi (strain Dardar).

The experimental data have confirmed that spider mites which have become resistant to organophosphorus acaricides can be successfully controlled with the DNBP esters mentioned above. Considerable differences in susceptibility to DNBP and its derivatives are found between the different strains of mites, indicated by the levels of LC_{50} values.

INTRODUCTION

Although many acaricides are available, the control of spider mites often fails because of the occurrence of resistance. Reports of spider mites developing resistance to organophosphorus acaricides and certain chlorinated hydrocarbon acaricides have been recorded for nearly twenty years from many of the fruit-producing countries (Helle, 1965).

It was first reported by Emmel (1960) and Emmel & Czech (1960) that an acaricide based on dinitro-alkyl phenyl acrylate, commonly called binapacryl, is equally effective for the control of resistant and non-resistant mite species. Since that time dinitrophenol derivatives have been of great interest for mite control since no conclusive evidence of resistance induction has been reported (Jeppson et al., 1962; Aller & Lippold, 1963; Helle, 1966). Repeated observations under laboratory conditions have shown that some spider mite species exhibit differential susceptibility to binapacryl. For this reason the susceptibility of four spider mite strains to DNBP and three DNBP esters as well as to dinocap was studied in separate experiments with the aim of defining the acaricidal effect of each compound by determining LC_{50} values. Particular consideration was given to Metatetranychus ulmi (Dardar strain) because this strain can tolerate binapacryl to a high degree.

METHOD AND MATERIALS

The dinitrophenol compounds 2-sec-butyl-4,6-dinitrophenyl-3,3-dimethylacrylate (binapacryl), 2-sec-butyl-4,6-dinitrophenyl-

isopropyl carbonate (dinobuton), 2-sec-butyl-4,6-dinitrophenyl-stearate, DNBP itself (dinoseb) and 2,4-dinitrocapryl-phenyl-crotonate (dinocap) were tested under standardized conditions against four strains of spider mites in several concentrations. The formulations used were emulsifiable concentrates. The phosphorus esters demeton and phenkapton were used for the exact toxicological characterisation of the resistant and non-resistant mite strains.

Dosage-mortality tests were made by using the slide-dip method described by Voss (1961). In all tests 50 mites (adult females) were fixed by the dorsum on double-faced "Scotch" tape attached to a microscope slide. The prepared slides were dipped in the toxicant solution and gently agitated for 5 seconds. Six to ten replications of 50 mites were run at each concentration. Excess toxicant then was blotted off the slide with filter paper. The treated samples were then kept in petri dishes in a constant temperature cabinet (22 to 23°C, 90 to 95% RH for 24 h. After this time mortality was checked under a stero-microscope.

Since natural mortality after 24 h only ranged from 2 to 4 %, the figures were not corrected by Abbott's formula. LC_{50} values were established following probit analysis.

The test animals were adult females of the same age and in a similar physiological state, taken from laboratory cultures. The normal susceptible population of Tetranychus urticae (Bad Soden strain) and the highly resistant Baardse strain were each reared in mass-culture rooms at 20 to 22°C and 40 to 50 % RH, on fully-grown primary leaves of Phaseolus vulgaris. They were kept out of any contact with acaricides. The multi-resistant Baardse strain shows not only a fixed resistance to organophosphorus and chlorinated hydrocarbon acaricides but also a natural and non-selected tolerance towards binapacryl.

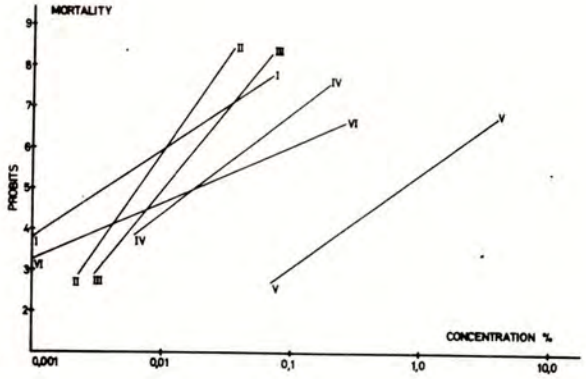
The red spider mite Tetranychus cinnabarinus which is cultured on carnation (Dianthus caryophyllus) under glasshouse conditions is resistant to organophosphorus compounds especially demeton.

The population of Metatetranychus ulmi (Dardar strain) originated from mites infesting apple trees in the Provence, South France. They have been reared in the laboratory in a mass culture on small potted apple trees at a room temperature of 22 to 24°C and 60 to 70 % RH, since 1963. The Dardar strain shows a high resistance to several organophosphorus esters which in some cases is stronger than that of the Baardse strain. Although the Dardar strain has been kept without any contact with DNBP esters and was never purposely exposed to DNBP acaricides, it shows a remarkable tolerance to binapacryl.

RESULTS

Figure 1 shows the log dosage-probit lines for the Bad Soden strain with dinoseb, the three DNBP esters, dinocap and phenkapton. The slopes and orientation of the regression lines indicate that this strain of T. urticae does not react uniformly to the dinitrophenol compounds. This strain is highly susceptible to dinoseb which has the lowest LC_{50} value (Table 1). However, the line I has a shallower slope which is comparable with that of the stearate and carbonate (dinobuton).

Fig. 1. Log dosage-probit lines for a population of T. urticae (Bad Soden strain) treated with: DNBP (I), binapacryl (II) dinobuton (III), DNBP-stearate (IV), dinocap (V), phenkapton (VI)



The phenkapton line VI has the least acute slope. The steep slope of line II indicates that binapacryl is the most effective dinitrophenol derivative tested for the control of this susceptible strain T. urticae. Binapacryl is followed by the carbonate and the stearate with nearly identical LC_{50} values. Dinocap is nearly uneffective against this spider mite species as line V shows.

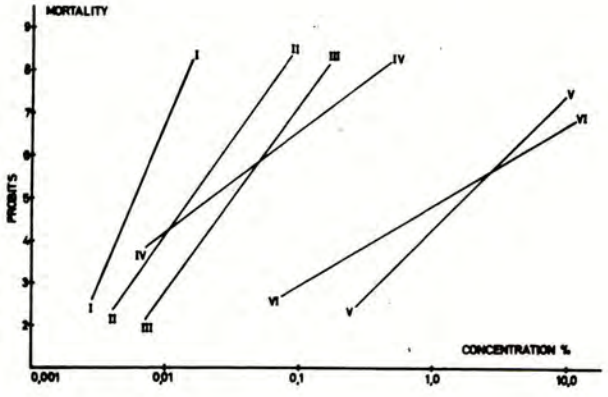
The results for the Baardse strain of T. urticae, (Fig. 2) show that by comparison with Fig. I, all the lines are shifted slightly to the right. This suggests a possible tolerance to dinoseb, binapacryl and dinobuton. The rather steep slope of line I indicates that this strain reacts uniformly to DNBP. The ratios of the LC_{50} values for the resistant strain over the susceptible strain are roughly 2 for dinoseb and binapacryl and 3 for dinobuton (see Table I).

Table I

LC_{50} values for four strains of spider mites treated with DNBP, DNBP esters, dinocap and two organophosphorus esters

Toxicant	T. urticae		M. ulmi	T. cinnabarinus
	strain Bad Soden	strain Baardse	strain Dardar	
DNBP	0.0036	0.0058	0.0018	0.0040
binapacryl	0.0064	0.0153	0.0760	0.00098
stearate	0.0172	0.0217	0.0192	0.0118
dinobuton	0.0102	0.0314	0.0637	0.0253
dinocap	0.6820	1.6666	0.0188	0.0950
phenkapton	0.0178	1.1716	0.3688	------
demeton	------	------	------	0.1727

Fig. 2. Log dosage-probit lines for a population of Tetranychus urticae (Baardse strain) treated with: DNBP (I), binapacryl (II), dinobuton (III), DNBP-stearate (IV), dinocap (V), phenkapton (VI)



The LC_{50} values and the slopes of the log dosage-probit line of the stearate for both strains are practically identical. Dinocap probably has no effect on the Baardse strain as the LC_{50} value of 1.666 % demonstrates. Line VI shows the Baardse strain to be highly resistant to phenkapton. The ratio of the LC_{50} values of the Bad Soden strain over the Baardse strain show that the latter is 66 times less susceptible to the organophosphorus ester. The fact that the Baardse strain maintained its resistance to the organophosphorus compound for several years without any selection, confirms that this resistance is clearly of the stable type.

Line I in Figure 3 shows that M. ulmi has a higher susceptibility to dinoseb, which is nearly twice as great as that with the susceptible strain and three times as great as with the resistant strain of T. urticae. On the other hand line II (binapacryl) and line III (dinobuton) are shifted to the right and the slope values have decreased markedly indicating a natural tolerance to several DNBP esters because this strain has been maintained without any selection.

The stearate in this case is the most effective DNBP derivative. Previous work with other methods of application has confirmed these results. The most astonishing effect is that M. ulmi is very susceptible to dinocap when the slide-dip method is used for application. Attempts to establish log dosage-probit lines with dinocap by the leaf-spray and leaf-dip methods produced insignificant mortality at relatively high dosages. The organophosphorus resistance of the Dardar strain is demonstrated by line VI.

Fig. 3. Log dosage-probit lines for a population of M.ulmi (Dardar strain) treated with: DNBP (I), binapacryl (II), dinobuton (III), DNBP-stearate(IV), dinocap (V), phenkapton (VI)

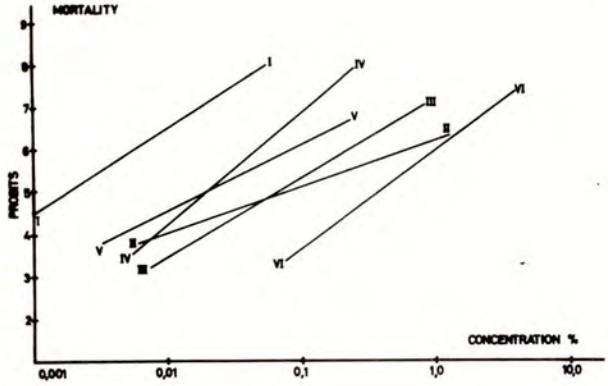


Fig. 4. Log dosage-probit lines for a population of T. cinnabarinus treated with: DNBP (I), binapacryl (II), dinobuton (III), DNBP-stearate (IV), dinocap (V), demeton (VI)

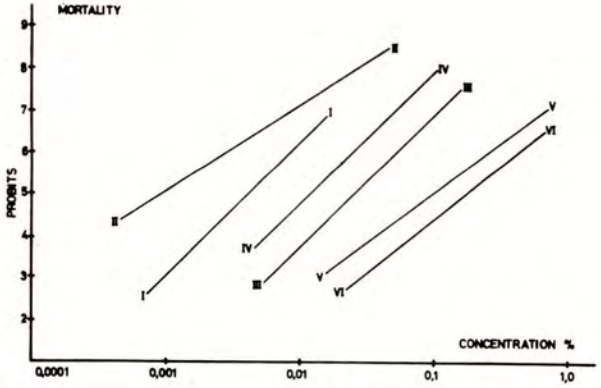


Figure 4 shows that T. cinnabarinus has a graduated susceptibility to the toxicants tested in this study. The log dosage-probit lines show no marked differences in slope. The well known efficacy of binapacryl for the control of this species is confirmed by line II which in this special case is in front of line I (dinoseb). This is a very surprising result. The dimethylacrylic ester of DNBP is 20 times as effective as DNBP itself.

Lines IV and III and their LC_{50} values indicate that the stearate is twice as effective than dinobuton. On the other hand T. cinnabarinus has a very low susceptibility to dinocap. The organophosphorus resistance of this spider mite strain is confirmed by line VI and the corresponding LC_{50} values (Table 1).

DISCUSSION

The results presented confirm that both organophosphorus-resistant and susceptible spider mite populations can be controlled successfully with DNBP and DNBP esters. However, considerable differences in susceptibility were found between species. As the LC_{50} values indicate all strains of spider mites tested show the highest susceptibility to dinoseb except that of T. cinnabarinus which has an even higher susceptibility to binapacryl. Dinoseb has a very small range of LC_{50} values (0.0018 to 0.0058 % a.i.) for both strains of T.urticae and for M.ulmi. These results are in agreement with those reported by Reichner et al. (1962) who also recorded 100 % mortality at very low dosages.

Binapacryl was the most effective DNBP ester tested. It gave as good control of the sensitive strain of T. urticae as the multi-resist-ant Baardse strain. The present experiments show that binapacryl is less effective against M. ulmi - four times less, from a comparison of the LC_{50} value for the latter with that for the highly resistant strain of T. urticae. In contrast, the tested strain of M. ulmi showed a remarkable susceptibility to the stearate which gave the best control of all the DNBP esters tested. The stearate was nearly equally toxic to all four strains of spider mites. Dinobuton in most cases was two to four times less effective than the stearate and binapacryl.

As can be deduced from the LC_{50} values, dinocap, which is normally used as a fungicide for the control of apple powdery mildew, has practically no penetration effect on spider mites when used in recommended dosages.

Because a 2 to 4-fold decrease in susceptibility may not be sufficient to indicate a sufficient difference attributable to resistance (Aller & Lippold, 1963), the data presented here suggest that the results of this work reflect morphological and/or physiological differences between the mites in their reaction to DNBP and its esters. Tolerance is indicated rather than differential resistance.

The results also indicate that the toxic effect must be due to the DNBP of which a critical concentration must be upon or in the body of the mites. Recognising that the uncoupling effect of this chemical influences many metabolic processes, it might be expected that the differences in susceptibility of the mites would be related in some way to metabolic processess, especially to oxidative phosphorylation, and to ATP-production. On the other hand pick-up and penetration depend largely upon the morphological and histochemical condition of the integument of the mite species and last and not

least on the physico-chemical properties of the toxicant itself (Gösswald et al. 1963).

At present it is a matter for speculation whether the date on the different susceptibilities of spider mite species to DNBP and DNBP derivatives are the results of differences in penetration of the toxicant into the body or the results of differences in metabolic activity of the mites. It would be interesting and quite useful to undertake further studies on this subject by using C^{14}-labelled DNBP esters. This work is being continued in our laboratories.

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DRAZOXOLON- A New Fungicide

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Summary

Drazoxolon [4(2-chlorophenylhydrazono)-3-methyl-5-isoxazolone] is a new type of fungicide with a broad spectrum of activity. It is especially effective against powdery mildews on apples, blackcurrants, cereals, chrysanthemums, cucurbits and roses. Also it has shown considerable promise as a seed dressing for control of root-rots and seedling blights on vegetable crops, grasses, maize and cotton.

INTRODUCTION

Drazoxolon [4-(2-chlorophenylhydrazono)-3-methyl-5-isoxazolone] was first synthesised at Jealott's Hill in 1960. It is highly active as a foliage fungicide, particularly against powdery mildew, and it is also effective against a number of seed and soil-borne diseases. Primarily it acts as a protectant, preventing the germination of fungal spores, but against powdery mildews it has a very marked eradicant action.

Drazoxolon is a yellow, crystalline solid with a faint odour, almost insoluble in water, acids and paraffin hydrocarbons. It is soluble in alkali, chloroform (ca.10%), aromatic hydrocarbons (4%), ketones (5%) and ethanol (1%). Its vapour pressure is 4×10^{-6} mm Hg at 30°C.

FORMULATIONS

Drazoxolon has been formulated as cols and dispersible powders for use as foliage sprays, as powders for dry or slurry seed dressings, as dusts for application to soil, and as grease formulations. Preliminary results suggest that col formulations give more persistent deposits on foliage.

TOXICITY TO MAMMALS

Acute oral toxicity The following values have been determined:-

Species	LD_{50}
Rat	126 mg/kg
Mouse	129 mg/kg
Hen	100 mg/kg
Cat	50-100 mg/kg
Rabbit	100-200 mg/kg
Guinea-pig	25 mg/kg
Dog	17 mg/kg
Sheep	(approx. 20 mg/kg)

The effect of drazoxolon is to cause convulsions, due to action within the brain not in the spinal cord or peripheral nerves or muscles. Animals dying after oral administration are in a state of coma for some hours before death.

Intraperitoneal toxicity This has been determined for technical drazoxolon in the female rat at LD_{50} 26 mg/kg. Toxic signs are similar to those produced by oral administration but are more rapid in onset.

Effects on skin and eyes Experiments have shown drazoxolon to have no marked irritant effects on either the skin or the eye. Some sensitisation reactions may, however, occur and prolonged contact with the concentrated solution should be avoided. Accidental splashes should be washed off immediately.

Inhalation effects The fume has some lung-irritant action. The saturated vapour is not likely to cause a hazard but the concentration of fume should not exceed 0·5 mg/m^3. A yellow colouration of urine will be a warning of excessive exposure.

Chronic toxicity This has been evaluated in rats and dogs in 90-day feeding studies.

In rats, no significant abnormalities occurred at a dietary level of 30 p.p.m. At higher rates (100 and 300 p.p.m.) animals failed to gain weight normally (partly at least due to unpalatibility of diet) but there were no clinical abnormalities except roughened coats at 300 p.p.m. At no level were any histological abnormalities observed.

In dogs given 2 mg/kg/day (approx. 73 p.p.m. in diet) there were no adverse effects. At 4 mg/kg/day (146 p.p.m.) two dogs in a group of four died after 3 doses.

TOXICITY TO FISH

Tests on brown trout have given the following results :-

Exposure period	MLD p.p.m. active ingredient
24 hours	1·95
48 hours	1·25
96 hours	0·55

TOXICITY TO BEES

An experiment, in which drazoxolon at eight different concentrations was fed to two replicate batches of ten worker bees of mixed ages, gave the following results:-

Concentration drazoxolon %	Mean % kill
4·0	20·0
3·0	0
2·5	0
2·0	1·4
1·5	10·0
1·0	0
0·25	0
0·063	0

Apart from the unexplained anomaly in the batch fed with 1·5% drazoxolon, the compound does not appear to have any appreciable toxicity to bees.

BIOLOGICAL ACTIVITY

Drazoxolon has been included in a large number of field trials in the United Kingdom and overseas and briefly the results are as follows:-

Apple Mildew and Scab

In glasshouse tests, drazoxolon gave excellent control of powdery mildew (Podosphaera leucotricha) and scab (Venturia inaequalis) on apple rootstocks, being as effective as dinocap and captan but drazoxolon gave steeper dosage response curves (Figs 1 and 2) and has a better eradicant effect than dinocap

on established infections of mildew. It desiccates the mycelium of powdery mildew and prevents sporulation.

No phytotoxicity was detected even after repeated applications at rates as high as 0·2% a.i. in high volume sprays.

In the field there was, generally, 20-25% less mildew on trees treated with 8 oz drazoxolon per acre per application in high or low volume sprays than on trees receiving a standard dinocap programme. Even better results were obtained when 32 oz were applied per acre up to petal fall followed by 8 oz throughout the rest of the season (Table 1). These trials were carried out on large blocks of trees because the advantage of a heavy initial application is reduced in single-tree plot trials where reinvasion by mildew from adjacent trees can readily occur. It appears that the heavier early sprays suppress or eradicate primary infections and thus reduce the initial spore inoculum which is produced at this time, while the later sprays maintain a high degree of protection.

Table 1.

Effect of Drazoxolon on Mildew on Apples
(Variety, Cox's Orange Pippin)

Treatment	Rate of application per acre approx. 10-day intervals		No. of shoots mildewed as percentage of all shoots		
	Until petal fall	After petal fall	11 Aug.	14 Sept.	6 Jan.
Drazoxolon	8 oz	8 oz	32·7	65·3	10·8
	32 "	8 "	17·1	46·1	6·5
Dinocap	8 oz throughout the season		54·5	68·3*	13·4

* The standard treatment was continued to mid-August with 3 more sprays than the drazoxolon treatment because of heavy mildew infection.

In trials where counts of primary mildew were possible in the following spring, the reduction of infection corresponded broadly to the suppression of secondary mildew.

Drazoxolon at 16 oz per acre per application throughout the season gave even better results but in some years on certain varieties, notably Cox's Orange Pippin, Laxton's Superb, Laxton's Exquisite and Jonathan, in some orchards, this rate induced a premature loss of rosette leaves and leaves on extension shoots. Other varieties, such as Worcester Pearmain, Laxton's Fortune and George Cave were unaffected. Susceptibility increases as the season progresses and the first signs of damage are generally seen in July. Phytotoxicity appears to the more pronounced in impoverished orchards and very young trees. Premature leaf fall was negligible or absent on all varieties when the rate did not exceed 8 oz per acre or when 32 oz per acre per application was used up to petal fall followed by 8 oz per acre for the rest of the season.

However, this year the spray programme based on 32 oz dropping to 8 oz per acre increased the incidence of russet in certain orchards, while fruit in other orchards had a good finish. This has been an exceptional season with late frosts and a very wet May followed by a long relatively dry period, and it is impossible to conclude the extent to which these climatic changes predisposed the fruit to injury by this chemical. The occurrence of russeting in this particular season, after five years of trials in which similar treatments did not increase it to any noticeable extent, is a perplexing problem which will be the subject of further investigation.

Because of the risk of damage at the higher rates drazoxolon is not being recommended for control of apple scab.

FIGURE I:

COMPARISON OF DINOCAP AND DRAZOXOLON

(APPLE MILDEW)

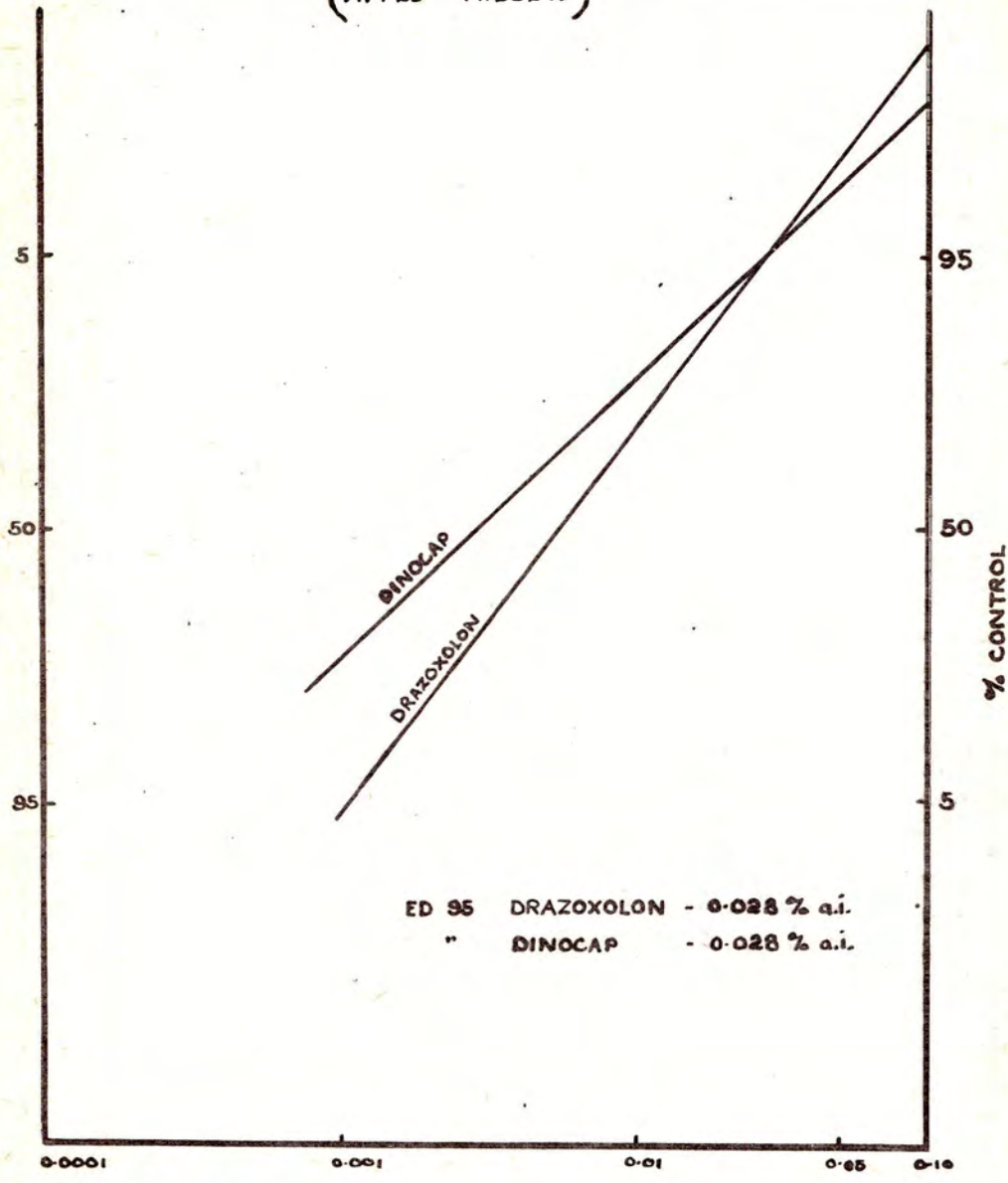
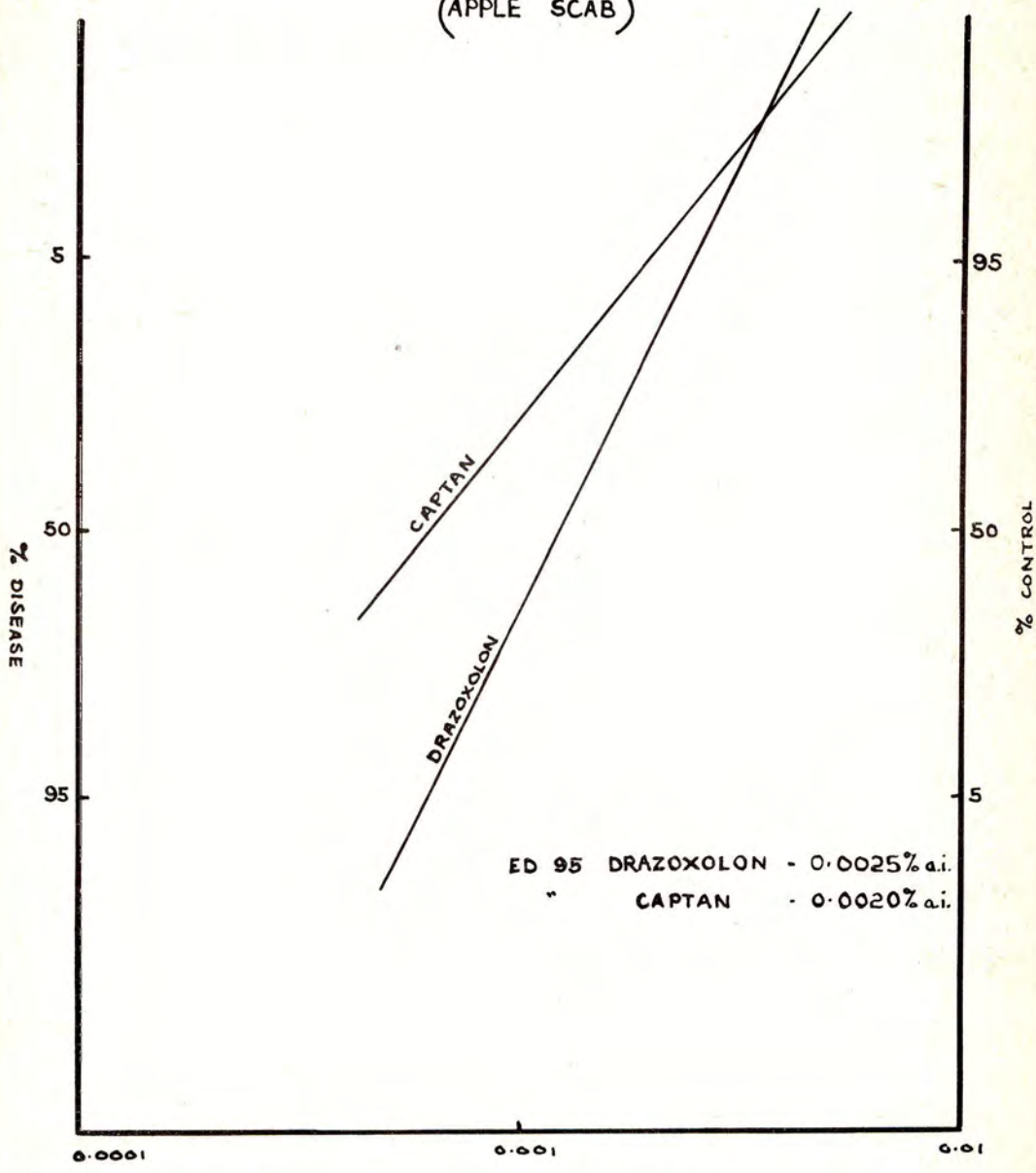


FIGURE 2:

COMPARISON OF CAPTAN AND DRAZOXOLON

(APPLE SCAB)



Mildew and Leaf-spot of Blackcurrants

Since 1960 the effect of drazoxolon on mildew (Sphaerotheca mors-uvae) on blackcurrants has been assessed in field trials at Long Ashton Research Station (Corke, 1965), at Luddington Experimental Horticultural Station (Ingram, 1967) and at Fernhurst Research Station

The results of all these trials show that drazoxolon at 16 oz per 100 gallons of spray has given excellent control of this disease, equal to or better than that given by the standard rate of quinomethionate, and also good control of leaf-spot (Pseudopeziza ribis). Some of these results are given in Table 2.

Table 2.

Some Results with Drazoxolon for Control of American Gooseberry Mildew and Leaf-spot of Blackcurrants

Treatment (oz./100 gallons)	Mildew % leaves infected		Leaf Spot % leaves infected
	Long Ashton 1965	Luddington 1966	Luddington 1966
Untreated	49·6	100	94
Drazoxolon 16	6·4	17	<5
Quinomethionate 4	29·8	21	<5
Dinocap 4	-	54	32
Lime sulphur 10 lbs	-	36	26

At present the use of drazoxolon is restricted to the post-harvest period or to non-fruiting bushes but it is hoped that when further residue data have been obtained it will be possible to use it on fruiting bushes also. Drazoxolon has been used without adverse effect on a wide range of blackcurrant varieties.

Cereal Mildew

In the United Kingdom 16 oz of drazoxolon applied as a tank-mix with the hormone weedkiller in 20 gallons of spray per acre markedly reduced the incidence of powdery mildew (Erysiphe graminis) in Proctor barley, and increased yields. A second spray one month later did not give a sufficient response to compensate for the damage caused by the tractor wheels. The results of a series of 10 trials carried out in 1966 are shown in Table 3. At each site a 5 acre square was treated and compared with a similar adjacent untreated area. Large plots were used to reduce the risk of reinfection from untreated areas.

Table 3.

Effect of Drazoxolon on Powdery Mildew in Barley

Treatment	Disease	Yield	
	% Leaves Diseased *	Cwts/acre	Increase (%)
Hormone weedkiller alone	62	33·3	
Hormone + Drazoxolon @ 16 oz/acre	48	36·0	8

* Assessed 6 weeks after treatment.

Good control of mildew and increases in yield were obtained by Podhradszky (1967) in trials in Hungary with drazoxolon on mildew-susceptible wheat varieties and on autumn and spring barley crops. In these trials drazoxolon was more effective than cycloheximide, binacryl, triamphos, dinocap and dichlone.

Powdery Mildew of Cucurbits

In glasshouse tests, high volume sprays containing 16 or 8 oz of drazoxolon per 100 gallons gave good control of powdery mildew (Erysiphe cichoracearum) on cucumbers, equal to quinomethionate and better than dinocap, but residues were unacceptably high. So far there are no residue data from trials on outdoor cucumbers but it is expected that weathering may reduce residues.

Powdery Mildew of Chrysanthemums

Drazoxolon has been used as a routine spray for control of powdery mildew (Oidium chrysanthemi) on chrysanthemums in greenhouses at Fernhurst. It has given excellent control when used at 5 oz in 3 gallons of water per acre.

Powdery and Downy Mildew on Hops

Drazoxolon at 0·05% in high volume sprays in preliminary trials in the United Kingdom was as good as dinocap for control of powdery mildew (Sphaerotheca humuli) on hops, and at 0·2% was equal to Bordeaux mixture for controlling downy mildew (Pseudoperonospora humuli). No phytotoxicity was detectable but spraying 3 weeks before harvest stained the cones slightly. Trials in Germany suggest that 0·1% drazoxolon sprays can give adequate control of hop downy mildew.

Powdery Mildew and Blackspot on Roses

Drazoxolon is particularly effective against mildew (Sphaerotheca pannosa), and suppresses blackspot (Diplocarpon rosae) on roses. It has been included in numerous trials on many varieties of roses in the United Kingdom and overseas and key results are given in the following tables:-

Table 4.

Effect of Drazoxolon on Powdery Mildew on Roses
(Var. Fashion)

Treatment	% a.i. in spray	Leaves infected (%) (7 days after the sixth spray)
Drazoxolon	0·1	33
"	0·05	23
Dinocap/captan	0·025*	96
Dinocap	0·025	96
Thiram	0·1	60
Control	-	87

* concentration of dinocap

Table 5.

Effect of Drazoxolon on Blackspot on Roses
(Var. The Queen)

Treatment	% a.i. in spray	Leaves infected (%) (2 days after the eighth spray)
Drazoxolon	0·1	11
"	0·05	20
Captan	0·1	82
Thiram	0·1	91
Control	-	100

Rust on Coffee

Following observations of *in vitro* activity against the rust pathogen (*Hemileia vastatrix*) drazoxolon proved in a field trial to be one of the best fungicides for the control of this disease, at least as effective as copper, and also there was a suggestion from the results that it might control coffee berry disease (*Colletotrichum coffeanum*) Hocking (1965 and 1967).

Seed-and Soil-Borne Diseases

Drazoxolon is active against a number of soil-borne pathogens including *Pythium ultimum*, *Fusarium* spp., *Rhizoctonia solani*, *Gibberella zeae* and *Rhizopus* spp.

In numerous greenhouse tests drazoxolon as a seed dressing at 500 p.p.m. was at least as active as thiram at 1000 p.p.m. on the seed for control of *Pythium ultimum* on peas. This was confirmed in field trials in 1963 (Table 6.)

Table 6.

Results of Rod-Row Trials to assess the Value of Drazoxolon as a Seed Dressing for Peas (Var. Kelvedon Wonder)

Treatment (p.p.m. a.i)		Emergence as percentage of untreated Sites 1	2	3	Means
Drazoxolon	1000	172	236	147	185
"	500	159	247	137	181
Thiram	1000	146	200	128	158
"	500	143	203	128	158
Captan	1000	151	230	148	176
"	500	143	236	145	175
Untreated		100	100	100	100

In preliminary market garden trials drazoxolon as a seed dressing was superior to thiram, measured by increased emergence of lettuce, celery, beans, brassicae and freesias. In conjunction with Scottish Agricultural Industries Ltd, field and glasshouse experiments have shown that drazoxolon at 0·25% w/w gives worthwhile increases in emergence of grasses and clovers. No phytotoxicity was observed at rates as high as 1·0% a.i. after one year's storage.

Against *Gibberella zeae* of maize drazoxolon showed great promise in greenhouse tests (Table 7), and in small scale field experiments in this country, Italy and the United States.

Table 7.

Effect of Seed Dressings on Emergence of Maize from Soil infected with Gibberella zeae in Glasshouse Tests

Treatments *	Emergence as percentage of control Jealott's Hill	Italy
Drazoxolon	288	133
Thiram	233	131
Captan	-	136
'Agrosan' GN	255	-
Untreated	100	100

* Each fungicide was applied at 1000 p.p.m. a.i. on the seed. At Jealott's Hill the soil was inoculated, but in Italy the seed was scratched to increase infection and planted in natural soil from an old maize field.

Further field trials are required to decide the optimum amount of chemical to apply to maize.

Drazoxolon has also given worthwhile control of root-rotting pathogens of cotton. In 1966, in two field trials in Spain there was a severe Rhizoctonia/Fusarium infection, drazoxolon as a seed dressing at 0·1% gave greater increases in emergence than PCNB applied as a seed dressing or as an "in-the-furrow treatment" (Table 8).

Table 8.

Effect of Different Treatments on Emergence of Cotton Field Trials in Spain 1966

Treatments		Emergence as percentage of untreated		
		Seville	Cordoba	Mean
PCNB	as seed dressings	90	125	108
Drazoxolon	0·1% a.i. on seed	237	163	201
PCNB		135	162	149
Drazoxolon + PCNB	16 oz a.i./acre applied in furrow	162	140	151
Captan + PCNB		130	131	131
Untreated		100	100	100

In a single box test at Jealott's Hill drazoxolon as a seed dressing at 1000 p.p.m. was superior to captan and difolotan but inferior to thiram for control of Rhizopus arrhizus on groundnuts (Table 9).

Table 9.

Effect of Seed Dressing on Emergence of Groundnuts from Soil Infected with Rhizopus arrhizus-Glasshouse Test

Treatments *	Disease Index
Drazoxolon	64·3
Thiram	55·7
Captan	74·0
Difolotan	71·9
Untreated	100·0

* Each fungicide was applied at 1000 p.p.m. on the seed.

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