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FIELD PERFORMANCE OF CELA S 2957 AND CELA K 673 UNDER TROPICAL CONDITIONS

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Summary CELA S 2957 (0-2,5-dichloro-4-(methylthio)-phenyl-0,0-diethyl-phosphorothioate) and CELA K 673 (0-2,5-dichloro-4-(methylthio)-phenyl-0-ethyl-methylphosphonothioate) have been tested on a wide range of crops and pests under tropical and subtropical climate conditions. Both compounds gave an effective control of a variety of pests on a number of crops at dosages well comparable with some of the standard materials. The effective dosages for the control of pests in tropical zones seem to be slightly higher than needed under temperate conditions. CELA K 673 gave excellent control of resistant mites on apples. Phytotoxic effects with 50% e.c. formulations of both compounds have never been observed.

INTRODUCTION

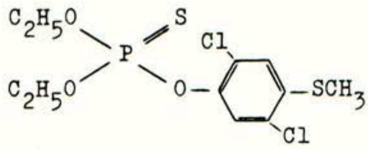
Sehring and Buck (1968) reported on the chemical and insecticidal aspects of the new organophosphorus compounds CELA S 2957 and CELA K 673. In another publication the relationship between constitution and biological activity of the two pesticides was described by Buck et al. (1971).

Results of field tests with the two compounds under temperate conditions were presented on the occasion of the meeting of the 38. Deutsche Pflanzenschutztagung 1971 at Berlin by Prokić-Immel and Adlung. Field trial data from tests carried out on pests in warmer climates are reviewed in this report.

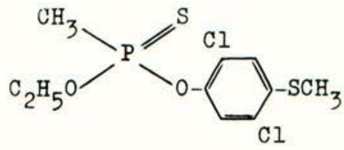
CHARACTERISTICS OF CELA S 2957 AND CELA K 673

Data are compiled in the following table.

Compound	CELA S 2957	CELA K 673
Chemical name	0-2,5-dichloro-4-(methylthio)-phenyl -0,0-diethyl-phosphorothioate	0-2,5-dichloro-4-(methylthio)-phenyl -0-ethyl-methylphos-phonothioate



$C_{11}H_{15}Cl_2O_3PS_2$



$C_{10}H_{13}Cl_2O_2PS_2$

	$C_{11}H_{15}Cl_2O_3PS_2$	$C_{10}H_{13}Cl_2O_2PS_2$
Molecular weight	361.25	331.24
Appearance		
pure grade	white crystals	white crystals
technical grade	yellow oil	yellow-brown oil
Boiling point	153-155° C, 0.05 mm Hg	167-168° C, 0.1 mm Hg
Melting point	39.0-39.5° C	43-44° C
Solubility		
soluble in	acetone, benzene, ethanol, methylenchloride	acetone, benzene, ether, methylenchloride
slightly soluble in	gasoline	gasoline
insoluble in	water	water
Acute toxicity		
oral LD_{50} rat	13 mg/kg	27 mg/kg
oral LD_{50} mouse	141 mg/kg	46 mg/kg
dermal LD_{50} rabbit	31 mg/kg	

RESULTS

An extensive field trial program on a number of crops and pests has been carried out in various countries, using mainly 50% e.c. formulations of both compounds.

Trials on soybeans, sorghum, maize and cotton in Colombia

The goal was to determine at what dosage the compounds under test produce an equal degree of control in terms of the effective dosage. It was hoped that this approach would best indicate the differences in biological activity between the products and reveal the specific potential of each product.

During 1970/71 the reported experiments were carried out on the well prepared test fields of the company's Research Station in Colombia. Tests were laid out in a randomized complete block system with four replications. Size of the plots was 24 m² for sorghum, 100 m² for cotton. Each compound to be tested was applied at several dosages, e.g. 250, 500, 750, 1000, 1250 g/ha a.i. in the first trial, thereafter number of dosages was reduced. The test compounds were sprayed by the high volume method, i.e. 600 - 1000 l./ha. For reason of statistical validity of counted insect numbers the timing of the spraying depended on the presence of a sufficient number of the particular insect species to be tested. In most of the tests the control efficiency was determined one to three days after spraying; there are also a few results available on residual efficacy. From the figures obtained mortality percentages were calculated. In addition the data were submitted to a statistical treatment using the chi^2-method. The result of one such test is presented as a model.

This example consists of 10 treatments (4 compounds and one untreated plot). 3 compounds were tested at 2 dosage levels and one at three rates. Table 1 shows the number of insects of the 10 treatments in 4 replications observed 3 days after application. Total numbers of insects are presented for each treatment and each block. Complete homogeneity of the insect distribution is assumed, the theoretical numbers of the insects per plot being 77.6. The orthogonal contrasts of the totals of the block as well as of the treatments were determined by means of chi^2-analysis as is employed in genetical research. The results are summarized in table 2. The inhomogeneity of residual was determined by means of the theoretical values estimated on the basis of the marginal values. The statistical significance is expressed at levels of $p = 0.05$ and 0.001.

Table 1 : Observed numbers of alive insects of the trial

No.	Treatments	Dosage g/ha a.i.	Blocks A	B	C	D	Total	Mortality %
1	S 2957	500	40	12	11	13	76	97
2	S 2957	1000	26	7	7	10	50	98
3	Monocrotophos	500	19	5	4	6	34	99
4	Monocrotophos	1000	16	6	5	4	31	99
5	K 673	500	82	22	17	26	147	94
6	K 673	1000	11	4	0	1	16	99
7	other materials		55	15	10	17	97	97
8			30	9	5	8	52	98
9			11	3	2	3	19	99
10	Untreated	0	1416	400	317	449	2582	
Total blocks			1706	483	378	537	3104	

Table 2 : Chi^2-analysis of dates presented in table 1

Contrasts evaluated	d.f.	chi^2	significance
treated - untreated	1	7.025,5714	+++
S_{500} - M_{500}	1	1,1366	
S_{500} - K_{500}	1	3,2481	
M_{500} - K_{500}	1	8,2275	++
S_{1000} - M_{1000}	1	0,2326	
S_{1000} - K_{1000}	1	0,7448	
M_{1000} - K_{1000}	1	0,1450	
S_{500} - S_{1000}	1	0,4356	
M_{500} - M_{1000}	1	0,0058	
K_{500} - K_{1000}	1	11,0573	+++
treatments	9	18.507,6108	+++
blocks	3	1.499,5103	+++
residual inhomogeneity	27	7,7355	NS

S = S 2957 K = K 673 M = Monocrotophos

As all of the original results cannot be presented due to space limitations, the following summary is compiled for each of the crops showing the number of trials and effective dosage (g/ha a.i.) required for control of several pests.

a) Sorghum

Species of	No. of trials	Effective Dosage g/ha a.i.		
		S 2957	K 673	Monocrotophos
Empoasca	6	500	500	500
Aphids	4	750	750	500
Diatrea	2	500	-	500
Diabrotica	3	500	-	750
Spodoptera	3	500	750	500

b) Maize

Species of	No. of trials	Effective Dosage g/ha a.i.		
		S 2957	K 673	Monocrotophos
Empoasca	11	500	500	500
Diabrotica	6	500	500	750
Spodoptera	7	750	750	750
Heliothis	1	750	750	750

c) Soybeans

Species of	No. of trials	Effective Dosage g/ha a.i.		
		S 2957	K 673	Monocrotophos
Empoasca	4	500	500	500
Nezara	6	500	500	500
White Flies	4	500	500	500
Anticarsia	5	500	500	500
Platynota	3	500	500	500
Diabrotica	6	500	500	500
Prodenia	2	500	500	500

d) Cotton

Species of	No. of trials	Effective Dosage g/ha a.i.		
		S 2957	K 673	Monocrotophos
Aphids	6	500	500	500
Alabama	7	500	500	500

Comparing the biological efficacy data of S 2957 and K 673 in respect to the species of various genera it can be stated that:

- S 2957 is slightly superior to K 673 in the control of Spodoptera
- K 673 is slightly superior to S 2957 in the control of aphids, Nezara
- both are more or less equal in the control of Alabama, Prodenia, Diabrotica, Empoasca, white flies, Anticarsia, Platynota.

The differences are not in all cases so significant as to warrant different application rates. There is also some evidence that the control efficiency depends upon the kind of crop as is indicated by higher dosages for the control of certain pests on sorghum.

Trials on apple pests in Italy

In previous trials the acaricidal efficiency on non-resistant tetranychid mites had been established on a number of crops for both S 2957 and K 673. In 1971 trials on resistant European Red Mites were carried out on apple trees in Italy, two of which are presented. A full report was delivered at the 38. Deutsche Pflanzenschutztagung 1971 in Berlin.

Trees of the variety Gravensteiner were sprayed to run-off. A randomized block system with 1 tree per plot in 4 replications was chosen. Mites of 25 leaves per plot were recorded three times after treatment. Table 3 shows the mortality data as totals for the 4 replications as the number of mites per 100 leaves for the total of the untreated plots.

Table 3 : Control of resistant mites on apples in Italy

Trial No.		I			II		
apple variety		Gravensteiner			Gravensteiner		
date of application		June 5, 1971			June 25, 1971		
		Percentage control at intervals days after treatment			Percentage control at intervals days after treatment		
Compound	Dose ppm	3	9	18	3	10	25
K 673	125	99	98	86	98	56	0
	250	99	99	96	99	95	44
	500	100	99	98	99	98	46
S 2957	500	75	39	17	80	28	0
	2500	95	96	84	98	48	44
Azinphosmethyl	5000	28	44	11	35	0	0
OMITE ®	570	99	97	97	98	97	93
Untreated [a]		2012	2562	1677	3402	6313	1953

[a] number of mites per 100 leaves

K 673 controlled the mite population at a rate of 125 ppm to the same extent as the standard compound OMITE. A remarkable residual effect was obtained by applying 250 ppm of K 673. Only at a ten times higher dosage was S 2957 able to match the performance of the 250 ppm rate of K 673. The low mortality percentages of 5000 ppm doses of azinphosmethyl indicate the extent of resistance.

Trials on cotton pests in Central America

During the season 1967 first dosage trials from cotton pests with S 2957 were carried out by a CELA expert. A spraying program of 15 applications revealed that S 2957 performed satisfactorily at a dosage of 800 g/ha a.i., while the results with a dosage of 1200 g/ha a.i. were very encouraging. Especially the control of the last instar larvae of Spodoptera which were aboundant during the later part of the season was remarkable.

In the 1968 trials, S 2957, which was sprayed at a dosage of 800 - 1200 g/ha a.i. during the season, was slightly superior to Monocrotophos at a rate of 390 - 780 g/ha a.i. as far as yield is concerned (Carchate et al. 1969). This was attributed to the good control of Heliothis and Spodoptera while the efficiency against Trichoplusia was not good as expected.

From the following seasons in which 900 - 1000 g/ha of S 2957 were applied good control of Heliothis sp., Anthonomus grandis Boh. and Trichoplusia ni Hbn. was reported. A significant increase of numbers of flowers was noted in comparison to some of the standard products. Also the yield data confirmed the results of previous years.

Trials on rice pests in Indonesia

Early in 1970 a trial was started to control the following pest complex of rice Cnaphalocrocis medinalis, Trypory innotata and Pachydiplosis oryzae. On plots of 50 m^2 which were replicated four times, six sprayings were carried out at 2 weeks interval.

At a dosage of 750 g/ha a.i. S 2957 produces very encouraging results. By this treatment the percentage of dead hearts was considerably reduced, the gallmidge and plant hoppers were well controlled. The yield was increased by 80% over that of the untreated plots. The overall performance of S 2957 was about equal to that of standard compounds. The trial work is continued. (Panoedjoe, 1970).

Trials on pistachio pests in Iran

Among the pests on pistachio Agonoscena targionii is most important. In randomized block system with three replications S 2957 was sprayed at a rate of 0.075% a.i. by the high volume method. Counts of the live larvae on the first, third and fifth day after application revealed mortality data of 99% on all three occasions. (Tagizadeh, 1970).

Crop tolerance

No phytotoxic effects of any kind have been reported from any of the experiments performed under tropical conditions. The same good tolerance toward crops like sugar beets, potatoes, top fruit and various vegetable varieties was noted in the trial reports from Europe. In some cases, e.g. cotton, fields treated with S 2957 showed improved development of the crop which resulted in a longer growing period and retarded harvest dates.

DISCUSSION

The reported field trials indicate that both developmental compounds S 2957 and K 673 revealed good biological activity against a number of pests of the tropics. Their field performance is comparable to that of modern broad spectrum compounds.

The field performance data demonstrate activity in a number of indications for both S 2957 and K 673. However, each one exhibits slight specific advantages as to the effective dosage: S 2957 in the control of Spodoptera species and K 673 against certain sucking insects.

Based on the available data it can be stated that generally higher rates of active material are needed for effective control of pests under tropical conditions than under temperate conditions as a comparison of the effective dosages for similar insect groups shows (cf. results reported by Prokić-Immel and Adlung 1971). In this connection allowance is made for the differences in crops and pests species.

In comparison with S 2957 K 673 has always shown a better acaricidal efficiency against normally susceptible spider mites as results of Prokić-Immel and Adlung (1971) indicate. The most striking difference of both compounds was found in the fact that the phosphonothioate K 673 is able to control the European Red Spider Mite which is resistant to the thiophosphates S 2957 and azinphosmethyl.

Also noteworthy is the fact that S 2957 is able to overcome the low degree of susceptibility of pistachio pest complex in Iran towards dimethoate which was successfully applied for a long time.

The improved condition of crops treated with S 2957 cannot be attributed only to good control of the pests. It is possible that a certain fungicidal and bactericidal side effect can be made responsible as these two properties have been found in laboratory tests (Buck et al. 1971). This observation has still to be confirmed by further testing in the field.

In addition we want to mention that another compound of this group has been synthesized: CELA S 4506, the chemical name of which is O-2,5-dichloro-4-(ethylthio)-phenyl-O,O-dimethyl-phosphorothioate. First results from Germany and Colombia indicate a good field performance coupled with a more favourable toxicity (LD_{50} oral on rat: 145 mg/kg).

Acknowledgement

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CONTROL OF PESTS OF COTTON, RICE AND MAIZE WITH EI 47470

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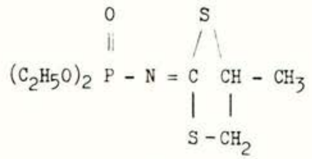
Summary Results of five years' experiments with EI 47470 (2-(diethoxyphosphinylimino)-4-methyl-1,3 dithiolane) are reviewed. On cotton, dosages of 0.75 to 1.04 kg a.i./ha (0.67 to 0.93 lb/ac) have given excellent control of leafworm (Spodoptera littoralis) in the Mid-East with long residual action. Control of spiny (Earias insulana) and pink (Pectinophora gossypiella) bollworms has been obtained in U.A.R. with sprays of EI 47470 at 0.9 kg a.i./ha (0.8 lb/ac) at two week intervals. EI 47470 was also effective on mites, whiteflies and bollworm (Heliothis spp). The control of rice pests in S.E. Asia with repeated applications of granular EI 47470 at 2 kg a.i./ha (1.8 lb/ac) is reviewed. It was effective on several species of leafhoppers, planthoppers and stemborers and gave outstanding control of rice gall midge (Pachydiplosis oryzae). On maize, several species of stemborers have been controlled with sprays or granular applications of EI 47470. For control of European corn borer (Ostrinia nubilalis) one or two applications of 1.0 to 1.5 kg a.i./ha (0.9 to 1.3 lb/ac) are recommended.

INTRODUCTION

EI 47470 (2-(diethoxyphosphinylimino)-4-methyl-1,3-dithiolane), a systemic broad-spectrum insecticide, was discovered in American Cyanamid laboratories several years ago.

It has the following structural formula:



The proposed common name is mephosfolan. It has been marketed under the trademark CYTROLANE*.

EI 47470 is available as an emulsifiable concentrate (25% and 50% w/v) and as granules (10%).

The acute toxicity of different formulations of EI 47470 is shown in table 1.

*Trademark of American Cyanamid Company

Table 1

Acute toxicity of EI 47470

Animal species	Formulation	Type of test	LD_{50} (mg/kg)
Rat, male	Technical	Oral	8.9
" "	25% e.c.	"	25
" "	50% e.c.	"	20
" "	10G	"	149
Rabbit, male	Technical	Dermal	9.7
" "	25% e.c.	"	27
" "	50% e.c.	"	16.8
" "	10G	"	340

EI 47470 has been under active development during the last five years, with emphasis on control of pests of cotton, rice and maize. Some of this work is reviewed in this paper.

COTTON

EI 47470 has been evaluated in U.A.R., U.S.A., Turkey, Israel, Mexico, Brazil and other countries. To date it has been used commercially on a limited cotton acreage in U.A.R.

Results are reviewed by pest.

Egyptian cotton leafworm (*Spodoptera littoralis*)

Laboratory tests. The toxicity of EI 47470 to leafworm larvae has been determined in U.A.R. Some typical results are shown in table 2.

Table 2

Laboratory Toxicity of EI 47470 to Leafworm Larvae

Application method	Larval instar	LD_{50} µg/larva	LD_{50} µg/g body weight	LD_{50} Conc. %
Topical	4th	0.35	9.2	-
Topical	5th	0.8	6.4	-
Sandwich	5th	15.0	54.7	-
Spray	4th	-	-	0.056

Results from several laboratories of topical LD_{50} to 4th- or 5th-instars varied from 6 to 13 µg/g body weight. Comparison with the stomach toxicity LD_{50} to 5th-instars determined by Toppozada et al's method (1964) at Alexandria University shows that EI 47470 is several times more toxic to leafworm larvae by contact than by stomach action.

Vermes (1967) applied EI 47470 25% e.c. at two dosages, using a Potter tower, to cotton leaves in petri dishes. At 0.625 mg a.i./cm^2, mortality was 40% of 3rd-/4th-instars and 10% of 5th-instar, whilst at 2.5 mg a.i./cm2 mortality was 93% and 40% respectively. It was less active than EI 47031 (2-(diethoxyphosphinylimino)-1,3-dithiolane).

Sprays were applied to one-day old egg masses by Mitri and Kamel (1970). EI 47470 at a concentration of 0.19% failed to prevent hatching whilst chlorphenamidine completely inhibited hatching at 0.375%. EI 47470 is not therefore an ovicide.

Field tests. Four publications relate to the effectiveness of EI 47470 on leafworm in U.A.R.

Zeid et al (1968) reported that EI 47470 was one of the most effective products for control of a mixed instar field infestation (table 3), with rapid action.

Table 3

Effectiveness of Insecticides against Cotton Leafworm in the Field

Treatment	Dosage (kg a.i./ha)	% larval reduction ... days after spraying	
		2 day	5 day
EI 47470 25% e.c.	0.89	90.1	87.1
EI 47031 25% e.c.	0.89	98.6	88.8
Endrin/dicrotophos 20/20% e.c.	1.2/1.2	88.5	82.7

The residual effectiveness of EI 47470 on leafworm has been determined by several workers. Leaves were collected from the field at intervals after spraying and bioassayed in the laboratory (table 4).

Table 4

Residual Effectiveness of EI 47470 at 0.89 kg a.i./ha against two instars of Cotton Leafworm

Reference	Mortality (%) ... days after treatment on 2nd instar				4th instar			
	0	3	6	9	0	3/4	6	8/9
Kamel & Mitri (1970	98	68	18	-	100	88	11	-
Zeid et al (1968)	-	-	-	-	87	65	-	37
Kamel et al (1969)	100	96	98	34	100	94	54	22

Mortality was variable between the tests probably due to differential plant growth and insecticide metabolism. The results show that EI 47470 is relatively persistent against both larval instars.

During 1971 tests were made in U.A.R. to compare the efficacy on leafworm of sprays of 0.89 and 1.04 kg a.i. ha of EI 47470 with the standard treatment of EI 47031 at 0.89 kg a.i./ha. Results of one of the tests are shown in table 5 (El-Beheiri, 1971).

Table 5

Residual Effectiveness of Insecticides against two instars of Cotton Leafworm. 1971.

Treatment	Dosage (kg a.i./ha)	Mortality (%) ... days after treatment on 2nd instar				4th instar			
		0	4	6	10	0	4	6	10
EI 47031 25% e.c.	0.89	94	94	82	72	92	90	68	68
EI 47470 25% e.c.	0.89	92	94	70	52	92	92	80	62
EI 47470 25% e.c.	1.04	100	98	86	80	100	96	88	78

Results of the 1971 field trials in U.A.R. have shown an enhanced residual effect of the higher dosage of EI 47470 on leafworm, comparable to the best insecticides in the tests. It will be recommended at this higher dose (1.04 kg a.i./ha) in U.A.R. in 1972.

Field tests in Turkey by Tayakisi (1970) demonstrated over 90% control of leafworm when counted 2, 4 and 6 days after spraying with 1.0 kg a.i./ha whilst Seval (1970) reported excellent control with 0.75 kg of mixed instars over a 12-day period.

In Israel, Tadmor (1970) found that 0.75 kg a.i./ha of EI 47470 controlled a heavy field infestation of leafworm. Newly hatched larvae were found after 12 days.

Bollworm (*Heliothis* spp)

Cowan & Davis (1968) found that eight weekly applications of EI 47470 sprays at 1.12 kg a.i./ha gave significant reduction of bolls damaged by bollworm (*Heliothis virescens*) and tobacco budworm (*H. virescens*) and increased yield of seed cotton over the untreated. It was however less effective than monocrotophos at 0.84 kg a.i./ha.

The topical LD_{50} of EI 47470 was determined as 0.25 µg/g body weight to bollworm and 0.21 µg/g to tobacco budworm (Wolfenbarger *et al*, 1970).

In Israel, light infestations of Old World bollworm (*Heliothis armigera*) were controlled by 0.9 kg a.i./ha in 1968 (Luz, 1968) and in 1970 by 0.75 kg a.i./ha (Tadmor, 1970). In Iran, a H.V. spray of 0.025% gave 72% mortality of Old World bollworm in a preliminary trial.

Mites

Kavut (1970) applied a foliar spray of EI 47470 at 0.5 kg a.i./ha to cotton infested with two spotted mite (*Tetranychus urticae*) in Turkey. Control after 3 days, 7 days and 14 days was respectively 98.2%, 90.3% and 91.5%.

In Brazil, Cavalcante *et al* (1970) obtained good initial control and effective residual action against two spotted mite with 0.25 and 0.375 kg a.i./ha and of tropical mite (*Polyphagotarsonemus latus*) with 0.375 kg a.i./ha.

In a 1969 Cyanamid trial in Turkey, 0.5 kg a.i./ha gave as good control of mites on cotton as 0.4 kg dimethoate. EI 47470 is registered in Turkey for control of mites using 0.5 kg a.i./ha.

Whitefly

Hassan (1971) reported that EI 47470 at 0.54 kg a.i./ha gave better control of whitefly (*Bemisia tabaci*) in Sudan than the standard dimethoate/DDT (0.45/1.08 kg a.i./ha).

Spiny and pink bollworms

Field trials have been carried out at several locations in U.A.R. for control of spiny (*Earias insulana*) and pink bollworms (*Pectinophora gossypiella*) since 1967. None of these data have yet been published. Results from a trial at Alexandria University in 1969 are shown in table 6 (Zeid, 1969). EI 47470 gave good results, particularly on spiny bollworm, in this and other field trials. In 1971, EI 47470 was used in U.A.R. for control of bollworms on a limited area.

Table 6

Control of Pink and Spiny Bollworms on Cotton with Insecticide Treatments, 1969.

Treatment[1]	Dosage (kg a.i./ha)	Mean bollworm larvae/100 bolls[2]		Mean bollworm control (%)	
		Pink	Spiny	Pink	Spiny
EI 47470 25% e.c.	0.89	10.0	1.5	81.4	90.3
Carbaryl 85% w.p.	3.1	8.5	2.25	84.2	85.5
Monocrotophos 40% e.c	1.44	13.0	4.25	75.8	72.6
Endrin/dicrotophos 20/20% e.c.	1.2/1.2	13.5	5.5	74.9	64.5
EI 47031 25% e.c.	0.89	34.5	12.25	35.8	21.0
Untreated	-	53.75	15.5	0	0

1 Sprays applied August 23rd and September 9th.

2 Assessments made on 25 bolls from each of four replicates 7 and 14 days after each spray.

Badawi (1971), applying three sprays of EI 47470 at 0.54 kg a.i./ha at 20 day intervals obtained only partial control of pink and spiny bollworms. The percentage of damaged bolls at the end of the season was reduced from 21.3% to 13.0%. This dosage rate was lower than recommended, and the intervals between sprays longer than optimal.

Field trials in Turkey carried out in 1968 and 1970 have shown that EI 47470 gives variable control of spiny bollworm. Control at 1.0 kg a.i./ha was promising (Seval, 1970). Further tests were scheduled during 1971, the results of which will be published in due course.

Conclusions

EI 47470 can be recommended for control of the following cotton pests:

Pest	Dosage (kg a.i./ha)
Aphids, whitefly, leafhoppers	0.5
Mites	0.375 to 0.5
Bollworms (*Heliothis* spp.)	0.75
Leafworm	0.75 to 1.0
Spiny bollworm	0.875 to 1.0
Pink bollworm	0.875 to 1.0

RICE

Field tests at the International Rice Research Institute (Annual Report 1967) showed good control of leafhoppers and planthoppers (Inazuma dorsalis, Nilaparvata lugens and Nephotettix spp), leaf whorl maggot (Hydrellia spp) and stem borers (Tryporyza incertulas , Chilo suppressalis and Sesamia inferens) with EI 47470 10% G applied at 20 day intervals at 2 kg a.i./ha. There was a low incidence of white heads and of virus. Yield was increased.

In other trials in the Philippines (Sanchez et al, 1968) EI 47470 10% G, applied three times at 2 kg a.i./ha (10, 40 and 70 days after transplanting), gave very good control of seedling maggot (Hydrellia philippina) and yellow rice stem borer (T. incertulas). Yield was increased by 0.57 tons/ha.

In 1970 Sanchez et al (1970) compared applications of granules at 1 and 2 kg a.i./ha at the same timing as in the previous experiment. The 2 kg dosage gave more effective stem borer control than when EI 47470 was applied at 1 kg but yield increase (1.15 tons/ha) was highest with the lower dosage. However, in these tests carbaryl, phorate, lindane, and phorate/lindane granules were superior to EI 47470 for control of leaf and planthoppers.

Rice gall midge (Pachydiplosis oryzae) was very effectively controlled with two applications of granules at 3.92 kg a.i./ha, two and four weeks after transplanting (Cantelo and Kovitvadhi, 1967).

The All India Coordinated Rice Improvement Project evaluated EI 47470 in 1968 and 1969 (Progress Reports 1968 and 1969). Three applications (15, 35 and 55 days after transplanting) of EI 47470 10% G at 2 kg a.i./ha at several locations in both years, gave better overall insect control than standard granular materials with high yields of rice. Control of gall midge was outstanding.

Isa (1970) reported promising control of Chilo agamemnon in U.A.R. with three applications of EI 47470 10% G at 1.1 kg a.i./ha.

EI 47470 is registered in Mexico for control of rice stem borers (Chilo spp) by application of 2 kg/ha.

EI 47470 has successfully controlled all major pests of rice in S.E. Asia with 3 to 4 applications of 2 kg a.i./ha. Development work is continuing at reduced application rates and in combinations with other insecticides.

MAIZE

Harding et al (1968) found EI 47470 10% G highly effective on the first-generation of European corn borer (Ostrinia nubilalis), assessed 30 days after topical foliar application of 1.12 kg a.i./ha.

Munson et al (1970) using a similar treatment of EI 47470, obtained good control of an artificial infestation of European corn borer and also of western corn rootworm (Diabrotica virgiferae) attacking the roots. They discussed the possibility of timing applications to control both pests simultaneously in parts of Iowa and Missouri.

A field trial in Italy during 1970 compared spray and granular application of EI 47470 with carbaryl sprays. Control of first-generation European corn borer with EI 47470 was superior to carbaryl (table 7).

Table 7

European Corn Borer Control - Italy, 1970.

Treatment	Dosage/application (kg a.i./ha)	Date applications	No. borer exit holes/100 plants on 6th August
Carbaryl 85% w.p.	3.1	7th & 29th July	34.5
EI 47470 25% e.c.	1.5	7th & 29th July	24.5
EI 47470 10% G	4.0	4th July	23.5
Untreated	-	-	59.0

Foliar applications of DDT and EI 47470 granules were compared in a French trial. EI 47470 gave effective larval control and increased yield (table 8).

Table 8

European Corn Borer Control - France, 1968.

Treatment	Dosage* (kg a.i./ha)	Live larvae/ 20 plants on 7th Oct.		Yield (ton/ha)
		No.	Log.	
DDT 5% G	1.5	15	1.09	116.3
EI 47470 10% G	4.0	17	1.14	112.2
Untreated	-	63	1.79	94.4
L.S.D. (P=0.05)		-	0.22	9.4

* applied 1st July

A comprehensive study on control of the three corn borers in U.A.R. has been carried out at Alexandria University. Preliminary trials in 1964 and 1965 showed that four sprays (20, 30, 45 and 60 days after planting) of carbaryl, DDT or EI 47470 gave effective control of Sesamia cretica, Chilo agamemnon and Ostrinia nubilalis, and increased yield (Saad, 1967). The dosage of EI 47470 was 1.2 kg a.i./ha for the first two sprays and 1.8 kg for the last two.

In 1967 and 1968, studies were made on the effect of numbers of sprays of carbaryl, DDT and EI 47470 on corn borer infestation and on yield (Saad et al, 1971). The data are shown in table 9.

Table 9

Control of Maize Borers - Alexandria, U.A.R. 1967.

Treatment[1]	No. of sprays[2]	Mean[3] % borer[4] infested plants	Yield (ton/ha)
Carbaryl 85% w.p.	2	44.7	8.98
	3	36.0	8.35
	4	31.3	10.10
EI 47470 25% e.c.	2	50.0	6.10
	3	39.3	7.60
	4	41.3	8.78
DDT 50% w.p.	2	58.0	8.32
	3	48.0	7.93
	4	47.3	9.42
Untreated	-	74.9	3.43

[1] Treatment

	kg a.i./ha applied ... days after planting 20&30	45&60
Carbaryl	2.53	3.05
EI 47470	0.89	1.19
DDT	2.38	3.57

[2] Spray timing 2 sprays 20 and 45 days after planting
3 sprays 20, 30 and 45 days after planting
4 sprays 20, 30, 45 and 60 days after planting.

[3] Mean of ccunts 30, 45, 60, 75 and 90 days after planting.

[4] Sesamia cretica, Chilo agamemnon, Ostrinia nubilalis.

Carbaryl was the most effective insecticide and gave the highest yields. Two sprays gave significant control of all three borer species and increased yield. EI 47470 was more effective than DDT in controlling borers but DDT gave higher yields.

In field trials in Central and South America topical foliar applications of EI 47470 granules on maize have controlled fall armyworm (Spodoptera frugiperda) as well as borers (Diatraea spp).

Further trials are required to establish the optimum dosage and timing of EI 47470 for control of European corn borer. One application of 1.0 - 1.5 kg a.i./ha before hatching of first-generation larvae is suggested, followed by a second application if control of second-generation attack is desired.

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A NEW FORMULATION OF ENDOSULFAN FOR CONTROL OF THE DAMSON-HOP APHID

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Summary The cyclic sulphurous acid ester endosulfan has given good control of all stages of Phorodon humuli in field trials on hops in the United Kingdom. This work has been verified during 1971 by extensive commercial usage of a 35% emulsion formulation applied at a rate of 24 fl. oz/ac up to six times per season.

Compared with current phosphoric ester and carbamate insecticides, endosulfan has a slower initial effect on the pest but provides a longer period of plant protection.

The effects of endosulfan on the pest are of major commercial significance, since it should prove an effective alternative to products of chemical groups with inherent resistance problems.

INTRODUCTION

Since its introduction to the U.K., endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a - hexahydro - 6,9 - methano - 2,4,3 - benzo(e) - dioxathiapin - 3 - oxide) has been confined to controlling a limited number of pests including Cecidophyopsis ribis and Steneotarsonemus pallidus when formulated as a 20% e.c. with high wetter content and, more recently, Ceutorhynchus assimilis and Dysaneura brassicae when formulated as a 35% e.c.

The product has, however, a wide application spectrum controlling numerous biting and sucking pests while exhibiting good selectivity to beneficial insects and insect predators.

The damson-hop aphid Phorodon humuli continues to present serious problems in hop gardens and although recent developments in the phosphoric ester and carbamate fields have eased the hitherto critical situation, the risk of resistance and cross-resistance to these materials remains high. For this reason field trials were initiated to investigate the effects of the new 35% e.c. formulation of endosulfan on the pest.

FIELD TRIALS

1970

In Kent, two randomised block trials were laid down on areas of first year OT/48 stock to compare the relative effects of several rates of endosulfan (as a 35% emulsion) with those of a standard methidathion programme (as a 40% emulsion). In combination with propineb, both products were applied at high volume on given dates.

Variations in alatae counts in all plots of both trials necessitated the assessment of total apterae only from the growing points and first three expanded leaves of bines selected at random within each plot. Results obtained during the season are presented below.

Table 1

Number of apterae/growing point

Site: Marden

Date of Assessment	Treatment - %					Standard Error (P=0.05)	Application Dates
	Endosulfan				Methidathion		
	0.075	0.100	0.125	0.150	0.113		
15/7	14.2	16.2	18.8	15.5	14.2	8.74	16/7
20/7	11.8	6.3	7.2	5.0	4.8	5.26	
5/8	5.7	4.5	4.8	2.7	3.1	1.16	5/8
10/8	4.7	3.0	2.1	0.9	0.6	1.29	
17/8	0.16	0.20	0.36	0.08	0.10	0.091	18/8
22/8	0.12	0.14	0.11	0.09	0.04	0.063	
25/8	0.09	0.03	0.07	0.10	0.03	0.030	26/8

Table 2

Number of apterae/growing point

Site: Horsmonden

Date of Assessment	Treatment - %						Standard Error (P=0.05)	Application Dates
	Endosulfan					Methidathion		
	0.075	0.100	0.125	0.150	0.200	0.113		
22/6	38.3	41.4	38.4	33.4	-	21.9	12.77	11/6
28/6	24.8	25.0	10.2	7.8	-	7.2	5.11	23/6
3/8	10.9	17.6	4.1	3.6	*54.1	1.6	11.65	16/7
10/8	8.3	6.0	3.0	3.2	4.6	0.9	2.48	5/8
14/8	2.6	1.8	1.1	1.9	2.3	0.1	1.35	14/8
20/8	1.62	0.80	1.29	1.43	0.07	0.10	1.322	26/8

*Originally ineffective o.p. treatment: sprayed with endosulfan on final 3 dates only

Both insecticides gave excellent control of aphid at the two sites and a fair degree of correlation was found between rate of use of endosulfan and pest incidence. The material proved comparable to, but much slower than methidathion in knockdown properties with full treatment effects only becoming apparent several days after a given spray application.

Three applications of 0.20% endosulfan were made to heavily infested plots at the Horsmonden site in the latter half of the trials programme. Results in Table 2 clearly demonstrate the efficacy of this treatment.

At Marden, an unreplicated block, grower sprayed trial was initially used to evaluate the product under commercial conditions.

Two treatments were laid down on a 1.2 acre block of first year 'Bullion' hops which had received methidathion at 18 fl. oz/ac on June 15. Treatment 1 received two applications of endosulfan at 24 fl. oz/ac on June 23 and July 13 and two applications at 32 fl. oz/ac on July 27 and August 10. Treatment 2 received one application of methidathion at 18 fl. oz/ac, two applications at 24 fl. oz/ac and one application at 36 fl. oz/ac on corresponding dates. The spray volume varied between 50 and 130 gal/ac during this period and Table 3 summarises results obtained.

Table 3

No. of aphids/growing point				Assessment Date
Treatment 1		Treatment 2		
Alatae	Apterae*	Alatae	Apterae*	
1.97	0.81	1.43	0.47	4/7
0.82	1.38	0.69	0.62	18/7
0.42	2.77	0.56	1.86	4/8
0.04	1.37	0.03	1.44	15/8

*All Stages

These data verified results from the smaller plot trials and suggested that a spray programme incorporating endosulfan would have commercial significance since other work had shown good spray compatibility and crop tolerance even at abnormally high rates of use.

1971

Endosulfan was made commercially available in 1971 and the subsequent extensive use of the product permitted an appraisal of its effects when used in large areas of hops.

In addition, intensive studies were made in specific trials in Kent and the West Midlands. At Ledbury, Hereford, a randomised block trial was laid down on a 0.5 acre block of cv. 'Fuggle'. A single application of insecticides was made as per Table 4 on 27/5. No further applications were made until 21/6 when the following results were obtained:-

Table 4

Incidence of aphid at Ledbury following one application of insecticide

Treatment	Mean no. of Aphids			
	Per Growing Point		Per Mature Leaf	
	Alatae	Apterae*	Alatae	Apterae*
Endosulfan 20 fl. oz/ac	9.3	13.4	4.3	5.7
Endosulfan 24 fl. oz/ac	4.8	12.3	2.5	4.3
Methidathion 18 fl. oz/100 gal	5.1	14.0	3.2	5.5
Untreated	11.8	100 +	8.9	11.4
S.E. (P=0.05)±	3.70	5.96	1.83	4.44

*All Stages

Following assessment of aphid incidence on May 27, plots having received the lower rate of endosulfan were sprayed at a rate of 40 fl. oz/ac, while the untreated control plots were oversprayed and discarded.

The high rate endosulfan treatment had significantly reduced the aphid populations by the time of the next assessment on June 3.

Table 5

Treatment	Mean no. of Aphids			
	Per Growing Point		Per Mature Leaf	
	Alatae	Apterae*	Alatae	Apterae*
Endosulfan 40 fl. oz/ac	1.0	2.8	0.0	1.1
Endosulfan 24 fl. oz/ac	2.3	3.4	0.0	1.7
Methidathion 18 fl. oz/100 gal	2.6	3.0	0.3	0.9
S.E. (P=0.05)±	0.62	0.20	0.14	0.66

*All Stages

Subsequent applications of materials at rates quoted above reduced aphid populations to insignificant levels.

In three grower sprayed trials, endosulfan was compared to propoxur, demeton-S-methyl or methidathion in unreplicated 2-acre blocks of hops. All materials were used at rates shown in Tables 6a and 6b on a spray concentration basis while endosulfan was used at a constant 24 fl. oz/ac (8.4 oz a.i./ac).

Table 6a

Site	Cultivar	Comparable Product	Spray Volume gal/ac	Spray Dates
1 Severn Stoke Worcester	Bullion	propoxur w.p. 2 lb/100 gal	100	14/5
			100	27/5
			100	3/6
			150	18/6
			150	26/6
			200	7/7
2 Ledbury Hereford	Fuggle	demeton-S-methyl e.c. 12 fl. oz/100 gal	100	1/6
			100	15/6
			150	22/6
			200	4/7
			200	14/7
			200	24/7
3 Cranbrook Kent	Northern Brewer	methidathion e.c. 18 fl. oz/100 gal	80	3/6
			80	14/6
			100	24/6
			130	6/7
			130	20/7

Table 6b

Incidence of Aphid

Site	Comparative Area Per Young Leaf		Comparative Area Per Mature Leaf		Endosulfan Area Per Young Leaf		Endosulfan Area Per Mature Leaf		Date Assessed
	Al.	Ap.	Al.	Ap.	Al.	Ap.	Al.	Ap.	
1	2.8	2.1	3.5	20.6	1.9	7.5	0.5	1.8	21/6
	0.4	1.5	0.0	0.2	0.2	0.0	0.0	0.1	12/7
2	0.7	11.7	0.3	2.7	0.9	12.0	0.3	12.0	21/6
	0.6	3.3	0.4	0.0	1.8	3.3	0.5	0.0	25/6
3	0.0	5.4	0.0	0.8	0.2	3.2	0.0	0.5	16/6
	0.1	2.6	0.0	0.6	0.2	2.3	0.0	0.3	5/7

Al. = Alatae

Ap. = Apterae (all stages)

Results obtained indicated that endosulfan was at least as effective as the other products, although demeton-S-methyl proved slightly more effective at Ledbury.

No data on the incidence of summer reproductive stages are presented for these trials, since only small numbers were found on all treatments and it was clear that all products were controlling these stages.

Studies of cone infestation at harvest have shown no significant differences to date.

DISCUSSION

The results from replicated trials and commercial areas have shown that the 35% emulsion formulation of endosulfan will give useful control of the damson-hop aphid, including those strains resistant to the effects of phosphoric esters, by repeated applications of rates equivalent to 24 fl. oz/ac or above.

Endosulfan is not systemic in effect yet aphids were apparently discouraged from settling on areas of bine not present at the time of application. In this respect earlier reports of repellent and anti feeding properties (unpublished) appear to have substance. In many cases greater numbers of beneficial insects, particularly Coccinellids, were found in the endosulfan-treated areas than in those receiving the phosphoric ester and carbamate insecticides. Further work in both spheres is planned to elucidate these observations.

Owing to the non-systemic effects of endosulfan, it requires accurate and thorough programmed applications to ensure results equivalent to those described. Provided recommendations are followed, such control levels should be easily obtained.

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THE FORMULATION OF TETRACHLORVINPHOS FOR ULV APPLICATIONS

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Summary Tetrachlorvinphos (sold for agriculture under Shell Registered Trademark Gardona Insecticide) is an insecticide with broad activity against lepidopterous and dipterous pests. It has a low mammalian and bird toxicity which allows it to be used not only in the agricultural, but also in the animal, public health and domestic fields and which fits it especially for ULV application in these outlets.

However tetrachlorvinphos is a crystalline solid which has a low solubility in common solvents used for ULV formulations, unless phenol is added.

The phytotoxicity to cotton of any solutions incorporating phenol was judged to be unacceptably high and formulations containing the tetrachlorvinphos in suspension were considered as alternatives to solutions.

Suspensions were first formed with air milled wettable powders in non-volatile carrier liquids. The 2-10 µm particles settled and formed clays. Suspensions have now been developed by a process of wet milling with a particle size of 1-2 µm which are stable. They have shown promise in trials and are available now for market development studies.

INTRODUCTION

In the field of ULV applications most progress has been made with pesticides that are either liquid per se and have a relatively low vapour pressure, or that can be readily dissolved to provide high concentrations in suitable solvents. The selection of solvents is critical; the two most important factors are those of volatility and phytotoxicity. The use of too volatile a solvent will lead to excessive evaporation of the spray drops and result in poor impaction and recovery of the spray on the target. The tendency to cause phytotoxicity is governed by the composition of the solvent, aromatic fractions being particularly prone to cause damage. Aromatic solvents used for more conventional spray formulations (xylenes, trimethyl benzenes) are unsuitable for ULV applications because of their relatively high volatility and risk of phytotoxicity. On the other hand non-aromatic, involatile oils are usually poor solvents for insecticides. Consequently a compromise has to be reached as shown by Coutts and Parish (1967) during a search for suitable solvents to enable application of DDT to cotton. They used a mixture of an aromatic but volatile hydrocarbon solvent to dissolve the DDT together with a low aromatic but involatile white oil. This oil, although a poor solvent for DDT, acted as a carrier in which the DDT remained in suspension after the volatile solvent had evaporated. Johnstone (1967) in similar trials found that heavy aromatic naphtha (HAN) was too volatile a solvent; however, the addition of the less volatile but somewhat aromatic DUTREX although reducing evaporation, caused phytotoxicity to the cotton.

Formulation of tetrachlorvinphos

Tetrachlorvinphos is a solid, with a vapour pressure of 4.2×10^{-8} mm Hg at 20°C. It has a low solubility in most common solvents, and is only reasonably soluble in some chlorinated solvents (40% w/v). Solubility of the toxicant in aromatic hydrocarbons can be increased by the addition of phenol which acts as a complexing agent. Preliminary tests were carried out on cotton with a range of solvents and formulations at different drop sizes and the results are shown in Tables 1 - 3. Table 1 shows the formulations and solvents used, Table 2 the scale used to assess the phytotoxicity, and Table 3 the damage observed with a range of three drop sizes.

Of the solutions, formulations at drop sizes below 180 µm and formed from the chlorinated solvents or from the polar solvent dimethyl sulphoxide did not give rise to any phytotoxic damage. However, none of these solvents is suitable for practical use - they are either too volatile, too toxic or too expensive.

The three formulations based on aromatic solvents with different boiling ranges and volatility all caused scorch at all the drop sizes tested. With the most volatile formulations (SHELLSOL AB, SHELLSOL E) this damage was probably due to the presence of phenol. With the less volatile DUTREX damage occurred even in the absence of phenol, and must be attributed to the solvent *per se*, as found by Johnstone.

Apart from solutions, suspensions of a finely milled tetrachlorvinphos w.p. in ethylene glycol or kerosene were tested, and caused no phytotoxicity. Moreover, these suspensions caused no nozzle blockage when sprayed through the fine nozzles used in ULV spraying equipment. These tests showed that fine particulate suspensions of solid toxicants can be used in ULV applications. As the only diluent, water is too volatile, but if a proportion of the water is replaced by ethylene glycol or glycerol spray evaporation will be minimised and a reasonable drop size maintained. Ethylene glycol also reduces the risk of the suspension claying in the spray-tank.

One disadvantage of suspending relatively high concentrations of w.ps in water/glycol in the field is that fairly efficient stirring equipment is needed to afford a rapid wetting out and breaking up of agglomerates when preparing the spray.

More recent work has led to the development of high toxicant content liquid concentrates of very fine particle size, prepared by wet-milling and based on water. The particles in these suspension concentrates are much smaller (1-2 µm) than those of air-milled w.ps (2-10 µm). Conditions of processing and the formulation recipes are very critical to ensure a good flowable product free from air-entrainment, which is readily dilutable in water and has a good shelf-life. For tetrachlorvinphos a 70% w/v suspension concentrate has been developed which can be readily diluted with glycol or a mixture of water and glycol immediately prior to ULV applications in the field. The product can of course also be diluted with water to any required concentration for conventional uses. The small particle size and the structure of the formulation minimise sedimentation and phase separation of the product, but if it does occur - e.g. under extreme conditions of temperature and storage - the product can be readily rehomogenised by gently shaking.

DISCUSSION

Tetrachlorvinphos is a toxicant highly suitable for use in ULV applications. It has an extremely low order of toxicity to mammals and can be used safely without any special precautions. It has also a low level of toxicity to birds and wild life in general. For this reason it can be used not only in agricultural outlets, where it has a particular spectrum of activity against lepidopterous and dipterous pests, but also in animal health, public health and domestic outlets.

<u>Table 1</u>

<u>Candidate solvents and formulations</u>

% w/v	A	B	C	D	E	F	G*	H*
Tetrachlorvinphos (a.m.)	50	40	40	20	40	40	20	30
Phenol	34	13.4	13.4					
Lubrizol 260								4
DUTREX 217	to 100% v							
SHELLSOL AB		to 100% v						
SHELLSOL E			to 100% v					
Dimethylsulphoxide				to 100% v				
Chloroform					to 100% v			
Methylene chloride						to 100% v		
Ethylene glycol							to 100% v	
SHELLSOL K (kerosine)								to 100% v

* These formulations were not true solutions but suspensions of a tetrachlorvinphos 50% w w.p in these solvents.

Table 2

Classification of phytotoxicity

Type	0	No marks
	A	Necrosis restricted to the area of the spray stain
	B	Necrosis spreading beyond the area of the stain
Severity	1.	Slight indentation of cuticle but no discolouration
	2.	Lighter green colour formed
	3.	Cuticle penetrated giving brown necrotic area
	4.	Severe scorch but no leaf wilting
	5.	Severe scorch and leaf wilt

In addition to the range of tetrachlorvinphos formulations already available (50% w and 75% w.ps, a 24% w/v e.c., granules and dusts) a suspension concentrate formulation has now been developed. Although this new formulation is specifically useful for ULV, it can be used for conventional spraying also.

So far, in ULV applications it has been used for the control of forestry pests (Pachynematus montanus, Porthetria dispar) in pasture (Dichroplus maculipennis and Scyllinops spp), in cotton (Prodenia spp, Heliothis Zea) and for the control of fruitflies on citrus and olives (Dacus oleae and Ceratitis capitata). No problems of phytotoxicity have been noted in these trials. In animal health, dairy cattle have been sprayed against horn flies (Haematobia irritans).

Quantities of tetrachlorvinphos suspension concentrates are now being produced for market development studies.

References

1. Coutts, H.H. and Paris, R.H. (1967) T.P.R.I., Arusha, Report No.611.

2. Johnstone, D.R. (1967) PANS 13, 82.

Table 3

Visual assessment of phytotoxicity on cotton

	Droplet diameter range (μm)		
	200-250	120-180	80-100
Solvents			
DUTREX Phenol 70/30	B5	B5	B4
DUTREX	B5	B3/2	B2
SHELLSOL AB	B4	0	0
SHELLSOL E/Phenol 70/30	B5	B5	B4
SHELLSOL E	B4	01	0
Dimethyl sulphoxide	A4	0	0
Ethylene glycol	0	0	0
SHELLSOL K (kerosine)	0	0	0
Formulations			
A (phenol and DUTREX)	B5	B4	B4
B (phenol and SHELLSOL AB)	B5	A4	A3
C (phenol and SHELLSOL E)	A4	A4	A3
D (dimethylsulphoxide)	A3	0	0
E (chloroform)	A2	0	0
F (methylenechloride)	A2	0	0
G (ethylene glycol) *	-	0	0
H (odourless kerosine) *	-	0	0

* Suspensions of tetrachlorvinphos w.p. in these solvents.

ULTRA LOW VOLUME AND ULTRA LOW DOSAGE SPRAYING WITH CHLOROPHTHALONIL

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Summary The protective action of five different ULV formulations of chlorophthalonil in oil against potato blight (Phytophthora infestans) has been examined in the laboratory. A simple formulation of micronised chlorophthalonil in Shell sol T alone was as effective, when applied at 6 oz a.i./ac, as a commercial wettable powder applied at 36 oz a.i./ac. The amount of chlorophthalonil deposited on the leaf after spraying with a ULV formulation in oil was proportionally greater than after spraying with the wettable powder. Chlorophthalonil in oil was also found to be at least as resistant to weathering as the wettable powder. These results were confirmed by field resistance studies and by a preliminary field study on grape vines designed to assess the degree of protection against vine mildew (Plasmopara viticola).

These results fully justify proceeding with extensive field trials employing ULV micronised chlorophthalonil in Shell sol T.

INTRODUCTION

Ultra low volume (ULV) spraying may be defined as the application of biocide formulations at rates of less than 3.6 pints per acre (5 litres per hectare). The principles and advantages of ULV and ultra low dosage (ULD) spraying has been enunciated by Bals (1969; 1971). Basically the biocide is formulated in a non-evaporating carrier, usually mineral oil, and applied as an aerosol of uniformly small droplets. This results in a much larger amount of the biocide reaching the target surface than with aqueous sprays and consequently, the rate of application can be proportionately reduced. Bals (1970) showed that the dose rate of insecticides on cotton and rice could be reduced by 90% and 97% respectively; similarly Joyce (1969) found that the dose rate for ULV spraying with phosphamidon against insect pests of rice paddy was between 10% and 50% that required in aqueous application.

The technology of ULV spraying has been described by Mass (1971). The advantages claimed for ULV spraying may be summarised as follows: the technique requires no water; it is much less effort to apply; it is three to four times faster than conventional spraying; the dose rate can be reduced by between 50% and 97%; and the material is not washed off by rain.

Heath (1969) found that ULV spraying with chlorophthalonil "Daconil"* at 3.25 oz a.i./ac (0.227 kg a.i./ha) was more effective against apple scab (Venturia inaequalis) than captan applied in water at 40 oz a.i./ac (2.8 kg a.i./ha). It was decided to study the control of potato blight (Phytophthora infestans) by ULV spraying with chlorophthalonil. Initial experiments showed that the formulation employed was phytotoxic and inactive against P. infestans. This phenomenon was

* A common name suggested for tetrachloroisophthalonitrile

found to be due in part to the oil used as a carrier and in part to certain ingredients in the formulation. Experiments were set up to select a carrier which would neither inactivate the chlorophthalonil nor render it phytotoxic. Shell sol T was selected as the most suitable material available.

In the experiments described below the ability of chlorophthalonil in five ULV formulations to control potato blight at dose rates between 3% and 45% that of the wettable powder formulation has been assessed. The resistance to weathering and field performance of one ULV formulation against potato blight and vine mildew (Plasmopara viticola) has also been examined.

METHODS AND MATERIALS

Laboratory Evaluations

i) Activity against potato blight, Phytophthora infestans

Pot grown potato plants of Arran Pilot raised in a glasshouse were employed and the experiments were conducted in infection cubicles in a pathological glasshouse. Chlorophthalonil was prepared in five formulations in Shell sol T. These were:

1. Micronised chlorphthalonil, 100 g ; edelex oil No. 17, 100 g.
2. Micronised chlorphthalonil, 100 g ; edelex oil No. 17, 99.2 g; bentone "38", 0.8 g.
3. Micronised chlorphthalonil, 100 g ; edelex oil No. 17, 92.2 g; bentone 38, 0.8 g ; boiled linseed oil, 6.8 g ; methanol, 0.2 g.
4. Micronised chlorphthalonil, 100 g.
5. Milled chlorophthalonil, 100 g.

Micronised chlorophthalonil had an average particle size of 4 µm, whilst the average particle size of milled chlorophthalonil was 11 µm. All formulations were further diluted in Shell sol T to give the required levels of application; these were 1, 4, 6, 8, 12 and 16 oz a.i./ac (0.07, 0.14, 0.28, 0.42, 0.56, 0.84 and 1.12 kg a.i./ha). The test formulations were applied to the test plants with a "Micron ULVA" sprayer which was passed over the test plants at approximately 2 m.p.h. (3.22 km.p.h.). Control treatments consisted of a commercial wettable powder formulation (Daconil 2787) which was applied with a "Beacon" hand sprayer until "run off" giving a rate of application equivalent to 36 oz a.i./ac (2.62 kg a.i./ha) and an untreated control. A standard suspension of zoospores and sporangia of P. infestans was applied to the experimental plants twenty four hours after spraying. The efficacy of each treatment was assessed by recording the percentage of infected leaflets on each plant.

ii) Resistance to Leaching

Nine plants were selected which had been sprayed two weeks earlier. Three had been treated with 6 oz/acre micronised chlorophthalonil in Shell sol T, three with 6 oz/acre micronised chlorophthalonil + edelex + bentone + linseed oil + methanol in Shell sol T and three had been treated with the commercial wettable powder formulation (36 oz/acre). All of these plants had been inoculated with P. infestans 24 hours after being sprayed and had then been kept in a moist atmosphere for 6 days. They had also been watered with a watering can eight days after the initial spraying with the chlorophthalonil formulations. Three untreated plants were used as controls.

The 12 experimental plants were placed in a tray and given the equivalent of three inches of rain in 2 hours through a rose on a watering can held 40 feet above them. The plants were allowed to dry for 12 hours and ten leaf discs (9 mm diam.) were taken at random from the leaves of each plant. New leaves which had grown since the application of the chlorphthalonil were not examined. The leaf discs from each plant were placed in 20 ml aliquots of acetone and incubated for 24 hours to allow the chlorophthalonil to go into solution. The acetone extracts were tested for the presence of chlorophthalonil by gas-liquid chromatography (GLC) using a 5 ft (1.524 m) glass column packed with 3% E 30 on 100-120 mesh diatomite QC.

Field Evaluations

i) Field resistance to potato blight

This experiment was conducted for 6 weeks during June, July and August 1971. Four small plots were selected for this experiment in a field of King Edward Potatoes. These plots were sprayed with micronised chlorophthalonil alone in Shell sol T at rates of 6, 9 and 12 oz a.i./ac (0.42, 0.63 and 0.84 kg a.i./ha) and the wettable powder formulation respectively. Twenty leaflets were removed from each plot after the first week and after each succeeding week for six weeks. The treatments were repeated after leaflet samples had been removed at the end of the third week. Five leaflets from each treatment were inoculated on the upper surface with one drop of the standard spore suspension of P. infestans and another five were similarly inoculated on the lower surface. These leaves were placed on moistened filter paper in petri dishes and incubated for seven days at 20°C. The leaves were then examined and the percentage of infected leaflets was recorded for each treatment. The remaining ten leaflets were used for a GLC analysis to obtain a comparative estimate of the quantity of chlorophthalonil remaining on the leaf surface after each treatment.

ii) Field evaluation resistance to downy mildew of vine, Plasmopara viticola

This was conducted in a vineyard containing "Saint Emilion des Charentes" vines at Saint Hilaire de Villefranche in the Cognac region of France. The same formulations were employed as had been used in the evaluation of field resistance. Each treatment was applied to a one metre segment in one row and a two metre segment in the opposite row of two rows of vines at one side of the vineyard. There were two replicates of each treatment. The vines were sprayed on seven occasions over the period during which vine mildew was expected to occur. Spraying took place on the following dates: May 17, June 2, June 20, July 4, July 28, August 13 and August 28; a commercial application of "Cuprosan Duper D" was used as a positive control. In deference to the farmer a "no treatment" control was not included. The level of infection was assessed by recording the percentage of mature infected leaves in a treatment sample of ten. Assessments were taken at random from each treatment on August 13, August 25 and September 3.

RESULTS
(a)

The performance of chlorophthalonil in five different ULV formulations is given in Table 1. The five formulations differed little in performance although C, which contained all the formulating ingredients used in these experiments, and D, which consisted of micronised chlorophthalonil and Shell sol T without any other ingredients were marginally most active. When these two formulations were examined at lower concentrations (Table 2) the simple formulation "D" was significantly more active than the complex formulation "C" at 6 oz a.i./ac and as effective as the standard wettable powder formulation applied at 36 oz a.i./ac. The resistance of the wettable powder and formulations C and D to leaching by simulated rain is is given in Table 3. Leaves treated with the wettable powder formulation carried only 26 ppm chlorophthalonil as opposed to an anticipated 60-66 ppm since the rate of application had been six times that of the ULV treated leaves. Both ULV formulations were considerably more resistant to leaching than the wettable powder formulation. The results show that 29% of the chlorophthalonil in formulation C and 47% in formulation D were lost by artificial weathering whereas 62% was lost from the wettable powder.

Following the promising results obtained from the laboratory studies, an experiment was established at Hatfield to determine the resistance of treated potato plants in the field to P. infestans. The number of leaves available for sampling was too small to allow a statistical analysis, however, the results (Tables 4a and b) indicate that ULV applications of chlorophthalonil are as effective against potato blight as the wettable powder. It was also noticeable that in all treatments the leaves received much less protection on the under surface.

Leaf samples from the field resistance plots were analysed by GLC to determine the quantity of chlorophthalonil remaining on them after weathering. The results of this show that after seven days the amount of material on the leaves given the three ULV treatments is not in proportional to the quantity applied. It is also noticeable that although no rain occurred during days 17-23 after the first spraying, chlorophthalonil was lost from the leaves and there was a concommitant decline in resistance to infection. The results also indicate that the amount of active ingredient on the leaves falls to a low level after three weeks and that the amount of material on leaves treated with 9 oz a.i./ac by ULV application and 36 oz a.i./ac by wettable powder treatment were similar throughout the experiment.

(b)

In the pilot field evaluation of ULV applications against vine mildew the area of the vineyard available for the experiment prevented sufficient replication of treatments for statistical analysis. Nevertheless there is again good evidence (Table 7) that chlorphthalonil when applied at 9 oz/ac on a ULV formulation is at least as effective as the wettable powder.

DISCUSSION

Experiments to assess the activity of five different chlorophthalonil in oil formulations against potato blight (Phytophthora infestans) showed that at the four rates of application used there was no significant difference in the degree of blight control. The most complex formulation containing micronised chlorophthalonil, edelex oil, bentone, linseed oil and methanol in Shell sol T was compared with the simple formulation consisting of micronised chlorophthalonil in Shell sol T alone. The simple formulation gave significantly more control of potato blight at 6 oz a.i./ac than the complex formulation. Of considerable interest is the fact that there was no significant difference between the performance of the simple micronised formulation applied at 6 oz a.i./ac and the commercial wettable powder formulation containing 36 oz a.i./ac.

Chlorophthalonil which had been applied in a ULV oil formulation appeared to be more resistant to leaching than that applied in a wettable powder formulation (Table 3). However, this distinction was not maintained in the field studies (Table 5). In both experiments in which the quantity of chlorophthalonil on the leaves has been assessed (Tables 3 and 5) the leaves treated with the wettable powder contained proportionally less than was expected, since the rate of application was 3-6X greater than the ULV formulations. This may be attributed to losses of the wettable powder due to run-off, whereas with ULV applications on the other hand every particle falling on the leaf remains there.

The ability of ULV micronised chlorophthalonil in oil to control potato blight in the field was demonstrated by the field resistance evaluation. This method has proved to be very useful especially in those areas where the occurrence of potato blight cannot be guaranteed. Treated leaves were inoculated with a dense spore suspension of P. infestans and incubated under conditions which favoured the pathogen and so were subject to a much greater biological stress than is encountered in the field. Hence any treatment which prevents infection in field resistance tests will also be effective in the field. No treatments were particularly effective in protecting the underside of the leaf; this is probably due to unsophisticated sprayers which were used for these experiments. Resistance to infection is maintained by leaves inoculated on their upper surface for between 7 and 13 days. The loss of chlorophthalonil from leaves in the field resistance experiment (Table 5) occurred irrespective of the amount of rain which suggests that some other factor, possibly sunlight, may also play an important role in the weathering of chlorophthalonil. Hence an evaluation of field resistance cannot replace a fully replicated field trial, although it does indicate the measure of success which could be expected from such a trial. Similarly the results of the preliminary field trial of resistance to vine mildew suggest that a full field trial should be undertaken.

On the basis of these results it is recommended that the efficacy of ULV chlorophthalonil in oil against potato blight and vine mildew be further evaluated in full scale field trials.

Table 1

Control of potato blight by five formulations of chlorophthalonil in ULV applications

Rate of application oz a.i./ac	Mean % infected leaflets Formulation*				
	A	B	C	D	E
4	11.9	11.2	8.0	5.3	14.0
8	9.8	8.8	8.9	2.2	10.8
12	6.0	8.0	5.7	5.4	6.2
16	1.6	3.8	4.3	5.3	7.9
Wettable powder control	2.7	2.7	2.7	2.7	2.7
No treatment	66.0	66.0	66.0	66.0	66.0
L.S.D. (P = 0.05)	5.7	6.0	7.0	3.5	6.5

* A - Micronised chlorophthalonil, edelex oil No. 17 and Shell sol T.
B - Micronised chlorophthalonil, edelex oil No. 17, bentone 38 and Shell sol T.
C - Micronised chlorophthalonil, edelex oil No. 17, bentone 38, boiled linseed oil and methanol.
D - Micronised chlorophthalonil and Shell sol T.
E - Milled chlorophthalonil and Shell sol T.

Table 2

Control of potato blight by two formulations of chlorophthalonil in ULV application

Rate of application oz a.i./ac	Mean % infected leaflets Formulation* C	D
1	24.4	21.8
2	19.1	18.5
4	16.8	14.2
6	15.5	6.5
Wettable powder control	3.2	3.2
No treatment	90.8	90.8
L.S.D. (P = 0.05)	1.7	5.1

* See Table 1

Table 3

Leaching of chlorophthalonil by simulated rain

Formulation	Chlorophthalonil concentration in acetone extract ppm: Before Leaching	After Leaching
Micronised chlorophthalonil in Shell sol T	11.3	6.0
Micronised chlorophthalonil + edelex + bentone + methanol + linseed oil in Shell sol T	10.5	7.5
Wettable powder	26.0	10.3
Untreated control	0.0	0.0

Table 4

Field resistance of leaflets sprayed with micronised chlorophthalonil in Shell sol T by ULV application

(a) Leaflets inoculated on top surface: % infected leaflets

Rate of application oz a.i./ac	Days after 1st application			Days after 2nd application		
	7	16	23	7	13	21
6	0	40	60	0	20	40
9	0	60	80	0	0	20
12	20	60	60	0	0	20
Wettable powder control	0	40	80	0	0	40
No treatment	100	100	100	100	100	80

(b) Leaflets inoculated on under surface: % infected leaflets

Rate of application oz a.i./ac	Days after 1st application			Days after 2nd application		
	7	16	23	7	13	21
6	40	20	100	40	60	40
9	20	20	100	20	20	40
12	40	40	80	20	20	20
Wettable powder	100	100	100	0	20	40
No treatment	100	100	100	100	100	80

Table 5

Chlorophthalonil remaining on potato leaflets after exposure to weathering: Concentration chlorophthalonil ppm in acetone extract

Rate of application oz a.i./ac	Days after 1st application			Days after 2nd application	
	7	16	23	7	13
6	13	<1	<1	13	3
9	27	8	<1	30	14
12	18	12	8	37	18
Wettable powder	15	7	<1	36	12
No treatment	0.0	0.0	0.0	0.0	0.0

Table 6

Rainfall and sunshine on experimental plots used for field resistance evaluation of ULV chlorophthalonil in oil formulations

Rate of application oz a.i./ac	Days after 1st application			Days after 2nd application		
	7	16	23	7	13	21
Rain (mm)	8.5	4.3	0.0	0.4	14.2	33.6
Total rain		12.8	12.8	0.4	14.6	48.2
Sunshine (hr.)	44.9	53.9	74.7	49.1	31.3	58.6
Total sun		98.8	173.5		80.4	139.0

Table 7

Control of downy mildew of vines by chlorophthalonil in ULV application: mean % infected leaves

Rate of application oz a.i./ac	Date of assessment 13.8.71	25.8.71	3.9.71
6	0.0	0.0	8.3
9	4.4	0.0	5.0
12	0.0	1.7	10.0
Cuprosan Super D	19.0	1.7	5.5

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CONTROL OF BRASSICA PESTS WITH A GRANULAR FORMULATION OF DIMETHOATE

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Summary Following promising results in 1968 and 1969 with solid dimethoate formulations applied to Brassica crops for control of cabbage root fly (*Erioischia brassicae*) and cabbage aphid (*Brevicoryne brassicae*) dimethoate granules based on limestone grit were tested on cauliflowers and Brussels sprouts during 1970.

On summer cauliflowers and "Jade Cross" sprouts spot applications effectively reduced damage by root fly and aphids, which resulted in increases in yields of marketable curds and sprouts.

On Brussels sprouts grown for market band applications of dimethoate granules were found to be adequate though less effective than spot applications for root fly and aphis control. With both methods numbers of aphid-infested plants were greatly reduced for a period of ten weeks after treatments in late May. Second applications over the foliage in August also reduced infestations of aphids in the lower sprouts at the time of first picking.

The merits and disadvantages of spot and band applications are discussed.

INTRODUCTION

Dimethoate was tested as a treatment for Cabbage Root Fly in 1968 and 1969. Experiments on cauliflowers and Brussels sprouts showed that 5% dust and granule formulations applied as spot treatments after planting out were superior to liquid drenches and compared favourably with recommended treatments with diazinon and chlorfenvinphos. Results also showed that during the period of the trial the dimethoate treatments gave complete control of cabbage aphid (*Brevicoryne brassicae*) against which the other two compounds were ineffective.

Further work in 1970 was undertaken in order to investigate the performance of granular formulations of dimethoate in cauliflowers and Brussels sprouts for control of both root fly and aphids. In one trial on Brussels sprouts for market machine-applied band treatments were compared with spot applicator treatments. Some results of these trials are presented and discussed.

METHOD AND MATERIALS

Small Plot Trials. Transplanted brassica crops, "All the Year Round" cauliflowers and "Jade Cross" sprouts, were given spot applications of insecticides on the day after planting out to control the first generation attack of cabbage root fly. Plots comprised 40 plants set out at two foot intervals on the square. Treatments were replicated four times in a randomised block design.

Half of each plot, 20 plants, was re-treated after two or three weeks using the same amounts of insecticide per plant as previously, but applied as a spot treatment over the foliage to control cabbage aphid.

The formulation of dimethoate granules used in these trials was based on limestone grit of particle size 0·3 to 0·6 mm diameter and contained 5% active ingredient. A commercial formulation of chlorfenvinphos granules containing 10% active ingredient was used as a standard and applied with a suitable applicator at the recommended rate of 0·17 gm of product per plant.

Assessments of root fly damage were made six weeks after planting on cauliflowers and at harvest, 5th November, on the Brussels sprouts using the root damage index, Coaker and Finch (1965). Aphid infestation of the cauliflower curds at harvest on 8th July was assessed by scoring each curd between 0 and 10, the higher the score the heavier the infestation. Damage to foliage by aphids on the Brussels sprouts was similarly scored on 8th August.

Mechanised Trial. In a larger scale trial on "market" Brussels sprouts a tractor with front-mounted Horstine Farmery granule applicator was used to apply dimethoate and chlorfenvinphos granules in 4 inch bands along the rows on 21st May, three days after planting out. Plots comprised two rows 25 yards long containing 60 plants and replicated eight times. Second applications were made on 7th August.

Assessments of aphid control on the foliage by counts of infested plants were made on three dates during the growing period and picked sprouts were assessed during the first pick in early September. Control of root fly was assessed by counts of wilted plants in mid June and root damage in September.

RESULTS

Cauliflowers. On cauliflowers planted out on 7th May during a period of warm dry weather in previously well irrigated sandy loam, spot applications of dimethoate granules applied once on 8th May gave excellent control of aphid and root fly and increased the yield of marketable curds on 8th July by 55-60%.

Aphid control (Table 1) was slightly better with 0·04 g per plant than with 0·02 g and two applications were marginally better than one at both levels.

Table 1

Control of Cabbage Aphid on Cauliflowers by spot applications of dimethoate granules on 8th May and 22nd May, 1970.

Chemical	Formulation	Dose a.i./plant	Aphid infestation scores 8th July 1 application*	2 applications
Dimethoate	5% granule	0·02 g	1·75	0·5
Dimethoate	5% granule	0·04 g	0·25	0·0
Chlorfenvinphos	10% granule	0·017 g	7·00	7·25
Untreated			9·25	8·25
S.E. ±			0·79	0·66

* 1 application refers to treatments on 8th May in Tables 1 - 4.

Root damage on 24th June was reduced effectively by all treatments and was not improved with the higher dosage nor by second applications two weeks after the first, (Table 2).

Table 2

Control of Cabbage Root Fly on Cauliflowers by spot applications on 8th May and 22nd May, 1970.

Chemical	Formulation	Dose a.i./plant	Root damage index 24th June 1 application angles	Root damage index 24th June 2 applications angles
Dimethoate	5% granule	0•02 g	20•3	19•9
Dimethoate	5% granule	0•04 g	19•5	18•4
Chlorfenvinphos	10% granule	0•017 g	18•2	18•5
Untreated			50•4	45•3
S.E. ±			2•64	2•11

The highest yields of marketable curds were obtained in the dimethoate plots and small increases resulted from both increased dosage and second applications, (Table 3).

Table 3

Control of Cabbage Root Fly and Aphids on Cauliflowers by spot applications on 8th May and 22nd May, 1970.

Chemical	Formulation	Dose a.i./plant	Yield of marketable curds 8th July			
			1 application		2 applications	
			Crates/ ha	Crates/ acre	Crates/ ha	Crates/ acre
Dimethoate	5% granule	0•02 g	820	332	961	389
Dimethoate	5% granule	0•04 g	840	340	1097	444
Chlorfenvinphos	10% granule	0•017 g	793	321	838	339
Untreated			526	213	516	209
S.E. ±			104	42	86	35

(Interaction treatment × application not significant).

Table 4

Control of Cabbage Root Fly and Aphids on Cauliflowers by spot applications on 8th May and 22nd May, 1970.

Chemical	Formulation	Dose a.i./ plant	Total Yield No. curds/ 40 plants	% Unmarket- able	% "Bracty"	% "Ricy"
1 application						
Dimethoate	5% granule	0·02 g	41	17·1	19·6	24·4
Dimethoate	5% granule	0·04 g	37	5·4	13·5	8·1
Chlorfenvinphos	10% granule	0·017g	36	19·4	25·0	11·1
Untreated			37	37·8	18·9	18·9
2 applications						
Dimethoate	5% granule	0·02 g	36	8·3	22·2	16·7
Dimethoate	5% granule	0·04 g	39	0	25·6	20·5
Chlorfenvinphos	10% granule	0·017g	39	10·5	25·6	13·1
Untreated			33	33·3	24·2	21·2

Table 4 shows the number of curds harvested in each treatment and the percentages which were unmarketable, "Bracty" and "Ricy". While dimethoate clearly reduced the proportion of unmarketable curds, particularly at the higher dose, it did not appear to affect the numbers of bracty and ricy curds.

Brussels sprouts. "Jade Cross" sprouts for processing planted out on 25th June on a fine sandy loam shortly after rain were treated the following day, and sub-plots retreated 19 days later. In early July the plants became heavily infested with cabbage aphid which retarded growth and caused leaf distortion in all plots except those treated with dimethoate granules.

The chlorfenvinphos plots and control plots were sprayed on 15th July to prevent losses of plants from the aphid attack. Dimethoate treated plots remained almost completely free of aphids throughout the growing period (Table 5).

Table 5

Control of Cabbage Aphid on Jade Cross sprouts by spot applications of dimethoate granules on 16th June and 15th July.

Chemical	Formulation	Dose a.i./plant	Aphid damage scores 6th August 1 application*	2 applications
Dimethoate	5% granule	0·02 g	1·00	0·50
Dimethoate	5% granule	0·04 g	0·75	0·00
Chlorfenvinphos	10% granule	0·017 g	2·75	2·75
Untreated			2·75	2·75
S.E. ±			0·23	0·26

Cabbage root fly was assessed on 5th November when the crop was harvested. All treatments reduced damage from the second generation attack, but chlorfenvinphos persisted longer than dimethoate, which failed to prevent a late infestation of the roots (Table 6).

Table 6

Control of Cabbage Root Fly on Jade Cross sprouts by spot application of dimethoate granules on 26th June and 15th July.

Chemical	Formulation	Dose a.i./plant	Root damage index (angles) 5th November	
			1 application	2 applications
Dimethoate	5% granule	0·02 g	52·8	45·5
Dimethoate	5% granule	0·04 g	41·8	26·6
Chlorfenvinphos	10% granule	0·017 g	8·2	2·0
Untreated			68·6	66·5
S.E. ±			4·7	4·7

The total crop of sprouts picked from 40 plants per plot was weighed and significant increases in yield found on dimethoate treatments. In sub-plots of 20 plants differences between one and two applications were not significant (Table 7).

* 1 application refers to treatments on 16th June in Tables 5 - 7.

Table 7

Control of Cabbage Root Fly and Aphids on Jade Cross sprouts by spot applications on 26th June and 15th July.

Chemical	Formulation	Dose a.i./plant	Yield of sprouts per plot (lb) 5th November 1 appl.	2 appl.	Mean
Dimethoate	5% granule	0·02 g	13·3	15·5	14·4
Dimethoate	5% granule	0·04 g	14·6	15·2	14·9
Chlorfenvinphos	10% granule	0·017 g	10·8	9·8	10·3
Untreated			10·4	8·4	9·4
S.E. ±			1·2	1·2	1·09
			(NS)	++	+

Market Brussels sprouts. Band applications of dimethoate and chlorfenvinphos on 21st May after planting out failed to reduce the number of wilting plants on 15th June significantly, whereas after spot applications of dimethoate few or no wilting plants were found (Table 8).

Table 8

Control of Cabbage Root Fly on Brussels sprouts for market by band and spot foliar applications of dimethoate granules on 21st May and 9th August.

Chemical	Formulation	Dose a.i./acre	Method	% Wilting Plants 15th June (angles)	R.D.I. 7th Sept. (angles)
Dimethoate	5% granule	0·75 lb	4" band	7·34	36·15
Dimethoate	5% granule	1·0 lb	4" band	4·38	32·0
Dimethoate	5% granule	0·25 lb	spot	2·62	33·2
Dimethoate	5% granule	0·5 lb	spot	0·0	36·1
Chlorfenvinphos	10% granule	0·67 lb	4" band	10·3	30·4
Untreated				7·7	36·2
S.E. ±				2·0	

Control of cabbage aphid in July was also better with spot applications though numbers were greatly reduced by the band treatment at the higher rate of 1 lb a.i. per acre and a persistence of ten weeks was obtained against aphids migrating in to the crop several weeks after application under very dry soil conditions (Table 9).

Table 9

Control of Cabbage Aphid on Brussels sprouts for market by band and spot foliar application of dimethoate granules on 21st May and 7th August.

Chemical	Formulation	Dose a.i./ acre	Method of Application	% plants infested (angles) after 1 appl. 31.7.71	after 2 appl. 13.8.71	28.8.71
Dimethoate	5% granule	0·75 lb	4" band	27·1	6·6	27·7
Dimethoate	5% granule	1 lb	4" band	10·4	7·6	17·5
Dimethoate	5% granule	0·25 lb	spot	11·7	14·4	19·2
Dimethoate	5% granule	0·5 lb	spot	9·2	0	26·3
Chlorfenvinphos	10% granule	0·67 lb	4" band	38·5	65·5	31·9
Untreated				36·1	66·9	32·0
S.E. ±				3·25	4·16	3·37

All treatments failed to prevent infestation of the lower sprouts in September, though a reduction of 51% was obtained with the foliar band application of 1 lb a.i./acre compared with untreated.

DISCUSSION

The results from spot applications of dimethoate granules to cauliflowers and processed sprouts show root fly control comparable with chlorfenvinphos and very effective control of cabbage aphid. On cauliflowers the treatments were successful in spite of almost complete absence of rain throughout the growing period. This suggested that the active ingredient is held in the region of the plant roots, and prolonged uptake by the plant occurs. Establishment of aphid colonies was prevented for up to nine weeks after treatment. The granules were clearly not affected adversely by the continuing hot, dry weather. Results from similar treatments under much wetter conditions in 1971 indicate that the performance of dimethoate in this formulation is satisfactory under both dry and wet conditions.

Where spot applications of dimethoate granules have been compared with surface band applications at realistic dosage levels, the former have invariably given better control of cabbage root fly and aphids when the the treatments were applied shortly after planting. This is due to the higher concentration of granules obtained close to the plant, and to the greater accuracy of placement with spot applicators.

Foliar applications of granules in August to control cabbage aphid on plants which are meeting in the row have been no less effective by band treatment than by spot treatment. By both methods the granules have been funnelled into the leaf axils or retained on the leaf surface, but the higher rates applied in a band have been fully utilised at this stage, whereas when the plants are small, as much as 90% of band applied granules may be wasted, depending on planting distance, due to being out of the effective range of action on the soil surface.

Acknowledgements

The assistance of Miss K. Bryan and Mr. A. Norton, who carried out most of the work involved in aphid and root fly assessments is gratefully acknowledged. I am also indebted to Dr. Q. A. Geering for his advice and help with the mechanised trial.

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WINTER TREATMENTS OF FRUIT TREES AND VINEYARDS WITH DINOTERB ACETATE

F. Colliot and B. Henrion
Pepro, Lyon, France

Summary During 1969, 1970 and 1971, large scale experiments have been carried out in France which have clearly shown the interest of dinoterb acetate applied during the dormant stage of the vegetation for the control of the winter insects and mites in fruit trees and vines.

Excellent results have been obtained, mainly on *Panonychus ulmi*, a parasite which it is becoming more and more difficult to control in arboriculture with dinitrocresols (DNOC) and oleoparathions.

Furthermore, dinoterb acetate has shown very good action on the eggs of aphids and mainly *Myzus persicae* on peach trees. Other parasites such as *Argyresthia ephipella*, the cherry fruit moth, *Operophtera brumata*, the winter moth, *Quadraspidiotus perniciosus*, the San José scale, etc., are also well controlled by this winter treatment.

In viticulture, the product has shown very good efficiency on *Eriophyes vitis* and *Phyllocoptes vitis*.

The rates recommended for these different applications are 400 to 600 g a.i./hl of the speciality in the form of an oily paste 40%.

Dinoterb acetate has shown itself to be perfectly selective and is more readily tolerated by the vegetation than the dinitrocresols (DNOC ammonium salt) and dinoterb (DNTB ammonium salt).

INTRODUCTION

DNTBP acetate is a new active ingredient, discovered and patented by MURPHY CHEMICAL LTD.

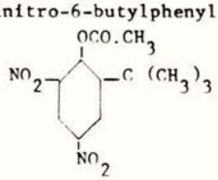
Our work has been carried out in close collaboration with Technicians from MURPHY CHEMICAL, whom we thank for the help they have given us and for the samples they have supplied to us.

MAIN CHARACTERISTICS OF DINOTERB ACETATE

1) Physical and Chemical Characteristics

Chemical Formula : 2,4 dinitro-6-butylphenyl acetate

Structural Formula :



Empirical Formula : $C_{12}H_{14}N_2O_6$

Molecular weight : 284

Appearance : Pale yellow crystals

Melting Point : 134-135°C

Solubility in water : 10 ppm

Also very soluble in acetone and xylene

Stability : Stable in dilute acids and for pH < 7
Unstable at high temperature in an acid medium
Unstable in an alkaline medium

Presentation : Oily paste containing 40% dinoterb acetate

2) Toxicity

Dinoterb acetate is less toxic than DNOC and dinoterb.

	DNOC	dinoterb	dinoterb acetate
Oral LD 50 Rat :	7 to 10 mg/kg	26 mg/kg	62 mg/kg
Oral LD 50 Mouse :	-	32 mg/kg	68 mg/kg

3) Preparations used

During our trials, we examined among others the following formulations which figure in the tables and graphs, in comparison to dinoterb acetate examined firstly in the form of a wettable powder 50% and secondly in the form of an oily paste 40%.

VERALINE CREME A : Cream containing 500 g/l dinoterb ammoniac salt

HERBOGIL CREME A : Cream containing 50% DNOC ammoniac salt

Oleoparathion 3% : Cream containing 3% ethyl parathion + 78% paraffin oil

TESTS AND RESULTS

1) ACARICIDAL ACTIVITY OF DINOTERB ACETATE ON WINTER MITES

We have carried out a good many tests against *P. ulmi*. During the hatching period of the winter eggs and after this period, we kept a check on the rate of hatching and the number of mites on the leaf, following the criteria given below :

a) Coefficient of hatching

From 25 spots where the eggs are laid and which have been located in advance, for each replicate the percentage of hatching of the winter eggs is assessed according to the following scale :

0 = no hatching	4 = 75% hatching
1 = 10% "	5 = 90% "
2 = 25% "	6 = 100% "
3 = 50% "	

b) Coefficient of infestation

From 50 leaves in each replicate chosen at random, the number of mites present (larvae and adults) can be assessed according to the following scale :

0 = no mites on leaves
1 = 1 to 5 mites
2 = 5 to 10 mites
3 = 11 to 20 mites
4 = 21 to 50 mites
5 = over 50 mites

(In order to simplify the interpretation of the graphs, the results have been given in number of mites. This number has been obtained by taking the average of the coefficients of infestation which are then transposed onto a graph).

In the first place, we shall study the efficiency of dinoterb acetate according to the formulation used : wettable powder 50% or oily paste 40%; and according to the stage of growth (Fleckinger system) at the time of treatment (see table I).

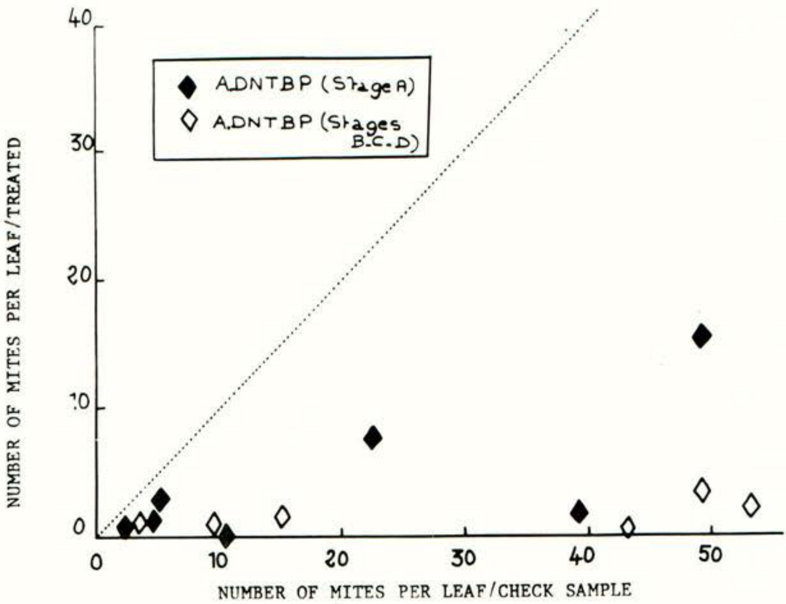
The good over-all efficiency of the product emerges from the 24 tests described. However, it is to be noted that with the wettable powder formulation (13 tests) the results are slightly inferior to those obtained with the oily paste and relatively insufficient in two applications carried out at stage A on apple trees, even with 600 and 750 g a.i./hl (graph I). The same thing can be seen with this formulation in the tests against other parasites and we have thus been led to prefer the oily paste.

TABLE I - Efficiency of dinoterb acetate against P. ulmi (according to the formulation and stage of treatment)

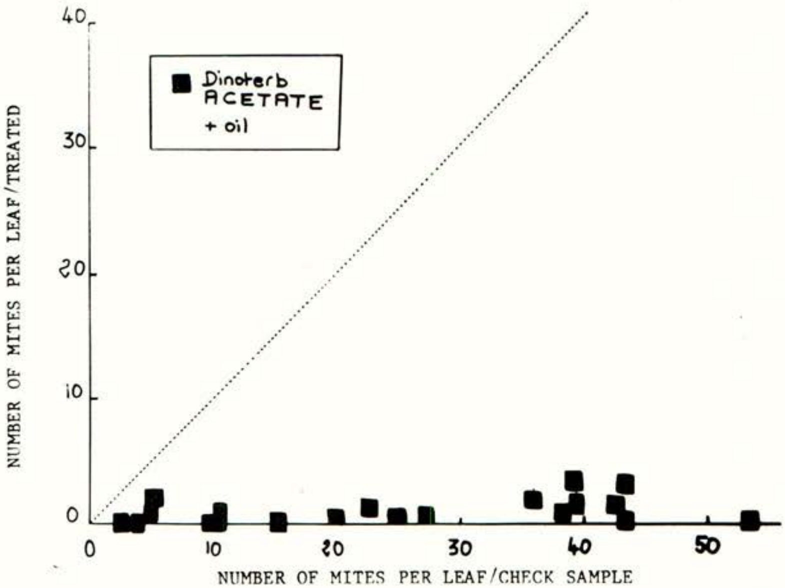
Coefficient of infestation on leaves (0 to 5)								
N° trial	Date of treatment	Stage	Rate g a.i./hl	DNTBP acetate WP	DNTBP acetate OP	Check sample	Date of control	Crop
3/69	20/2/69	A	750	-	1.1	4.4	6/5	Apple
10/69	27/2/69	A	750	-	0.2	2.4	30/4	"
9/69	21/1/69	A	750	-	1.2	4.2	30/4	"
D 47/70	24/3/70	B - C	400	0.4	0.1	1.2	4/5	"
D 40/70	19/3/70	B - C	750	0.5	0.1	3	8/5	"
D 39/70	17/3/70	B - C	750	0.1	0.1	2.4	8/5	"
D 49/70	25/3/70	C - C_3	400	0.4	0.1	2.3	8/5	"
De 6/70	11/3/70	A	750	2	0.5	3.4	4/5	"
Cha 2/70	11/3/70	A	600	3	-	4.7	12/5	"
Cha 2/70	13/4/70	C - D	400	1.1	-	4.7	12/5	"
B 2/70	6/3/70	A	750	0.5	0.3	1.4	13/5	"
D 48/70		C - D	400	0.2	0.3	2.4	8/5	"
De 2/70	23/2/70	A	750	0.6	0.7	4.2	11/8	"
De 2/70	6/4/70	C	400	0.8	0.1	4.9	11/5	"
De 14/70	14/4/70	D	400	0.2	0.2	4.4	21/5	Peach
D 28/70	26/1/70	A	600	0.9	0.8	1.5	14/5	"
D 29/70	27/1/70	A	600	0.1	0.1	2.4	20/5	"
D 30/70	27/1/70	A	600	0.3	0.1	0.9	28/4	"
De 16/71	19/3	B	600	-	0.75	4.46	26/4	Apple
D 40/71	24/3	C - D	600	-	0.25	3.2	24/5	"
R 15/71	23/3	A	600	-	1.84	3.34	27/5	"
De 21/71	1/4	B	600	-	0.32	3.56	22/4	"
De 13/71	11/3	A - B	600	-	1.04	4.05	6/5	Peach
D 16/71	4/2	A	600	-	0.56	4.27	28/4	"

WP = wettable powder   OP = oily paste

GRAPH I (WP FORMULATION)



GRAPH II

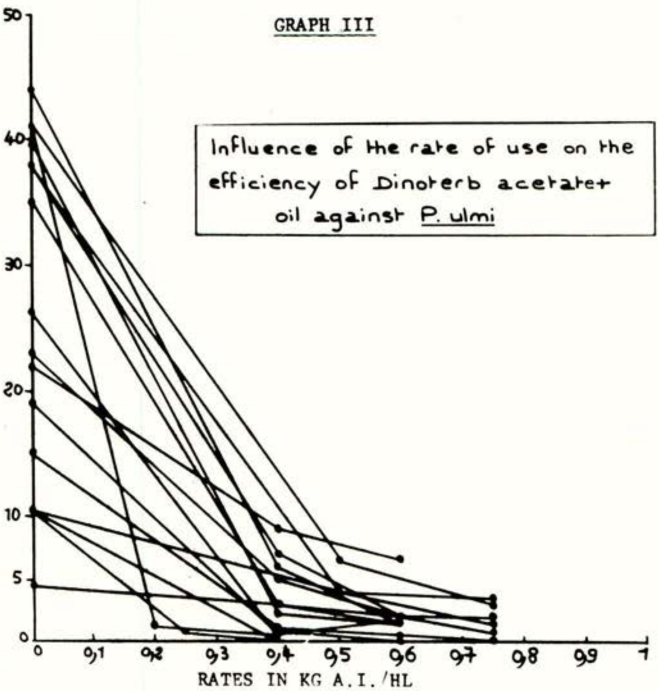


We shall then examine the influence of the rates of use on the efficiency of dinoterb acetate (oily paste) against the winter forms of P. ulmi (table II and graph III).

TABLE II - Influence of the rate of use on the efficiency of dinoterb acetate + oil against P. ulmi

N° Trial	Date of treatment	Stage	Coefficient of infestation on leaves treated with ADNTBP at the various rates + oil						Date of control	Crop
			0	250	400	500	600	750		
3/69	20/2/69	A	4.4			1.8		1.1	6/5	Apple
10/69	27/2/69	A	2.4			1.3		0.6	6/5	-
9/69	21/1/69	A	4.2			1.3		1.2	30/4	-
D 40/70	19/3/70	B - C	3		0.4		0.2	0.1	8/5	-
D 39/70	17/3/70	B - C	2.4		0		0.1	0.1	8/5	-
De 6/70	11/3/70	A	3.4		1.5		0.8	0.5	4/5	-
2/70	6/3/70	A	1.4		1.1		0.8	0.3	13/5	-
D 48/70		C - D	2.4	0.1	0.3				8/5	-
De 2/70	23/2/70	A	4.2		1.9		0.8	0.7	11/5	-
De 14/70	14/4/70	D	4.4	0.5	0.2				21/5	Peach
De 16/71	19/3/71	B	4.57		1.7		0.6		19/4	Apple
D 40/71	24/3	C - D	3.2		0.25		0.25		24/5	-
R 15/71	23/3	A	3.34		2.17		1.84		27/5	-
De 21/71	1/4	B	3.56		0.32		0.78		22/4	-
De 13/71	11/3	A - B	4.05		1.13		0.58		6/5	Peach
De 16/71	4/2	A	4.27		0.90		0.56		28/4	-

GRAPH III



For 16 tests thus analysed, we see :

- that somewhat incomplete results, whatever rate is used, are sometimes registered when the treatment is made too early in the season (dormant stage - stage A),

- a constant and excellent efficiency for the rate of 600 g a.i./hl at the very beginning of vegetation at the moment when the buds swell (stages A-B),

- a good efficiency at low rates of 400 g a.i./hl and even in some cases 250 g a.i./hl for applications made at stage C-D, but this late treatment cannot be envisaged in practice, because of the risks of phytotoxicity, in spite of the good selectivity of the product compared to DNOC and dinoterb.

Thus we retain the rate of 600 g a.i./hl at the very beginning of the vegetation at the swelling of the buds : stage A-B, but before they open and before the appearance of the very first green parts (sprouting leaves on the apple tree) and appearance of colour in the petals (peach tree).

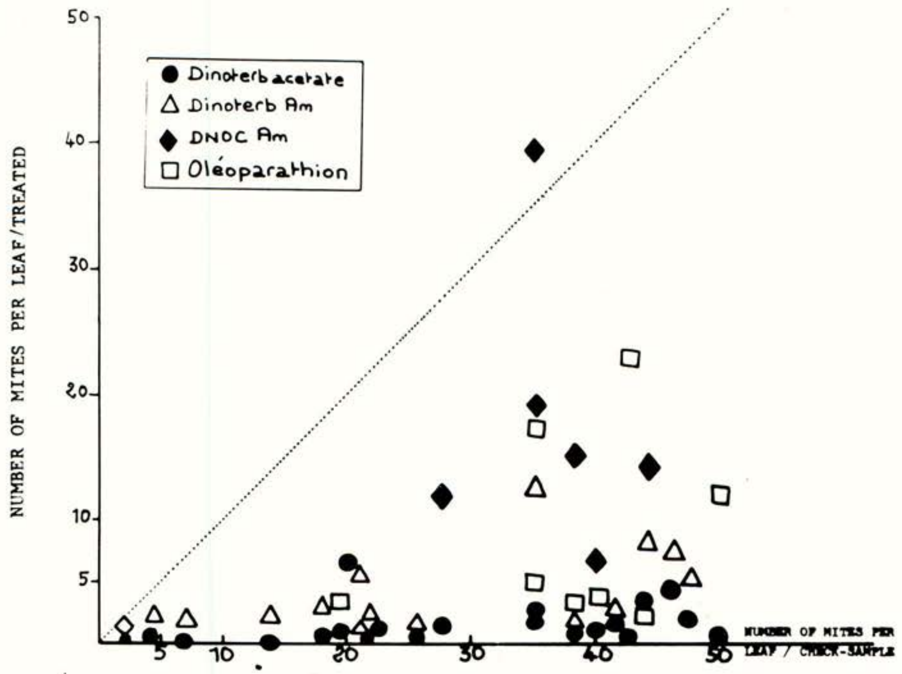
Finally, we shall compare the efficiency of dinoterb + oil, still on P. ulmi, to that of the controls already mentioned, i.e. :

- DNOC - dinoterb - oleoparathion (table III, graph IV)

TABLE III - Efficiency of dinoterb acetate + oil in comparison to the controls against P. ulmi

N°	Trial	Stage	Rate g a.i./hl	Coefficient of infestation from 0 to 5					Date of control	Crop
				DNOC	DNTBP	Check sample	DNTBP acetate + oil	Oleo-parathion		
	10/69	A	750	3	0.8	4.2	0.5	1.2	6/5	Apple
	3/69	A	750	3.2	2.7	4	0.8	1.4	14/5	-
	9/69	A	750	2.9	2.2	4.6	1.4	1	7/5	-
D	47/70	C	400	-	0.7	1.8	0.1	-	23/5	-
D	40/70	B - C	600	-	0.45	3.3	0.5	-	20/5	-
D	39/70	B - C	600	-	0.8	3.3	0.3	-	20/4	-
D	49/70	C	400	-	0.8	2.8	0.1	-	20/4	-
De	6/70	A	750	-	2.02	4.78	1.44	-	14/5	-
BB	2/70	A	750	-	0.97	1.37	0.27	-	13/5	-
D	48/70	C - D	400	-	-	3.20	0.3	1.2	20/5	-
Cha	5/70	C - D	400	-	-	5	0.1	2.6	12/5	-
De	2/70	A	600	-	1.5	4.8	0.8	-	11/5	Peach
De	14/70	D	400	-		4.56	0.18	3.4	21/5	-
D	29/70	A	600	2.60	-	3.65	0.60	-	20/4	-
D	30/70	A	600	0.43	-	0.86	0.11	-	28/4	-
De	16/71	B	600	-	1.05	4.46	0.75	-	26/4	Apple
D	40/71	C - D	600	-	1.22	3.2	0.25	-	24/5	-
R	15/71	A	600	-	1.67	3.34	1.84	-	27/5	-
De	21/71	B	600	-	0.52	3.56	0.32	0.67	22/4	-
De	13/71	A - B	600	4.36	-	4.05	1.04	3.12	6/5	Peach
D	16/71	A	600	1.77	-	4.27	0.56	1.37	28/4	-

GRAPH IV



It clearly appears that the product tested ensures the best results and, in all cases, when 20 to 25 mites per leaf can be counted in the untreated plots, allows the spring populations to be limited to less than 5 individuals and, in fact, generally to less than 2-3.

It can also be seen that while dinoterb ammoniac salt often gives satisfactory results, they are nevertheless less complete and sometimes rather insufficient.

DNOC does not ensure a sound protection and oleoparathion gives an irregular efficiency with definite cases of failure.

Comparison between the percentage of hatching of the eggs and the populations of mites actually present on the leaves

This comparison shows a certain discrepancy between the rate of hatching and the population of mites later found on the leaves.

This difference has been found in most of the tests carried out on peach, apple and pear trees. It shows that in order to appreciate the efficiency of a winter treatment, it is quite insufficient to merely evaluate the rate of hatching of the winter eggs and demonstrates the necessity to carry out, in particular, checks on the populations of mites actually present on the leaves.

2) EFFICIENCY OF DINOTERB ACETATE AGAINST OTHER PARASITES

Myzus persicae

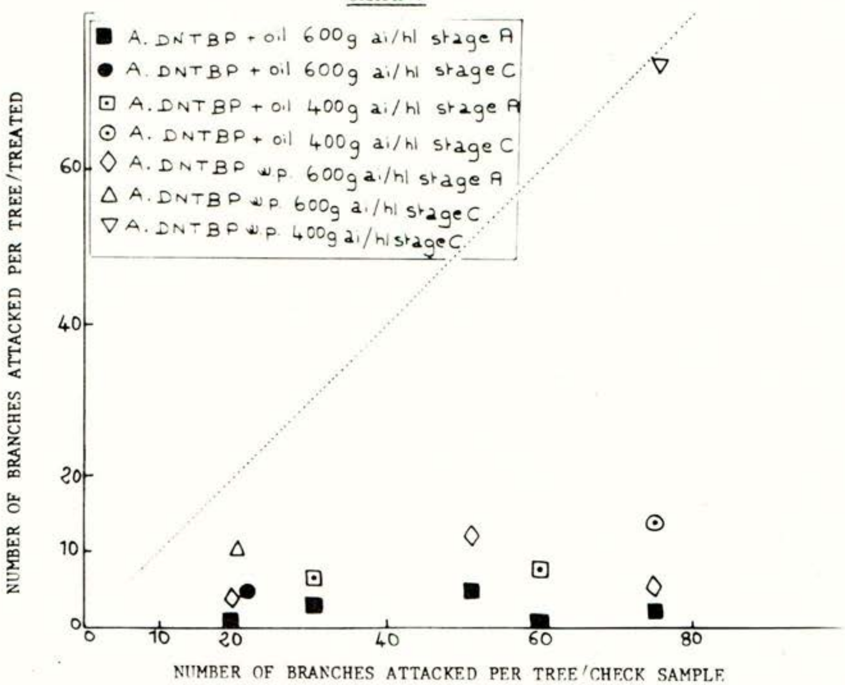
We have carried out several tests against the Green Peach Aphid, which is a parasite important in large fruit-growing regions such as the Rhone Valley and Languedoc-Roussillon and which it is becoming increasingly more difficult to control as a result of the cases of resistance which it shows towards a good number of common insecticides. These tests are summarised in table IV and graphs V and VI.

TABLE IV - Efficiency of dinoterb acetate against M. persicae

N° Trial	Date of treatment	Stage	Number of twigs attacked per tree: DNTBP acetate 400 g a.i./hl OP	DNTBP acetate 400 g a.i./hl WP	DNTBP acetate 600 g a.i./hl WP	DNTBP acetate 600 g a.i./hl WP	DNOC Am 600 g a.i./hl	Oleoparathion 3%	Check sample	Date of control
D 28/70	26/1	A			6.25	12.7	4.5	-	52.5	14/5
D 29/70	27/1	A			2 92	5.3	4	-	75/4	8/5
D 29/70	27/1	C	14	74					75/4	8/5
D 30/70	27/1	A			1.3	3.8	0		19/5	28/4
D 30/70		C			5.4	10			19/5	28/4
D 18/71	4/2	A	8		1.16		1.66	13.25	60.5	28/4
D 17/71	4/2	A-B	6.4		3.9		2.5	15.4	30	14/5

WP = wettable powder OP = oily paste

GRAPH V



In graph V which demonstrates the influence of the rate of use, of the stage of application and of the formulations, we see that :

- 600 g a.i./hl applied at stage A, in the form of an oily paste, give the best results,

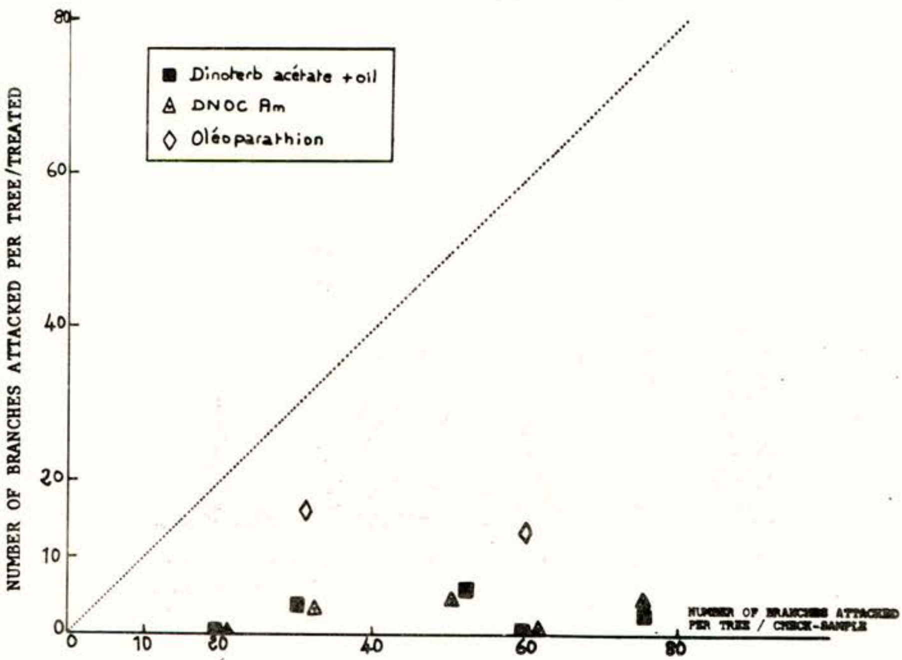
- the same formulation used at 400 g a.i./hl, at either stage A or C, ensures a lower and somewhat inadequate efficiency,

- the wettable powder shows itself to be inferior.

In graph VI, we compare the efficiency of the dinoterb acetate + oil (this formulation having been thus retained), with that of the controls (DNOC and oleoparathion). It can be seen that the experimental formula gives excellent results, equivalent to those obtained with DNOC ammoniac salt, which is the best product currently used on M. persicae.

It can also be seen that the efficiency of oleoparathion is clearly not as good.

GRAPH VI



3) SELECTIVITY OF DINOTERB ACETATE

We have chosen to illustrate the good selectivity of dinoterb acetate, taking as an example the results recorded on peach trees, since this is the type of fruit tree which has shown itself to be the most susceptible to nitrated dyes in winter treatments.

We thus see in table V and graph VII that where the dinoterb ammonium salt is severely phytotoxic, the acetate in the form of an oil is not very different from that of the check sample and, in every case, it is clearly more readily supported than DNOC which is nevertheless of classic use on this crop.

In the same way, DNTBP acetate demonstrates a perfect selectivity on the other species of fruit trees, apple, pear, cherry.....and on vine.

GRAPH VII

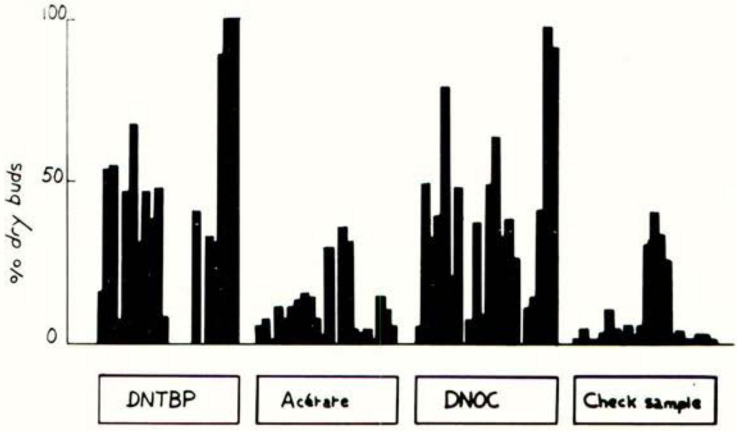


TABLE V - Selectivity of dinoterb acetate on peach trees

N° Trial	Date of treatment	Peach tree variety	Stage	Percentage of dry buds at the rate of 750 g/hl								N° treatment
				Buds (wood)				Buds (flowers)				
				dinoterb 750/600 g/l	dinoterb acetate 600 g/l	DNOC 750/600 g/l	Check sample	dinoterb 750/600 g/l	dinoterb acetate 600 g/l	DNOC 750/600 g/l	Check sample	
8/69	25/2/69	Red Haven	D - E	30	5	6	2	16	5	5	1	1
		Ch. Ingouf	D	85	15	40	9	54	7	49	4	2
		Red Wing	E	85	6	40	10	55	1	32	1	3
		Dixired	D	77	8	13	2	7	11	39	1	4
		Southland	E - F	68	12	60	5	47	7	79	3	5
2/69	13/1/69	Springtime	-	82	12	16	12	68	11	20	10	6
		Dixired	-	56	7	48	10	31	13	48	4	7
		Blanche	-	60	14	-	2	47	15	-	2	8
		Cardinal	-	50	10	12	10	38	14	7	5	9
		Red Haven	-	73	9	29	12	48	7	37	2	10
4/69	20/2/69	Mayflower	A	47	1	7	2	8	2	8	5	11
9/68	14/2/68	Southland	A	-	0 *	0	0	-	29 *	49	30	12
		Fairhaven	A	-	-	10	0	-	-	63	40	13
		Ch. Ingouf	A	-	0 *	0	0	-	35 *	32	33	14
		Red Haven	A	-	0 *	0	0	-	31 *	38	25	15
De 3/70	4/3/70	Springtime	A	12	12	17	1 (					
		Dixired	A	3	4	46	0 (					
		Cardinal	A	16	1	9	0 (	41	4	26	2	16
		Red haven	A	75	2	23	0 (					
		Coiron	A	59	14	49	0 (					
"	15/4/70	Dixired	C	-	23	-	2 (					
		Cardinal	C	-	5	-	1 (	-	3	-	3	17
		Red Haven	C	-	5	-	1 (					
		Coiron	C	-	2	-	1 (					
Mo 2/70	-	Red Haven	A - B	-	-	-	-	33	4	11	1	18
		Ch. Ingouf	" "	-	-	-	-	31	1	14	1	19
		Southland	" "	-	-	-	-	89	14	41	2	20
-	-	Red Haven	C	*	*	*	-	100 *	10 *	97 *	2	21
		Ch. Ingouf	C	*	*	*	-	100 *	5 *	91 *	1	22

* = treatment at 400 g a.i./hl

We have also carried out numerous tests against other phytoparasitic arthropods and in this case too, dinoterb acetate has given excellent results. Although the space to discuss them in detail is lacking, we can nevertheless mention :

Aphis pomi, the Green Apple Aphid and Dysaphis plantaginea, the Pear-bedstraw Aphid, against which the rate of 600 g a.i./hl, applied at the end of the dormant stage of the vegetation, shows itself to be quite efficient.

Operophtera brumata, the Winter Fruit Moth, which is controlled in an interesting way by the product

Argyresthia ephipella, the Cherry Fruit Moth, is destroyed in a satisfactory way as from the rate of 450 g a.i./hl, still for treatments carried out at stage A-B.

Dinoterb acetate + oil is also efficient on insects such as Quadraspidiotus perniciosus, the San José Scale.

Finally, the series of tests carried out on vine have shown that, for the most part, as from 400 g a.i./hl, in addition to its efficiency against P. ulmi, the product had an excellent control over the attacks of Grape Bud Mite and Rust Mite. Its action on Eotetranychus carpini, the Yellow Spider, has also been demonstrated.

CONCLUSIONS

Dinoterb acetate in the form of an oily paste and applied at the swelling of the buds (stage A-B) of fruit trees and vine, demonstrates a remarkable efficiency against P. ulmi at the rate of 600 g a.i./hl.

This application allows the orchard to be entirely protected from the early and often serious attacks of Red Spiders and then, in the season, allows specific acaricide treatments to be undertaken with the greatest possible chance of success

The efficiency of dinoterb acetate on another troublesome parasite, M. persicae, the Green Peach Aphid, also shows itself to be excellent; the same rate of 600 g a.i./hl is to be retained for applications made at the end of the dormant stage of the vegetation.

The product again demonstrates its good efficiency on the other winter arthropods in the orchard and vineyard.

It has a perfect selectivity which means that it can be used on all species.

Dinoterb acetate thus proves itself to be more interesting than dinoterb ammonium salt (which cannot be used on peach trees due to an absence of selectivity) and DNOC (which has a mediocre efficiency on mites).

It should permit an exceptional improvement in the efficiency and polyvalence of winter treatments and play a large part in achieving good sanitary conditions in the orchard and vineyard.

Dinoterb acetate, in the form of an oily paste, has been granted a temporary Sales Licence in France, for use at the rate of 600 g a.i./hl, for the winter treatment of fruit trees and vine.

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PROPERTIES OF CHLORDANE WHICH JUSTIFY NEW EXPERIMENTS WITH THE ALPHA- AND GAMMA-ISOMERS

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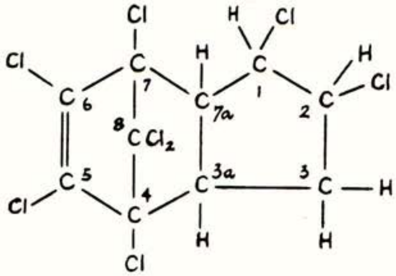
Summary Use of technical chlordane in agriculture leaves alpha- and gamma-chlordane as principal terminal residues on plants. Review of the literature shows that these isomers metabolise in mammals mainly to hydrophilic products that can be excreted. In the food chain there is little possibility of amplification of these residues, and in this respect a pure form of chlordane may be unique amongst kn own organochlorine insecticides.

Technical chlordane contains many organochlorine compounds including heptachlor; consequently complications may arise in research with it. AG chlordane is much purer containing 70% alpha- and 25% gamma-isomers with less than 1% heptachlor. Used experimentally for protection of sugar beet, fodder beet, maize against soil insects, residues in crops and soil are correlated with application rates. Though beyond our scope precisely to determine efficacy, good soil disinfecti on probably needs 5-6 kg a.i./ha at which rate residues are likely to fall within safe limits.

INTRODUCTION

Technical chlordane was introduced in 1945 and is believed to be the second oldest organochlorine insecticide in extensive commercial use being preceded only by DDT. Its production involves the Diels-Alder reaction of hexachlorocyclopentadiene with cyclopentadiene to yield chlordene which is chlorinated to yield technical chlordane. These reactions lead principally to the synthesis of isomers of octachloro-4, 7-methanotetrahydroindane (in more precise nomenclature isomers of 1, 2, 4, 5, 6, 7, 8, 8-octachloro-3a, 4, 7, 7a-tetrahydro-4, 7-methanoindane)



There are many possibilities for such reactions to deviate from the desired route, and so the technical chlordane produced in this way comprises a viscous liquid mixture of organo-chlorine compounds of composition approximately as follows :-

Isomers of octachloro-4, 7-methanotetrahydroindane		47%
Chlordene and its isomers		21%
Heptachloro-4, 7-methanotetrahydroindene (heptachlor)		10%
Various $C_{10}H_{7-8}Cl_{6-7}$ compounds		8%
Nonachlor (enneachlor)		7%
Diels-Alder adducts to pentachlorocyclopentadiene		2%
Hexachlorocyclopentadiene	less than	1%
Other compounds		5%
		100

During the first five years of manufacture a small proportion of the intermediate hexachlorocyclopentadiene remained unreacted in the end product. When it was discovered that the presence of hexachlorocyclopentadiene constituted a toxicological hazard in the agricultural use of early technical chlordane, the process of manufacture was adjusted so that its content could be limited to a maximum of 1% (U.S. Federal Register of Interpretation, 1955); in fact, since 1951, the occurrence of hexachlorocyclopentadiene has seldom even approached 1% in technical chlordane.

Beginning in 1950 with the work of R.B. March and his subsequent publication (1952), attempts have continually been made to improve our knowledge of the composition of technical chlordane. The techniques of physico-analytical research including infra-red absorption spectroscopy, gas chromatography, nuclear magnetic resonance, and mass spectroscopy have been applied; but, even at the present time, the configurations of some isomers of the minor constituents have not been elucidated.

In the structure of the molecule of octachloro-4, 7-methanotetrahydroindane shown in the first paragraph, a possibility of stereoisomerism arises because the chlorine and hydrogen atoms attached to carbon atoms 1 and 2 in the five-membered ring may be situated relatively either in cis- or trans- positions. In technical chlordane the trans-isomer which is now known as gamma-chlordane predominates over the cis-isomer which is known as alpha-chlordane, the gamma-chlordane content being typically 1.25 times greater than the alpha-chlordane content. Throughout the remainder of this paper these short names will be used to identify the stereoisomers of octachloro-4, 7-methanotetrahydroindane when there is no possibility in the context of confusing them with the technical chlordane of commerce, whose principal components have been enumerated in the first introductory paragraph.

More particularly because of the complex composition of technical chlordane, it is essential to establish a standard of reference in the form of a stock of homogeneous technical chlordane whose physico-chemical properties have been accurately measured and whose toxicology and biological properties can be accepted as typical of production which is expertly managed. When authorities, formulators, and research scientists request technical chlordane, such is the stock from which they are supplied; and this also constitutes the standard to which each successive works batch must be strictly comparable. When the reference stock eventually becomes depleted and a new stock is proposed, the retiring and the new stocks must be compared in terms of the most detailed analytical chemistry and of bioassay with respect to at least six insect species on a variety of plants or grasses, and finally by a comparison of toxicological properties. Experience shows that, as a con-sequence of such care, it becomes possible even over the span of many years to discriminate between production batches that would have significantly different performances in commerce and agriculture. So the fact that ordinary technical chlordane is a complex mixture does not lead to insuperable difficulty in characterising or specifying this product, but it does lead to two important questions which must be considered next.

First, when technical chlordane is applied to the protection of field crops, it becomes necessary to consider both qualitatively and quantitatively whether residues on the crop may have the same compositions as that of technical chlordane; or otherwise, whether some components of technical chlordane may disappear perhaps more slowly or perhaps more quickly than the alpha- or gamma-isomers of chlordane. It has been demonstrated that when residues are extracted from the crop within a few hours after spraying technical chlordane their gas-chromatogram is essentially similar to that of the technical chlordane applied; whereas, if the residues are extracted at a time between three and six days after spraying, the chromatogram may still show peaks due to alpha- and/or gamma-chlordane, but those peaks which characterise the respective associated organochlorine compounds become proportionately smaller with elapse of time. So it can be demonstrated that most of those organochlorine compounds present in technical chlordane do decompose in plants or weather away more quickly than either alpha- or gamma-chlordane which are the principal terminal residues. Consequently, if the quantitative result for residues should be calculated by reference to the principal peaks of alpha- and gamma-chlordane and then reported in terms of technical chlordane, the numerical result would be consciously biased on the high side, although it is implicit that here is a safeguard against misuse of technical chlordane.

Secondly, because of the presence of heptachloro-4, 7-methanotetrahydroindene (heptachlor) in technical chlordane, the possibility has to be considered that residues of heptachlor or of heptachlor epoxide might occur on or in plants which have been treated with technical chlordane. In nearly every known instance, any residues which could be attributed to the heptachlor impurity of technical chlordane are much smaller than the terminal residues of alpha- or gamma-chlordane; even so, their long-term significance may be important because heptachlor and its epoxide share a propensity to be accumulative in body fat whereas, as shown by Korte (1967), residues of octachloro-4, 7-methanotetrahydroindane are for the most part metabolised in animals to hydrophilic substances.

These considerations suggest that in order to gain more precise data concerning residues of alpha- and gamma-chlordane in plants, and in order to be able more specifically to assess the significance of such residues free from the complication of concomitant organochlorine compounds, it has become desirable to produce and to arrange field trials with octachloro-4, 7-methanotetrahydroindane of a purity far exceeding that of the technical chlordane characterised at the start of this Introduction.

METHOD AND MATERIALS

The new product to which the common name "AG Chlordane" has been given contains not less than 95% of the isomers of octachloro-4, 7-methanotetrahydroindane. This percentage typically comprises 70% of alpha-chlordane and 25% of gamma-chlordane. The remainder consists of the various organochlorine compounds which were listed as constituents of technical chlordane, but with hexachlorocyclopentadiene and heptachlor each restricted to 1% maximum. Being a much purer product than technical chlordane, AG chlordane is a wax-like solid.

AG chlordane is very soluble in aromatic liquids: about 142 g of it dissolve in 100 g of xylene, and about 180 g are soluble in 100 g of high aromatic naphtha at $30^{o}C$. Accordingly, by well known principles (British Insecticide and Fungicide Council, 1965) and by techniques similar to those described in detail by Velsicol (1970), AG chlordane is readily formulated either as emulsifiable concentrate 480 g a.i./l or as granules containing up to 33.3 % a.i. It is practicable to formulate also emulsifiable concentrates containing both AG chlordane and methyl parathion.

The pure individual isomers of alpha- and gamma-chlordane have melting points of 107°C and 105°C respectively. They have been prepared mainly for use in analytical chemistry, in toxicological feeding studies, and in studies of metabolism. For these isomers the respective values of acute oral toxicity in the albino rat are not significantly different, and the acute oral toxicity LD50 of AG chlordane 95% a.i. as manufactured is 241 ± 50 mg/kg in albino rats.

During an extended research beginning 1958 Korte (1967) studied the metabolism of certain organochlorine insecticides in which carbon atoms of the hexachlorocyclopentene ring had been isotopically labelled with ^{14}C. The isomer of chlordane used in those experiments was designated alpha-chlordane, but it was the trans-isomer and must therefore have been identical in structure with the isomer designated in this paper as gamma-chlordane. He investigated the metabolism of chlordane by the fungus Aspergillus flavus, by mosquito larvae Aedes aegypti, and in much greater detail by male rabbits. He demonstrated that living cells of these three species could convert gamma-chlordane (our nomenclature) to water-soluble metabolites.

Among his detailed studies on male rabbits Korte (1967) had fed to each animal 100 mg of ^{14}C-labelled gamma-chlordane each week for a period of ten weeks. During these same weeks 47.2% and 22.7% of the isotopic carbon were excreted in urine and faeces respectively. At autopsy the fatty tissues stored only 4% of all the isotope which had been fed, whilst in many other tissues almost all the remainder of the isotopic carbon was located and shown to consist mainly of water-soluble metabolites of gamma-chlordane.

These and other tracer measurements by Korte (1967) suggest that, after an extended period of feeding gamma-chlordane at a steady rate, a certain asymptotic concentration of chlordane and of its metabolites is approached in body-fat. The asymptote by no means represents a saturated solution in the physico-chemical sense of that adjective; instead, it seems that the concentration of this solution in fat stands indirectly in a sluggish equilibrium with the instantaneous concentration of hydrophilic metabolites of chlordane which are being re-currently excreted from watery tissues of the body. And so it would follow, as Korte indeed observed, that, upon terminating the feeding of chlordane in the diet, the concentration of chlordane and of its metabolites in all important tissues must gradually diminish.

These views are supported by more recent research in metabolite isolation and synthesis by Schwemmer, Cochrane, and Polen (1970) and, more particularly, by the long term feeding studies reported by Polen, Hester, and Benziger (1970). Having first identified oxychlordane as a lipophilic metabolite in vivo resulting from the long term feeding of either alpha- or gamma-chlordane, they proceeded to feed alpha- and/or gamma-chlordane to rats and dogs for one and two years respectively, also to pigs for shorter intervals. They demonstrated that the concentration of oxychlordane in body fat rises gradually to a value which is in equilibrium with the chlordane content of the diet; and for diets containing up to so much as 150 ppm of chlordane this relationship is nearly linear and the proportionality constant approximately unity.

Thus, on present evidence which reveals no amplification factor, it seems probable that neither chlordane nor its metabolites could accumulate in food chains. In this respect chlordane may be unique amongst known organochlorine insecticides, and it is for this reason that AG chlordane stands in need of evaluation in agriculture with special reference to its residues in crops and soils.

To assist such a research by our collaborators in many European countries, a trial programme was prepared, outline of which is as follows :-

Insecticide for trial — AG chlordane

Formulations available — Granule 10% a.i. (1970)
Granule 33.3%a.i. (1971)
e.c. 480 g a.i./l. (1970-1971)

Crops — For 1970 trials mainly as soil insecticide in sugar beet, potato, maize

Seed bed — Soil free from heptachlor and residues of other organochlorine insecticides, fine even tilth to depth 20 cm

Rates of application as soil insecticide — Granules or e.c. at rates within the range 3.5 - 16 kg a.i./ha. Spray e.c. in 200 l. water/ha. Incorporate into soil to depth 2 - 5 cm

Plots			
	Sugar beet	25 m^2	Replicates 4 altogether
	Potato	25 m^2	" " "
	Maize	50 m^2	" " "

Depth of seed		
	Sugar beet	3 cm
	Potato	10 cm
	Maize	3 cm

Other insecticides, fungicides — Use on foliage as needed, but avoid use of other persistent organochlorine compounds

Evaluation

(a) At normal harvest time statistically examine roots of sugar beet and potato for damage by Melolontha spp., Blaniulus guttulatus, Archiboreoiulus pallidus, Elateridae, (Agriotes spp) and other soil insects. Check maize for speed of growth, ultimate height, and yield of grain.

(b) For residue analysis reserve samples as follows :

Soil	0-2-5 cm depth
Soil	5-10-15 cm depth
Sugar beet	roots and foliage separately
Potato	tubers
Maize	grain, stalk, foliage separately

Residue analysis for alpha- and gamma-chlordane by Velsicol method AM-0494

Weathering of soil — After harvesting these crops from 1971 trials, reserve the plots until autumn 1971 or spring 1972 and at the normal season plant early carrots or late carrots. Harvest the carrots at normal time, and analyse for residues of alpha- and gamma-chlordane by Velsicol method AM-0494

Our collaborators amended the programme as necessary to suit the local conditions with which only they could be familiar, although this general procedure has been followed in Belgium on sugar beet, in France on fodder beet, in Italy on sugar beet, potato, maize, and in Austria on maize during 1970. Trials of similar kinds are being made during 1971, also in other countries and on other crops and against insects of various orders.

RESULTS

The experiments so far carried out in Europe indicate, relative to given rates of AG chlordane application, the ranges in which residues of alpha- and gamma-chlordane are likely to be found in the root and leaf of sugar beet, in fodder beet and in the soil profile, and in grain, stalk, and leaf of maize. Tables 1, 2, 3 present data, from the sources specified, which are typical of a wider variety of research which we hope others will publish. Residue analyses have been completed also on tubers of potato protected in the soil by AG chlordane, but these data are not yet so numerous that we can be certain of a typical range or average residue content.

Just as the alpha-isomer preponderates in AG chlordane, so in most instances the residues of alpha- exceed those of gamma-chlordane; and wherever this is not so, as among certain data of Table 2, our findings may perhaps be attributed to what appears to be a trace of gamma-chlordane in the seed bed before this experiment was started.

So far we have not progressed to the final stage of the trials programme which is concerned with the growing of carrots in these same seed beds to ascertain the uptake of chlordane from the soil in successive seasons.

Also, the work reported here has not shown with precision at what ranges of chlordane concentration in upper layers of the soil specific insect species can be controlled. This is partly because samples of AG chlordane reached our collaborators after the normal crop planting time when normal populations of soil insects had seasonally declined. Such tentative observations as we already have suggest that AG chlordane would need to be applied at about 5 kg a.i./ha to provide commercially adequate soil disinfection. However, to decide what is adequate needs long experience of competitive products at the site in question, and the detail of such comparisons falls outside the scope of this paper.

Table 1

Residues of alpha- and gamma-chlordane in sugar beet

Gembloux, Belgium

Data from Van Steyvoort, Zenon-Roland, Martens (1971)

AG chlordane	Residues of chlordane isomers ppm			
	in roots		in stalks and leaves	
kg a.i./ha	alpha-	gamma-	alpha-	gamma-
4.5	0.016	<0.010	0.016	<0.010
4.5	0.021	<0.010	0.021	<0.010
9.0	0.050	0.013	0.072	0.023
9.0	0.030	<0.010	0.032	0.018

Table 2

Residues of alpha- and gamma-chlordane in soils and fodder beet

Domaine de Jonville, 77 Ponthierry, near Paris

30 May 1970	AG chlordane granules 10% a.i. broadcast and incorporated to depth 2-5 cm
30 May 1970	Fodder beet seeded at depth 3 cm
Rainfall	June 53 mm July 51 mm August 40 mm September 20 mm
8 October 1971	Harvest and soil sampling

AG chlordane	Residues of chlordane isomers ppm							
	in soil profile				in root		in leaf	
	surface layer		depth 5-10cm					
kg a.i./ha	alpha-	gamma-	alpha-	gamma-	alpha-	gamma-	alpha-	gamma-
4	7.9	3.5	0.38	1.30	0.034	0.080	0.007	0.057
8	19.2	6.6	0.29	1.24	0.058	0.072	0.006	0.037
16	20.3	7.1	1.13	0.48	0.174	0.116	0.019	0.080
untreated	nd	0.8	nd	0.86	nd	0.081	nd	nd

nd = not detected

Table 3

Residues of alpha- and gamma-chlordane in grain, stalk, and leaf of maize

Data from Bundesanstalt für Pflanzenschutz, Wien II

10 June 1970	AG chlordane granules 10% a.i. applied at rate corresponding to 3.5 kg a.i./ha and incorporated to depth 5 cm
10 June 1970	Seed of maize drilled at depth 3 cm immediately after incorporating AG chlordane
16 September 1970	Harvest

Portion of maize plant	Residues of chlordane isomers ppm	
	alpha-	gamma-
Grain	< 0.005	< 0.005
Stalk	< 0.005	< 0.005
Leaf	0.021	0.012

DISCUSSION

Experiments in Belgium by Van Steyvoort (1965) and in France by Durgeat and Lhoste (1965) had demonstrated how heptachlor should be used at 3 - 3.5 kg a.i. per overall hectare for control of the millipedes Blaniulus guttulatus and Archiboreoiulus pallidus in crops of sugar beet, and their researches have been vindicated by years of satisfactory commercial experience with heptachlor. To find corresponding data for chlordane is exceedingly difficult, but some farmers in Belgium and France found by experience that to provide a similar degree of soil disinfection 8 kg of technical chlordane/ha were needed, - and this would contain alpha- and gamma-chlordane and heptachlor totalling 4.6 kg besides other organochlorine compounds. So, although there is no documentary evidence and although the proportions of the two chlordane isomers differ as between technical chlordane and AG chlordane, there were prima facie reasons for supposing that 5 kg or slightly more of AG chlordane/ha would be needed for the protection of Belgian and French sugar beet.

The 1970 work reported here is the most tentative introduction to the use of AG chlordane as a soil insecticide in Europe. If, by its extension, it can be shown that about 5 kg a.i./ha will regularly provide good protection for sugar beet, fodder beet, and maize there would be good prospects that residues of alpha- and gamma-chlordane would fall within the tabulated ranges. In the meantime a wealth of supporting data is being accumulated by colleagues in Velsicol, Chicago, and chronic toxicological studies are proceeding satisfactorily.

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ORGANOTHIOCYANATES AS SYNERGISTS FOR INSECTICIDES

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Summary: The joint effect of the two organothiocyanates ("Thanite" and "Lethane 384") was studied with insecticides from different groups at 5:1 ratios. "Thanite" (isobornyl thiocyanoacetate) proved to be synergistic for carbaryl, UC 10854 (*m*-isopropylphenyl *N*-methylcarbamate) and "Zectran" (4-dimethylamino-3.5-XYLYL N-methylcarbamate) from the carbamates and trichlorphon from the organophosphorus compounds against the susceptible strain of housefly. Thanite continued to be synergistic for these compounds and also for pyrethrins against R-DDT, R-malathion and R-carbaryl strains of houseflies. "Lethane 384" (2-butoxy-2'-thiocyanodiethyl ether) was synergistic only with carbaryl against the susceptible strain. Thanite synergized UC 10854, pyrethrins and trichlorphon against the cotton leafworm larvae while Lethane was able to synergize only carbaryl and pyrethrins against the same larvae. A set of data was planned to express the joint effect or an isobologram from which the joint action ratio was measured and calculated.

INTRODUCTION

Organothiocyanates have been known as a class of synthetic insecticides for more than three decades. Yet only recently El-Sebae *et al.*(1964) have found that a number of organothiocyanates, including Thanite (isobornyl thiocyanoacetate), are capable of synergizing a number of carbamates against susceptible and carbamate resistant strains of houseflies. Bakry *et al.*(1968) concluded that a series of para-substituted benzyl thiocyanates had a synergistic activity to carbaryl against houseflies showing an excellent correlation with the electron withdrawing properties of the substituents as measured by Hammett's sigma values.

El-Sebae (1969) found that Lethane 384 was as potent as Thanite in inducing higher synergism with DDT and carbaryl more than with azinphos-ethyl.

Hewlett (1969_b) recorded potentiation between Thanite and propoxur in their action on susceptible houseflies.

The present investigation was directed to study comparatively the synergistic effect of Thanite and Lethane 384 when mixed with various insecticides of different chemical structures against insects of different susceptibilities.

METHODS AND MATERIALS

A standard susceptible strain of housefly supplied by W.H.O. was used. Three resistant strains of houseflies, a DDT-resistant strain of 250 fold, a malathion-resistant strain of 50 fold, and a carbaryl-resistant strain of more than 50-fold were also tested. All insect strains were maintained at 25 ± 2°C. and 65 ± 5% relative humidity and were reared as described by Toppozada _et al_.(1967). Acetone solutions of compounds used alone or in mixtures with synergists were applied topically to female flies of 3-4 days old. In addition a susceptible laboratory colony of the Egyptian cotton leafworm (_Spodoptera littoralis_) was treated using the residual film method by applying one ml acetone solutions in petri dishes of 90 mm diameter. Three replicates each of ten insects were used for every concentration including the control throughout all the joint action tests.

Technical samples of _p,p_-DDT, lindane, toxaphene, endrin, carbaryl, "Isolan" trichlorphon, UC 10854 (3-isopropylphenyl _N_-methyl--carbamate), piperonyl butoxide, "Lethane 384" (53% 2-butoxy-2-thiocyanodiethyl ether), and "Thanite" (82% isobornyl thiocyano-acetate), were tested alone or in combinations.

RESULTS

Thanite and Lethane 384 were compared with piperonyl butoxide in synergizing or potentiating different insecticides. Preliminary tests on the susceptible strain of houseflies showed that piperonyl was able to synergize the four carbamates carbaryl, "Isolan", "Zectran" and "UC 10854", pyrethrins and trichlorphon while causing only very slight synergism with DDT, lindane, camphechlor, endrin; an additive effect only was found with malathion, parathion-methyl and dioxathion.

Thanite caused synergism of carbaryl, "Zectran", "UC 10854", trichlorphon, slight synergism of lindane, while causing antagonism for the rest of the tested insecticides. Meanwhile Lethane 384 was synergistic only for carbaryl. The percentage of synergism or antagonism at the LD_{50} level was calculated for all mixtures according to Hewlett (1960) AND El-Sabae _et al_.(1964). The quantitative data deduced from the regression lines are shown in Table I.

The successful synergistic mixtures were retested against resistant strains: R-carbaryl, R-DDT and R-malathion as shown in Table 2. The potency of the same synergistic mixtures were tested against the cotton leafworm larvae as shown in Table 3.

The potentiation data of Thanite when mixed with Zectran and UC 10854 were expressed in the form of isobolograms as suggested by Hewlett (1969_b) as shown in Figures 1 and 2.

Table 1

Synergism of different insecticides by Thanite and Lethane at 1:5 ratio against the susceptible houseflies.

Compound or mixture	LD_{50} ug/♀	Confidence limits of LD_{50} ug/♀	Slope	Synergistic ratio (SR)	% synergism at LD_{50} level
Thanite	5.00	3.18-8.16	2.47		
Lethane	3.60	2.48-5.22	2.44		
Carbaryl	2.00	1.61-2.73	4.30		
Carbaryl + Thanite	0.55	0.28-1.05	1.70	3.45	49.4
Carbaryl + Lethane	0.40	0.20-0.56	2.24	5.00	48.0
Isolan	1.30	0.88-1.91	2.54		
Isolan + Thanite	0.84	0.68-1.03	5.73	--	-47.0
Zectran	0.55	0.35-0.85	2.51		
Zectran + Thanite	0.22	0.16-0.30	3.65	2.4	49.6
UC 10854	0.88	0.56-1.13	2.01		
UC 1054 + Thanite	0.097	0.02-1.75	6.07	9.07	100.0
Pyrethrins	0.95	0.50-1.80	1.53		
Pyrethrins+Thanite	0.75	0.41-1.35	1.64	-	-68.0
Pyrethrins+Lethane	0.45	0.23-0.87	1.42	-	-20.0
Trichlorphon	0.175	0.12-0.24	3.46		
Trichlorphon+Thanite	0.064	0.05-0.07	5.90	2.73	87.0

(-) is a measurement of antagonism.

Table 2

Synergism of different insecticides against three resistant strains of houseflies by different synergists at 1:5 ratio

Compound or Mixture	R-DDT		R-malathion		R-carbaryl	
	LD_{50} ug/♀	%Synergism at LD_{50}	LD_{50} ug/♀	%Synergism at LD_{50}	LD_{50} ug/♀	%Synergism at LD_{50}
Pb	>10	-	>10	-	60.0	-
Thanite	4.8	-	3.30	-	9.0	-
Lethane	-	-	3.40	-	-	-
Carbaryl	>100	-	>50	-	>100	-
Isolan	2.15	-	1.30	-	2.10	-
Isolan + Pb	0.31	100	0.20	100.0	0.31	100.0
Isolan + Thanite	-	-	0.62	-26.0	-	-
Zectran	2.75	-	2.70	-	1.00	-
Zectran + Pb	0.32	83.0	0.24	100.0	0.20	76.0
Zectran+Thanite	0.35	40.0	0.23	68.0	0.30	63.0
UC 10854	1.30	-	1.30	-	1.65	-
UC 10854 + Pb	0.28	100.0	0.15	100.0	0.36	100.0
UC 10854 + Thanite	0.50	42.0	0.16	100.0	0.51	89.0
Pyrethrins	2.75	-	1.83	-	4.50	-
Pyrethrins + Pb	0.26	100.0	0.12	100.0	0.42	90.0
Pyrethrins+Thanite	-	-	0.60	10.0	0.63	66.0
Pyrethrins+Lethane	-	-	0.35	79.0	-	-
Trichlorphon	-	-	-	-	0.39	-
Trichlorphon + Thanite	-	-	-	-	0.29	30.0

(-) is a measurement of antagonism

Table 3

Synergism of carbamate insecticides with organothiocyanates and Pb as synergists against S. littoralis larvae at 5:1 ratio

Compound or mixture	LC_{50} mg/ml (mg/Petri dish)	Confidence limits of LC_{50} mg/ml	Slope	% Synergism at LC_{50} level
Pb	4.70	4.05 - 5.45	8.07	-
Thanite	4.70	3.98 - 5.54	17.00	-
Lethane 384	5.10	4.32 - 6.01	8.27	-
Carbaryl	>50	———	-	-
Carbaryl + Lethane	0.96	0.75 - 1.21	3.59	100.0
UC 10854	>10	———	-	-
UC 10854 + Pb	0.23	0.10 - 0.50	1.20	100.0
UC 10854 + Thanite	0.26	0.17 - 0.37	3.06	100.0

The joint action ratio (R) was measured from the isobolograms and the measured values were compared with the calculated ones as shown in Table 4.

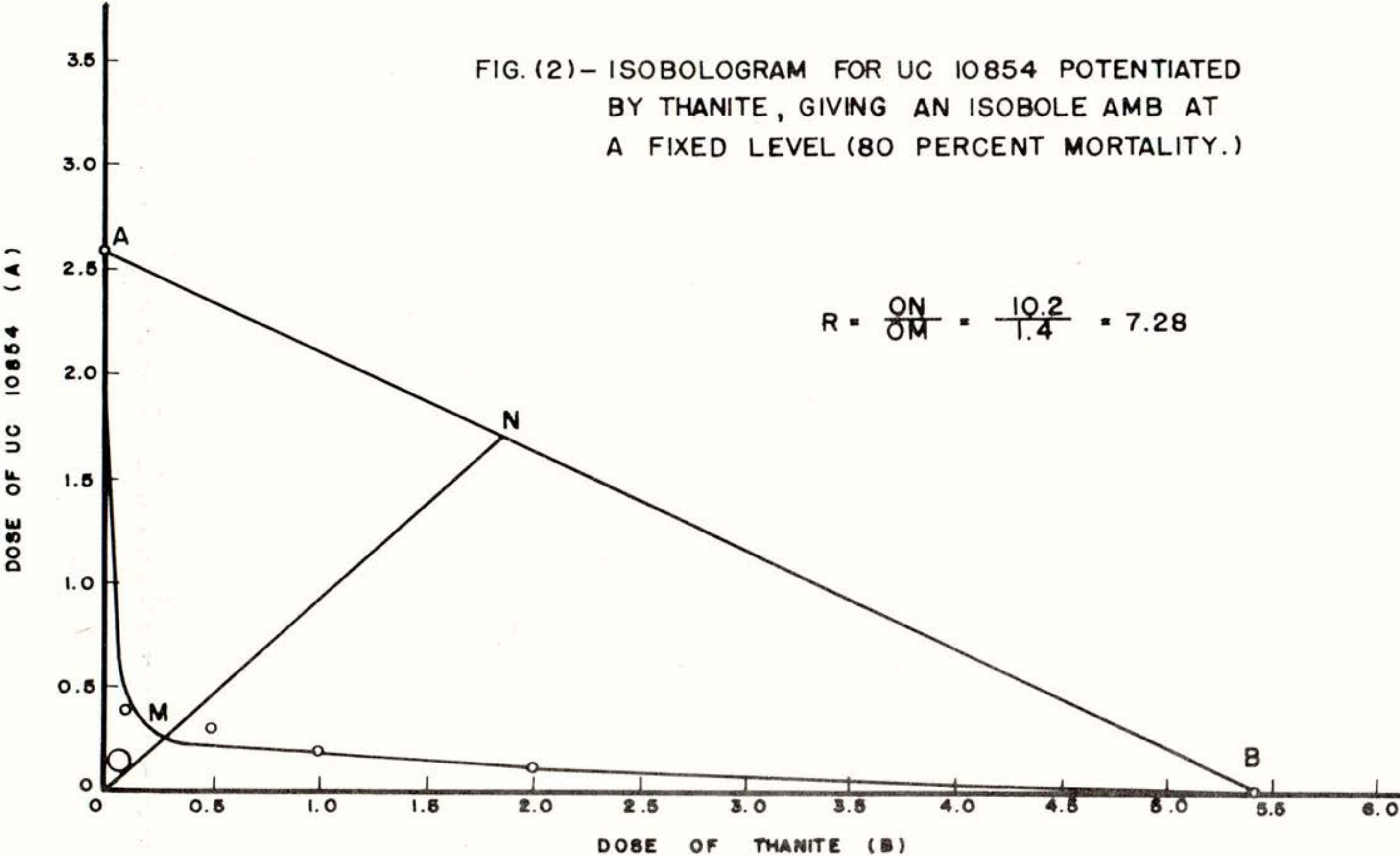

FIG. (2)– ISOBOLOGRAM FOR UC 10854 POTENTIATED BY THANITE, GIVING AN ISOBOLE AMB AT A FIXED LEVEL (80 PERCENT MORTALITY.)

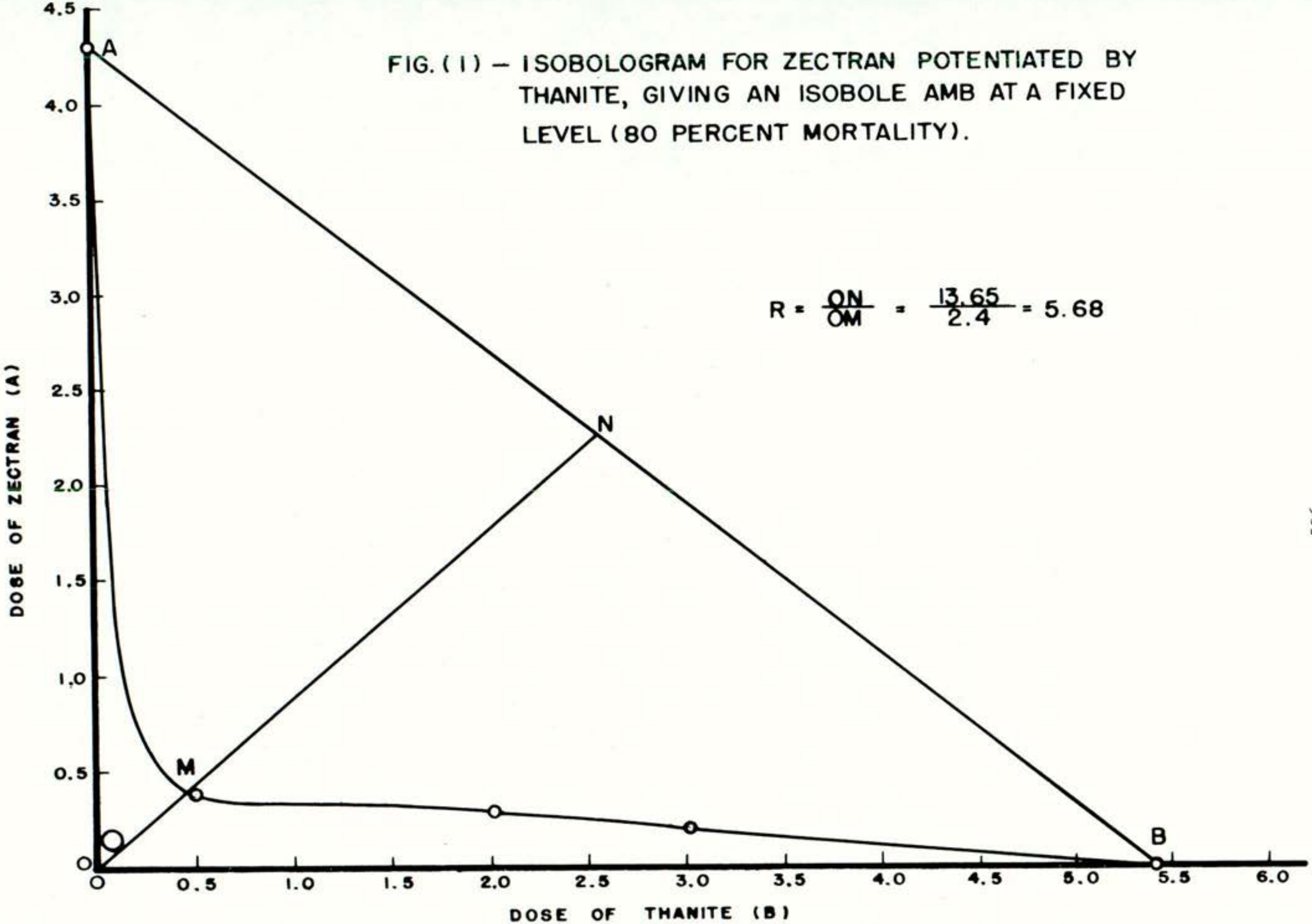

FIG. (1) – ISOBOLOGRAM FOR ZECTRAN POTENTIATED BY THANITE, GIVING AN ISOBOLE AMB AT A FIXED LEVEL (80 PERCENT MORTALITY).

Table 4

Measured and calculated values of joint action ratio (R) for Zectran and UC 10854 combinations with Thanite against R-malathion strain of houseflies at 80% level of mortality.

Parameters	Thanite + Zectran	Thanite + UC 10854
V_1 (Thanite)	0.0926	0.0185
V_2	0.0930	0.1540
$V_1 + V_2$	0.1856	0.1725
K	8.6600	15.5000
R (calculated)	5.3300	8.7500
R (measured from isobole)	5.6800	7.2800

DISCUSSION

The present results are in agreement with the results of Georghio (1962) concerning the effect of piperonyl butoxide (Pb) as a synergist. Thanite was less effective than Pb in the number of compounds synergized and in the degree of synergism. Lethane 384 was the least effective one.

Antagonistic effects were noticed especially for parathion-methyl, dioxathion and malathion when mixed with either Pb, Thanite or Lethane 384.

Such cases can be attributed to an interference by the added compounds with the metabolic oxidative intoxication processes. This assumption is confirmed by the observation that most of the antagonized compounds were phosphorothioates which are liable to be oxidized to the more in vivo potent oxygen analogues. These results agree with those mentioned in the literature by Sun and Johnson (1960 a & b) and Dyte & Rowlands (1970). Bakry et al.(1968) found antagonism for parathion-methyl when mixed with 4-nitrobenzyl thiocyanates which was an active synergist for carbaryl. They also found no synergism for DDT with this thiocyanate.

The important role of oxidases in the detoxification of carbamates was supported by the results of Kuhr (1969) who believed that cytochrome P-450 might be involved in the metabolism of carbaryl and perhaps pther carbamates by housefly microsomes. Metcalf et al.(1966) came to the conclusion that methylenedioxyphenyl compounds synergize carbamates by acting as inhibitors of phenolase enzymes which detoxify these carbamates largely by ring hydroxylation. The capability of Thanite to inhibit the activity of oxidative enzymes was also supported by the results of Nomeir (1969) which showed that Thanite inhibited completely the oxidation of methyl group in fly homogenates. Similarly, Khamis et al. (1971) proved that Thanite was able to inhibit the oxidative detoxication of carbaryl and α-naphthol by the *Pericularia oryzae* enzyme preparation. Thus, it can be suggested that the mechanism of mode of action of organothiocyanates as carbamate synergists is through its ability to inhibit the mixed function oxidative enzymes which are responsible of detoxifying carbamates mainly by ring and N-hydroxylation (Balba et al., 1968).

Thanite synergized carbamates against the resistant strains of insects. Since it is known that the development of resistance in insects is characterized by increasing rates of detoxification mainly by oxidation and hydrolysis, thus it is reasonable to expect more response with synergized mixtures against the resistant strains. El-Aziz et al. (1969) recorded that the RMIP resistant strain of houseflies has about twice as much catecholase activity as the NAIDM susceptible strain. The data of Bakry et al. (1968) that organothiocyanates can inhibit phenolases might explain the ability of Thanite to synergize UC 10854 (MIP) and Zectran even against the carbaryl-resistant strain.

The ability of Thanite to synergize trichlorphon against the susceptible and carbaryl-resistant strains of houseflies can be considered parallel to the results of Sun and Johnson (1960b) showing that Pb was cabable to synergizing a number of organic phosphorus compounds which do not contain thio-groups.

The present data indicated that Thanite and Lethane synergized pyrethrins against cotton leafworm larvae and against the malathion-resistant strain of houseflies. However, the two compounds failed to synergize the pyrethrins against the susceptible strain and the DDT-resistant strain of houseflies. On the other hand Thanite only synergized pyrethrins against the carbaryl-resistant strain.

Casida & Lykken (1969). and Lykken & Casida (1969) concluded that the limiting factor in breaking down and detoxifying pyrethrins is through the successive oxidation steps. The two organothiocyanates (Thanite and Lethane) are capable of inhibiting the mixed function oxidases. Thus it can be postulated that Thanite and Lethane induce their synergism for pyrethrins by blocking the detoxifying oxidative enzymes.

The dosage mortality data for Zectran and UC 10854 in combination with Thanite against the malathion-resistant strain of houseflies were expressed in the form of isobolograms. This strain was preferred because Thanite gave the highest potentiation for carbamates against it. The fixed level of response was taken at 80 per cent mortality. It was observed that the two isoboles were not straight lines (Figs. 1 and 2) as the isoboles sweeps nearer to the origin, thus indicating the presence of potentiation. These results are in agreement with the previous ones (Table 2). In addition, the parameter joint action ratio (R) was suggested by Hewlett (1969_c) to be $R = \frac{ON}{OM}$. The R value was evaluated and was found to exceed one as it was 5.68 and 7.28, for Zectran and UC 10854, in combinations with Thanite, respectively. The greater the R value the greater is the potentiation (Hewlett, 1969_c). Thus, it could be said that UC 10854 was potentiated more than Zectran in the presence of Thanite.

These measured values were compared with the calculated values of R. For computation of the degree of potentiation, Hewlett and Biol (1968) and Hewlett (1969_a) suggested a number of relations for calculating mathematical models for which the following equations were adopted for estimating R value.

$$V_1 + K\,(V_1V_2)^{\frac{1}{2}} + V_2 = 1 \qquad (1)$$

where K = Finney's coefficient of synergism, and $V = \frac{z}{Z}$.

The joint action ratio (R) as suggested by Hewlett and Biol (1968) can be calculated as follows:

$$R = 1 + \tfrac{1}{2}\,K. \qquad (2)$$

Both values of K and R were calculated for the combinations of UC 10854 and Zectran with Thanite at 80% level of response. A quite sufficient degree of reproducibility was found for joint action ratio (R) values either measured from the isobole or calculated (Table 4).

The advantage of the joint action ratio (R) is that it does not depend on an arbitrary choice of doses or ratio of dose, whereas the cotoxicity coefficient and the synergistic ratio do.

The point M on the two isobolograms represent the most economic point where the least amount of each constituent are sufficient to induce the same level of toxicity. Hewlett and Biol (1968) therefore suggested the use of isobolograms in the determination of insecticide - synergist mixtures of minimum cost.

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ARYLHYDRAZINES AS CARBAMATE SYNERGISTS AND MONOAMINE OXIDASE INHIBITORS IN INSECTS

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Summary: Three phenylhydrazine derivatives proved to be synergistic for "Isolan" (1-isopropyl-3-methyl-5-pyrazolyl dimethylcarbamate) and UC 10854 (m-isopropylphenyl N-methylcarbamate) against susceptible and R-carbaryl and R-malathion strains of houseflies. The most powerful synergist (N-acetyl-N-phenylhydrazine) was also synergistic for carbaryl and "Isolan" against cotton leafworm larvae. It is suggested that these hydrazines act as carbamate synergists by inhibiting the monoamine oxidase (MAO). The presence of MAO in housefly adults homogenate was proved quantitatively. MAO was higher in the females, the abdomen and in the susceptible strain the I_{50} of syn. II was determined as 3.3×10^{-4}M. The kinetics and factors affecting in vitro activity of MAO were investigated.

INTRODUCTION

Zeller (1962) demonstrated that monoamine oxidase (MAO) plays a role in the metabolism of catecholamines and other amines. The oxidation was found to be connected with the biological functions of these amines as in the operation of the C.N.S., in the regulation of blood pressure, and in the permeability of capillaries.

Yamad and Yasundobu (1962) demonstrated that plasma MAO was a copper enzyme and that the copper was involved in enzyme activity. Kaliman (1964) demonstrated that phenylhydrazine derivatives inhibited tyramine oxidation by liver oxidase.

El-Sebae et al. (1971) studied the biological effects of twelve synthesized derivatives. The compounds were toxic against mosquito *Culex pipiens* larvae. They were nontoxic when applied topically to houseflies. However they were capable of synergizing UC 10854 (m-isopropylphenyl N-methylcarbamate) against houseflies. The trend of relative synergism was parallel to that of mosquito larvicidal toxicity. These hydrazine derivatives were proved to be MAO inhibitors.

The aim of the present investigation is to study the synergistic effect of some arylhydrazine derivatives with different carbamates against insects of different susceptibilities. Besides the MAO activity was detected and measured in housefly tissues, and the effect of the most synergistic hydrazine derivative as a MAO inhibitor was studied.

METHOD AND MATERIALS

A standard susceptible strain, a malathion-resistant strain of 50-fold, and a carbaryl-resistant strain of more than 50-fold of houseflies were used. In addition a susceptible colony of they Egyptian cotton leafworm, Spodoptera littoralis, was included in the tests of synergistic effects. All the insect cultures were maintained at 25 ± 2°C. and 65 ± 5% relative humidity. Three to four days old females were topically applied by one microlitre acetone solution droplet. The third instan leafworm larvae were confined on the surface deposit left on the internal side of petri dishes from one ml actone solution. Three replicates each of ten insects were used including the control. The LD_{50} and LC_{50} values were interpolated from probit mortality regression lines. The synthesized 2,4-dinitrophenylhydrazine (syn.I), acetone 2,4-dinitrophenylhydrazine (syn.II), and N-acetyl-N-phenylhydrazine (syn.III) were tested for their synergistic potency with technical Isolan, and UC 10854 (m-isopropylphenyl N-methylcarbamate).

The flies homogenate was prepared by weighing 32 g of 3-4 days old flies after being anaesthetized by ether, and then mixed with 60 ml redistilled water for homogenization. The homogenate was centrifuged at 5000-6000 rev/min for twenty minutes. The supernatant was taken and the monoamine oxidase (MAO) preparation was proceeded as described by Mostafa (1971). The enzyme activity was evaluated by the Tabor spectrophotometric method as described by Colowick and Kaplan (1963) using benzylamine as a substrate. Readings on a Unicam SP 500 spectrophotometer were recorded initially and every minute for 5 minutes. The MAO activity was measured in total homogenate for different strains of houseflies and also in homogenates of different sexes and different parts of the fly body. Extrinsic factors affecting the MAO activity were studied, especially the substrate concentration, and the pH. The quantitative inhibition of MAO by the different inhibitors was studied to determine the I_{50}. These inhibitors were adopted because they showed the highest degree of synergism of carbamate in the tested mixtures.

RESULTS

The joint effect of the three hydrazines with carbamates against the susceptible standard strain is shown in Table 1. The three hydrazines were nontoxic alone at the used concentrations. The joint effect with the same carbamate against the two resistant strains is shown in Table 2. Synergist II was shown to synergize carbaryl and Isolan against the cotton leafworm larvae (Table 3).

Table 1

Synergism of carbamate insecticids by arylhydrazine derivatives I, II and III synergists at 1:5 ratio against susceptible houseflies.

Compound or mixture	LD_{50} ug/♀	Confidence limits of LD_{50} ug/♀	Slope	Synergistic ratio (SR)	% synergism at LD_{50} level
Isolan	1.30	0.88 - 1.91	2.54		
Isolan+syn.1	1.22	1.62 - 5.16	1.56	1.06	10.0
Isolan+syn.11	1.77	0.34 - 1.73	3.80	1.68	46.0
Isolan+syn.111	1.22	0.85 - 1.73	3.95	1.06	10.0
UC 1085	0.80	0.56 - 1.13	2.01		
UC 1085+syn.1	0.50	0.30 - 0.31	3.06	1.60	40.0
UC 1085+sun.11	0.29	0.16 - 0.51	1.71	1.72	74.0
UC 1085+syn.111	0.50	0.27 - 0.92	4.06	1.60	40.0

where: syn.1 = Synergist 1 (2,4-dinitrophenylhydrazine).
syn.11= Synergist 11 (N-acetyl-N-phenylhydrazine).
syn.111=Synergist 111 (acetone 2,4-dinitrophenylhydrazine).

Table 2

Synergism of carbamate insecticides by arylhydrazines

at 1:5 ratio against two resistant

strains of houseflies.

Compound or mixture	R-carbaryl strain		R-malathion strain	
	LD_{50} ug/♀	Synergistic ratio (SR)	LD_{50} ug/♀	Synergistic ratio (SR)
Syn.1	nontoxic		nontoxic	
Syn.11	" "		" "	
Syn.111	" "		" "	
Isolan	2.10	-	1.30	
Isolan + 1	0.63	3.33	0.49	2.65
Isolan + 11	0.27	7.77	0.54	2.40
Isolan + 111	-	-	0.88	1.36
UC 10854	1.65	-	1.30	
UC 10854+syn.1	-	-	0.55	2.36
" +syn.11	0.99	1.66	0.85	1.51
" +syn.111	0.76	2.17	0.72	1.80

Table 3

Synergism of carbamate insecticides with synergist 11

(N-acetyl-N-phenylhydrazine) against S. Littoralis

larvae at 1:5 ratio.

Compound or mixture	LC_{50} mg/ml (Mg/petri-dish)	Confidence limits of LC_{50} mg/ml	Slope	Synerg-istic ratio (SR)	% synerg-ism at LC_{50} level
Syn.11	10	- -	-	-	-
Carbaryl	50	- -	-	-	-
Carbaryl+syn.11	3.10	1.00 - 9.60	1.00	16.12	100.00
Isolan	0.68	0.52 - 0.88	4.15	-	-
Isolan+syn.11	0.33	0.26 - 1.04	1.66	1.23	34.00

The reaction constants and velocities of MAO activity in oxidative deamination of benzylamine from homogenates of different sexes and parts of flies are shown in Table 4. The optimum pH value of the buffer medium for MAO activity was found to be 7.3 at 3.3 mM benzylamine concentration. The optimum benzylamine concentration as a substrate was interpolated as 3.3 mM under the specified experimental conditions described by Mostafa (1971). The kinetics of the MAO activity was studied using the optimum pH and substrate concentration.

Table 4

Comparison of in vitro activity in different strains, sexes and parts of houseflies

Homogenate of	Rate constant (K) x 10^3	Velocity (V) umole min^{-1}
Total body of both sexes from susceptible strain	4.55	14.16
Total body of both sexes from R-DDT strain	3.20	10.84
Total body of both sexes from R-carbaryl strain	3.25	10.83
Total body of both sexes from R-malathion strain	3.25	10.83
Total body of males from R-malathion strain	1.79	6.25
Total body of females from R-malathion strain	2.43	8.08
Heads of males from R-malathion strain	7.71	22.50
Thoraxes of males from R-malathion strain	6.01	20.58
Abdomens of males from R-malathion strain	9.82	27.08

and the amount of crude enzyme was 0.002 mg (0.666 mgm per liter). The Michaelis constant (Km) and maximum velocity (V_{max}) were graphically derived from Lineweaver-Burk resiprocal plots as shown in (Fig.1). The V_{max} was found to be $6.6x10^{-6}$ M min and the Michaelis constant (Km) was calculated as $8.33x10^{-3}$ M. The I_{50} value of syn.11 was interpolated to be $3.33x10^{-4}$ M.

DISCUSSION

Arylhydrazines as carbamate synergists:

The three tested hydrazine derivatives were nontoxic to houseflies when tested alone. Yet they were capable of enhancing the toxicity of the two carbamates Isolan and UC 10854 against the susceptible standard strain of houseflies as shown in Table 1. The activation effect is a complete synergistic effect as the hydrazines were nontoxic to the flies at the used concentrations (Metcalf, 1967). UC 10854 was more synergized than Isolan. This might be attributed to the high similarity between UC 10854 and adrenaline which is one of the main substrates of MAO in animal tissues (El-Sebae et al., 1971). However synergist II was the best synergist for Isolan as well as UC 10854. This might be parallel to its inhibitory effect on MAO.

The three arylhydrazines which showed different degrees of synergism with the susceptible strain were also effective in synergizing both Isolan and UC 10854 against R-carbaryl and R-malathion strains. It was interesting to note a negative cross tolerance of the Isolan-synergized combinations with the three hydrazines against the two resistant strains of houseflies. This suggests an important practical application for such synergistic mixtures in being specifically effective against such resistant strains.

Synergist II was the only one to show synergism when mixed with carbaryl or Isolan against the cotton leafworm larvae. The combinations with UC 10854 were not tested due to the need for high concentrations exceeding the degree of solubility of this hydrazine derivative. The highest response was in the combination of carbaryl andsyn. II. The hydrazine derivative was nontoxic at the tested concentrations; carbaryl alone was of very low toxicity against this strain of cotton leafworm. It is probable that this low susceptibility to carbaryl holds for this higher response to this synergist which may act as an inhibitor for detoxifying systems.

It is postulated that the arylhydrazines act as carbamate synergists by blocking the MAO activity which is believed to play an important role in the oxidative detoxication of the carbamate moiety. This hypothesis is supported by the known inhibitory effect of MAO by arylhydrazines and also by the noticed similarity between N-methyl carbamate moiety and the methylaminoethanol side chain in adrenalin which is a substrate of MAO.

To confirm this assumption, preliminary tests proved the presence of MAO activity in insect tissues. In addition the most effective carbamate synergist II was shown to inhibit MAO activity. The quantitative data for assaying the MAO activity in homogenates of different strains, sexes and body parts are shown in Table 4. It was noticed that the total body homogenate of both sexes of the susceptible strain showed more in vitro MAO activity than the three resistant strains. This might reflect certain biochemical specific characteristics for the susceptible strain. However, no clear variation in the MAO activity was observed between the three resistant strains although the chemicals used in selections belong to different chemical groups. This favours the general belief of the broad spectrum detoxification systems in different resistant strains.

No similar data were recorded in the literature. The superiority of MAO activity in the susceptible strain might explain its lower susceptibility to the hydrazine carbamate combinations as described above.

The MAO activity in total body male homogenate was less than the corresponding weight of the females. This result coincides with the known phenomenon of lower susceptibility in female insects. However the MAO activity in sexed adults was less than the activity of both sexes homogenate when mixed. This could be due to more enhanced enzyme activity on mixing the extract of both sexes by providing more favourable medium or cofactors.

Comparisons of MAO activity in homogenates from different body parts showed that the most active site was the intestines followed by the heads while the thorax showed the least activity. This can be explained in the light of the reported facts that high concentrations are incorporated in the intestines to help in detoxifying liberated toxic amines during digestion and putrefaction of nitrogenous compounds (Warner, 1965 and Cheymol and Boissier, 1968).

Graphically, the MAO activity data showed an obvious linear relation between time and amount of benzaldehyde formed as a result of the oxidative deamination of benzylamine. This indicates a typical enzymatic reaction (Neilands and Stumpf 1958).

The optimum pH value was found to be 7.3, which agrees with the data mentioned by Morris (1968) and McEwen (1965). The optimum concentration of benzylamine as a substrate was found to be 3.3 m<u>M</u>. It was noticed that after this concentration the MAO activity decreased on increasing the benzylamine concentration. This might be attributed to the inhibitory effect of the benzylamine when in excess of the optimum concentration (Takagi and Gomi, 1967 and McEwen, 1965).

In addition, the data of Fig. 1 indicated that the oxidative deamination of benzylamine is substrate concentration dependent; thus it represents a first order enzymatic reaction. This conclusion agrees with that of Khamis <u>et</u> <u>al</u>. (1971).

The I_{50} values of syn. II was found to be 3.3×10^{-4} <u>M</u>. This value reveals that syn. II is a median inhibitor if compared with I_{50} values of other inhibitors recorded in the literature. Thus in further studies we can expect getting more powerful synergists for carbamates from the MAO inhibitors.

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2,2-DIMETHYL-1,3-BENZODIOXOL-4-YL N-METHYLCARBAMATE, A NEW BROAD SPECTRUM EXPERIMENTAL INSECTICIDE

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Summary 2,2-dimethyl-1,3-benzodioxol-4-yl N-methylcarbamate (NC 6897) is an experimental insecticide with high residual and contact activity against a wide range of insects. It is of fairly high acute oral toxicity but of low cumulative and dermal toxicity.

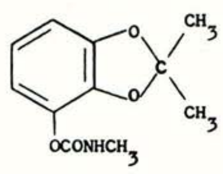
It shows particular promise in trials as an insecticide for use against cockroaches and other insect pests of buildings, with good flushing activity and long residual control.

In crop protection trials, excellent results have been obtained against a number of soil pests including corn rootworm, (*Diabrotica* spp.). Foliar pests showing high levels of susceptibility include a number of coleopterous species, grasshoppers and pests of rice. Very few instances of phytotoxicity have been recorded. A systemic effect has been demonstrated against some species.

Although high levels of activity were apparent in initial laboratory tests on veterinary species, NC 6897 was relatively inactive against resistant strains of the cattle tick (*Boophilus microplus*) and was of short persistence on fleece.

INTRODUCTION

2,2-dimethyl-1,3-benzodioxol-4-yl N-methylcarbamate, code number NC 6897, has the following structural formula :



It was discovered at the Research Centre of Fisons Ltd., Agrochemical Division in 1967. It has since been extensively tested as an experimental insecticide both in the U.K. and overseas for control of pests in crop protection and public health. Recently published references to the activity of the compound include Grayson (1970) who found NC 6897 to be the most effective of ten new materials included in the 1969 cockroach control programme at Virginia Polytechnic Institute, giving excellent residual control of susceptible and resistant German cockroaches (*Blattella germanica*). Barlow and Hadaway (1970) demonstrated that NC 6897 was highly toxic to adult mosquitoes both by topical application and as a residual spray on plywood and plaster.

The effectiveness of NC 6897 against cabbage root fly was referred to by Rolfe (1969).

PHYSICAL AND CHEMICAL PROPERTIES

Molecular weight :	223.23
Appearance :	White crystalline solid
Melting point :	129-130°C
Solubility at 25°C, w/v :	in water 40 ppm, in hexane 350 ppm, in odourless kerosene 0.1%, in o-xylene 1.2%, in benzene 4%, in chloroform 20%, in ethanol 4%, in acetone 20%.
Hydrolysis half-life :	64 hours in 0.01 N sodium carbonate buffer in 20% v/v aqueous ethanol at 25°C and at pH 7.
Vapour pressure :	5.0×10^{-6} mm Hg at 25°C (= 66 ug/m^3) (no toxic vapour at normal working temperatures).
Odour :	None.

MODE OF ACTION

NC 6897 is a contact and stomach poison which is effective by cholinesterase inhibition.

TOXICOLOGY

NC 6897 is of moderately high acute oral toxicity to most mammalian species (LD50 35-100 mg/kg) and of low dermal toxicity (LD50 >400->1000 mg/kg). It is of low cumulative and dietary toxicity to rats, and cats continuously exposed for 33 days in a room treated with 100 mg/m^2 NC 6897 showed no evidence of toxicity or blood cholinesterase inhibition.

BIOLOGICAL ACTIVITY

Initial laboratory screening tests showed that NC 6897 had a broad-spectrum of insecticidal activity. The results indicated that further evaluation would be worthwhile against pest species in all the major insect orders and also against ticks. Only very slight activity was recorded against spider mites.

Further laboratory investigations and field trials have been conducted mainly in the domestic and public health and crop protection fields of use although initially some work was carried out against veterinary species.

Domestic and Public health pests

Particular emphasis has been placed on cockroach control. Screening results indicated a very high level of activity against these insects with excellent persistence. Subsequent laboratory tests in the U.K., U.S.A. and Japan have shown that NC 6897 is very effective in giving rapid and long term control of the following species : German cockroach (*Blattella germanica*), Oriental cockroach (*Blatta orientalis*), American cockroach (*Periplaneta americana*), Japanese cockroach

(*Periplaneta japonica*) and the Brown-banded cockroach (*Supella longipalpa*).

Knockdown is rapid but the rate of knockdown varies with the formulation used. In the laboratory at Chesterford Park knockdown of German cockroaches started 10 minutes after exposure to a deposit of 1 g/m^2 a.i., applied as 80% w.p. on glass, and was complete within 20 minutes.

NC 6897 has some flushing activity. Cockroaches, on first contact with the insecticide deposit, become increasingly agitated until muscular co-ordination is lost and knockdown occurs.

Residual activity is dependent upon the type of surface sprayed and the extent to which deposits have been eroded. Laboratory tests (Table 1) have shown that a deposit of 0.1 g/m^2 a.i., applied as a 1% a.i. suspension of wettable powder, persists best on impervious surfaces such as glass and ceramic tile, but is of relatively short persistence on fresh concrete, as a result of alkaline hydrolysis, and on vinyl floor tiles and gloss paint as a result of absorption of the active ingredient. In practice much heavier deposits are invariably applied so that longer persistence is obtained (see Table 2).

Table 1

The Persistence of 0.1 g/m^2 NC 6897 against Blattella germanica on various surfaces in the laboratory

Surface sprayed	Percent. cockroach mortality (24 hours) at days indicated after spraying: 1	8	29
Glass	100	100	100
Ceramic tile	100	100	80
Brick	100	100	20
Vinyl-coated paper	100	100	5
Hardboard	100	84	5
Polyurethane-coated wood tiles	100	80	5
Emulsion	100	33	5
Vinyl floor tiles	100	12	
Gloss paint	70	30	
Concrete	30		

In U.K. "field" trials many types of premises have been treated including hotels, restaurants, schools, hospitals, private houses, bakeries, supermarkets and office blocks. The 80% w.p. was used throughout the trials and a range of spray concentrations was employed. Frequent observations on numbers of freshly killed cockroaches were made after treatment and a note was made of any live insects. In this way it was possible to assess the duration of effective control, effective control being defined as the achievement of very low numbers of roaches in comparison with the pre-spray numbers. Breakdown of effective control was defined as the point at which live pests re-appeared, or numbers rose significantly. Table 2 gives the mean period of effective control for each concentration employed.

Table 2

Duration of cockroach control with NC 6897 in "field" trials

Conc. NC 6897 as % a.i.	No. of weeks of effective control Range	Mean	No. of Trials
1.0	5-33	20	9
0.8	18-28	20	7
0.5	6-23	11	8
0.4	-	10	1
0.25	5-14	10	10
0.125	3-8	4	4

In general, the higher the concentration the longer the residual action. However, if spray deposits were undisturbed, even a low concentration gave effective control for many weeks.

Trials have also been carried out in East Africa, South Africa, India and Australia and have given results which are in good agreement with those obtained in the U.K.

On the basis of results obtained, for trial purposes, 0.25% has been selected as the recommended spray concentration or, where a longer period of control is required and spray deposits will not be quickly removed by cleaning, a special rate of 0.5% is recommended.

NC 6897 has also been shown to be effective against a wide range of other insect pests of buildings including Pharaoh's ant (*Monomorium pharaonis*), the garden ant (*Lasius niger*), fleas (e.g. *Xenopsylla cheopis*), bed bugs (*Cimex lectularius*), silverfish (*Lepisma saccharina*), the house cricket (*Acheta domesticus*), earwigs (*Forficula* spp.), Wasps (*Vespula* spp.) and the housefly (*Musca domestica*). A number of non-insect species are also controlled by NC 6897 including spiders, woodlice, centipedes and millipedes.

High levels of activity have been demonstrated against a number of mosquito species in the laboratory but no field work has yet been carried out.

Crop pests

Primary screening tests at Chesterford Park Research Station showed that NC 6897 had good persistence on sprayed plants against the vetch aphid (*Megoura viciae*), larvae of the diamondback moth (*Plutella maculipennis*) and large white butterfly (*Pieris brassicae*) and larvae of the mustard beetle (*Phaedon cochleariae*). Tests for systemicity showed that soil treatment controlled vetch aphids for long periods and was also effective against larvae of the large white butterfly and the mustard beetle. Diamondback moth larvae were only moderately well controlled.

Further laboratory and field tests against soil and foliar pest species have been carried out on a wide range of crops in the U.K., Southern France, U.S.A., Japan, South Africa and East Africa. Throughout these trials very vew instances of phytotoxicity have been recorded.

Soil pests

A laboratory test against larvae of the cabbage root fly (Erioischia brassicae) indicated that NC 6897 was slightly less active than the standard chlorfenvinphos, but was of sufficient interest for field trials to be carried out. In U.K. trials on "All the Year Round" cauliflowers in 1970, spot treatments of a 5% granule formulation applied at 0.02 g and 0.04 g a.i. per plant were compared with dimethoate granules applied at the same rates, and chlorfenvinphos granules applied at the standard rate of 0.017 g a.i. per plant. All three treatments were effective in reducing root fly damage, improving growth and vigour and increasing yield of curds. None of them controlled an infestation of caterpillars and only dimethoate controlled a heavy aphid infestation which occurred six weeks after treatment. Chlorfenvinphos gave slightly better control of root fly damage than either dimethoate or NC 6897 but dimethoate produced higher yields of marketable curds mainly because of aphid control. In U.S.A. trials only mediocre results were obtained with NC 6897 where large numbers of cabbage root fly were present.

Extensive field trials have been carried out in the U.S.A. against corn rootworm particularly the Northern corn rootworm (Diabrotica longicornis). At 0.5 and 0.75 lb a.i./ac NC 6897 was as effective as most of the registered insecticides such as carbofuran, mostly applied at higher rates, giving very high yield increases. Excellent control was also obtained at 0.25 lb a.i./ac but in these trials only moderate infestation occurred in the untreated plots. Topical application tests on corn rootworm in the laboratory confirmed activity equal to that of carbofuran. Other soil pests against which high levels of activity have been demonstrated are the onion fly (Delia antiqua), seed corn maggot (Hylemya cilicrura), the banded cucumber beetle (Diabrotica balteata) and symphylids.

Foliar pests

In laboratory tests against the migratory grasshopper (Melanoplus sanguinipes) NC 6897 was more than 8 times as toxic as the standard malathion. Similarly very high levels of activity have been d monstrated against the desert locust (Schistocerca gregaria) and the migratory locust (Locusta migratoria).

Direct spray tests against four aphid species in the laboratory indicated that the bean aphid (Aphis fabae) and the vetch aphid were both highly sesceptible to NC 6897, while the pea aphid (Acyrthosiphon pisum) and the peach potato aphid (Myzus persicae) were relatively insensitive. Field trials have since shown that NC 6897 is ineffective against a number of aphid species and therefore has limited potential as an aphicide.

Field trials in South Africa against the mealy bug (Planococcus citri) on vines have demonstrated that at 0.05%, two sprays at 3-week intervals give superior control to that obtained with the standard parathion. NC 6897 was less effective than the standard methidathion, however, in controlling citrus red scale (Aonidiella aurantii).

In Japan, field trials have shown that NC 6897 is extremely effective against the rice leaf hopper (Nephotettix cincticeps).

Tests against lepidopterous larvae on fruit in the U.K. have shown high levels of activity against Tortrix spp. but relatively poor control of looper caterpillars such as the winter moth (Operaphtera brunata). In Japan, field trials have shown that NC 6897 is very effective against the rice stem borer (Chilo suppressalis). Laboratory tests in the U.S.A. indicated only low levels of activity against the bollworm (Heliothis zea).

High levels of activity have been shown against a number of leaf-feeding beetles. Trials in the U.S.A. and France have demonstrated that NC 6897 gives excellent control of the Colorado potato beetle (Leptinotarsa decemlineata): 0.5 lb a.i./ac has given complete control with good persistence. Promising results have also been obtained against the potato flea beetle (Epitrix hirtipennis), cereal leaf beetle (Oulema melanopus) and the alfalfa weevil (Hypera postica), activity comparing favourably with standard treatments. In laboratory tests in the U.S.A. the boll weevil (Anthonomus grandis) was shown to be relatively insensitive to NC 6897.

Veterinary pests

Initial screening results indicated high levels of activity against the sheep blowfly (Lucilia sericata) and the cattle tick (Boophilus microplus). Persistence of NC 6897 against sheep blowfly on fleece was poor, however, compared with the standard diazinon.

Although high levels of activity were recorded against the susceptible Yeerongpilly strain of the cattle tick, the compound proved to be much less active against the Biarra, Ridgelands and Mackay resistant strains from Australia.

Bee and Fish toxicity

Bee toxicity of NC 6897 was found to be very high. Bee tests showed that direct spray toxicity is approximately ten times greater than that of carbaryl while the toxicity of sprayed clover flowers and of stomach poisoning treatments in honey is approximately five times that of carbaryl.

The LC_{50} of NC 6897 to fish (guppies) at 24 hours was 0.5 ppm and rapid mortality was noted.

DISCUSSION

In experimental trials so far completed NC 6897 shows particular promise as an insecticide for use against cockroaches and other insect pests found in and around buildings. Mosquitoes have also shown high levels of susceptibility in the laboratory and field testing would be worthwhile. NC 6897 has shown a broad-spectrum of activity against crop pest species but because of its high production cost further work in the near future will be limited to testing lower dosage rates.

FORMULATIONS

Experimental formulations are available as follows :- 25% w.p. for use on crops; 80% w.p. (Ficam 80W) for cockroach control; 5% granules for use against soil pests.

Acknowledgements

I wish to acknowledge the work of many of my colleagues at Chesterford Park Research Station whose results have contributed to this paper in particular Mr. K.O. Storey's work on cockroach control. The Staff of the Overseas Research Units and Subsidiary Companies of Fisons Agrochemical Division have provided additional information, and the Kureha Chemical Company Ltd., Japan obtained the results on rice pests.

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THE EVALUATION OF "CELA W 524", A SYSTEMIC FUNGICIDE, FOR THE CONTROL OF POWDERY MILDEW AND APPLE SCAB

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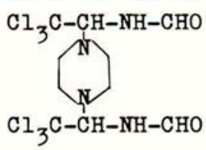
__Summary__ "Cela W 524" was tested in comprehensive field experiments during 197o and 1971 in Europe and South Africa at concentrations between 15o and 3oo ppm a.i.

The product was demonstrated to be promising for the control of powdery mildew and apple scab on foliage and fruit. 13 apple varieties were included in the test program. Occasionally in Golden Delicious russeting was observed under certain conditions. The material also showed a remarkable side effect against O-P-resistent and susceptible spider mites.

INTRODUCTION

At Brighton in 1969 Schicke and Veen first reported on the fungicidal effects of "CELA W 524" the proposed common name of which is triforine [piperazine-1.4-diyl-bis-1-(2.2.2-trichloroethyl)formamide]. Detailed information on the control of horticultural diseases was given by Schicke and Arndt (1971) and on the control of cereal diseases by Schicke __et al.__ (1971). Further information on the efficiency of triforine was published by Drandarevski (1971); Ebenebe (1971); Fuchs __et al__. (197o); Fuchs __et al__. (1971a); Fuchs __et al__. (1971b); Ost __et al.__ (1971); Schlüter and Weltzien (1971). In 197o and 1971 we conducted a series of field tests on the control of apple diseases. The tests included totally 15 apple varieties and 8 important apple growing regions of Germany, Italy and South Africa.



METHODS AND MATERIALS

In all tests triforine was used as a 2o % e.c. 4 to 2o trees per single treatment were sprayed in 4 to 8 randomized blocks. We used a high pressure transportable pump and 2.ooo to 3.ooo l of water per hectar.

Spraying started with the green tip stage of buds and ended about 14 days before harvest. Spraying intervals varied between 1o and 14 days according to local recommendations.

The results on powdery mildew and apple scab were valued at the end of the shoot growth. 1oo to 2oo shoots per treatment were evaluated by a 1-9 scale (BBA 1966; 1=uninfested, 2=<2,5 %, 3=<5 %, 4=<1o % 5=<15 %, 6=<25 %, 7=<33 %, 8=<66 %, 9= 66-1oo % infestation). Fruit

Table 1

Powdery mildew infestation on apple leaves (1-9 scale)

country		Germany				Italy		
region		north	west	southwest	south	north	average	
tests		1	7		2	4	14	%
197o	check	5.4	5.7		5.7	5.6	5.6	2o.o
	triforine 25o ppm	1.7	2.1		3.4	1.1	2.o	2.5
	benomyl 25o ppm	2.1	2.6		3.1	2.3	2.5	3.7
	binapacryl 5oo ppm	2.6	2.7		3.9		3.2	6.o
	dinocap 25o ppm	2.o	2.5		3.7	2.8	2.7	4.3
tests		2	13	4	4	5	28	%
1971	check	5.3	6.9	7.2	5.9	5.8	6.4	29.o
	triforine 25o ppm	1.6	2.2	1.8	1.8	1.3	1.9	2.5
	benomyl 25o ppm	2.4	2.8	3.o	2.4	1.6	2.5	3.5
	dinocap 25o ppm	2.9	2.8	2.8	2.o	2.4	2.6	4.o

sign. diff. p o.o5 : o.4 p o.ol : o.5

varieties: Cox Orange, Golden Delicious, Weißer Klarapfel, Lodi, Goldparmaine, Stark's Early, Imperatore, Jonathan, Boscoop scab and russeting were noted by a 1-4 scale (BBA 1966 scab: 1=uninfested, 2=<o,25 cm^2, 3=<1 cm^2, 4=>1 cm^2; russeting: 1= none, 2=slight, 3= medium, 4= severe). 4oo apples per treatment were checked.

RESULTS

Powdery mildew (Podosphaera leucotricha): 47 tests with medium and severe infestations proved the highly potent effect of triforine against powdery mildew. Experiments with different dosages proved that 15o ppm a.i. was as efficient as dinocap at 25o ppm. 2oo ppm of triforine was as effective as benomyl at 25o ppm. 25o ppm of triforine reduced mildew infestations better than binapacryl (5oo ppm), dinocap (25o ppm) and benomyl (25o ppm) (tables 1, 2, 3 and 4).

Table 2

Powdery mildew infestation on apple-leaves; 1-9 scale

country	South Africa 197o/71					average	
variety *	1	1	2	3	4	1-9	%
check	4.3	7.5	6.9	5.3	4.2	5.7	22
triforine 25o ppm	1.2	1.9	1.5	1.4	1.5	1.5	1.3
benomyl 25o ppm	1.7	4.3	4.3	2.o	1.6	2.8	4.5
binapacryl 5oo ppm	1.7	3.2	3.7	1.9	2.3	2.6	3.8
sign.diff.	p oo5: o4 ; p oo1: o5						

*varieties: 1 - Golden Delicious; 2 - Rokewood; 3 - White Winterpearmaine; 4 - Granny Smith

Treatments with triforine also had a very positive effect on the reduction of primary infections of powdery mildew. In late 1971 we observed that second shoots (August growth) were much less infested when treated with triforine than shoots of untreated plots (table 3).

Table 3

Primary infections of powdery mildew on second shoots, (% shoots of 7 tests)

check	triforine			benomyl	dinocap
-	15o	2oo	25o ppm	25o ppm	25o ppm
55 %	27	15	1o %	14 %	25 %

Spider mites: In several tests, exspecially in Italy, the trees were infested by spider mites being susceptible and resistant to o-p-insecticides. The treatments with triforine reduced the spider mite populations to 10-30 % of those counted in check plots which were only sprayed with o-p-insecticides. Further investigations are in progress.

Table 4

Influence of dosages on the control of mildew and scab; average infestation %; 1971

	triforine 150	triforine 200	triforine 250	triforine 300	benomyl 250	dinocap 250	captan 1000	check	No of tests
powdery mildew	4.0	3.5	2.5	2.5	3.5	4.0		29	33
scab; foliage	4.5	2.0	1.5	1.0	1.5		1.5	33	17
scab; fruits	10.0	2.0	6.0	4.0	4.0		8.0	67	6

Apple scab (Venturia inaequalis): 26 tests with medium to severe scab infestations on leaves were evaluated. In 1970 triforine reduced the infestations as well as benomyl, both at 250 ppm a.i. Captan was less effective. In 1971 triforine worked as well as benomyl (250 ppm) and captan (1.000 ppm). Triforine also performed well at 200 ppm; 150 ppm seemed to be insufficient (tables 4 and 5).

Triforine at 250 ppm, benomyl at 250 ppm, and captan at 1.000 ppm gave the same reduction of fruit scab infestations. Differences between single treatments were not significant. Tests with different dosages in 1971 demonstrate that 200 - 300 ppm of triforine performed as well as 250 ppm of benomyl and slightly better than captan at 1.000 ppm. (Italy).

FACTORS OF EFFICIENCY

Spraying intervals: As mentioned above spraying intervals were 10 to 14 days. In single tests in 1970 intervals of 7 and 14 days were compared. The results are listed in table 6. When extending from 7 to 14 days sufficient control of powdery mildew was obtained by triforine and benomyl at 250 ppm, contrary to plots sprayed with binapacryl (500 ppm) and dinocap (250 ppm) which showed increased infestation. Prolonging the spraying interval from 7 to 14 days reduced the effect against scab with triforine as well as with benomyl, captan and dodine.

Application: Control of scab was achieved with low volume spray too. However as with other fungicides evidence was found that powdery mildew control was improved by using high volume spray.

Climate: In Italy and South Africa corresponding to higher temperatures triforine in 1970 had a better efficiency against powdery mildew and apple scab than in Germany (table 7). Comparing the results of Germany from 1970 (cool, rainy spray season) and 1971 (dry,

Table 5

Scab infestation on apple (leaves: 1 - 9 scale; fruits: % infested fruits)

object		leaf scab						fruit scab				
country			Germany		Italy			Germany		Italy		
region		north	west	south	north	average		west	south	north	average	
tests		1	4	3	2	10	%	4	3	2	9	%
1970	check	5.7	5.8	5.8	9.0	6.4	29.0	48	51	100		61
	triforine 250 ppm	2.3	2.7	3.5	1.5	2.6	4.0	11	11	8		10
	benomyl 250 ppm	3.3	3.1	3.0	2.9	3.0	5.0	11	6	7		8
	captan 1000 ppm	4.5	2.7	3.5	3.3	3.3	7.0	10	9	14		11
tests		1	5	6	4	16	%	6	8	2	16	%
1971	check	5.5	7.4	7.0	5.8	7.1	37.0	43	85	70		65
	triforine 250 ppm	1.5	1.8	1.3	1.3	1.4	1.5	3	2	11		3
	benomyl 250 ppm	1.4	1.5	1.3	1.8	1.5	1.5	0	1	7		1
	captan 1000 ppm	1.5	1.5	1.4	1.7	1.5	1.5	2	1	17		3
sign. diff.		p 0.05:0.3		p 0.01:0.3				p 0.05: 10; p 0.01: 13				

varieties: Cox Orange, Golden Delicious, Weißer Klarapfel, Lodi, Goldparmaine, Imperatore, James Grieve, Boscoop, Jonathan warm spray season) it becomes obvious that the fungicidal effect of triforine and comparative compounds is increased by suitable climatic conditions. Best control of powdery mildew was obtained in Italy and South Africa in 197o (tables 1+2), and the best scab control in the Po valley of Italy with its extreme temperatures and scab infestation.

Table 6

Powdery mildew and apple scab control by prolonged spraying intervals 197o; %Effect of treatment averaged of no of tests

treatment		powdery mildew		apple scab foliage		apple scab fruits	
ppm		7 days	14 days	7 days	14 days	7 days	14 days
triforine	25o	83	84	82	69	83	78
benomyl	25o	78	77	79	71	94	83
dinocap	25o	78	66				
binapacryl	15oo	78	73				
captan	1ooo			82	76	93	88
dodine	45o			82	69	86	74
no of tests		8		7		4	

Table 7

Powdery mildew and scab infestation on apple under different climatic conditions; % effect of treatment

year	1970		1970		1971		1971	
country	Germany		Italy, S.A.*		Germany		Italy	
treatment	mildew	scab	mildew	scab	mildew	scab	mildew	scab
triforine 250 ppm	79	75	95	99	94	96	96	96
benomyl 250 ppm	67	78	79	95	87	97	87	92
dinocap 250 ppm	65		85		87		60	
captan 1000 ppm		75		93		98		93
average temp. °C	13.6		17.9		15.5		18.0	

* season 1970/71

PLANT COMPATIBILITY

Phytotoxicity: Treatments with triforine and other compounds sprayed in our tests caused some burning of the petals when sprayed into full blossom under conditions of extreme temperatures and insolation. Counts of the fruit setting proved that this effect had no negative influence for the final yield.

Young leaves of Golden Delicious when sprayed shortly after petal fall under similar unfavorable weather conditions showed small reddish spots, especially when treatments were combined with liquid mineral fertilizing.

Russeting: (table 8) In areas with frequent russeting in 1970 slightly lower russeting of Golden Delicious was observed when sprayed with triforine than with other tested compounds.

Table 8

Russeting on Golden Delicious; % of fruits injured

country	Germany				Italy	S.Africa	average
region	north	west	s.west	south	north		
tests 1970	1	3			1	2	7
check		44			78	11	39
triforine, 250 ppm	58	26			46	15	30
benomyl, 250 ppm	79	34			39	14	39
captan + dinocap 1000 + 250 ppm	71	38					47
mancozeb+binapacr. 675 + 500 ppm						11	
dodine, 450 ppm		41					
tests 1971	3	4	2	4	3		16
check	61	66	66	69	66		67
triforine, 250 ppm	62	26	4	32	51		36
benomyl, 250 ppm	61	21	10	24	61		35
dinocap, 250 ppm	60	8	9	7	72		27
captan, 1000 ppm	40	26	4	17	50		26
captan + dinocap 1000 + 250 ppm	56	9	9	34	53		35
mancozeb, 675 ppm			34				

In 1971 russeting of untreated Golden Delicious was much more severe than 1970. Russeting of treated plots was much less frequent. In this year fruits treated with triforine showed a slightly higher degree of russeting than fruits sprayed with only captan or dinocap (table 8).

Plant growth: In 1970 we first observed that trees sprayed with triforine produced longer shoots, larger leaves with dark green colour and obviously bigger fruits. Some measurements gave following results (table 9).

Table 9

Influence of triforine on the growth of apple in %; check = 100

country	Germany							Italy	
region	west							north	
variety*	1	2	1	2	4	2	4	2	3
	shoot length		fruit weight		shoot length			foliage seize	fruit weight
check	100	100	100	100	100	100	100	100	100
triforine 250 ppm	117	122	108	110	131	111	119	121	232
benomyl 250 ppm	108	111	100	108	118	106		106	171
capt+dinocap 1000+250 ppm	108	104	100	117	115	113	100	114	184
sign. p 0.05	12		*			12		11	33
diff. p 0.01	17					17		15	48

*varieties: 1-Cox Orange; 2-Golden Delicious;
3-Imperatore; 4-Jonathan

RESIDUES

Residues in apples after a normal series of 8 to 12 treatments and after a period of two weeks between the last treatment and harvest were found below 0.5 ppm. Considering the results of 90-days-feeding studies on dogs and rats which proved the low toxicity of the substance, these residues are not toxicologically relevant. 2 years feeding studies on rats and dogs as well as teratogenic and 3 generation studies on rats are in progress.

DISCUSSION

The above results prove the usefulness of Triforine for the control of apple diseases.

The fungicide demonstrates an excellent efficacy against powdery mildew and apple scab; the effect against powdery mildew is more distinct. The results obtained with triforine against powdery mildew in both years and all regions were significantly superior to the comparison products being on the market already. The effect against scab equalled that of other materials used.

The relation between success of the treatment and the climatic conditions is interesting. It is evident that the effect at higher is better than that at lower temperatures. This is concluded from the comparison between the warmer year 1971 and the colder one 1970 in Germany and from a comparison of the warmer regions Italy and South Africa with the more temperate region Germany. This finding is valid for all fungicides being used. The observation of russetings in the field in 1970 and 1971 suggests a dependence of russeting from biotop and climatic conditions in a distinct stage of growth of the fruit.

Trees treated with triforine show a stimulation of foliage and stem growth correlated with good fungitoxic efficacy. In this connection triforine was superior to products used in comparison.

The good biological properties of triforine linked with favourable toxicological findings and results of residue studies seem to open excellent practical possibilities for the use of triforine in controlling apple diseases.

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

RESULTS WITH "BAS 3270 F" (76 943)

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Abt. Entwicklung u. Anwendung

Pflanzenschutz, Limburgerhof

Summary:

This report deals with trials with the seed dressing BAS 3270 F, which contains 50% active ingredient (2,5 dimethyl-furane-3-carboxylic acid cyclohexylamide). The range of effectiveness of BAS 3270 F is mainly against the Basidiomycetes, such as Ustilago nuda, U. tritici and Tilletia tritici. The efficacy against Fusarium nivale and Helminthosporium spp. is somewhat poorer. Combination with other fungicides effective against Fusarium and Helminthosporium is necessary in order to make BAS 3270 F a good universal mercury-free seed dressing. One of these combinations, 2,5-dimethyl-furane-3-carboxylic acid cyclohexylamide with maneb, is currently being tested under the code name BAS 3302 F.

INTRODUCTION

For many years two economically important cereal diseases of barley and wheat, Ustilago nuda and U. tritici, have been controlled by hot-water treatment of the seed, although this method is rather time-consuming and somewhat risky due to possible damage to the germ.

The lack of a systemic fungicide for the control of these diseases was not for want of trying, but not until 1966 was hot-water treatment of cereals superseded by the introduction of oxathiin-derivatives (by UniRoyal Inc.). However, the efficacy of oxathiin-derivatives is in principle restricted to the Basidiomycetes, and pathogens of other classes, such as Fusarium spp. and Helminthosporium spp., have to be controlled by the addition of other effective fungicides. The use of methanol (Ustilgon), applied as wet seed treatment, is said to be successful only on spring barley (Schlör 1970). In the last few years many trials have been carried out with benomyl. According to observations available to date, this product is not fully effective in the case of quite heavy infestation with loose smut. Its total lack of efficacy against Helminthosporium is an additional disadvantage.

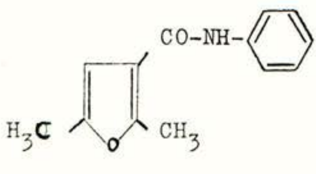
We have worked with seed dressings for several years and examined among other fungicides the derivatives of the furane carboxylic acid. We reported on the results of the trials with these derivatives on the occasion of the International Plant

Protection Conference (Paris, 1970). As the tolerance of this substance on some varieties of barley and under certain climatic conditions was somewhat critical, we carried out trials with further furane carboxylic acid derivatives. Of these, the 2,5-dimethyl-furane-3-carboxylic acid-cyclohexylamide ++ was tolerated even by sensitive varieties of barley.

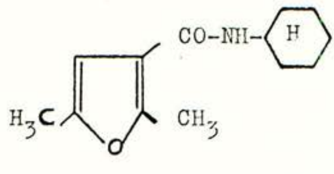
The results of the trials with these derivatives in the laboratory, greenhouse and out of doors are as follows.

MATERIAL AND METHODS

The trials with the aforementioned 2,5-dimethyl-furane-3-carboxylic acid-anilide were carried out under the code name BAS 3191 F. It is a seed dressing with 50% active ingredient.



BAS 3191 F



BAS 3270 F

The seed dressing of 2,5-dimethyl-furane-3-carboxylic acid cyclohexylamide is code-named BAS 3270 F.

Footnote: ++ This compound was synthetized by Dr. H. Distler and Dr. R. Widder, of Badische Anilin-u. Soda-Fabrik AG, Ludwigshafen.

The melting-point of 2,5-dimethyl-furane-3-carboxylic acid cyclohexylamide is 104-105°C. The active ingredient is practically insoluble in water, but soluble in most organic solvents. The mammalian toxicity is low: the acute oral LD 50 amounts to > 6400 mg/kg for rats and > 8000 mg/kg for rabbits.

The trials with BAS 3270 F for the control of loose and covered smut were carried out with 3 replicates on outdoor plots of 1.5 $m.^2$ We used naturally infected seed of barley and wheat for the loose smut trials, and as far as covered smut trials were concerned the wheat was inoculated before the trials with 2 g spores per kg. seed. In the greenhouse, we examined the efficacy of BAS 3270 F against snow mould (*Fusarium nivale*) on naturally-infected rye according to the method issued by the BBA (Federal Institute of Biology, Berlin, 1966). The trials for the assessment of the germinating power and thriving capacity were also carried out according to the regulations of the BBA. We examined BAS 3270 F in comparison with BAS 3191 F on different varieties of spring barley and at different temperatures, in order to gain knowledge on the influence of BAS 3270 F on the germination and the foliar development.

In these trials 100 grains of barley, which had been seed-treated, were sown into steamed compost (in pots for transplanting) at a depth of 2 cm and at temperatures from 8 to 10°C, 20 to 22°C and 25 to 27°C. Assessment of germination was done every 3 days and the length of the leaves was measured. The trials at temperatures between 8 and 10°C ran for 27 days and the trials at 20 to 22°C and 25 to 27°C for 15 days.

In vitro tests were carried out to determine whether the active ingredient of BAS 3270F is also effective against other economically important diseases.

We set up a series of dilutions of the compound in acetone, and these were admixed with dextrose-potato agar in petri dishes to give final concentrations of 3, 6, 12, 25, 50 and 100 ppm per plate. Each fungus was tested against all 6 concentrations, and the tests were set up in triplicate. The agar plates were inoculated in the centre with a 0.5 ccm section of mycelium from a culture that was a few days old. The fungi tested were Fusarium nivale, F. culmorum, Helminthosporium gramineum, H. sativum, H. teres, Septoria nodorum, Cercosporella herpotrichoides and Ophiobolus graminis.

Depending on speed of growth at 25°C the diameter of the mycelia was measured after a growth period of 6 to 12 days and we ascertained those concentrations of active ingredient which are 50% fungistatic in comparison with the growth of fungi in the control without active ingredient additive.

RESULTS AND DISCUSSION

In table I, we describe the efficacy of BAS 3270 F against Ustilago nuda in barley, U. tritici in wheat and Tilletia tritici in wheat. The rates of application were 250 g seed dressing per 100 kg seed. The results show that the control was very good for a moderate infection of loose smut and for a heavy infection of covered smut. When assessing the yields of the trial plots, there was no unfavourable influence on the 1000-grain weight and the size of grain in comparison with the control plots. Trials carried out on rye against snow mould in the "cold" greenhouse from 22.12.70 to 11.3.71 reveal that 3270 F effects a 75% reduction of infection compared with the control plot, but the results are not as good as with fuberidazole and benomyl.

Table 2

Since BAS 3270 F is intended for use in various cereals, 3 varieties of barley and wheat and one variety of rye were treated with 250 g of the seed dressing per 100 kg seed, and the influence on the germinating power and thriving capacity was examined. Results are given in table 3. They prove that the active ingredient is tolerated well by the types of cereals tested.

Table 3

As mentioned in the introduction, spring barley did not tolerate BAS 3191 F in some cases. This resulted in delayed emergence and stunted growth. As we believe that this is mainly due to temperature, we carried out germination trials on 4 varieties of spring barley at lower and higher temperatures. In order to present table 4 more clearly, we have given only the final results of two temperatures.

Table 4

At higher temperatures no differences were observed between the emergence and foliar growth with BAS 3270 F and BAS 3191 F. While BAS 3270 F did not have any adverse effect on any of the varieties examined, even at the relatively low temperatures of germination, BAS 3191 F caused considerable delay and checking of foliar growth. The decrease amounts to as much as 36% in the variety Amsel. At lower temperatures we observed a delay in emergence of only 3 days compared with the control.

In connection with the efficacy against Ustilago and Tilletia, we were interested to determine whether and to what extent the active ingredient of BAS 3270 F is effective against other economically important pathogens of cereals. The effectiveness of 2,5-dimethyl-furane-3-carboxylic acid cyclohexylamide was ascertained in an agar plate test.

Table 5

Table 5 shows that even comparatively small quantities of active ingredient can inhibit fungi of cereals which do not belong to the Basidiomycetes. We can see its efficacy against Fusarium nivale, already confirmed by the trial in the greenhouse. The active ingredient of BAS 3270 F is less effective against leaf stripe of barley, caused by Helminthosporium gramineum.

In order to achieve a good control of a heavy infestation of Fusarium nivale in rye, or Helminthosporium gramineum in barley, it is necessary to combine BAS 3270 F with oxine-copper, dithiocarbamates, thiuramdisulphides, captan or other fungicides that are effective against Fusarium and Helminthosporium. A combination of 2,5-dimethyl-furane-3-carboxylic acid cyclohexylamide with maneb is being tested under the code name BAS 3302.

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TABLE I

Effectiveness of "BAS 3270 F" against Ustilago nuda on barley and U. tritici and Tilletia tritici on wheat (outdoor trials on small plots)

Variety	Disease	Date of: a) Seed treatment b) Assessment	Loose/covered smut in.......% Rate of application 250 g/100 kg of grain	
			BAS 3270 F	Control
Barley susceptible strain	Ustilago nuda	26.10.70 28. 5.71	0	6,7
Winter wheat var. "Capelle"	Ustilago tritici	9.11.70 21. 6.71	0	5,6
Winter wheat var. "Heine VII"	Tilletia tritici	3.11.70 20. 7.71	0	44

TABLE II

Effectiveness of BAS 3270 F against Fusarium nivale on rye
(var. Carstens winter rye)

Seed dressing Rate of application 250 g/100 kg of grain	% Fusarium-infected plants
"BAS 3270"	7
Fuberidazole	3
Benomyl	1
Control	28

Seed treatment: 22.12.70. Assessment: 11.3.71.

TABLE III

Influence of BAS 3270 F on the germinating power and thriving capacity of different varieties of cereals.

Seed dressing

Rate of application 250 g/100 kg of grain	Type of cereal	Germinating power......%	Thriving capacity......%
		Average	
		after 10 days.	after 15 days.
BAS 3270 F	Barley	98	95
Control		98	94,5
BAS 3270 F	Wheat	99	97
Control		99	98
BAS 3270 F	Rye	96	92
Control		95	92

TABLE IV

Influence of temperature on the development of spring barley after seed treatment with BAS 3270 F.

Variety of spring barley	Germination and growth at.......°C					
	8-10°C			20-22°C		
	Control	BAS 3270 F	BAS 3191 F	Control	BAS 3270 F	BAS 3191 F
Firlbeck's Union	16 cm=100	100	75	21 cm=100	114	114
Rika	13 cm=100	107	77	22 cm=100	108	108
Iris	13 cm=100	100	77	23 cm=100	100	113
Amsel	14 cm=100	100	64	24 cm=100	100	95
	Assessment after 27 days			Assessment after 15 days		

595

TABLE V

Effect of 2,5-dimethyl-furane-3-carboxylic acid-cyclohexylamide on the growth of the mycelium of various fungi.

Agar plate test

Fungus	Amount of a.i. required to achieve 50% fungistatic effect on various fungi in cereals 3 6 12 25 50 100 ppm
Fusarium nivale	=====
Fusarium culmorum	==================
Helminthosporium gramineum	===============
Helminthosporium sativum	==========
Helminthosporium teres.	===============
Septoria nodorum	=======================
Cercosporella herpotrichoides	===============
Ophiobolus graminis	==========

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

"HOE 2960", 2-(O,O-DIETHYL THIONOPHOSPHORYL)-4-PHENYL-1,3,4-TRIAZOL, A NEW ACARICIDE AND INSECTICIDE

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Agricultural Chemicals Department, Hoechst U.K. Limited

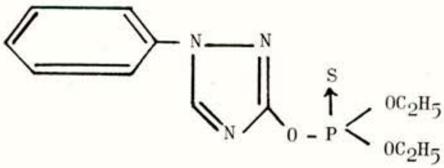
Summary Hoe 2960 is the trials code number for a new acaricide and insecticide discovered by Farbwerke Hoechst A.G. in 1967 and introduced to the United Kingdom experimentally in 1969.

Initial field work with a 40% emulsion was concentrated on the control of Panonychus ulmi on apple, the product proving highly effective against summer stages of all strains tested, including those resistant to other organophosphates, at rates down to 0.02 a.i.

INTRODUCTION

Physical and Chemical Properties

2-(O,O-diethyl thionophosphoryl)-4-phenyl-1,3,4-triazol has the following structure:



HOE 2960

$C_{12}H_{16}O_3N_3PS$ M.W. 313

Physical Form:	Light brown-yellow liquid
Solubility:	Soluble in almost all organic polar solvents Solubility in water: 39 ppm at 23°C.
Analysis:	By gas chromatography without decomposition
Formulations:	40% e.c., 2% and 5% granules and 5% oil formulation

Toxicology

Table 1

Route	Animal	LD50, Hoe 2960 a.i., (mg/kg)
Acute oral	Rat	82
	Dog	320-350
Dermal	Rabbit	280
Acute intraperitoneale	Rat	107 (1 application)
	Rat	160 (5 applications of 32)

An LC100 of 0.1 - 0.2 ppm was established after 48 hours at a water temperature of 22-24°C for Lebistes reticulatus (Guppy).

Mode of Action

Hoe 2960 acts as a contact and stomach poison. It penetrates deeply into plant tissue without being systemic. Initial effects are recognisable several hours after application while the duration of action is, as a rule, 2 - 3 weeks.

Vulić et al. (1970) reported on 500 field trials throughout the world covering more than 120 species of pest in over 70 crops. Trials conducted with 46 commercial preparations from the groups of chlorinated hydrocarbons, phosphoric esters and carbamates showed that Hoe 2960 would stand comparison with any.

In the United Kingdom, observations in extension trials suggest that Hoe 2960 is highly effective against other pests of fruit including Laspeyresia pomonella, Archips podana and hemipterous pests of which Eriosoma lanigerum is highly susceptible.

Field trials with the granular formulations have also given good control of a range of soil active pests including Erioischia brassicae, Pemphigus bursarius and Psila rosae.

FIELD TRIALS

1969

A single trial was carried out at a site in Essex where spiders were known to be resistant to phosphoric esters. Materials were applied on 11.9.69 and assessment of living motile stages made on 17.9.69.

Table 2

Site: Fingringhoe

Cv. Worcester

Treatment %	Mean no. of Mites/Leaf
Untreated	46.9
Hoe 2960 0.04 a.i.	0.4
" 0.40 a.i.	0.2
S.E. (P=0.05) ±	8.72

The very high rate of chemical caused no phytotoxicity to the foliage of the test trees.

1970

Hoe 2960 was included in trials at two heavily infested sites at Cliffe and Higham, Kent. The sites received blanket applications of an experimental, non-acaricidal O.P. fungicide "Hoe 2873" (2-(0,0-diethyl thionophosphoryl)-5-methyl-6-carbethoxy-pyrazolo-(1,5,a) pyrimidine) at 0.015 a.i. on 8 - 10 day mildew control programmes commencing pink bud. Acaricides were applied to randomised plots in these areas, programmed applications of binapacryl and dinocap being included for reference purposes.

Regular assessments of surviving mites yielded the following results:

Table 3

Site: Cliffe

Cv. George Cave

Treatment %	Mean no. of Mites/Leaf					
	Date					
	14/5	26/5	10/6	25/6	9/7	27/7
Binapacryl 0.05*	12.3	15.0	16.9	18.5	27.0	32.7
Dinocap 0.03*	14.6	15.3	18.0	21.1	30.4	34.6
Hoe 2960 0.02 a.i.**	22.8	5.7	4.8	10.9	19.8	10.8
" 0.04 a.i.**	18.6	3.7	2.4	9.4	14.0	3.4
" 0.06 a.i.**	19.5	1.9	0.9	3.6	6.0	1.0
Tetradifon 0.01**	22.7	4.2	10.3	18.5	23.8	7.4
S.E. (P=0.05)±	3.78	1.84	1.53	2.89	6.25	2.13

*Applied May 9+9+12+9+9+10+13 days

**Applied May 18, June 8 and July 10

Table 4

Site: Higham

Cv. Cox/Lambourne

Treatment %	Mean no. of Mites/Leaf				
	Date				
	9/6	17/6	27/6	10/7	27/7
Binapacryl 0 05*	19.8	26.7	34.2	46.0	-
Dinocap 0.03*	13.4	19.4	35.9	49.4	-
Hoe 2960 0.02 a.i.**	7.2	11.8	13.4	58.5	14.4
" 0.04 a.i.**	4.6	8.1	7.0	29.0	6.8
" 0.06 a.i.**	1.7	4.9	2.5	23.3	3.2
Tetradifon 0.01**	7.3	19.4	13.7	43.1	12.8
S.E. (P=0.05)±	2.73	2.00	4.87	11.13	6.01

*Applied May 7+8+8+10+9+9+11+12

**Applied May 18, June 8, July 10

Despite the very high infestation pressure at both sites and the non-contributory effects of the Hoe 2873 mildew programme, Hoe 2960 gave good control of the pest particularly at the higher rates of use.

1971

Further studies were made of the efficacy of Hoe 2960 at several sites in Kent and East Anglia with emphasis placed on elucidating the knockdown and residual effects observed in the earlier trials.

Comparisons were often drawn with the equivalent effects of dicofol at sites where no resistance was known to exist to the latter.

Results computed to date are presented in Table 5.

Table 5

Site	Treatment %	Mean no. of Mites/Leaf					
		Days after application*					
		0	5	9	21	32	50
Linton Kent	Untreated	7.2	8.2	9.3	10.5	10.0	17.6
	Hoe 2960 0.02 a.i.	7.8	0.4	0.3	0.6	0.9	4.7
	" 0.04 a.i.	5.7	0.1	0.1	0.3	0.5	2.9
	Dicofol 0.04	7.5	0.7	0.5	0.7	1.1	6.1
	S.E. (P=0.05)±	-	0.51	0.17	0.43	0.34	2.10
Hartlip Kent	Hoe 2960 0.02 a.i.**	5.0	0.2	0.3	8.1	10.7	21.1
	" 0.04 a.i.	11.1	0.3	0.2	2.1	4.0	9.3
	Dicofol 0.04	11.5	0.4	0.1	2.7	4.9	14.2
	S.E. (P=0.05)±	-	0.26	0.02	0.72	0.86	3.04

*Applied 24/6 at Linton: 22/7 at Hartlip

**Repeat of application on 26/5

Data presented in Table 5 indicate that 0.04 a.i. Hoe 2960 was at least equivalent to dicofol in initial kill effect and gave better control over extended periods of time. The 0.02 a.i. rate, although good in initial effect, lacked the residual power of the higher rate and some pockets of foliar bronzing were observed at the 50 day post treatment stage. This was also true of the dicofol treatments but no damage was noted where 0.04 a.i. Hoe 2960 had been used.

In a smaller, unreplicated plot trial at Newington, Kent, good correlation was found between the application rate of Hoe 2960, initial mite mortality and subsequent plant protection as shown in Table 6. Even at the highest rate of use no phytotoxicity was noted on foliage or fruit of test trees. Figures are the mean of counts on 6 trees/plot.

Table 6

Site: Newington

Treatment %	Mean no. of Mites/Leaf — Days after application on 22/7: 0	2	4	11	21	28	40	Cv.
Untreated	8.8	9.0	11.0	13.1	16.4	18.0	23.6	Cox
	9.6	10.2	10.0	11.3	16.9	21.3	26.0	Worc.
Hoe 2960 0.02 a.i.	6.8	1.4	0.1	0.1	0.6	3.2	10.0	Cox
	10.2	1.8	0.2	0.4	2.6	5.4	11.8	Worc
" 0.04 a.i.	5.8	0.3	0.0	0.0	0.3	1.7	6.3	Cox
	13.1	2.1	0.3	0.0	0.5	2.1	8.9	Worc.
" 0.08 a.i.	11.2	0.1	0.0	0.0	0.1	0.7	2.5	Cox
	7.4	0.0	0.0	0.0	0.2	1.3	4.4	Worc.
" 0.12 a.i.	6.5	0.0	0.0	0.0	0.0	0.3	1.3	Cox
	6.8	0.0	0.0	0.0	0.1	0.4	0.9	Worc.

DISCUSSION

In field and laboratory experiments in the United Kingdom Hoe 2960 has been tested against strains of Panonychus ulmi known to exhibit low levels of response to one or more of a range of current products including phosphoric esters. There has been no evidence to suggest that such resistance traits have impaired the activity of the material which has consistently given rapid and prolonged kill under diverse conditions.

These preliminary results suggest that good control of red spider mite would be obtained if a rate of approximately 0.04 a.i. (equivalent to 16 fl. oz of commercial product/100 gal) were employed at any time from early season. At this concentration some significant levels of mite mortality have often been observed up to six weeks after application.

Hoe 2960 is not only unique in its ability to control O.P. resistant and O.P. susceptible pests with almost equal effect (Vulić et al. 1970) but also in its spectrum of activity. Work throughout the world has shown the following to be highly susceptible to the effects of the material:

Tetranychidae	Agromyzidae	Tenthredinidae
Aphididae	Anthomyiidae	Noctuidae
Psyllidae	Gracilariidae	Lymantriidae
Cicadina	Hyponomeutidae	Pieridae
Heteroptera	Tortricidae	Coleoptera-Nitidulidae
Cecidomyidae	Coccoidea	Coleoptera-Chrysomelidae

Work in the United Kingdom has shown Hoe 2960 to be non-phytotoxic to a wide range of crops when applied by itself or in combination with current fungicides. The material appears safe on all major apple and pear cultivars even at abnormally high rates of use and in this sphere Hoe 2960 should prove invaluable on a commercial level.

Acknowledgements

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METHOMYL - A NEW CARBAMATE INSECTICIDE FOR USE ON HOPS

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Summary Methomyl, a new systemic insecticide, has shown good activity against organophosphorus resistant strains of damson-hop aphid (*Phorodon humuli*) now infesting hop gardens. In replicated and grower trials carried out in 1970 and 1971 foliar sprays of methomyl at 8 oz a.i./100 gal (226g/455 l.) HV generally gave superior control to that given by other compounds.

INTRODUCTION

The damson-hop aphid (*Phorodon humuli*) is the most serious insect pest of hops and, when not controlled, can cause heavy annual losses.

Migration of winged aphids (alatae) from their overwintering hosts, *Prunus* spp., occurs between late May and the end of June. The aphids settle on the underside of the hop leaves where they produce wingless females (apterae), popularly referred to in their early stages of development as 'nits' and in their later stages as 'cows'. The cows breed rapidly to produce more nits and several generations of cows and nits may occur during the season. These weaken the hop plant by their extraction of its sap and their secretion of honeydew results in the growth of sooty moulds which impair the photosynthetic activity of the leaves. The aphids may also infest the cones, greatly reducing their value.

Hop growers have had difficulty in controlling the damson-hop aphid with organophosphorus insecticides since 1962 (Gould 1965). Dicker and Muir (1971) reported that aphids collected from wild hops in Yorkshire were much more susceptible to demeton-S-methyl than were aphids from cultivated hops in Kent and Herefordshire. In recent years attention has been turned to compounds of the carbamate group to evaluate their effectiveness in controlling organophosphorus resistant aphids.

Methomyl (*S*-methyl *N* -[(methylcarbamoyl)oxy] thioacetimidate) is a carbamate insecticide with systemic and contact properties. This compound was first evaluated in the U.K. in 1967 as a 90% s.p. under the code number D.P. 1179 and in 1970 as a s.c. (3 lb a.i. per U.S. gal). In 1971 trial work on hops was carried out with a 25% w.p. formulation, available commercially in Europe as Lannate methomyl insecticide for the control of a broad spectrum of pests on grapes, top fruit, sugar beet, cotton, ornamentals, potatoes and a number of other vegetable crops.

PROPERTIES

Structural formula: $CH_3-\underset{\substack{|\\ S-CH_3}}{C}=NO\overset{\substack{O\\ \|}}{C}-NHCH_3$

Characteristics: White crystalline solid with slightly sulphurous odour.

Melting point: 78° - $79^{\circ}C$

Specific gravity: 1.2946

Vapour pressure: 5×10^{-5} mm of Hg @ $25^{\circ}C$
1.6×10^{-4} mm of Hg @ $40^{\circ}C$

Solubility at $25^{\circ}C$

Solvent	Grams/100 grams solvent
methanol	100
acetone	73
ethanol	42
isopropanol	22
water	5.8
toluene	3

Stability: Stable in solid form and in aqueous solution.

Toxicity: The LD50 for methomyl by oral administration to fasted male and female white rats is 17 and 24 mg/kg, respectively. Eye irritation studies with rabbits showed only mild conjunctivitis and no corneal injury. The Approximate Lethal Dose (ALD) by skin absorption with rabbits was greater than 1500 mg/kg.

DEVELOPMENT OF METHOMYL

The aphicidal activity of methomyl was first demonstrated on hops by Dicker (1969) in 1968. He found that 0.5 g a.i. per hill as a soil drench gave maximum control of damson-hop aphid about five days after treatment and control remained at this level for about ten days. However mortality did not reach the 100% levels achieved by dimefox drench treatments. The following year Dicker (1970) again evaluated dimefox at 0.5 g a.i. per hill but compared it with methomyl at the increased rate of 1 g a.i. per hill. These rates gave comparable control, giving over 80% mortality for four weeks. In addition, Dicker found that foliar sprays of methomyl gave effective control of aphids in small-scale field trials.

It was indicated that methomyl would be effective at concentrations around 0.05% a.i. In 1970 replicated small plot trials were conducted to determine effectiveness of various rates around this concentration. Additionally some larger unreplicated treatments were carried out.

1970 TRIALS

a) <u>Replicated trials with foliar spray</u>

<u>Method and materials:</u>

Two trials were carried out in mid Kent. Treatments were as follows:-

1. Methomyl 4 oz a.i./100 gal (113g/455 l.)
2. Methomyl 8 oz a.i./100 gal (226g/455 l.)
3. Methomyl 12 oz a.i./100 gal (340g/455 l.)
4. D.P. 1642 S-methyl N-(carbamoyloxy) thioacetimidate 8 oz a.i./100 gal (226g/455 l.)
5. Demeton-S-methyl 58% w/v 12 fl. oz product/100 gal (341cc/455 l.)
6. Propoxur 50% 1½ lb product/100 gal (679g/455 l.)
7. Untreated.

All chemical treatments were applied HV to run off. Treatments were replicated three times in randomised blocks. In one trial (DP 6) spacing was 6'6" ($2m^2$) square and each plot contained 16 hills. In the other trials (DP 7) spacing was 6'6" x 3'3" ($2 \times 1m^2$) and plots contained 24 hills. Trials were assessed by counting aphids on 25 young leaves per plot.

<u>Results:</u>

Log (x + 1) transformations were made on aphid numbers. Results and analyses are given in Table 1.

b) <u>Unreplicated trials with foliar sprays</u>

<u>Method and materials:</u>

Methomyl was applied to 3 plots in mid Kent. Spraying was done with growers' machinery and each plot was 1 acre. In all cases the remainder of the garden was treated with methidathion. Applications of methomyl were as follows:-

Site 1: 8 oz a.i./100 gal, 2 applications at 100 gal/ac plus 3 applications at 150 gal/ac (1680 l./ha)
Site 2: 8 oz a.i./100 gal, 3 applications at 75 gal/ac
Site 3: 12 oz a.i./100 gal, 1 application at 75 gal/ac

<u>Results:</u>

Growers were pleased with the results of methomyl treatments at 8 oz a.i./100 gal and considered results were equivalent to or better than those given by methidathion. The single application of 12 oz a.i./100 gal gave absolute control of a severe infestation and the hops did not need respraying for 3 weeks.

c) <u>Replicated trials with soil drenches</u>

<u>Method and materials:</u>

Two trials were carried out in mid Kent. Treatments were as follows:-

1. Methomyl 1 g a.i. per hill, 4 fl oz drench per hill
2. Methomyl 1.5 g a.i. per hill, 4 fl oz drench per hill
3. Methomyl 2 g a.i. per hill, 4 fl oz drench per hill
4. Dimefox 50% w/v 1% drench, 4 fl oz drench per hill
5. Untreated

Drenches were applied to the crown of each hill. Treatments were replicated four times in randomised blocks. Plots contained 16 hills.

Table 1

Log (x + 1) transformations on mean number of nits and cows per 25 young leaves

(means for cows shown in parenthesis)

Trial reference	DP 7	DP 7	DP 6	DP 6	DP 6	DP 6
Date assessed	17th June	30th June	22-23rd June	1st July	7th July	17th July
Days from spraying	5	18	6-7	15	6	16
1. Methomyl (4 oz)	2.83 (1.34)	3.16 (2.50)	2.18 (1.05)	2.80 (1.50)	0.36 (0.10)	1.54 (0.30)
2. Methomyl (8 oz)	2.09 (0.84)	2.70 (2.07)	1.98 (0.73)	2.60 (0.86)	0.26 (0)	1.31 (0)
3. Methomyl (12 oz)	0.77 (0)	2.27 (1.24)	1.82 (0.10)	2.54 (0.98)	0.10 (0)	1.25 (0.10)
4. D.P. 1642	2.55 (1.45)	3.50 (2.81)	2.15 (1.07)	2.53 (0.69)	0.10 (0)	1.14 (0.16)
5. Demeton-S-methyl	2.68 (1.37)	3.46 (2.72)	2.40 (1.14)	2.93 (1.54)	0.86 (0.30)	1.69 (0.72)
6. Propoxur	2.68 (1.45)	3.07 (2.61)	1.90 (0.30)	2.67 (1.18)	0.42 (0)	1.31 (0.26)
7. Untreated	4.17 (2.79)	- -	3.36 (2.56)	3.72 (2.84)	3.17 (2.35)	3.67 (2.66)
L.S.D. (+ 0.05)	1.25 (1.04)	0.83 (1.05)	0.53 (1.00)	0.59 (1.08)	0.93 (0.41)	0.50 (0.56)

Results:

Dimefox and methomyl failed to give adequate control, probably due to the drought conditions. In one trial dimefox gave best control whereas in the other methomyl was superior at all rates. Treatment means were not significantly different.

Conclusions from 1970 trials

Trials with foliar sprays showed that methomyl at 12 oz a.i./100 gal was the best treatment followed by methomyl at 8 oz a.i./100 gal. The results of replicated and unreplicated trials indicated that methomyl at 8 oz a.i./100 gal HV was superior to the other compounds used at recommended rates. In view of the high level of control achieved by this treatment it was decided that this should be the standard rate for further work in 1971.

Drench treatments of dimefox and methomyl were unreliable under dry conditions and it was apparent that work in the immediate future should be concentrated on further evaluation of foliar sprays.

1971 TRIALS

Method and Materials:

18 growers were asked to apply methomyl through their own sprayers on 1-2 acres of hops in order to compare it with the standard aphicide(s) used on the remainder of the garden. 17 sites were in Kent and 1 in Herefordshire. Growers were requested to apply methomyl at 8 oz a.i. per 100 gal (226g/455 l.) of water on the same days as the comparative treatments were made. Comparative products were applied according to their label recommendations. The timing of the sprays and the quantity of spray mix per acre were left to the growers'discretion. Treatments were applied on average every 10 days and spray volumes ranged from 40 to 200 gal/ac (448-2240 l./ha). Generally, the first one or two applications were made at 50 gal/ac (560 l./ha) and thereafter at 100 gal/ac (1120 l./ha) or more.

All growers used methidathion, an organophosphorus compound, in at least part of their spray programmes and consequently the majority of comparisons were with this compound. Other compounds used by growers included endosulfan, an organochlorine, and propoxur, a carbamate. The remainder were organophosphorus compounds (as listed in table 2).

Aphid infestations occurred at all sites. Immigration of alatae began about the first week in June, reached a peak at the end of June and tailed off be the end of July. Occasional flies were seen in August. Assessments of aphid infestations were made in the trials immediately before each spraying when possible. Nits and cows were counted on 40 or 50 leaves randomly selected from different hills. Leaves from the 2nd, 3rd and 4th newest pairs of leaves were taken from hop shoots.

Results:

Table 2 shows the comparative results of methomyl and standard aphicide treatments. In the table, monthly means have been derived from between 1 and 4 assessments carried out each month at every site. It was observed that methomyl achieved excellent initial knockdown of aphids.

Table 2

Monthly mean aphid numbers of nits and cows/10 leaves
(numbers of cows given in parenthesis)

M = methomyl C = comparative product

Site ref.	Locality	June M	June C	July M	July C	August M	August C
a) C= methidathion							
P1	Yalding	35 (0)	183 (0.9)	18 (0)	30 (0.1)	0 (0)	0 (0)
P2	Canterbury	3 (0.2)	9 (0.2)	72 (0)	91 (0.4)†	-	-
P3	Selling	451 (0.3)	222 (0)	461 (24.2)	374 (28.1)†	-	-
P5	Selling	-	-	18 (0.1)	146 (1.5)	0 (0)	0 (0)
P6	Paddock Wood	370 (10.3)	975 (45.3)	51 (0)	85 (0.8)†	-	-
P7	Goudhurst	114 (2.1)	527 (16.2)	528 (17.3)	391 (13.0)	11 (0.3)	37 (3.3)
P8	Goudhurst	58 (0)	157 (0.1)	3 (0)	33 (0.2)	1 (0)	4 (0.3)
P9	Hereford	7 (0)	1041 (1.5)	138 (0)	821 (110.8)†	-	-
F1	Lamberhurst	-	-	201 (6.1)	121 (0.1)	39 (0.4)	22 (5.0)
F2	Marden	-	-	88 (10.5)	124 (12.6)†	-	-
F3	Paddock Wood	6 (2.0)	10 (2.0)	49 (1.0)	144 (8.2)	10 (0.2)	11 (1.2)
F4	Hadlow	-	-	394 (39.8)	182 (33.6)	0 (0)	12 (2.8)
F6A	Staplehurst	-	-	-	-	4 (0.3)	6 (0.7)
F8	Faversham	3 (0)	10 (0)	46 (0.3)	104 (5.9)	0 (0)	<1 (0)
F9	Faversham	5 (0)	4 (0)	217 (7.7)	281 (10.3)	85 (4.8)	420 (50.2)
b) C= propoxur							
P5	Selling	-	-	18 (0.1)	90 (0.1)	0 (0)	0 (0)
c) C= endosulfan							
P6	Paddock Wood	370 (10.3)	623 (23)	51 (0)	307 (6.4)†	-	-
F6	Staplehurst	-	-	60 (1.4)	104 (0.8)	-	-
d) C= demephion							
P4	Canterbury	89 (0)	254 (2.0)	84 (0)	509 (5.5)†	-	-
e) C= demeton-S-methyl + TEPP							
F10	Canterbury	-	-	82 (20.2)	97 (37.0)†	-	-
f) C= mevinphos							
P9	Hereford	-	-	7 (0)	25 (0.8)	10 (0)	38 (19.5)
g) C= dimefox*							
F7	Tenterden	40 (0)	278 (0)	-	-	-	-

* methomyl and dimefox were applied 1 and 15 days respectively before assessment.

† further assessment not carried out because of change in spray programme.

Table 3 shows the relative performances, derived from 80 assessments of nit numbers, of methomyl and standard aphicides in commercial use.

Table 3

Number of assessments indicating relative performance of methomyl to standard aphicides

	Methomyl superior	Methomyl inferior	Methomyl equal	Total no. of assessments per month
June	17	2	1	20
July	32	6	12	50
August	8	2	0	10
Total	57	10	13	80

DISCUSSION

Systemic activity of methomyl in hops was first observed in 1968 by Dicker when the material was applied as a soil drench. In the following year he found that methomyl also controlled aphids when applied as a foliar spray. Until 1962 demeton-S-methyl was used extensively by growers to control aphids. Since then growers have found it increasingly difficult to achieve satisfactory control with this organophosphorus compound, due to the aphids developing resistance to it. As methomyl is a non-organophosphorus compound Dicker's results indicated that it could become a valuable spray for use against organophosphorus-resistant aphids.

Replicated trials carried out in 1970 showed that methomyl sprays gave good aphid control. The general order of effectiveness of treatments as determined from 6 assessments of these trials was: methomyl 12 oz a.i. rate, methomyl 8 oz a.i. rate, propoxur, methomyl 4 oz a.i. rate, demeton-S-methyl, untreated. All the compounds significantly reduced aphid numbers and the high rate of methomyl was significantly better than demeton-S-methyl. Other differences were mostly insignificant. Assessments indicated that although the highest rate of methomyl gave best control, the middle rate also gave excellent control of cow aphids. Good control of the latter is necessary because survivors can breed rapidly resulting in quick reinfestation of hop gardens.

The organophosphorus compound methidathion was widely used to control aphids on hops in 1971. This product was not included in the comparative treatments in the 1970 replicated trials, but many comparisons were made in grower trials in 1971. Of 80 assessments made of nit numbers, methomyl was superior to methidathion on 41 occasions and only inferior on 10 occasions. In comparison with other products in grower trials methomyl always gave better control.

In summary, the trials have shown that a programme of methomyl treatments at 8 oz a.i./100 gal HV throughout the seasons contained aphid infestation to commercially acceptable levels, resulting in clean hops at the end of the season.

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A HORMONE-LIKE MOSQUITO LARVICIDE WITH FAVORABLE ENVIRONMENTAL PROPERTIES

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Summary The metamorphosis of all instars of mosquito larvae was blocked at the early pupal stage by exposure to 0.1 ppm aqueous solution of 2,6-di-t-butyl-4-(α,α-dimethylbenzyl) phenol. Fourth-stage larvae are most sensitive: 92 percent were killed as early pupae after being exposed to the chemical for one hour at 1.0 ppm. It is suggested that the chemical is acting by interfering with the normal oxygen utilization of the pupae.

The compound is highly selective to mosquito larvae; it is non-toxic to fish and mammals and is biodegradable.

INTRODUCTION

The harmful effects of persistent chemicals on the environment prompted investigators to look for new, safe and biodegradable insecticides. Among the new groups of chemicals, insect hormone mimics have shown promise as insect control agents (Bower et al., 1966; Bower, 1968 Wigglesworth, 1969). For the most part, these chemicals are analogs of the authentic hormone and act in a similar manner (e.g., Spielman and Williams, 1966; Srivastava and Gilbert, 1968). This paper presents a new chemical with a juvenile hormone-type activity which is chemically unrelated to the insect hormone. The chemical, designated as MON-0585, is a highly selective, non-toxic and biodegradable mosquito larvicide. In contrast to many hormone analogs that lack potential utility as pesticides, experiments with 2,6-di-t-butyl-4-(α,α-dimethylbenzyl)phenol indicate that it may well have practical importance as a new type of insecticide (Sacher, 1971).

METHOD AND MATERIALS

Bioassay - Yellow fever mosquito larvae (*Aedes aegypti*) were used for all the laboratory evaluations. The procedure (Sacher, 1971) consisted of continuous exposure of second instar mosquito larvae to the chemical through the larval and pupal stages. Variation of the test included the use of third and fourth instar larvae and short-term exposure to the chemical followed by transfer to distilled water.

Tyrosinase Studies - To study the effect of MON-0585 on purified mushroom tyrosinase (Sigma Chem. Co., St. Louis, Mo.) and to determine the levels of tyrosinase activity in treated and untreated mosquitoes pupae, we use the chronometric method described by Miller and Dawson (1941) and by Wilkinson (1965).

Approximately 200 fourth instar larvae were treated with 2.0 ppm of MON-0585. When 70% of the larvae started to pupate (and die), we rinsed them several times with distilled water.

Eight grams of mosquito pupae were ground one minute in a Waring blender with 10 ml of cold distilled water. The crude homogenate was filtered through muslin cloth and the filtrate was spun at 3200 xg for 10 minutes in a refrigerated centriguge (0-4°C). The supernatant was collected, diluted to 30 ml and stored in an ice box until ready to use.

RESULTS

Slide 1 will show the morphogenetic effects of MON-0585 on *Aedes aegypti* larvae. All stages of mosquito larvae, when exposed to the chemical, seem to develop normally through their larval instars. However, metamorphosis is blocked in the early stages of pupation and the resulting prepupae die in a characteristic compact, stalky and unmelanized form.

The results of variation in the exposure time of mosquito larvae to MON-0585 are shown in Figure 2. In this test larvae were exposed to the chemical for a specific length of time and were subsequently transferred to distilled water for completion of their life cycle.

The LC_{50} for 2nd, 3rd and early 4th instar larvae under continuous exposure to MON-0585 were calculated as 0.065, 0.05 and 0.04 ppm, respectively.

The results of the inhibition studies with mushroom tyrosinase are summarized in Table 1 and in Figure 3. The I_{50} of MON-0585 was calculated from the graph to be 1.7×10^{-4} M.

A comparison between the tyrosinase titer in treated and untreated yellow fever mosquito pupae is shown in Table 2. The mean value of tyrosinase in the treated and untreated mosquitoes was 24.6 and 21.4 units per gram fresh weight, respectively.

DISCUSSION

Activity of MON-0585 against Mosquitoes - Sacher (1971) has noted that regard less of the stage in which the mosquito larvae were treated with MON-0585, identical response was obtained in all cases; complete larval development followed by early pupal death. Furthermore, in contrast to conventional insecticides, late instars were as susceptible to the chemical as early instar. It is interesting to note that even at the high dose level, little or no mortality occurred at the larval stages.

The results of the variation in exposure time (Figure 2) show that fourth instar larvae are most sensitive to the chemical and as little as one hour exposure at 1.0 ppm was sufficient to elicit 92% response. Early instar larvae show a gradation of response, thus as much as 24 hours exposure were required to show the above effect in the second instar.

Favorable Environmental Properties - In light of the recent concern about the harmful effects of the persistent, non-specific and toxic pesticides to the environment, MON-0585 appears to have many properties which are of great importance for a modern insecticide.

MON-0585 is a highly selective mosquito larvicide. The chemical is equally effective against susceptible, organo-chlorine resistant and organophosphate resistant mosquitoes. However, MON-0585 is practically inactive against other organisms including non-target aquatic and terrestrial species. Furthermore, it is practically non-toxic to fish and is extremely safe to mammals (Table 3)

The acute rat oral LD_{50} is 1890 mg/kg and the rabbit dermal minimum lethal dose is greater than 3160 mg/kg.

MON-0585 contains only carbon, hydrogen and oxygen, thus the chances of environmental persistence and contamination are very small. Indeed, both bioassay and gas chromatography have shown that the half-life of 10 ppm MON-0585 in soil was approximately two days. The chemical has, however, sufficient hydrolytic and photostability to control mosquito larvae in the field. Preliminary small plot field tests indicate that MON-0585 gives complete control of mosquito larvae at approximately 1.0 lb/A.

Studies on the Mode of Action of MON-0585 - In an attempt to determine the mode of action of MON-0585 we have suggested a variety of mechanisms for its action, one of these being the inhibition of the enzyme tyrosinase which is known to be a key factor in the hardening and darkening of the insect cuticle (Karlson and Sekeris, 1964). Furthermore, it was shown that the titer of this enzyme increases in insects during their larval stage with a peak of activity in the early pupal stage (Aziz, 1967). The fact that MON-0585 manifests its effect in the early pupal stages and the occurrence of the unmelanized (white) pupae suggested that tyrosinase might be affected by this chemical.

The principle of the assay is to determine the catecholase activity of tyrosinase by measuring the length of time required to liberate a sufficient amount of O-benzoquinone to oxidize a known amount of ascorbic acid. O-benzoquinone is reduced immediately in the presence of ascorbic acid. When the ascorbic acid is completely oxidized, the O-benzoquinone remains unchanged and its first appearance can be measured by means of a starch iodine indicator solution.

The molar concentration for 50% inhibition of mushroom tyrosinase (I_{50}) by MON-0585 was calculated as 1.7×10^{-4} M. Although this level of activity is not very high, it is possible that tyrosinase is one of the biochemical lesions of this chemical.

Contrary to the results anticipated, a comparison between the tyrosinase titer in MON-0585 treated and untreated yellow fever mosquito pupae shows a somewhat higher enzyme concentration in the former than in the latter. The mean value of tyrosinase in the treated and untreated mosquitoes was 24.6 and 21.4 units per gram fresh weight, respectively.

The interaction between oxygen and MON-0585 was studied to determine if the chemical interferes with the normal oxygen utilization by the larvae and the early pupae. It was noted that affected pupae seemed to have difficulties staying at the water surface and breathing normally. Consequently, we suggested that high oxygen concentration in the treated medium might reverse the effects of MON-0585.

Fourth instar larvae were treated with 0.08 ppm (approximately LC_{90}) of MON-0585 under continuous bubbling of oxygen. In contrast to the expected typical white pupae resulting from the exposure to the chemical, these pupae melanized prior to their death. These results might indicate that the lack of melanization and possibly the lethal effects of MON-0585 are connected with interference of oxygen utilization by the pupae.

In summary, MON-0585 is a specific, low toxicity material for control of mosquito larvae at their breeding sources. It is a new type of material which exhibits a different mode of action against immature mosquitoes. The compound appears to have a relatively low order of environmental persistence and a potential for minimal environmental contamination.

It is suggested that MON-0585 acts by interfering with the normal oxygen metabolism by the pupae.

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Table 1

Inhibition of mushroom tyrosinase by MON-0585

I	Ascorbic Acid	(Q) x 10^5	(1/Q) x 10^{-4}	t	(1/t) x 10^3
0	1.0	0.57	17.6	21.9"	45.6
	2.0	1.14	8.77	51.2"	19.5
	2.5	1.42	7.04	71.1"	14.1
	3.0	1.70	5.88	93.4"	10.7
	5.0	2.84	3.52	303.1"	3.3
7.5 x 10^{-5}	2.0	1.14	8.77	54.6"	18.1
	3.0	1.70	5.88	92.6"	10.8
	5.0	2.84	3.52	303.0"	2.9
1 x 10^{-4}	2.0	1.14	8.77	50.6"	16.6
	2.6	1.42	7.04	56.0"	12.6
	3.0	1.70	5.88	82.9"	10.9
1.7 x 10^{-4}	2.0	1.14	8.77	88.1"	11.4
	2.5	1.42	7.04	111.0"	9.0
	3.0	1.70	5.88	133.3"	7.4
2.7 x 10^{-4}	2.0	1.14	8.77	132.1"	7.5
	2.5	1.42	7.04	166.7"	6.0
	3.0	1.70	5.88	194.3"	5.2

I	(S) x 10^{-6}	(1/S) x 10^7	% Inhibition
0	3.24	3.08	0
7.5 x 10^{-5}	3.44	2.91	7
1.0 x 10^{-4}	4.08	2.44	20
1.7 x 10^{-4}	6.65	1.51	51
2.7 x 10^{-4}	11.12	0.90	71

I = Molar concentration of MON-0585.
Q = Moles of O-quinone produced.
t = Time to end point in seconds.
S = Slope of reciprocal plot.
1/S = Initial reaction velocity.

Table 2

Tyrosinase activity from MON-0585 treated and untreated Aedes aegypti pupae

O-quinone produced (Moles x 10^5)	Time to end point (sec.)	Initial velocity	Catecholase activity+ units/grams fresh wt.
CONTROL MOSQUITO			
0.14	11.8		
0.28	17.5		
0.57	23.3		
0.85	33.8		
		2.5×10^{-7}	21.4
TREATED MOSQUITO			
0.14	9.5		
0.57	21.2		
0.85	29.4		
		2.94×10^{-7}	24.6

+ "Catecholase unit" = amount of enzyme required to take up 10 mm^3 of oxygen per minute during the oxidation process.

Table 3

Toxicology of MON-0585

Fish	LD_{50}	MLD[a]
Rainbow trout		10 ppm[b]
Black bullhead		10 ppm
Guppy		10 ppm
Carp		10 ppm
Green sunfish		50 ppm
Goldfish		50 ppm
Bluegill		50 ppm
Gambusia	ca. 15 ppm	
Mammals		
Rat acute oral	1890 mg/kg	
Rabbit acute dermal		3160 mg/kg

(a) Minimum lethal dose.

(b) Twenty-four hours reading for trout, 96 hours for other fish.

FIGURE 2

EFFECT OF VARIATION OF EXPOSURE TIME OF MOSQUITO LARVAE TO MON-0585

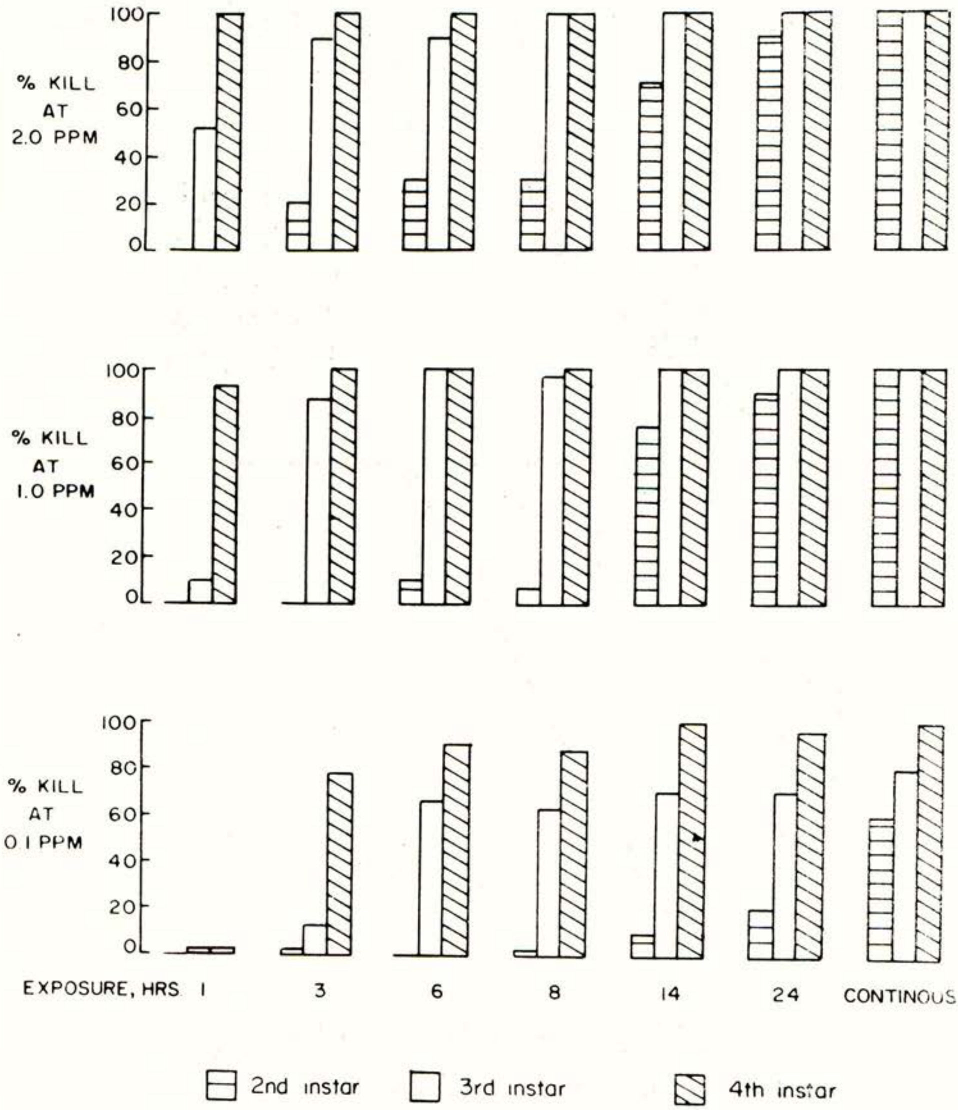
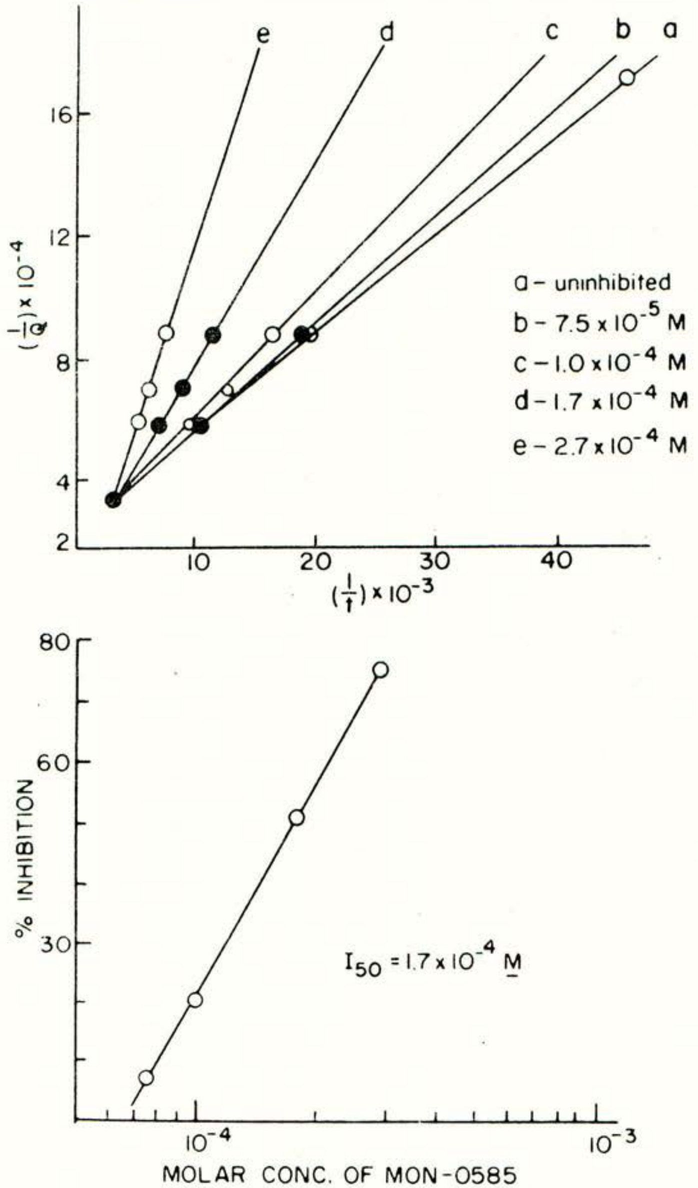


FIGURE 3

INHIBITION OF MUSHROOM TYROSINASE BY MON-0585



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A PRELIMINARY INVESTIGATION OF A POTENTIAL NEW ALGICIDE

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Summary One of the major problems facing mankind is the pollution of "economic water". A particular aspect of this, known as eutrophication, is increased algal growth resulting from the higher levels of nutrients in water. A possible method of combating this is the use of algicides. The potential of chlorophthalonil as an algicide is examined. It is shown that this compound is effective against a range of algae including *Chlorella*, *Chlamydomonas*, *Ulothrix*, *Anabaena*, *Oscillatoria* and *Microcystis* at low concentrations - often less than 0.001 ppm. It is also effective on natural populations of algae obtained from lakes, rivers and reservoirs. The effect is generally less after 300 hours than after 150 hours and is dependent upon the size of the initial cell inoculum. The work carried out is evaluated by comparison with a list of criteria for an "ideal algicide".

INTRODUCTION

Amongst the many problems facing mankind one which is receiving a great amount of attention is that of pollution. Governments in many countries are setting up Departments of the Environment, Environmental Protection Agencies, Councils of Environmental quality and so on. In the United Kingdom the Royal Commission of Environmental Pollution published its first report earlier this year and singled out certain priorities for action and enquiry. The first of the listed priorities for action was "provision to meet the increased demand for water .....".

The pollution of water involves not only water supply for domestic and industrial purposes but also its amenity value. A particular aspect of pollution of water which occurs in some countries concerns increased growth of algae. This problem, which is known as eutrophication, is due to the higher levels of nutrients in water over the last twenty or thirty years. The main nutrients concerned are believed to be nitrogen, phosphorous, organic matter and vitamins especially vitamin B_{12} (see for example Rohlich (1969) and Milway (1970)). It is important to distinguish between the major problem of gross pollution arising from untreated sewage, industrial and agricultural waste products etc. and the lesser problem of eutrophication. However eutrophication is undoubtedly increasing. In the water industry it leads to such unwanted effects as filter blockage in reservoirs, fish death as a result of toxin production, fish death as a result of deoxygenation of water due either to algal respiration, or during bacterial decay of algal cells, tainting of water, and development of foul odours.

Many expedients have been proposed to accomplish a reduction in algal growth and some are being tried. These include some which will, in our opinion, be ineffective. For instance it has been calculated that removal of phosphates from detergents will not decrease levels of phosphate in water to those which will limit algal growth (Anon (1968)). Other suggestions including avoiding the use of chemical fertilizers and reverting to an organic system of agriculture would seem to be impractical.

We have been approaching the question of excess algal growth from a different point of view - that is to find out if it can be controlled by the use of algicides at concentrations which will be harmless in all other ways.

Algae which are a nuisance in water are many and varied. Some of the major problem organisms are outlinedin Table 1. The unicellular green algae rarely cause great difficulty but filamentous ones such as *Cladophora* and *Ulothrix* often cause filter blockage and are also a problem in sewage works. Increased growth of diatoms has not been a particular feature of recent interest in eutrophication probably because their growth is limited by silica which has not been an element considered important as a cause of eutrophication. Nevertheless, large spring and autumn blooms of diatoms occur in most bodies of fresh water which may cause filter blockage when at their peak. Without much doubt the major group of problem organisms are the blue-green algae which "bloom" in tremendous quantities during summer months. They are responsible not only for filter blockage but also for odour and taste production, toxin production, and deoxygenation of water.

Table 1

Major algal nuisances in the water industry

Alga	Type	Main Problems Caused
Chlorella *Chlamydomonas*	Unicellular green	
Cladophora *Ulothrix* *Spirogyra*	Filamentous green	Filter blockage
Diatoma *Asterionella* *Tabellaria* *Fragilaria*	Diatoms	
Anabaena *Oscillatoria* *Microcystis* *Aphanizomenon*	Blue green	Filter blockage Odour production Toxin production Deoxygenation

Since the problem of increased algal growth involves such a wide range of species any successful algicide should have as wide a range of activity as possible. In particular it must be active against blue-green algae. This is but one of a large number of criteria which should be satisfied before an algicide is used on a wide scale (Table 2). Since algae are vital in aquatic ecosystems, being the primary producers, it is important that an algicide should really act more as an algistatic agent slowing down growth and spreading out an algal "bloom" over a longer time period. If this could be achieved water treatment plants could cope with the problem.

It may well be impossible for any compound active against algae to live up to all the idealised criteria given in Table 2 but nevertheless all of them should be considered in the screening of potential algicides.

Table 2

Criteria for an ideal algicide

1. Must be active against as broad a spectrum of Algae as possible but particularly against Blue-Green Algae.
2. Should preferably be algistatic as opposed to Algicidal.
3. Must be active sufficiently long to be effective and then be degraded leaving no toxic end products.
4. Must be easy to store, handle and apply.
5. Must be easily assayable.
6. Must be non-toxic to fish at active concentration.
7. Must be non-toxic to Zooplankton.
8. Must be non-accumulative either in the environment or in tissues of higher organisms.
9. Must produce no taste or odour problems in treated water.
10. Must be non corrosive and harmless to equipment.
11. Must be effective in widely different weather conditions.
12. Must be economical in use.

This paper describes some of the work so far carried out in examining the a: cidal properties of the compound chlorophthalonil and indicates the potential of this compound as an additional means of combating eutrophication.

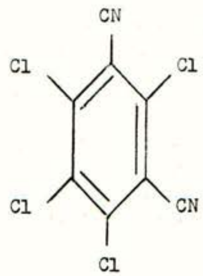
MATERIALS AND METHODS

1. The potential algicide

Chlorophthalonil (Fig. 1) is a white crystalline solid which is both odourless and tasteless. It is thermally stable at normal temperatures and is chemically stable in alkaline and acidic aqueous media. It is marketed as a fungici by the Diamond-Shamrock Co. under the trade name "Daconil" (see British Patent 1,058,557). In the current work it was used as a solution in acetone.

Figure 1

CHLOROPHTHALONIL *



* a suggested common name for tetrachloroisophthalonitrile

2. The effect of Chlorophthalonil on growth of algae in laboratory culture

The algae used were pure cultures of Chlorella pyrenoidosa, Anabaena variabilis, Chlamydomonas dysosmos, Oscillatoria sp, Microcystis sp and a mixed culture containing at least 95% Ulothrix sp. The first two algae were grown in simple inorganic media (Goulding and Merrett (1966)) and the others in inorganic medium supplemented with 0.1% yeast extract.

Aliquots (50 ml) of sterile medium contained in 250 ml cotton wool stoppered flasks were aseptically inoculated with the alga, obtained from stock cultures, to give an initial cell density of 500 cells mm^3. Cell numbers were estimated on a haemocytometer. Chlorophthalonil was added to give a final concentration ranging from 1 to 0.0001 ppm. Controls received an equivalent amount of acetone. The cultures were incubated at 25^o with constant shaking and constant illumination at 5,000 lux. Growth was assessed after 150 and 300 hours by withdrawing samples and estimating cell numbers. All experiments were carried out at least in triplicate and repeated at least once.

3. The effect of Chlorophthalonil on growth of algae in samples of natural waters

The effect of chlorphthalonil on growth of natural populations of algae was assessed by collecting samples from reservoirs, lakes and rivers. Within three hours of collection 1500 ml samples (four replicates) were dispensed into 2000 ml beakers and chlorophthalonil added as above. The beakers were placed outdoors in a fully exposed position. Samples were taken at two or three day intervals for estimation of cell numbers.

4. The effect of Chlorophthalonil on $^{14}CO_2$ fixation by natural populations of algae

Aliquots (10 ml) of reservoir or lake waters were placed in Thunberg tubes and chlorophthalonil added. The samples were illuminated for six hours at 25^o (8000 lux) and then kept in the dark for 18 hours. The tubes were shaken, 10 µmoles of sodium bicarbonate containing 2 µci of ^{14}C added and the samples reilluminated.

After two hours the cells were removed by centrifugation, acidified to expel excess bicarbonate, washed, boiled and transferred to a liquid scintillation counting vial and radioactivity determined. Experiments were carried out in duplicate and repeated.

RESULTS

The effect of chlorophthalonil on the growth of Chlorella, Chlamydomonas and Ulothrix is shown in Table 3. All concentrations tested except 0.0001 ppm had an effect on the growth of each alga. With Chlorella 50% inhibition was recorded at 0.1 ppm after 150 hours, but inhibition was less after 300 hours. Chlamydomonas reacted slightly differently to Chlorella in that it was more resistent to lower concentrations of chlorophthalonil. On the other hand 1 ppm caused complete inhibition. Microscopic examination showed that Chlamydomonas underwent drastic morphological change typified by cell lysis, when treated with 1 ppm chlorophthalonil for 18 hours.

Ulothrix being a filamentous alga was rather more difficult to handle, particularly in obtaining accurate cell counts. However the results (Table 3) show that inhibition was total at 1 ppm and it was observed that at this concentration the filament rapidly yellowed and died. Unlike any of the other species tested inhibition was greater after 300 hours than after 150 hours. The reason for this is not obvious but may reflect the lower metabolic rate of filamentous algae compared to unicellular forms.

The effect of chlorophthalonil on the growth of Anabaena, Microcystis and Oscillatoria is shown in Table 4. These blue green algae were all more susceptible than Chlorella, the most sensitive of the green algae. Microcystis was totally inhibited by 0.0001 ppm after 150 hours and 95% inhibited after 300 hours. Anabaena was almost totally inhibited at all concentrations tested after 150 hours but showed more resistance after 300 hours. Even then 0.1 ppm produced total inhibition of growth. Similarly Oscillatoria was totally inhibited by 0.1 ppm. This organism was the most resistant of the blue green algae but once again inhibition was less after 300 hours than after 150 hours.

Since these studies were carried out using cultures starting with a low initial inoculum it was decided to repeat the experiments using much higher concentrations of cells to start with. These experiments were designed to see if such high initial numbers would lead to a rapid absorption of the low levels of chlorophthalonil, apparently effective in controlling algal growth, with consequent loss or lessening of effect.

The results obtained using cultures of Anabaena variabilis starting with inocula of 500 cells/mm^3 or 5000 cells/mm^3 are shown in Table 5. They show a marked difference depending on initial inoculum of cells. Thus at 500 cells mm^3 growth was totally inhibited after 150 hours at concentrations of 0.001 ppm and above and 95% inhibited at 0.0001 ppm. In contrast, using an initial inocula of 5000 cells mm^3, 0.01 ppm only caused 35% inhibition after 150 hours and 25% after 300 hours. No inhibition whatsoever was observed after 300 hours at 0.001 ppm and below. Inoculum size therefore is critical with Anabaena. Similar results have been obtained with Microcystis, Chlorella and Oscillatoria.

Table 3

The effect of Chlorophthalonil on the growth of three green algae

(Figures are % inhibition to the nearest 5% and are means of three replicates)

Alga	Time of Exposure (hours)	Concentration of Chlorophthalonil (ppm)				
		0.0001	0.001	0.01	0.1	1.0
Chlorella	150	0	15	35	50	75
	300	0	15	40	40	40
Chlamydomonas	150	0	15	15	65	100
	300	0	35	50	65	100
Ulothrix	150	0	5	55	80	100
	300	15	30	50	75	100

Table 4

The effect of Chlorophthalonil on the growth of three blue green algae

(Figures are % inhibition to the nearest 5% and are means of three replicates)

Alga	Time of Exposure (hours)	Concentration of Chlorophthalonil (ppm)				
		0.0001	0.001	0.01	0.1	1.0
Anabaena	150	95	100	100	100	100
	300	55	60	95	100	100
Oscillatoria	150	50	75	85	100	100
	300	25	50	70	100	100
Microcystis	150	100	100	100	100	100
	300	95	100	100	100	100

Table 5

The effect of inoculum size on Chlorophthalonil inhibition of Anabaena variabilis

(Figures are % inhibition to the nearest 5% and are means of three replicates)

Time of Exposure (hours)	Inoculum Size (cells/mm^3)	Concentration of Chlorophthalonil (ppm)				
		0.0001	0.001	0.01	0.1	1.0
150	500	95	100	100	100	100
	5000	0	15	35	100	100
300	500	55	60	95	100	100
	5000	0	0	25	100	100

All the results so far given refer to work done with "unnatural" systems using artificial culture medium and culture conditions and it was obvious that these studies should be extended to natural populations of algae. Some preliminary studies have already been carried out using water from five locations. These include 2 reservoirs, a natural lake, a man-made lake and a highly polluted river.

Table 6 shows that results of a typical experiment involving a reservoir sample. This sample contained mostly blue green algae but a few diatoms were present. Rapid growth occurred in the controls such that a "bloom" of a blue green algae occurred which reached a peak in four days. This growth was controlled by chlorophthalonil but by 13 days all the samples had reached the control maximum value.

Table 6

The effect of Chlorophthalonil on growth of natural populations of algae

(Figures are % inhibition to the nearest 5% and are means of four replicates)

Concentration of Chlorophthalonil (ppm)	Time from start of experiment (days)					
	2	4	6	8	11	13
0.0001	80	50	20	0	0	0
0.001	90	80	40	0	0	0
0.01	100	85	50	15	0	0
0.1	100	90	70	35	0	0
1.0	100	100	65	50	20	0

Since this type of study is relatively long term, the possibility of evaluating the effect of chlorophthalonil on algae in natural water by a more rapid method was sought. One such method involved the use of $^{14}CO_2$ fixation over a two hour period in the light as outlined in the materials and methods section. Table 7 shows the result of an experiment using water from a natural lake. They show that the results obtained by the $^{14}CO_2$ method are very similar to those obtained by the cell number method after seven days exposure. The potential of this technique is being examined further.

Table 7

A comparison of cell number method and $^{14}CO_2$ method for estimating effectiveness of Chlorophthalonil

(Figures are % inhibition to the nearest 5% and are means of two replicates)

Concentration of Chlorophthalonil (ppm)	Cell Number Method	$^{14}CO_2$ Method
0.0001	10	0
0.001	25	20
0.01	70	65
0.1	95	85
1.0	100	95

DISCUSSION

Table 2 outlines a series of idealised criteria for an effective algicide. An attempt has been made in the current work to examine some of these points with respect to the compound chlorophthalonil. This compound has been shown to be effective against a broad range of algae including unicellular and filamentous green algae (Table 3) and blue green algae (Table 4). In particular it is effective against blue green algae the major problem organisms in the water industry. Chlamydomonas and Ulothrix were the least affected organisms but even with these organisms growth was inhibited by 50% after 150 hours by 0.01 ppm. This concentration is way below the quoted figure of 0.25 ppm which is the estimated LC50 for rainbow trout (Diamond-Shamrock Daconil specification).

In nearly all cases chlorophthalonil was less effective after 300 hours than after 150 hours. This may seem at first sight to be a deficiency in the property of the compound as an algicide. However, as indicated previously, it is important that algae is a water supply are not eliminated but rather their growth spread out over a longer period of time. This would seem to be the case in the studies so far carried out. This includes the work with natural algal populations (Table 6) in which growth was spread over 8 to 13 days depending on concentration compared to 4 days for the control. This may not be a long enough spreading effect and it could be necessary to use repeat doses. Such studies are now being undertaken.

The observation that inoculum size makes a marked difference in the results obtained (Table 5) is an important one since it will, to some extent, dictate how chlorophthalonil can be used as an algicide. Algal blooms develop rapidly from low initial numbers over about four weeks. Fortunately one can predict to within a few weeks when an algal bloom will develop and careful monitoring of numbers of a given species at this time will reveal when a bloom is commencing. If chlorophthalonil is eventually used as an algicide it will be necessary to apply it early in the development of a bloom. It will be of little use attempting to treat a bloom when it has fully developed except at relatively high concentrations which could be dangerous to fish and in any case would be much less economical.

Concerning other points in Table 2 it is known that chlorophthalonil is easy to store, handle, apply and detect. Its mammalian toxicity as determined in acute oral LD50 determinations for rats, dogs, rabbits and Mallard ducklings is extremely low (Diamond-Shamrock Daconil Specification). It is odourless, tasteless and non-corrosive and because it is active at such low concentrations it would be economical in use.

Thus such data as at present available certainly indicates the possibilities that chlorphthalonil could be a very valuable tool in combating the effects of eutrophication.

Acknowledgements

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