THE ACTION OF BENOMYL ON GERMINATING CONIDIA OF BOTRYTIS FABAE

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Summary An electron microscopical study has been made of the morphological changes induced by benomyl in germinating conidia of *Botrytis fabae*. Both freeze-etched replicas and chemically fixed sections were examined. There is less development of endoplasmic reticulum and an increase in vacuolation in treated cells. Endoplasmic reticulum tends to occur in treated cells as vesicular elements close to the plasmalemma rather than as multiple strands surrounding the nuclei. The swollen and distorted germ tubes produced by sub-lethal concentrations of benomyl are attributed less to a specific action on the cell wall than to an inhibition of other metabolic processes.

INTRODUCTION

Benomyl [methyl-*N*-benzimidazol-2-yl-*N*-(butylcarbamoyl) carbamate] is a promising new fungicide which has systemic activity against verticillium wilt of cotton (Erwin, 1969), and also controls apple scab and apple mildew (Byrde *et al*, 1969). Although the compound is active against a wide range of fungi it does not control diseases caused by phycomycetes (Delp and Klopping, 1968).

Clemons and Sisler (1969) have recently shown that benomyl is broken down rapidly in aqueous solution to methyl-*N*-benzimidazol-2-yl-carbamate, a stable compound as toxic to *Neurospora crassa* and *Rhizoctonia solani* as the original compound. Toxicant concentrations that ultimately prevent mycelial growth do not prevent germination nor do they appreciably inhibit initial increase of dry weight.

To investigate the site of action of the fungicide the cytological changes occurring in benomyl-treated germinating conidia of *Botrytis fabae* have been investigated by electron microscopy using both chemically fixed sections and freeze-etched replicas.

MATERIALS AND METHODS

Fungus Conidia from 7-9 day cultures of *B. fabae* were collected and washed as previously described (Fisher and Richmond, 1969). Germinating conidia were obtained by shaking a conidial suspension (5×10^6/ml) in 0.05% sucrose solution at 25°C for 18 h.

Transmission electron microscopy Conidia were fixed in 2% permanganate, dehydrated through a graded acetone series and embedded in vestopal. Sections were stained with lead citrate (Reynolds, 1963) and viewed in an AEI EM6B microscope.

Freeze-etching Conidia were suspended in 15% glycerol for 1 h, frozen in liquid Freon 12 and then treated as described by Moor (1966).

Scanning electron microscopy Suspensions of germinating conidia on small cover slips were fixed in 2% OsO_4 for 30 min, dehydrated in a graded acetone series and allowed to dry. The cover slip was then stuck to the metal specimen holder, coated under vacuum with gold-palladium and viewed in the Stereoscan electron microscope.

Benomyl A sample was prepared by recrystallization with ethanol from material kindly suppled by the Du Pont Co., (U.K.) Ltd.

RESULTS

Appearance of germinating conidia under the scanning electron microscope

When conidia are incubated with sub-lethal concentrations of benomyl (0.1-0.3 μg/ml) swollen and distorted germ tubes are produced (Figs. 1 and 2). Germination also occurs in more than one part of the conidia with greater frequency than in untreated conidia.

The wrinkled appearance of the conidia may be due to distortion or shrinking of soft material during the vacuum treatment. A similar effect has been observed by Greenhalgh and Evans (1968) in ascospores of *Hypoxylon rubiginosum*.

Chemically-fixed spores

Sections of resting conidia (Fig. 3) show features similar to those observed in *Botrytis cinerea* (Hawker and Hendy, 1963; Buckley *et al*, 1966). All the normal organelles are present, but the storage bodies observed by Buckley *et al*, (1966) were not present in *B. fabae*. Instead rosette-shaped aggregates of electron-dense material resembling glycogen (Young, 1969) are found. Endoplasmic reticulum is rather poorly developed and often occurs as cisternae and vesicles. Lipid bodies and several small vacuoles are present. In germinating spores (Fig. 4) there is an increase in the size of the vacuoles, the glycogen granules disappear and the cytoplasm appears more or less homogeneous although areas of varying electron density remain. There is a striking development in endoplasmic reticulum, the long strands frequently almost completely surrounding the nucleus.

The characteristic distorted germ tube produced when conidia germinate in the presence of benomyl is clearly seen in Fig. 5. There is less development of endoplasmic reticulum in treated cells than in germinating controls. Fragments of endoplasmic reticulum frequently occur as a meshwork or vesicles associated with the cell wall rather than as long strands about the nuclei. In this respect treated cells resemble resting controls. At a later stage vacuolation increases until finally the cell contents become one large vacuole with the cell organelles at the periphery.

Freeze-etching

The cytoplasm of cross-fractured replicas of resting spores (Fig. 6) appears homogenous and the aggregates of glycogen seen in chemically-treated sections cannot be detected. A conspicuous feature is the rounded vacuoles covered with small particles. More vacuoles are visible in freeze-etched than in chemically-fixed material. The reason for this is unknown, but the same effect has been observed by Remsen, *et al*, (1967). Mitochondria and lipid bodies are present as well as short strands of endoplasmic reticulum, one of which is visible in surface view. The most striking feature of germinating spores (Fig. 7) is the great increase in endoplasmic reticulum which is now present as large parallel sheets, four of which can be seen close to a nucleus. A vesicle is being formed by one of the strands of endoplasmic reticulum. The nucleus has a fractured nuclear membrane and nuclear pores can be seen. Small vesicles occur close to the plasmalemma. After treatment with benomyl (Fig. 8) the plasmalemma appears intact and unaltered, the small vesicles are now very distinct and one appears to be penetrating the cell wall. The most striking effect of benomyl treatment is the large increase in the number of small vacuoles present.

DISCUSSION

The distortion produced in germ tubes of *B. fabae* by benomyl treatment (Fig. 2) gives little guide to mode of action since compounds as diverse as griseofulvin (Brian, 1949), phenyl mercury compounds (Strzelczyk, 1968) and tecnazine (Esuruoso *et al*, 1968) have all been reported to give this effect.

The absence of long strands of endoplasmic reticulum surrounding the nuclei of benomyl-treated cells and the presence instead of vesicular endoplasmic reticulum close to the cell wall (Fig. 5)suggests an inbalance in cell metabolism. Endoplasmic reticulum is concerned with protein synthesis and the translocation of material within the cell (Frey-Wyssling and Muhlethaler, 1965): vesicular endoplasmic reticulum at the apex of vegetative hyphae is probably associated with cell wall synthesis (Marchant *et al*, 1967).

In benomyl-treated cells a shunt in metabolism may occur from the synthesis of protein and other cell constituents to wall synthesis. Local thickening of the cell wall was sometimes noted in benomyl-treated germ tubes (Fig. 5); this effect was not general. Treated hyphal walls were, however, found to stand up to vacuum coating for the stereoscan better than control hyphae. Griseofulvin which is thought to act by inhibiting protein and nucleic acid synthesis (El-Nakeeb, *et al*, 1965) also produces thickened and stronger hyphal cell walls in *Aspergillus niger* (Evans and White, 1967), while chloramphenicol, a specific inhibitor of protein synthesis in bacteria and blue-green algae (Vazquez, 1966) produces extreme local thickening in the yeast *Rhodotorula* glutinis (Smith and Marchant, 1969). The distortion of germ tubes by benomyl is therefore probably not due to a specific action on the cell wall but rather to an inhibition of other metabolic processes.

The vesicles seen close to the plasmalemma in Fig. 8 may be concerned in swelling of the cell wall prior to germination. Moor (1967) showed that vesicles formed from the endoplasmic reticulum released their contents into the yeast cell-wall to induce bud formation. Benomyl may stimulate the formation of related vesicles in germinating conidia of B.fabae thus encouraging multiple germ tube emergence.

The increased vacuolation in benomyl treated spores (Fig. 8) may be a specific response to the toxicant or, more likely, may simply be an indication of accelerated ageing prior to the death of the cell.

Although comprehensive biochemical studies are needed before a definite mode of action of benomyl can be postulated ultrastructural changes suggest that the fungicide may inhibit membrane synthesis. A rapid increase in endoplasmic reticulum occurs on germination of many fungal spores but germinating sporangiospores of Mucor rouxii (Bartnicki-Garcia et al, 1968) and Rhizopus stolonifer (Buckley et al, 1968) produce very little endoplasmic reticulum. This may explain why benomyl is ineffective against Phycomycetes (Delp and Klopping, 1968).

Acknowledgments

The authors wish to thank the Biochemistry Department, University of Bristol for the use of their electron microscope and freeze-etching apparatus, also Mr A. Brittain of the Biochemistry Department for assistance with freeze etching and Miss Elizabeth Hull for the Stereoscan photographs.

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Explanation of figures

Scale line indicates 1µ.

Fig. 1. Germinating conidia observed with the scanning electron microscope x 500.

Fig. 2. Conidia germinating in the presence of benomyl x 1,200.

Fig. 3. Section through resting conidium showing glycogen granules, nuclei, mitochondria and small strands of endoplasmic reticulum x 11,000.

Fig. 4. Section through germinating control conidium shows long strands of endoplasmic reticulum surrounding the nuclei and prominent vacuoles x 7,000.

Fig. 5. Section through conidium germinating in the presence of benomyl showing distorted germ tube and vesicular endoplasmic reticulum close to the plasmalemma.x 6,000.

Fig. 6. Replica of resting conidium with vacuoles and mitochondria in surface and section x 14,000.

Fig. 7. Replica of germinating conidium showing sheets of endoplasmic reticulum close to a nucleus. The nucleus shows fractured nuclear membrane and nuclear pores x 30,000.

Fig. 8. Replica of benomyl-treated germinating conidium showing increased vacuolation. Note vesicles close to Plasmalemma x 12,000.

Abbreviations:

er endoplasmic reticulum

g glycogen-like granule

l lipid granule

m mitochondrion

n nucleus

np nuclear pore

p plasmalemma

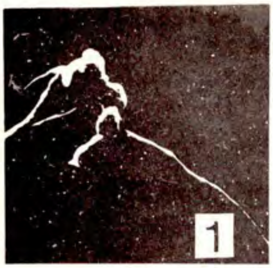
sp septal pore

v vacuole

ve vesicle

w woronin body

The arrows indicate direction of shadow



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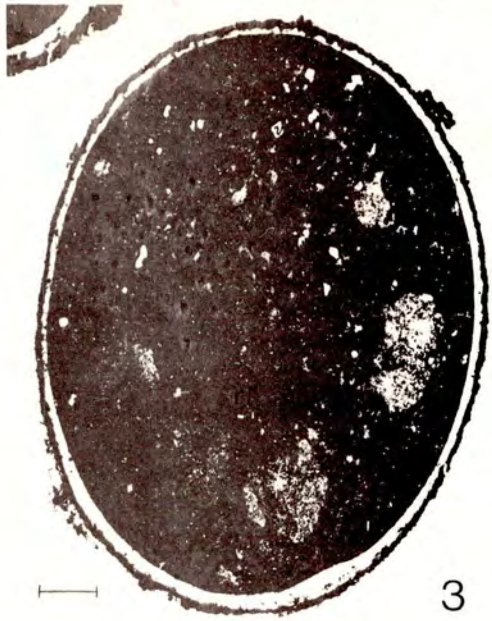

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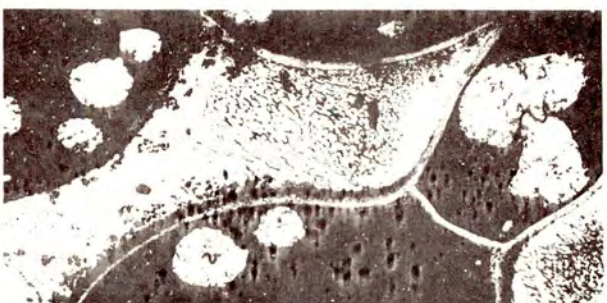

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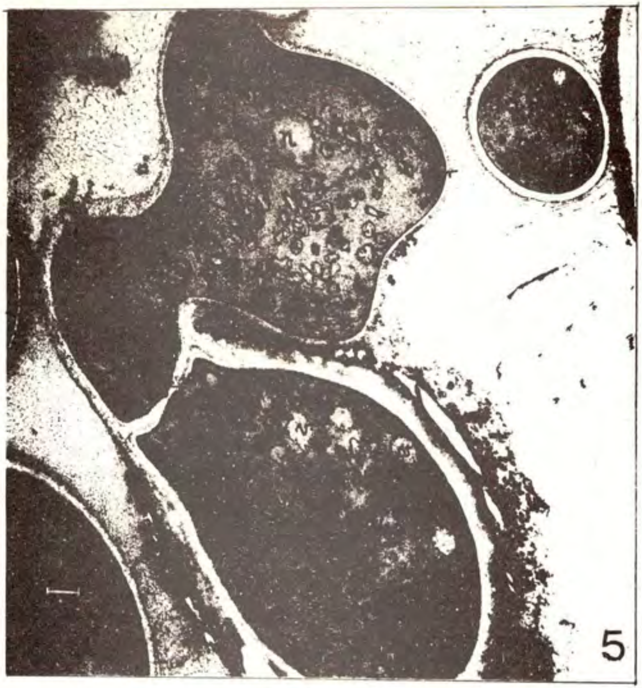
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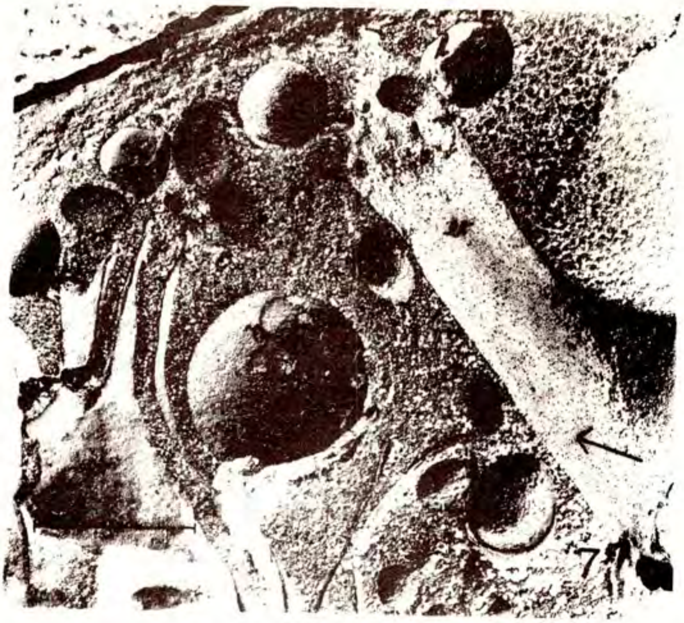
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Proc. 5th Br. Insectic. Fungic. Conf. (1969)

THE EFFECT OF FRUIT TREE RED SPIDER MITE ON YIELDS OF APPLE

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Summary Since 1961, apple yields have been compared from a grassed-down, irrigated orchard of Worcester Pearmain and Cox's Orange Pippin in Warwickshire. Fruit tree red spider mite (Panonychus ulmi) infestations were allowed to build up on some trees but were controlled on others by acaricides. In the first trial (1961-63) mite infestations caused no yield reduction in the first year, but a significant reduction of 31 per cent (Worcester) occurred in the second year. In the third year, when mites were controlled on all trees, there was a rapid recovery in yield from previously infested trees.

In the second trial (1964-65) there were again no differences in yield in the first year, but in the following year mite infestations significantly reduced yields by 43 per cent (Worcester) and 34 per cent (Cox).

In the third trial (1966-69) the reduction in annual yield due to continuous mite infestations varied between 11 and 76 per cent (Worcester). Where previously uninfested trees were allowed to become infested, it was not until the third year, 1968, that yield differences began to appear. Where previously infested trees were kept clean of mites by the use of acaricides, yields did not fully recover till the third year of acaricide application. Spring frost in 1967 seriously reduced yields, especially on Cox.

INTRODUCTION

For such an important pest of top fruit there is a dearth of reliable data on the extent of losses in yield following attacks by fruit tree red spider mite (Panonychus ulmi). Gould (1965) showed that where Cox trees were being grown on the regulated hedge system in a heavy soil overlying chalk, not ideal for fruit growing, heavy mite infestations had a direct effect on yield causing losses as great as 34 per cent. Colleagues of the N.A.A.S. at Wye reported (1965) significant increases in yield of 19 per cent on trees kept clean of fruit tree red spider mite. Complementary trials were started at Luddington Experimental Horticulture Station, Stratford-on-Avon, Warwickshire and the results obtained are described in this paper.

METHOD AND MATERIALS

The trees used in the trial were worked on M II rootstocks and planted as maidens in the winter of 1952-53. Equal numbers of the two cultivars, Worcester Pearmain and Cox's Orange Pippin, were planted alternately in each row. The site was grassed down and, being a sandy soil, irrigation had always been applied when there was a three inch soil moisture deficit. A randomised block layout was used on the 72 trees with either twelve single tree replicates (trial 1) or eighteen replicates (trial 2) of each cultivar, and acaricides were applied to maintain 'clean' (uninfested) trees by high-volume sprays from within a tractor-mounted screen. There were no guard trees between the plots.

Throughout the trial all the Worcester trees were sprayed with a captan fungicidal programme against scab, while the Cox trees received a dispersible sulphur programme to control scab and mildew with the minimum effect on spider mites.

On 'clean' trees demeton-methyl, later demeton-S-methyl, was applied each year at petal fall for control of apple sawfly and fruit tree red spider mite, except in 1969 when it was not used because Briggs and Avery (1968) had shown that it tended to promote extension growth. Sprays of phenkapton in 1961-63 and 1966, tetradifon in 1964-65 and 1967, or dicofol in 1968-69 were applied after petal fall to prevent a build-up in mite infestations. For sawfly control on trees where infestations of mites were not controlled, lindane was applied at petal fall. All trees were sprayed with DDT, or in later years carbaryl, for codling moth control.

RESULTS

<u>Levels of mite infestation</u> Except in 1961 when mites were counted in the orchard, a leaf-brushing machine was used to estimate the number of eggs and active mites on 24 leaves per tree. Samples were taken on three to five occasions between June and September. A large difference in the mite populations of the differently treated trees was maintained, as was shown by the striking contrast in the amount of leaf bronzing to be seen each September. Only in 1966, when relatively few mites were present, was there no leaf discolouration on the infested trees.

The number of active mites on leaves of Worcester was usually greater than on those of Cox. Although the leaves of Worcester trees were on average fifty per cent larger in area, there were more mites on Worcester leaves than on Cox leaves of similar area; this observation agrees with the fact that the foliage of Worcester became more bronzed.

With few exceptions the active mite population on 'clean' trees was not allowed to rise above one per leaf. The maximum number of mites on the infested trees varied from year to year, as shown by the following figures which refer only to Worcester: in 1961 the maximum was 14 mites per leaf, while in 1962 it rose to 212. In 1963 all the trees were sprayed to control mites and the population level remained below one mite per leaf. Populations were not recorded in 1964, although differences in the amount of leaf bronzing in the autumn showed that mites had been active. In 1965 mites were prevalent, up to 81 per leaf, while 1966 was a year of low infestations with a maximum of only 10 mites per leaf. Populations increased in 1967 and 1968, reaching levels of 43 and 65 mites per leaf respectively, and fell to a maximum of 34 mites in 1969. On infested trees the peak population in any one year was usually in July.

<u>Yields</u> Results from the original trial led to changes in the layout and it is convenient to consider the three trials separately.

<u>Trial 1 (1961-63)</u>

The yields from trees infested for one or two years were compared with those from continuously 'clean' trees and are shown in Table 1.

Table 1

Apple yields in relation to fruit tree red spider mite infestations - Trial 1

Presence of mites			Yield in bushels (40 lb) per acre of picked fruit					
			Worcester			Cox		
1961	1962	1963	1961	1962	1963	1961	1962	1963
i	c	c	353	430	448	173	319	501
i	i	c	350	348	421	160	294	429
c	c	c	364	506	467	204	370	461

c = 'clean'   i = infested

Fruit tree red spider mite did not influence yields of either cultivar in the first year, but after two successive years' infestation on Worcester there was a significant reduction in yield of 31 per cent compared with 'clean' trees. Where trees had been infested for one year only, there was a significant reduction in yield in the following year due to a residual effect of the first year's infestation. Although there was a reduction of 20 per cent in yield from Cox trees infested for two years, this was not significant.

In 1963 when all the trees were maintained clean of mites, yields were similar, indicating a rapid recovery in vigour.

Trial 2 (1964-65)

Although yield differences due to mite attacks had been shown, they were not significant on Cox. It was decided to simplify the treatments and maintain trees either 'clean' or infested until a significant difference in yield appeared on both cultivars. Accordingly these two treatments were re-randomised among the same 72 trees.

As in Trial 1 there were no yield differences in the first year, but in the second year mite infestations caused significant reductions of 43 per cent on Worcester and 34 per cent on Cox.

Table 2

Apple yields in relation to fruit tree red spider mite infestations - Trial 2

Presence of mites	Yield in bushels (40 lb) per acre of picked fruit			
	Worcester		Cox	
	1964	1965	1964	1965
infested	632	351	632	346
'clean'	691	612	623	523

Trial 3 (1966-69)

Each of the basic treatments was now split into two, i.e. 'clean' and infested, so giving four treatments, the available yields from which are given in Table 3.

Table 3

Apple yields in relation to fruit tree red spider mite infestations - Trial 3

Presence of mites		Yield in bushels (40 lb) per acre of picked fruit					
		Worcester				Cox	
1964-65	1966-69	1966	1967*	1968	1969	1966	1968
c	c	374	129	396	494	424	104
c	i	406	124	335	413	479	87
i	c	313	108	381	449	388	104
i	i	283	31	351	347	359	78

c = 'clean'   i = infested   * yields reduced by spring frosts   No 1967 crop because of spring frosts. 1969 crop not yet picked

Although the results have still to be analysed, the increase in yield of both Worcester and Cox in 1966, when a low infestation (maximum of 0.14 mites per leaf) was allowed to develop on previously 'clean' trees, agrees with work at East Malling Research Station which showed that low densities of mites stimulate the growth of infested plants while moderate and high densities reduce it. In all four years, yields of Worcester apples from continuously infested trees were lower than those from continuously 'clean' trees: by 24 per cent (1966), 76 per cent (1967), 11 per cent (1968) and 30 per cent (1969). The reduction in 1967 was exceptionally large, especially as infestations in the previous year were low. Spring frosts reduced yields generally in 1967 and they may have had a greater effect on trees whose vigour was reduced as a result of mite injury.

When previously infested trees were sprayed to keep them 'clean' there was a steady improvement in yields, although it was not until 1968, i.e., the third year, that they approached those from continuously 'clean' trees. From 1966 onwards mite populations were allowed to build up on some previously 'clean' trees; the effect of this did not appear until 1968 when there was a depression in yield following the high mite infestation the previous year.

The only available results from Cox are in 1966 and 1968 when the reduction in yield from continuously infested trees was 15 per cent and 25 per cent respectively.

DISCUSSION

Gould (1965) found that there were no obvious differences in the size and quality of the fruit picked from 'clean' and infested trees: the yield loss was caused by a decrease in the total number of apples produced. Similarly at Luddington the size and quality of the fruit were not affected by mite infestations, nor were there any significant differences in the percentage fruit set. However, whereas Gould recorded loss in the same year as the mite attacks, at Luddington differences in yield did not appear until the second harvest. It is probable that this delay was a result of the more suitable growing conditions at Luddington which enabled the trees to withstand one year's mite infestation without any effect on yield.

Although it has been shown that fruit tree red spider mite causes loss, sometimes severe, in apple yields, this reduction is brought about by successive, uncontrolled infestations which are so severe that they would not be tolerated in any commercial orchard. It would appear that in the absence of other stresses on the tree, for example those caused by thin soils or water shortage, the influence of low mite populations in any one year would have little effect on yield.

Acknowledgments

I am indebted to Mr. D. A. Holland of East Malling Research Station for the experimental design and statistical analyses, Mr. J. Ingram and other staff at Luddington E.H.S., and Mr. N. Jacob for the mite assessments.

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Proc. 5th Br. Insectic. Fungic. Conf. (1969)

RECENT WORK ON WHEAT BULB FLY

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Summary The growth of individual plants of Cappelle wheat attacked by wheat bulb fly larvae was compared with that of unattacked plants growing in the same crop. Attacked plants were slow to grow and had fewer shoots and ears than unattacked plants. Plants that died were killed directly by larvae, not by competition with unattacked plants. Only unattacked plants showed compensatory growth and in late-sown crops the numbers of ears/undamaged plant was negatively correlated with the density of the plant population surviving attack.

INTRODUCTION

A study of how larvae of wheat bulb fly (Leptohylemyia coarctata) affect the growth and yield of plant populations (Bardner, 1968) showed that the principal effect of large larval populations is to decrease the number of ear-bearing shoots, which in an unattacked crop is about 1.5 to 2 million/ac (3.7 to 4.9 million/h), depending on the season. In unattacked crops of winter wheat, the number of shoots is greatest during late April and early May, when there are about twice as many shoots as at harvest. This is also the time when larvae of wheat bulb fly cease their attacks and pupate. Because the maximum number of shoots usually exceeds the number of ears some compensation for the effects of a light attack is possible. However yield is diminished when the number of shoots (and hence ears) surviving attack is less than the number of ears in a comparable unattacked crop. The effects of attack upon the growth and yield of individual attacked plants are reported here.

Results obtained by other workers are somewhat contradictory. Gemmil (1927) suggested that attacked plants produce more shoots than unattacked plants, whereas Gough (1946) found that the numbers of shoots produced by attacked plants varied with the site. In one field, attacked plants had on average more shoots than unattacked plants but the reverse situation was found in five other fields. Long and Morris (in Mellanby 1960, 1961 and unpublished) marked and recorded the growth of individual wheat plants growing in an infested crop. Attacked plants grew slowly, and although they later produced more buds and shoots than unattacked plants a larger proportion of the attacked plants died before they could be harvested, and those that survived yielded less than unattacked plants. Most of these results were obtained with a variety no longer grown (Squarehead Master) or on infertile soil in the exhaustion experiment at Rothamsted. We used a modern variety (Cappelle) on land of reasonable fertility. In general our results confirm those of Long and Morris, although we found no evidence that attacked plants produced extra shoots. Compensatory growth was confined to unattacked plants which produced extra ears in response to low plant densities.

METHOD

The measurements were made at Rothamsted between 1965 and 1968 on crops of Cappelle winter wheat sown at approximately 3 bu/ac (200 kg/h) on land with larval populations of between 0.1 and 0.7 million/ac (0.2 to 1.7 million/h). In 1965, 1966 and 1967 data were obtained from the experiment and site described previously (Bardner, 1968) to compare the effects of wheat bulb fly on plants sown at different dates, there being three to eight replicates for each date of sowing. In 1965 and 1966 the experiments were sampled several times between late February and early May, eight to 12 samples of 6 in (15.2 cm) lengths of row being removed at each sampling; all the plants were dissected for signs of attack. In 1967, four one yd (0.91 m) lengths of row were selected at random in each plot. All the plants in these samples were marked by placing numbered and plastic coated wire rings round the stems at soil level, and the presence or absence of external signs of injury was noted. At harvest each plant was pulled up, the ears and stems were counted, grain size and stem length measured and threshed grain weighed. Grain was dried for 24 hr at 90°C.

In 1968 further observations were made on marked plants in a commercial crop of winter wheat sown by broadcasting. Most of the field was sown with seed treated with a dieldrin seed dressing, but a strip was also sown with seed free from insecticide. Five small plots each measuring 0.5m x 0.5m (0.55 x 0.55 yd) were located at random on each of the treated and untreated areas. Plants were marked and examined as in 1967. Table 1 gives details of cultural treatments and populations of plants and larvae.

Table 1
Details of Field Experiments

	Sowing date	Harvesting date	Eggs* million/ac	Larvae* million/ac	Plants* million/ac	Yield† cwt/ac
1965	Early 27.Oct.64	13.Sep.65	1.15	0.58	1.25	38.5
	Mid 25.Nov.64	"	"	0.50	0.69	36.5
	Late 22.Dec.64	"	"	0.28	0.35	30.3
1966	Early 2.Nov.65	24.Aug.66	1.67	0.74	0.86	47.4
	Late 8.Jan.66	"	"	0.21	0.48	39.1
1967	Early 28.Oct.66	30.Aug.67	0.90	0.14	0.69	49.6
	Mid 29.Nov.66	"	"	0.03	0.23	39.5
	Late 21.Dec.66	"	"	0.01	0.19	27.4
1968	7.Dec.67	13.Aug.68	0.33	0.33	0.88	29.4

* Acres to hectares x 2.47
† 1 cwt/ac = 125.5 kg/h

RESULTS

a) 1965 & 1966 Experiments

Tables 2 and 3 show the mean number of shoots/plant for injured and for uninjured plants at each sampling. Attacked plants grew more slowly than unattacked plants. Where unattacked plants had a mean of more than one shoot comparable attacked plants had less, most of these differences being significant.

Table 2
1965 Experiment

	Mean numbers of Shoots/plant 25.Feb.	11.Mar.	25.Mar.	12.Apl.	13.May
Early attacked	1.29	1.58	2.03	2.76	3.20
unattacked	1.35	1.64	2.30	2.89	3.22
Mid attacked	1.00	1.00	1.00	1.68	2.93
unattacked	1.00	1.00	1.04	2.56	4.51
Late attacked	-	1.00	1.00	1.58	3.03
unattacked	-	1.00	1.00	2.71	4.78
S.E. of differences between attacked and unattacked	0.055	0.051	0.034	0.397	0.476

Table 3
1966 Experiment

	Mean numbers of Shoots/plant 8.Mar.	22.Mar.	5.Apl.	26.Apl.	11.May
Early attacked	1.37	1.81	2.50	2.96	3.26
unattacked	1.72	2.40	3.25	4.42	4.50
S.E. of difference	0.059	0.065	0.103	0.325	0.256
Late attacked	1.00	1.00	1.10	1.86	3.50
unattacked	1.00	1.00	1.49	2.98	4.11
S.E. of difference	0.041	0.046	0.072	0.230	0.181

b) 1967 Experiment (Table 4)

Table 4
1967 Experiment

	Plants Marked	Plants Harvested	Shoots/plant (18-21. May 67)	Ears/plant	Yield/plant (g)	Yield/Ear (g)
Early Unattacked	148	143	4.3	2.9	4.1	1.5
Attacked before 28.Mar.	131	63	2.4	1.3	1.6	1.2
" after 28.Mar.	62	60	5.1	3.2	4.0	1.4
Mid Unattacked	91	87	7.2	4.6	8.2	1.8
Attacked before 28.Mar.	27	9	2.6	1.4	1.8	1.3
" after 28.Mar.	17	12	9.0	6.6	12.3	1.9
Late Unattacked	101	97	6.0	7.0	8.4	1.4
Attacked before 28.Mar.	19	6	3.3	5.1	6.3	1.3
" after 28.Mar.	10	3	1.5	2.0	3.3	1.6
S.E. of difference for any comparison	-	-	1.9	2.3	3.0	0.2

As in the 1965 and 1966 experiments the plant populations at the start of the attack were largest in the earliest sowings as poor germinating conditions and damage by birds decreased the plant populations in the later sowings. Large plant populations increase the chances of newly-hatched larvae finding plants (Raw,1954,1960)

In the 1965 and 1966 experiments, there was a positive correlation between the number of plants and the number of larvae infesting them, so it was not surprising to find considerably more injured plants in the early sowing.

In 1967, the marked lengths of row were inspected on 28 March and 28 April for signs of injury. The percentages of plants attacked were: early-sown 57, mid-sown 33 and late-sown 22. In all three sowings, fewer than 5% of marked unattacked plants disappeared before harvest; by contrast losses of attacked plants were substantial, more than half the plants usually disappearing before harvest. An exception was with early-sown plants attacked late (symptoms not present before 28 March), of which 60 out of 62 marked plants were found at harvest.

Most plants that disappeared did so before 18 and 21 May, as a count made at this time showed. Plants that developed symptoms before 28 March had fewer shoots/plant than uninjured plants when examined between 18 and 21 May, after attacks had ceased. Injured plants also had fewer ears and a smaller weight of grain/ear, although few of the differences were significant, as standard errors were large.

The numbers of ears borne by late-sown unattacked plants were negatively and significantly correlated with the total number of plants ($r = -0.7885$, 7 d.f.). The regression equation was: Number of ears = 10.53 - [(0.448 ± 0.157) total plants] There was no significant correlation between the numbers of unattacked plants and total plants in the early or mid sowing, or between the mean number of ears/attacked plant and the total number of plants in any of the sowings.

c) 1968 Experiment (Table 5)

Table 5
1968 Experiment

	Stem length (cm)	Weight of one seed (mg)	Ears/plant	Yield/plant (g)	Yield/ear (g)
Unattacked	3.22	38.2	2.23	2.25	1.08
Attacked	2.97	35.1	1.56	1.25	0.81
S.E. of difference	1.46	2.0	0.26	0.29	0.15

On each of the strips sown with dieldrin-treated and untreated seed, three plots were marked on 10 April and a further two on 23 April. Though there was a considerable variation in the yield/plot there was no significant difference in the mean yields of comparable plants marked at different times (or in different strips), so the results from all the plots were combined for analysis. The date of the initial attack by larvae was assessed by dissecting plants from near the plots. Larvae had finished entering the plants by early March, when the plants were still in the single-shoot stage with about two leaves each. In all, 642 plants were marked of which 32.2% had symptoms of attack. At harvest 95.2% of the unattacked plants were present, but only 34.1% of the attacked plants.

The mean yields of attacked plants still present at harvest was 56% of the yield of unattacked plants, a significant difference. Attacked plants also had significantly fewer ears/plant and less grain/ear than unattacked plants. The attacked plants also had shorter straws and smaller seeds than unattacked plants, although these differences were not significant. As in the 1967 late-sown treatment, uninjured plants responded to lessened competition by producing extra ears, the mean number of ears/unattacked plant being negatively correlated with the total plants harvested ($r = -0.878$, 11 d.f. significant at $P < 0.01$). The regression equation was: Number of ears = 3.800 - [(0.03185 ± 0.00599) total plants]. For attacked plants the correlation coefficient was not significant ($r = +0.060$).

DISCUSSION

a) Survival of plants

In both 1967 and 1968, more than 95% of the unattacked plants were still present at harvest, compared with 22 to 48% of the attacked plants; exceptions to this generalisation were early-sown plants first showing symptoms of attack between 28 March and 28 April, 96% of which were found at harvest. It has long been known that older plants, (such as those sown early or attacked late) survive attack by wheat bulb fly larvae better than young ones. Griffiths and Scott (1969) showed that plants in the late two-leaf to early three-leaf stage survive the destruction of the main shoot. In the field it usually takes a month for all larvae to hatch and enter plants and it is rarely possible to tell when and at what stage of growth a plant was entered by a larva. In 1967 most plants in the early sowing were in the three-leaf stage by 7 February, when about half the eventual number of larvae had entered. It seems that attacked plants are killed by larvae and not by competition with other plants, since most attacked plants died before mid-May, which was before ear-bearing shoots started to elongate and compete for space.

b) Growth and yield of attacked and unattacked plants

Attacked plants that were not immediately killed by larvae grew more slowly and had fewer and smaller ears than undamaged plants. In contrast to undamaged plants they did not respond to the decreased density of plants by producing extra ears/plant. The amount of compensatory growth of extra ears by unattacked plants is probably limited because damaged plants are of course not evenly spaced and therefore, even with heavy attacks, many of the unattacked plants will still be close enough to compete with each other for space.

Because attacked plants that survive have a lessened yield, methods of control which kill larvae only after they enter plants (such as insecticide sprays) are unlikely to prevent some loss of yield in heavily-infested crops.

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Proc. 5th Br. Insectic. Fungic. Conf. (1969)

DAMAGE ASSESSMENT ASPECTS OF APHIS FABAE ON SPRING SOWN FIELD BEANS

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Summary Surveys of the incidence of bean aphid on field beans during the last four years showed that the heaviest attacks occurred in 1967 followed by negligible infestations in 1968. Damage assessment aspects of aphid infestation were studied in three ways; by making use of survey results, in large plot insecticide trials and by studying the effects of different levels of aphid infestation on the growth and yield of single plants.

In 1967 the results from field surveys and a large plot replicated trial showed a similar relationship between bean aphid infestations and reduction of yield. In 1969 measurements of the effects of five categories of bean aphid infestation on several parameters of growth and yield of individual plants were consistent over six unsprayed fields.

INTRODUCTION

New varieties of field beans (*Vicia faba*) and new methods of husbandry have resulted in a potential yield of about 30 cwt/ac for spring-sown crops which are being increasingly grown as a break crop from cereals e.g. in the English southern counties of Berks, Bucks, Hants and Oxon the bean acreage increased from 1,500 to 18,000 acres between 1964 and 1968.

Bean aphid (*Aphis fabae*) is probably the most important pest of spring beans and can be a limiting factor in crop production. (Way, Smith and Potter 1954; Way, Bardner, Van Baer and Aitkenhead 1958).

Several aspects of the problem of bean aphid on spring-sown field beans, including the effect on yield, have been studied in the South of England. Since 1966 surveys have been made of the incidence and importance of bean aphid. Large scale trials have been done to determine the most satisfactory method of control (Gould and Graham 1969) and a number of methods have been used to investigate the effect of aphid infestations on crop yield.

Throughout the work aphid infestations were assessed by means of the method described by Gould and Graham (1969) in which the plants are classified into one of five categories related to the size and distribution of the aphid colony. The number of aphids in each of these categories has also been estimated (Table 1).

Table 1

Aphid Categories and Population assessment

Infestation Category	Nil (C)	Very Slight (VS)	Slight (S)	Moderate (M)	Heavy (H)
Mean No of Aphids	0	4	500	1,200	3,500
* Range	-	1-10	200-600	950-1,600	2,200-6,000

* Based on a sample of 20 "typical" plants for each category

These categories have been used to assess the level of aphid infestation as the results obtained also show the pattern of infestation in the crop whereas the value for mean aphids/plant may fail to distinguish between quite different patterns of infestation.

SURVEYS

In the four years 1966-1969 surveys have been made of bean aphid infestations on field crops in Berks, Bucks, Hants and Oxon. Farms growing over five acres of field beans were selected at random from county lists of bean growers and the number of fields was stratified according to the bean acreage in each county.

The fields were visited during the peak period of aphid infestation in the first and second week of July. On each field the infestation was recorded on 24 plants along a south-west facing headland and 24 plants along a parallel traverse within the field. The results are shown in table 2.

Table 2

Results of bean aphid surveys 1966-1969

Year	Acreage Surveyed		No. of Fields	Percentage plants in each category: Headland Samples			Field Samples		
				C + VS	S	M + H	C + VS	S	M + H
1966	850	Treated	36	96	2	2	97	2	1
		Untreated	10	72	8	20	90	3	7
1967	1,600	Treated	60	63	18	19	67	16	17
		Untreated	7	8	17	75	25	17	58
1968	1,000	Treated	29	100	0	0	100	0	0
		Untreated	11	99	0	1	100	0	0
1969	2,500	Treated	59	91	5	4	94	4	2
		Untreated	14	65	15	19	82	11	7

During the survey details were also obtained of the pesticides used, method and time of application and these results will be reported elsewhere.

Over the four year period, very heavy aphid infestations developed throughout the area surveyed only in 1967 and this was followed by a year of negligible attack in 1968. Infestations in 1966 and 1969 were similar and much more uneven with some very heavily infested fields.

DAMAGE ASSESSMENT

Three methods have been used to obtain information on the losses caused by bean aphid. These have involved the use of survey data, large plot yield trials and studies of the effects of aphid infestations on single plants.

a. Surveys

The 1967 survey of aphid incidence provided a wide range of infestation levels on different fields and farm estimates of yields were obtained for 38 fields. These showed a significant correlation ($R = 0.56$; 36 DF) with the peak aphid infestations recorded during the survey (Table 2). The following regression equation was obtained from the data:-

$$Y = - 0.13 X + 27.5$$

(Where Y = yield in cwt/ac and X = Angular transformation of % of total plants with moderate and heavy aphid colonies at the peak of infestation.)

Using the regression it was estimated that, in 1967, an average yield of 27.5 cwt/ac could be expected in the absence of heavy aphid infestations. On the unsprayed fields an average yield of 21 cwt/ac could be expected when on average 58% of plants were heavily infested.

As farmers' estimates of yield were used, these results can only be considered as an indication of the effect of aphids on the crop but they suggest that in 1967 bean aphid was a significant factor affecting yield.

b. Large plot trials

During 1966-1968 three large plot trials suitable for combine harvesting were done primarily to investigate the efficiency of pesticides used as preventive or eradicant treatments for the control of bean aphid (Gould and Graham 1969). These trials also provided information on the effect of aphid infestations on yield. The variety Blue Rock sown in 7 inch drills was used throughout and the plots were 40-45 yd long and 5-6 yd wide with four replicates of each treatment. Sprays were applied with a tractor-mounted boom sprayer at 25 gal/ac. The loss of crop resulting from wheel damage caused by a standard 11 in wheel tractor was assessed in 1966 and 1967 by driving the tractor through an extra set of control plots when late eradicant sprays were applied. Aphid assessments were made at regular intervals by examining 40 plants/plot. Strips 8 ft 6 in wide and 120 ft long were harvested from each plot. Yield data were also obtained from an unreplicated strip trial in 1967.

The results in Table 3 show the aphid infestation and yields obtained on the best preventive treatment and the control plots in each trial.

The results again confirmed the effect on yield of the heavy aphid infestations which developed in 1967. Untreated plots at Site a. had over 90% of plants with moderate or heavy colonies at the peak of aphid infestation with, on average 2,900 aphid/plant. This resulted in a 23% loss of yield (sig. $p = 0.05$) compared with a calculated 24% loss of yield for untreated fields in the 1967 survey. At Site b. where the mean peak aphid infestation was estimated as 3,500 aphids/plant a 35% loss of yield occurred on untreated strips.

Lower infestations occurred in 1966 with 45% of plants on untreated plots having moderate or heavy colonies and a 6% loss of yield was recorded at harvest. Negligible infestations developed in 1968 with no significant effect on yield.

The results also showed the value of preventive and eradicant treatments and gave some information on the effect of wheelings on yield when late eradicant treatments are applied.

These losses are considerably lower than those reported by Way et al (1958) but their work was done with older varieties of field bean grown in 16 in or 22 in drills.

c. Single Plant Studies

Judenko, Johnson and Taylor (1952) described a method for studying the effect of bean aphid on the growth and yield of field beans using data from a large number of

Table 3

<u>Bean aphid infestation and yields</u>

Year	Treatment	Date	Peak aphid infestation: % plants in each category in angular transformation C + VS	M + H	Estimated Mean aphids/ plant	Yield cwt/ac	Increase/ Decrease in yield cwt/ac
1966	Menazon, 10 June	7 July	84	5	22	23.3	+ 1.5
	Control (wheeled)		66	44	650	18.7	- 3.1
	Control		55	49	1,000	21.8	
	S.E.		4.2	3.2		1.8	
1967a	Menazon, 13 June	5 July	60	0	160	32.8	+ 7.6
	Control (wheeled)		5	84	3,400	24.1	- 1.1
	Control		0	84	2,900	25.2	
	S.E.		4	5		1.3	
1967b*	Phorate, 15 June	6 July	74	13	135	36.5	+ 12.9
	Control		0	84.3	3,500	23.6	
1968	Disulfoton, 11 June	20 July	84	0	0	29.4	+ 0.2
	Control		79	6	27	29.2	
	S.E.					1.4	

* Unreplicated strip trial

NB - Results for 'Slight' category not included single plants, some of which were kept clean by removing aphids by hand. The plants were grown at 2 ft spacing with 2 ft between the rows and the results obtained were unlikely to be applicable to field crops grown in 7 in drills where competition factors would be different. Way et al (1958) used a similar technique on sprayed and unsprayed plants which were also grown in wide rows at 20 in spacing.

A modification of this single plant technique has been used to obtain information on the effect of aphids on yield within infested, unsprayed fields of beans. Natural aphid infestations are usually patchy and a range of infestations, related to the date of primary colonization, can often be found in a relatively small area. This method, therefore, uses infestations developing in a natural pattern with normal competition factors influencing the results, at random.

In 1969 observations were made on six unsprayed fields. The variety was Maris Bead on five of the fields and Blue Rock on the other. On each field 20 plants in each of the five infestation categories previously described, were marked with coloured tapes in the second week of July at the peak of aphid infestation. All marked plants were situated within an area of about 100 sq. yd. at each site. Plants recorded as clean may have subsequently become infested by secondary migrants but this growing-point infestation would be too late to affect pod set and by late July a natural decline of aphid colonies occurred. The plants were collected during the first week of September and the length, number of trusses set, number of pods, number and oven-dry weight of seed were recorded. The results are shown in Table 4.

Table 4

The effect (Means of six sites 1969; i.e. 120 plants/category) of aphid infestations on growth and yield of field beans

		% Reduction compared with clean plants			
Infestation Category Mean aphids/plant	Clean 0	Very Slight 4	Slight 500	Moderate 1,200	Heavy 3,500 +
Length of stem	118 ± 2.0 cm	4%	8%	12%	16%
No of trusses set/plant	6 ± 0.3	15%	33%	44%	78%
No of pods/plant	12.7 ± 0.9 g	14%	36%	53%	82%
No of seeds/plant	37.2 ± 3.5	17%	38%	56%	80%
Wt of seed/plant	11.7 ± 1.0 g	17%	38%	65%	88%

The results showed a similar relationship between growth and aphid infestation at all six sites. Aphid infestations reduced stem length, number of trusses and number of pods set per plant, and the weight of seed produced. The seed was also smaller when aphid infestations were heavy. These results relate to one season only, and the relationship may change depending on weather conditions, the date of peak infestation and the subsequent collapse of colonies. These dates, however, have been very similar in the south-east over the last four years.

An increase in yield of 2-3 cwt/ac (i.e. 7-10%) is required to recover the cost of ground or aerial application of pesticides for aphid control. Using the data obtained from the single plant observations where moderate and heavy colonies gave on average 75% loss of yield this could have been expected on fields where, for example, 13% of plants had moderate or heavy colonies in early July. Similarly, using the regression obtained in the 1967 survey, a loss of 3 cwt could be expected where 15% of plants have moderate or heavy colonies in early July.

It may be possible to use these statistics for predicting whether or not control measures are justified if the number of plants colonized by primary migrants in early June is related to the number of plants with moderate or heavy colonies at peak infestation in July.

Accurate work on damage assessment is clearly difficult because of the various environmental factors affecting pest incidence and there may be no simple relationship between aphid infestation and losses from year to year. However, there was apparently good agreement between the data obtained from field experiments and surveys in 1967 and those from individual plants in 1969. Further observations are required to confirm the value of the data obtained.

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Proc. 5th Br. Insectic. Fungic. Conf. (1969)

ASSESSMENT OF LEATHERJACKET DAMAGE TO GRASSLAND AND ECONOMIC ASPECTS OF CONTROL

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This paper is a contribution from the Leatherjacket Working Party of the Closed Conference of Advisory Entomologists of the National Agricultural Advisory Service, based mainly on trials conducted in England and Wales during the last four years.

Summary The results are given of experiments designed to estimate the damage caused by known populations of leatherjackets to grassland. These refer mainly to permanent pasture and established leys and may not be directly applicable to intensively managed pasture.

With less than 1 million leatherjackets/ac in early spring it is cheaper to apply extra nitrogen to offset the damage than to apply an insecticide, unless maximum economic quantities of nitrogen are already being used. Above 1 million/ac (23/ft^2) an insecticide treatment is likely to be economically worthwhile.

If maximum amounts of nitrogen are being applied the use of an insecticide is probably justified above a leatherjacket density of 350,000/ac (8/ft^2).

Leatherjacket populations fluctuate widely from year to year and, although densities of over 1 million/ac may occur in a few fields every year, they are likely to be found in only 2-4% of fields even in 'peak' leatherjacket years.

NOTE

The treatment of grassland with DDT is not advocated even though it may be justified economically, because of the well known persistence and residue problems associated with its use. Work on alternative chemicals for leatherjacket control on grassland is in progress.

INTRODUCTION

Leatherjackets damage most arable crops but attention is generally focussed on the damage they cause to spring cereals, since these normally follow ploughed out grass and, in years of high populations, losses may be widespread and severe. Directly reseeded grass is frequently thinned but the losses caused by this pest in its normal habitat, established grassland, go largely unnoticed and until recently have received little attention in this country. Lange (1964) reports that in North-west Germany damage to grassland is likely if autumn and early winter infestations exceed 400,000/ac (9/ft^2) and that severe damage occurs if infestations exceed 1,200,000/ac (27/ft^2). White and French (1968) point out that where severe damage to established grassland is recorded the leatherjacket population is generally 2-2.5 million/ac.

In an observation trial on an established ley in Wales in 1962, Williams (personal communication) obtained an increase of 6.0 cwt d.m./ac (171%) in early bite and 17.8 cwt d.m./ac (70%) in hay yield by controlling a population of 1,045,000 leatherjackets/ac.

Grennan (1966) recorded a total reduction from 3 cuts of approximately 1 ton d.m./ac (35%) in 1965 compared to 1964, in a long term fertiliser experiment in a ley sown on virgin bog in 1961 and attributed the loss to an average leatherjacket population of 650,000/ac when assessed in May.

Kort (personal communication) recorded increases in hay yield of 25% and 73% when 1 million and 2 million leatherjackets/ac respectively were controlled.

In order to assess more accurately the damage caused by leatherjackets to grassland, replicated trials were laid down by N.A.A.S. Entomologists in the Northern Region in 1963 and 1964 (White and French, 1964). Since then, leatherjacket damage assessment on grassland has been co-ordinated by the Leatherjacket Working Party of the Conference of Advisory Entomologists and a further 10 experiments have been completed. The object of these trials was to study the interaction of leatherjacket populations and nitrogen application and to determine the population levels that justify insecticide treatment at different levels of nitrogen usage.

METHOD AND MATERIALS

Leatherjackets were controlled on half the plots in any experiment by applying DDT, and treated and untreated plots, which were 10 yd x 10 yd in size, were replicated 6-8 times in randomised blocks. The leatherjacket population was assessed before treatment and at intervals after treatment and the yield was measured at one or more crop stages corresponding to 'early bite' and cuts of silage, hay or second silage.

DDT was applied at the rate of 1 lb a.i. in 35-50 gal water/ac. It was usually applied in February or March but on one occasion (1966, Trial B) it was applied on 6th January. Normally, one application resulted in a virtual elimination of leatherjackets within one month, although in two experiments (1968, Trials B & C) a second application was needed. Low soil temperatures or the presence of a matted sward are probably the main factors reducing the efficiency of DDT.

The method used for assessing leatherjacket numbers was either:-

(a) Dawson's (1932) orthodichlorobenzene (O.D.C.B.) technique as modified by Milne et al and by N.A.A.S. Entomologists and described by George (1966), or

(b) Mayor and Browne's (1964) wet extraction method whereby leatherjackets are extracted from soil cores taken from individual plots.

Yields were assessed by cutting two 9 x 1 yd strips from each plot with an Autoscythe. Different strips were harvested at each cut but in the 1968A trial the grass which regrew in the early bite strip was cut again when silage and when hay cuts were taken. The yield of dry matter was determined in every trial; samples were analysed for crude protein and crude fibre in the 1963, 1964 and 1968A experiments.

Fertilisers were used in all except the 1963 and 1964 trials. Low nitrogen rates (21-36 units N./ac) were used in four trials in the Northern Region (1965, 1966A, 1966B and 1967A experiments). Later, following a trial in Wolverhampton (1967B) where five different nitrogen levels (0, 80, 120, 160 and 200 units N./ac) were used, five experiments were laid down using a standard design. In these three nitrogen levels (0, 120 and 200 units/ac) were combined with an insecticide treatment in a split plot design, so that half the plots at each nitrogen level were treated with DDT. 50 units/ac of phosphate and 100 units/ac of potash were also applied as an overall treatment to ensure that there would be no deficiency of these nutrients which could limit the response to nitrogen. 120 units of nitrogen was chosen as the highest nitrogen level up to which the yield response was linear (Whitehead and Cowling, 1967). Further details of the trials are given in Table 1.

Table 1

<u>Details of leatherjacket damage assessment experiments</u>

Year	Site	Soil Texture	Sward	Fertiliser applied (units)	Leatherjacket Population (000's/acre) Pre Treatment	Post Treatment	Early Bite	Yield Records Silage Cut	Hay Cut	Second Silage Cut
1963	Blagdon N'umberland	Loam	3 year ley	0	1,740 (27 Mar)	1,783 (23 Apr)	-	29 May	8 July	15 Aug
1964	Mitford N'umberland	Loam	Permanent Pasture	0	1,800 (4 Feb)	1,280 (8 Apr)	-	-	3 July	16 Sept
1965	Longtown Cumberland	Loam	Permanent Pasture	0, 21 N	385 (19 Mar)	275 (22 Apr)	-	11 June	5 July	-
1966 A	Cocklepark N'umberland	Clay loam	2 year ley	0, 36 N	25 (8 Mar)	9 (18 Apr)	-	-	15 June	-
1966 B	Walton Cumberland	Organic sandy loam	6 year ley	0, 36 N	900 (6 Jan)	600 (9 Mar)	-	-	24 June	-
1967 A	Penton Cumberland	Loam	Permanent Pasture	0, 36 N	661 (14 Feb)	388 (14 Mar)	8 June	22 June	8 July	-
1967 B	Drayton Warwicks	Clay loam	6 year ley	0, 80, 120, 160, 200 N	700 (1 Feb)	244 (17 Mar)	-	25 May	-	-
1968 A	Wivelsfield Green, E.Sx	Silt loam	9 year ley	0, 120, 200 N (50 P, 100 K)	950 (28 Feb)	1,300 (26 Mar)	9 May	27 May	13 June	-
1968 B	Penton Cumberland	Fine sandy loam	Permanent Pasture	0, 120, 200 N (50 P, 100 K)	700 (14 Feb)	475 (18 Mar)	28 May	11 June	24 June	-
1968 C	Churcham Glos.	Clay loam	Permanent Pasture	0, 120, 200 N (50 P, 100 K)	1,500 (15 Feb)	814 (4 Apr)	1 May	21 May	-	-
1968 D	Grittleton Wilts.	Clay loam	Permanent Pasture	0, 120, 200 N (50 P, 100 K)	800 (28 Feb)	172 (8 Apr)	7 May	20 May	-	-
1969	Stocksfield N'umberland	Clay loam	Permanent Pasture	0, 120, 200 N (50 P, 100 K)	1,024 (10 Mar)	1,044 (8 Apr)	29 May	12 June	26 June	-

RESULTS

The results of the 1963 and 1964 experiments (Fig. 1) showed the effect of DDT spray on yield of grass at silage, hay and second silage cuts, when the leatherjacket population in March was high, about 1.75 million/ac ($40/ft^2$).

One other trial (1968A) had a comparable leatherjacket population. Fig. 2 shows that DDT treatment doubled the yield of grass for each level of nitrogen at the first cut (early bite) and gave increases at the low, medium and high rates of nitrogen of 128%, 83% and 53% respectively at the silage cut and 75%, 48% and 16% respectively at the hay cut. The grass which grew after the first cut was recut twice and highly significant increases were recorded in the DDT treated areas; there was of course by this time little or no response to the nitrogen treatments. There seems little doubt that at these high densities leatherjackets cause heavy losses in grassland at all levels of nitrogen manuring.

The results of the 1966A trial, where the leatherjacket population was very low (less than 25,000/ac), showed that there was no response to DDT, suggesting that yield increases were not the result of growth stimulation by DDT.

The results of six of the other trials (1965, 1966B, 1967A, 1967B, 1968B and 1969) are shown in Figs. 3a and 3b. There were small but fairly consistent yield increases from DDT at all levels of nitrogen except in 1966B, where there was little response to DDT when no nitrogen had been applied.

Two other experiments (1968C and 1968D, Fig. 3c) did not conform to the pattern of the other trials. They showed no response to DDT in spite of 1,500,000 and 800,000 leatherjackets/ac being present at time of treatment (February). In the 1968D trial 800,000 leatherjackets had dropped to 172,000 (a drop of 78%) during March and this small number would be expected to have little effect on yield. In other experiments the reduction in numbers from pre- to post-treatment counts varied from nothing to about 50%. It is difficult to understand the lack of response in the 1968C experiment since 814,000 leatherjackets/ac were present at the beginning of April. A mat was present in this old permanent pasture and the leatherjackets may not have been feeding on the productive part of the sward. More work however is required to explain such variations in losses caused by leatherjackets.

Leatherjackets continue feeding until mid-June (Laughlin, 1967) and the full effect of damage is probably best measured by the loss of hay yield. Fig. 4 shows the regression of yield response of hay to DDT on leatherjacket numbers where no nitrogen was applied. The correlation coefficient ($r = 0.86$) is significant at $p = 0.001$. Fig. 5, with a correlation coefficient ($r = 0.71$) which is significant at $p = 0.001$, shows the same regression for all nitrogen levels. The lines are almost identical and show that on average 0.5 million, 1 million and 1.5 million leatherjackets/ac cause a loss of yield of about 2.5, 6 and 9.5 cwt d.m./ac respectively. Fig. 6 shows the regression of yield response of silage to DDT on leatherjacket numbers at all nitrogen levels. The correlation coefficient ($r = 0.49$) is significant at $p = 0.05$. The loss of yield is about 3.5 cwt d.m./ac when the leatherjacket population is 1 million/ac. The 2.5 cwt/ac difference in response between silage and hay cuts may be an estimate of the damage leatherjackets cause in the last few weeks of feeding, during which they double their weight (Laughlin, 1967) or it may result from earlier damage.

Fig. 1

Effect of a DDT treatment on yield of grass

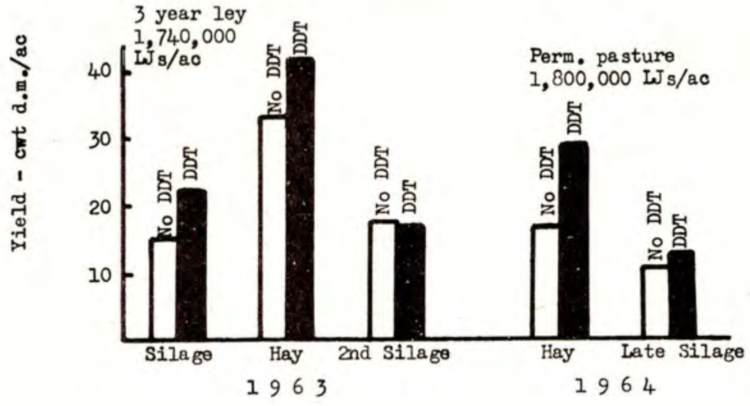


Fig. 2

Effect of DDT and nitrogen treatments on yield of grass

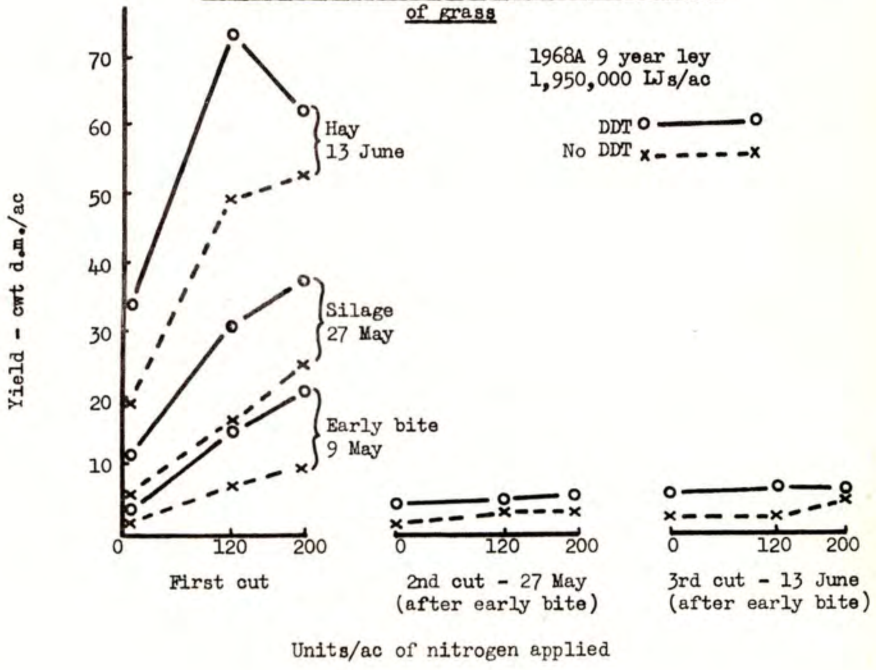


Fig. 3a, b and c

Effect of DDT and nitrogen treatments on yield of grass

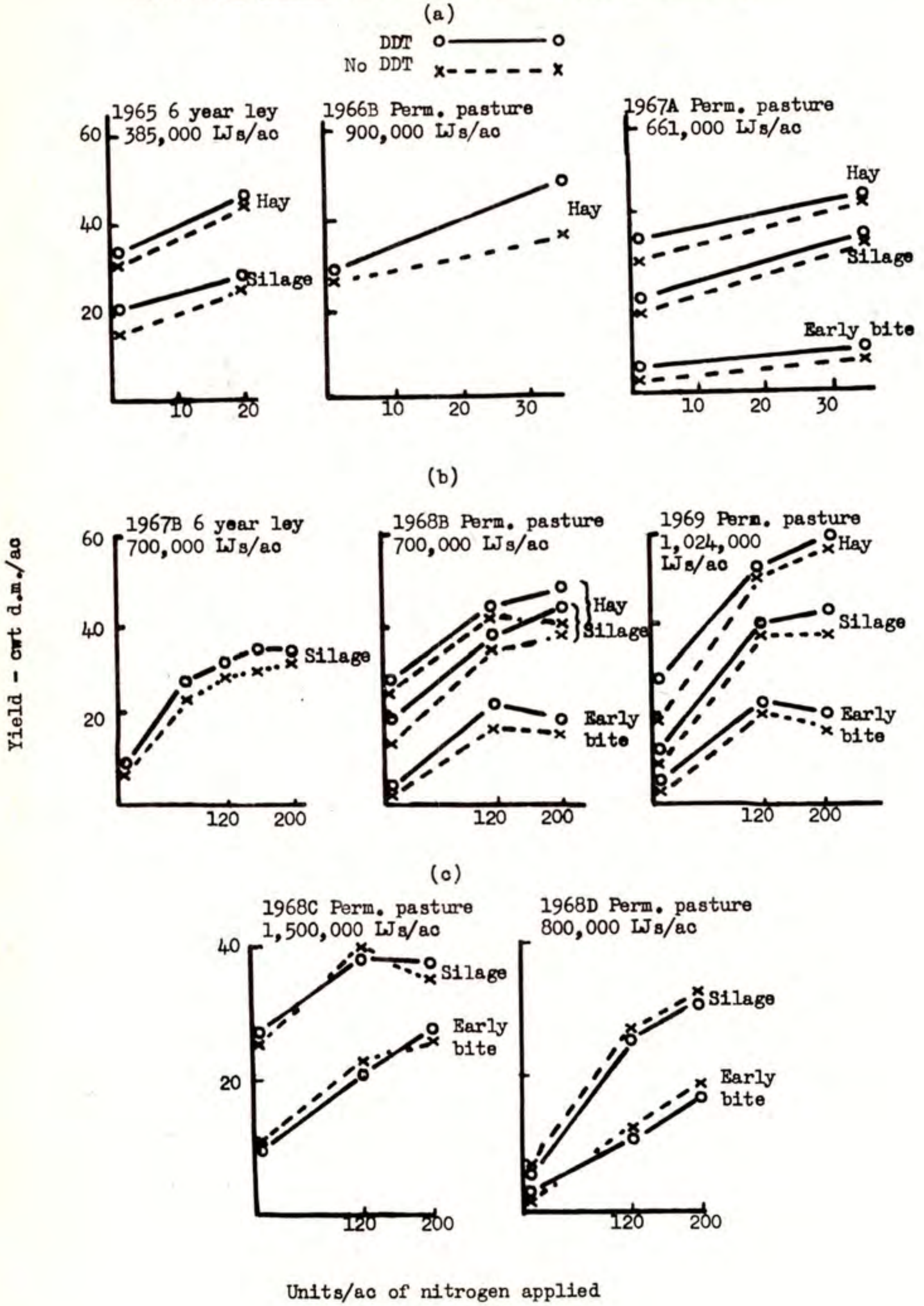
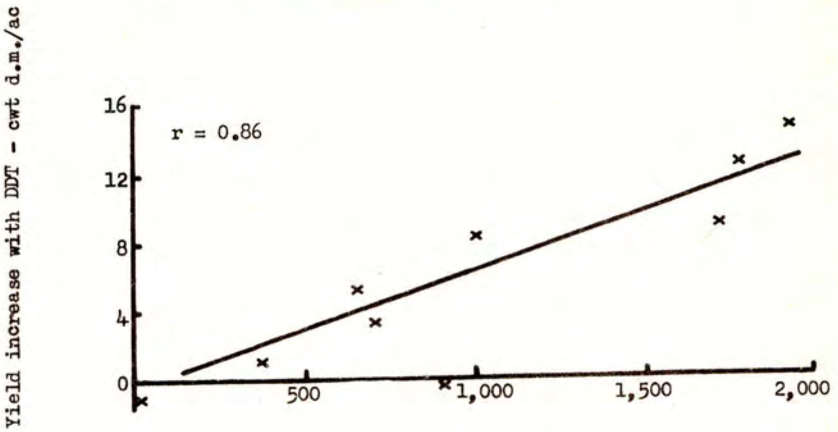


Fig. 4

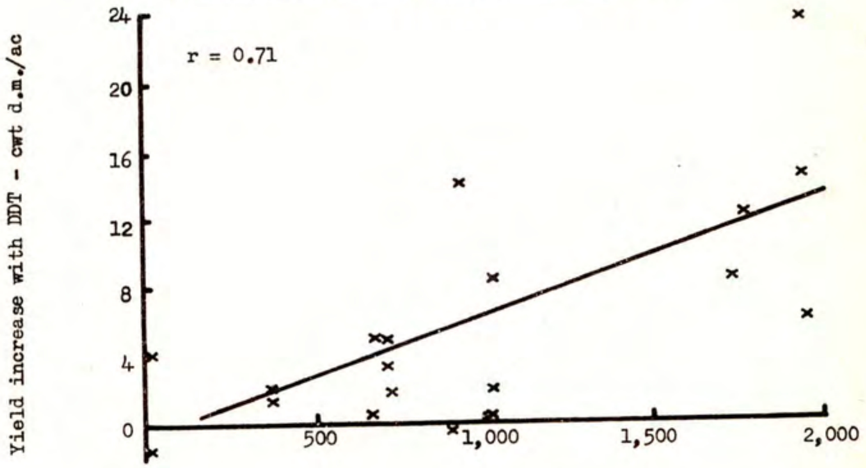
Regression of yield response of hay to DDT on leatherjacket numbers where no nitrogen was applied



Number of leatherjackets/ac (000's) before treatment (Feb-March)

Fig. 5

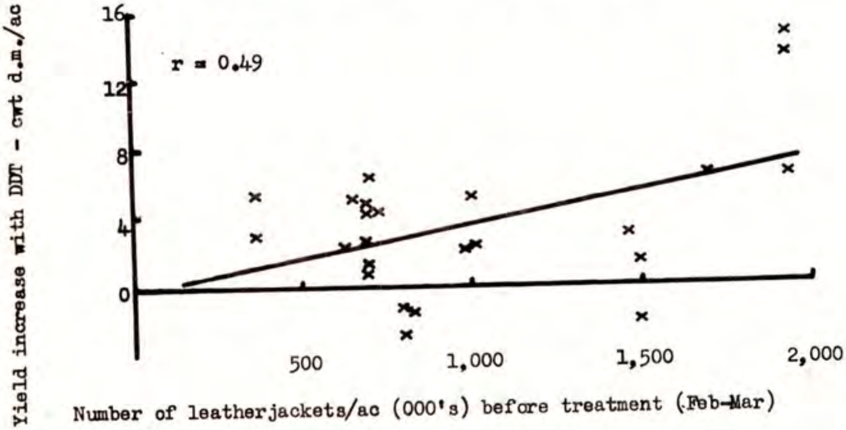
Regression of yield response of hay to DDT on leatherjacket numbers at all nitrogen levels



Number of leatherjackets/ac (000's) before treatment (Feb-March)

Fig. 6

Regression of yield response of silage to DDT on leatherjacket numbers at all nitrogen levels



DISCUSSION

Variations in yield response

Variation in response to DDT in different trials may be due to factors such as weather, soil texture, compaction, pasture management or sward characteristics. In these experiments the soil textures were generally medium to heavy loams. Two trials on lighter soils did not show consistent differences in response. Compaction may be an important factor which was not assessed in these experiments. Loose soil conditions would allow freer leatherjacket movement resulting in more root damage and slow recovery of the sward. Very severe damage to pasture has occurred on a loose peaty soil.

Sward composition and thickness of mat which would be influenced by management may affect the response. White and French (1968) showed that the yield of white clover and timothy was significantly reduced but that of perennial ryegrass was not. Golightly (personal communication) reported that in an observation study there was no loss of yield from a predominantly cocksfoot ley with 1,600,000 leatherjackets/ac. Thin pastures or new leys may suffer more severe loss than swards with a thick mat.

Chemical analysis of herbage samples

Analysis of samples from all cuts in 1963 and 1964 experiments showed no difference between protein content or starch equivalent of the treated and untreated areas. In the 1968 experiment DDT resulted in a slight increase in crude protein, especially at the lower nitrogen levels, which could be ascribed to a higher proportion of clover. At the higher nitrogen levels, where the proportion of clover was reduced, the crude protein content of the treated and untreated areas was similar.

Economics of controlling leatherjackets

Examination of the costs of applying nitrogen or DDT and the returns from these suggests that the optimum treatment will vary under different circumstances.

It can be seen from Figs. 2, 3a and 3b that the nitrogen response curve with DDT is generally parallel to and above the nitrogen curve without DDT. Addition of nitrogen will make good this difference up to a certain level of damage. Above this level additional nitrogen alone cannot bring the yield of grass up to the level of the nitrogen plus DDT curve (Fig. 2). Provided maximum economic rates of nitrogen are not already being used, it is cheaper to use extra nitrogen up to the point where the cost of nitrogen required to make good the loss caused by leatherjackets is no longer less than the cost of DDT. Assuming that 1 unit of nitrogen applied costs 10d then the cost of the DDT treatment (19/- applied) would buy 22.8 units of nitrogen. One unit of nitrogen produces 30 lb d.m./ac in the spring, therefore 22.8 units would produce 6.1 cwt d.m./ac. It can be seen from the regression lines (Fig. 4 & 5) that 6.1 cwt d.m./ac loss in yield of hay is caused by about 1 million leatherjackets/ac ($23/ft^2$). Therefore, below this leatherjacket level it is cheaper to apply nitrogen and above it is cheaper to apply DDT.

It should be pointed out, however, that the nitrogen response curve with DDT maintains its position above the one without DDT so that if maximum economic quantities of nitrogen are already being used an insecticide should be applied to obtain maximum yields. Assuming the value of 1 cwt d.m. of grass is about 12/-, then 1.5 cwt increase in yield will pay for a DDT treatment. The regression lines (Figs. 4 & 5) show that the average leatherjacket population in March causing 1.5 cwt d.m./ac loss is about 350,000/ac ($8/ft^2$). Few farms, if any, would in practice use more than 60 units of nitrogen/ac for hay because of the difficulty of handling and drying the extra herbage produced and in these circumstances leatherjacket numbers should exceed 1 million/ac before insecticides are applied.

The loss caused by leatherjackets to grassland under intensive grazing systems, where up to 100 units of nitrogen may be applied after each grazing, is not known and requires investigation. The losses in the first and second recuts in the 1968A experiment however suggest that leatherjacket damage to regrowth continues into June and this is supported by Loughlin (1967) who recorded that leatherjackets double in weight in the last month of feeding which ends in mid-June. Under these conditions of high nitrogen usage it may not be feasible to increase nitrogen rates and insecticide treatment may be justified at a lower level (350,000/ac).

Leatherjacket populations in grassland

Before any assessment can be made of the importance of leatherjackets in grassland in this country, it is necessary to know the level of populations occurring. Cohen (1953) and White (1963) estimated that peak years occur on average one year in five and Milne et al (1965) concluded that large drops in population are mainly caused by dry conditions in September leading to desiccation of eggs and young larvae.

Table 2, giving the autumn leatherjacket densities in grass fields in the Northern Region since 1953, shows that there are large seasonal variations in numbers. The same fields sampled each year had populations of over 1 million/ac in only two seasons (1962-63 and 1963-64). Table 3 shows the level of populations where routine samples were taken in the Northern and South-east Regions and in western parts of the country over the last four years. A relatively small percentage of fields have high populations even in 'peak' leatherjacket years such as 1968 and 1969. There are, however, 9.5 million acres of permanent grass and leys in these areas and high populations may give considerable losses in these years. For example, if 1 million leatherjackets/ac is used as the critical level, in 1968-69 approximately 380,000 acres would have suffered some damage.

Table 2

Leatherjacket populations per acre in permanent grass fields in Northern region sampled during autumn from 1953-68

Year	Total Fields	Percentage of fields within each category 0-250,000	250,000-500,000	500,000-1,000,000	1,000,000+
1953	18	95	5	0	0
1954	20	95	0	5	0
1955	10	70	20	10	0
1956	16	63	32	5	0
1957	21	100	0	0	0
1958	27	100	0	0	0
1959	2	100	0	0	0
1960	15	100	0	0	0
1961	22	95	5	0	0
1962	35	49	28	14	9
1963	35	63	28	6	3
1964	34	91	9	0	0
1965	30	93	4	3	0
1966	33	91	9	0	0
1967	29	96	4	0	0
1968	33	64	24	12	0

Table 3

Leatherjacket populations per acre in grass fields in England and Wales sampled during autumn and winter 1965-66 to 1968-69

Year	Total Fields	Percentage of fields within each category 0-250,000	250,000-500,000	500,000-1,000,000	1,000,000+
1965-66	135	92	5	3	0
1966-67	123	85	11	4	0
1967-68	154	78	14	6	2
1968-69	297	71	14	11	4

The detection of the small proportion of fields with high populations would be extremely difficult for anyone but the farmer. Some farmers, who are already making full use of their grassland, have expressed interest in the O.D.C.B. technique which would be the easiest method for them to measure leatherjacket infestations. Entomologists in the N.A.A.S. already forecast likely abundance of leatherjackets on a regional basis. In years of high infestations it would be feasible for interested farmers to sample their own land and apply appropriate treatments.

Kort (personal communication) reports that, following a general warning from The Agricultural Advisory Service, farmers in the Netherlands sample their own fields by immersing cores of turf in brine.

This series of trials has made it possible to forecast more accurately the loss which known populations of leatherjackets are likely to cause to grassland. The total yield loss is probably best measured by loss of yield of hay and the trials have shown that on grassland for conservation for hay, where less than maximum rates of nitrogen are normally applied, extra nitrogen will more cheaply offset the loss than an insecticide treatment unless populations exceed 1 million/ac - a rare occurrence even in peak leatherjacket years.

In a well managed grazing enterprise, where maximum use is normally made of nitrogen fertilisers, an insecticide treatment may be worthwhile at lower leatherjacket levels (about 350,000/ac) though more work is required on this aspect of damage assessment.

Acknowledgments

I thank my colleagues in different N.A.A.S. Regions for their co-operation in supplying much of the data in this paper and farmers who were inconvenienced by trials on their land. Thanks are also due to Mr. J. J. W. Williams, Mr. J. M. Rayner and Dr. W. H. Golightly for allowing me to quote unpublished results, to Mr. W. I. St.G. Light for help with statistics, to Mr. C. Dibb for much useful discussion and assistance with crop data, to Mr. P. Carden and Mr. B. D. Moreton for helpful comments on the draft of this paper and to Mr. B. Emmett and Mr. F. A. B. Ludlam for preparation of the figures.

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FORMETANATE - A NEW ACARICIDE

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Summary Formetanate is the common name for a new acaricide, 3-((dimethyl aminomethylene)imino)phenyl-N-methylcarbamate, discovered by Schering A.G., Berlin, and developed in the United Kingdom by Boots Pure Drug Co. Ltd. Since the compound is of low solubility, it is formulated as a soluble hydrochloride salt and all experimental work in the U.K. has been carried out with the salt formulation. Mammalian acute oral LD50 varies between 18 - 24 mg/kg. and dermal toxicity to rats is more than 5600 mg/kg.

During 1967, laboratory tests confirmed that formetanate has a pronounced effect on all stages of glasshouse red spider mite (*Tetranychus urticae*), and was of very high potency on strains resistant to organophosphorus compounds.

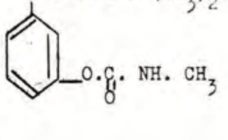
Extensive field trials over three years have demonstrated that formetanate is effective on resistant and susceptible strains of fruit tree red spider mite (*Panonychus ulmi*). Commercially acceptable control is obtained on apples by application of two sprays at 6 oz a.i./acre (L.V.) or 3 oz a.i./100 gal (H.V.) with no damage to the crop. So far no significant residues have been found in apples.

On resistant glasshouse red spider mite on roses formetanate has produced satisfactory control with one spray of 4 oz a.i./100 gal and in a single trial on chrysanthemums with two sprays of 7.5 oz a.i./100 gal, with no phytotoxicity to the crops.

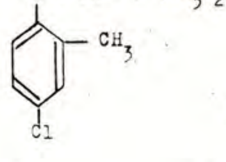
INTRODUCTION

Acaricidal resistance among phytophagous spider mites is a well documented phenomenon, having been known and studied in many countries of the world for the past twenty years (Helle 1965). Multi-chemical resistance is now a common feature in many populations of spider mites and for this reason there is an urgent need for compounds of novel chemical structure.

A few years ago, Schering A.G., Berlin, discovered an entirely new group of active acaricides. One of the most active members of this group was S.36056, 3-((dimethylaminomethylene)imino)phenyl-N-methylcarbamate* which has been given the common name, formetanate. The structure of formetanate is given below (1):-



(1)



(2)

* or N,N-dimethyl-N'-(3-(N-methylcarbamoyloxy)phenyl)formamidine

A second member of the group is chlorphenamidine (II), but the acaricidal activity of the two compounds is somewhat different. Formetanate is more potent on active stages than eggs but the reverse is the case with chlorphenamidine.

FORMULATIONS

Since formetanate is of low solubility, it is formulated as the water soluble hydrochloride salt, and (unless otherwise stated) this formulation has been used in all tests, but the dose is given as the active base in all cases.

CHEMICAL AND PHYSICAL PROPERTIES

	Base	HCl salt
Molecular weight:	221.3	257.7
Empirical Formula:	$C_{11}H_{15}N_3O_2$	$C_{11}H_{16}ClN_3O_2$
Appearance:	slightly yellowish crystalline substance	white powder
Melting point:	102 - 103°C	200 - 202°C (decomposition)
Volatility:	not volatile	not volatile
Solubility:	in water < 0.1% in benzene ~ 0.1% in acetone ~ 10% in chloroform ~ 10% in methanol < 20%	in water > 50% in benzene < 0.1% in acetone < 0.1% in chloroform 0.2% in methanol ~ 25% in hexane < 0.1%
Stability:	to hydrolysis: half-life in water/methanol solution 95 : 5 pH 4 130 hours pH 7 16 hours pH 9 100 minutes	to hydrolysis: see base pH 4 very slow hydrolysis
	to temperature: no changes observed when stored dry for 5 days at 60°C	to temperature: no changes observed when stored for 7 days at 60°C

TOXICOLOGY

Acute oral toxicity:	LD50	Rat -	base	24 mg/kg
		-	hydrochloride	20 mg/kg
		Mouse -		18 mg/kg
		Beagle dog -		19 mg/kg
		Chicken -		21.5 mg/kg
				(no neurotoxic properties observed)
Acute dermal toxicity:	LD50	Rat -	more than 5600 mg/kg	
		Rabbit -	more than 10,200 mg/kg	
Inhalation toxicity:	LC50 (4 hour exposure)		0.29 mg/l	
Bird toxicity (7 day cumulative):	LC50	Pheasant	- > 4640 mg/kg	
		Duck	- 6810 mg/kg	
		Quail	- > 4640 mg/kg	

Fish toxicity (4 day average tolerance limit):

Rainbow trout	- 2.8 ppm
Black bullhead	-75.0 ppm
Blue gill	-20.0 ppm

Chronic toxicity tests over one year have revealed no histo-pathological effects in dogs and rats when fed daily diets containing 100 ppm and 200 ppm respectively of formetanate hydrochloride. Formetanate is a cholinesterase inhibitor but no cumulative effect on cholinesterase activity was recorded in 90 day feeding tests. Toxicological data on formetanate has been submitted for clearance under the Pesticides Safety Precautions Scheme.

BIOLOGICAL ACTIVITY

In laboratory and glasshouse tests, formetanate has shown good activity on all stages of glasshouse red spider mite (Tetranychus urticae) (Table 1).

Table 1

Comparative laboratory activity of formetanate on glasshouse red spider mite

Toxicant	LD 50 ppm *		
	Adults	Larvae/Eggs	Eggs
Formetanate	80	12	84
Chlorphenamidine	280	17	20
Demeton-S-methyl	2	14	
Diazinon	38	340	
Malathion	56	625	
Dimethoate	4	11	

* Sprayed leaf disc technique.

A particularly interesting feature of the acaricidal activity of formetanate is its enhanced potency on resistant strains of mites (Table 2). Steinhausen (1968) has studied this effect in detail and his data indicate that laboratory strains of glasshouse red spider mite resistant to organophosphorus compounds are more susceptible to formetanate and therefore clearly fit Ascher's model of "resistance-induced enhanced susceptibility". Chlorphenamidine similarly has a more pronounced effect on organophosphorus strains of glasshouse red spider mite (Dittrich 1969). The phenomenon would therefore appear to be a property of formamidine acaricides.

Table 2

Formetanate - activity on adult glasshouse red spider mite, resistant and susceptible strains, in comparison with organophosphorus compounds

Toxicant	LC50 ppm.* Normal strain	OP resistant strain
Formetanate	2.80	0.35
Dimethoate	0.60	300
Demeton-S-methyl	0.75	450
Prothoate	0.80	800
Azinphos methyl	0.95	280
Diazinon	13.0	-
Methiocarb	17.0	55

* Slide dip technique.

FIELD TRIALS

Fruit tree red spider mite on apples

Replicated field trials in the U.K. on fruit tree red spider mite were carried out during 1967-68, comparing formetanate alone and in admixture with chlorphenamidine, with other commercial acaricides in a traditional two spray programme (Tables 3 - 4).

Table 3

Comparison of formetanate with other acaricides for the control of fruit tree red spider mite, 1967

Treatment oz a.i./100 gal (H.V.)	Mobile mites per 10 leaves 12/6	10/7	10/8	13/9
Formetanate				
4	44	0	56	45
Formetanate/ Chlorphenamidine 1:2				
16	18	1	38	35
8	49	19	102	46
Chlorphenamidine				
16	19	1	97	104
8	93	21	213	296
Phenkapton				
3.2	10	6	48	74
Tetradifon				
2	33	1	166	112
Untreated	109	450	1500	2430

Site details: Crop - apples variety - Worcester Pearmain
Spray dates - 26/5
16/6

Table 4

Comparison of formetanate with other acaricides for the control of fruit tree red spider mite, 1968

Treatment oz a.i./100 gal (H.V.)	Site 1 12/6	3/7	31/7	27/8	Site 2 20/6	16/7	14/8	12/9
	Mobile mites per 10 leaves							
Formetanate								
8	-	-	-	-	10	27	13	34
4	23	4	1	24	10	33	0	27
2	39	5	15	15	-	-	-	-
Formetanate/ Chlorphenamidine 1:2								
8	34	2	1	12	40	30	0	70
4.8	-	-	-	-	17	57	23	7
4	28	4	2	40	-	-	-	-
Phenkapton								
3.2	5	5	0	26	43	27	33	103
Tetradifon								
2	75	14	1	56	35	45	55	490
Untreated	284	361	157	-	1690	2220	-	3645

Site details: Crop - apples variety - Worcester Pearmain

Spray dates:- Site 1: 29/5, 19/6; Site 2: 7/6, 2/7

Table 5

Comparison of formetanate with other acaricides for the control of fruit tree red spider mite, 1969

Total mites per 10 leaves

Treatment oz a.i./100 gal (H.V.)	Site 1 26/6	14/7	1/8	Site 2 3/7	24/7	26/8	Site 3 3/7	24/7	21/8
Formetanate									
4	20	16	23	4	16	13	145	4	10
3	99	31	30	7	15	12	183	10	20
2	28	35	21	10	40	31	257	12	36
1	69	62	149	4	48	75	-	-	-
Phenkapton									
3.2	42	31	101	4	30	31	71	9	28
Tetradifon									
2	89	176	72	8	107	122	353	13	64
Untreated	269	511	501	70	365	215	520	488	1390

Site details: Crops - apples variety - Worcester Pearmain (1 and 3)
Discovery (2)

Spray dates (1) 9/6, 4/7 (2) 11/6, 10/7 (3) 19/6, 11/7

Resistance to tetradifon is indicated in these populations.

Table 6

Comparison of formetanate with other acaricides for the control of glasshouse red spider mite on roses, 1969

Treatment oz a.i./100 gal (H.V.)	% age infected leaves Site 1					Site 2		Site 3			
	19/6	27/6	3/7	10/7	22/7	10/7	27/7	19/8	26/8	2/9	10/9
Formetanate											
8	75	3.5	0	8.5	0	72	6.5	-	-	-	-
4	-	-	-	-	-	57	5.0	65	8	13	3
4 x 2	-	-	-	-	-	-	-	-	-	0	2
* "Pentac"											
8	73	21.5	10.0	16.5	50.0	65	25	67	72	53	57
8 x 2	-	-	-	-	-	-	-	-	-	38	33
Untreated	62	33.5	51.5	48.5	83.0	80	41.5	71	73	68	60

Site details: varieties - (1) Baccara (2) Super Star (3) Pink Sensation

Spray dates (1) 19/6 (2) 10/7 (3) 19/8 and for x2, 19/8 and 26/8

Wetter was added to all treatments.

* Bis (pentachlorocyclopentadienyl)

Although a formetanate/chlorphenamidine mixture is available for commercial use on the continent, no advantage can be seen in the combination under U.K. conditions. The activity of chlorphenamidine, at least in part, is associated with its vapour phase and therefore differences in temperatures between the U.K. and the continent may account for the decreased effectiveness of the compound in this country.

During 1969, formetanate was evaluated further in replicated trials using a wider range of doses than previously (Table 5). A high level of control was achieved again at rates down to 2 oz a.i./100 gal (H.V.), but at the lower rate of 1 oz a.i./100 gal (H.V.) control begins to break down.

An extensive programme of farm reliability trials has been carried out where single large plots of apples were sprayed by growers following instructions, and assessment made by trained staff. Two rates were examined, 4 and 6 oz a.i./acre (L.V.) or 2 and 3 oz/100 gal (H.V.), in a programme of two sprays, the first applied at petal fall and the second, 3 weeks later. Adjacent plots were sprayed with suitable standard acaricides. Normal susceptible mites and populations resistant to bridgeddiphenyl and organophosphorus compounds were included in these trials, and results showed that a very high level of control was obtained in all cases with the high rate of application but the low rate was not adequate. Where resistance was not a problem, control with the high rate of formetanate was equal or better than that obtained with tetradifon, dicofol, azinphos-methyl or varied programmes of tetradifon/dicofol, demeton-s-methyl/tetradifon and demeton-s-methyl/vamidothion/dicofol.

Glasshouse red spider mite on roses and chrysanthemums

In replicated trials carried out in S.E. England by the N.A.A.S.(French 1969), formetanate produced excellent control of resistant glasshouse red spider mite on roses (Table 6). A single spray of 4 oz a.i./100 gal gave satisfactory control but two sprays with a 7-day interval produced a dramatic reduction in the numbers of infested leaves.

On A.Y.R. chrysanthemums, excellent control of resistant mites was obtained with two sprays of 7.5 oz a.i./100 gal at a 7-day interval (Table 7).

Table 7

Comparison of formetanate with other acaricides for the control of glasshouse red spider mites on chrysanthemums

Treatment oz a.i./100 gal (H.V.)	Mean no. of mites and eggs on 3 leaves per plant 21/7		28/7	
	Mites	Eggs	Mites	Eggs
Formetanate 7.5	0	56	1	6
Dicofol 8	67	109	46	110
Tetradifon 2	81	172	25	163

Site details: Crop - chrysanthemums
Spray dates - 14/7, 21/7.
Population resistant to dicofol, tetradifon and other standard acaricides.

CROP SAFETY

Formetanate has been applied to sixteen apple varieties, alone and in admixture with pertinent insecticides and fungicides, both double and triple mixtures, and no damaging effects have been noted at the recommended rate of application.

No phytotoxicity was observed in the trials on roses or chrysanthemums (French 1969).

RESIDUES

Residue data are being obtained for apples treated in the U.K. but no results are available at present. Data are however available from overseas trials, and show that there is a significant decrease in the level of formetanate with time (Table 8).

Table 8

Degradation of formetanate on apples

days after treatment	ppm of formetanate
1	1.5
7	0.5
14	0.2
28	0.3

Variety - Cox's Orange Pippin
Origin - Germany
Rate of application - 8 oz a.i./100 gal (H.V.)

INSECTICIDAL ACTIVITY

Formetanate is active on various insects and slugs but its insecticidal and molluscicidal efficacy is still under evaluation and will be reported elsewhere.

CONCLUSIONS

Formetanate has shown good activity on all stages of red spider mites, and exhibits greater potency on laboratory strains of glasshouse red spider mites resistant to organophosphorus compounds than normal susceptible strains.

Field trials have indicated that two sprays of formetanate, the first applied at petal fall and the second, three weeks later, will give a commercially satisfactory control of fruit tree red spider mite at 6 oz a.i./acre (L.V.) or 3 oz a.i./100 gal (H.V.), and is effective on resistant as well as normal susceptible populations. This rate has been used safely on apples, both alone and in admixture with relevant insecticides and fungicides.

In replicated trials on resistant glasshouse red spider mite on roses, formetanate gave good control with one spray of 4 oz a.i./100 gal, and in a single trial on A.Y.R. chrysanthemums with two sprays of 7.5 oz a.i./100 gal, with no phytotoxicity to the crops.

Acknowledgments.

The authors wish to acknowledge the help and collaboration from staff of Schering A.G., Berlin who discovered formetanate. Thanks are also expressed to our many colleagues of Lenton Research Station and Boots Farm Sales who assisted with the trials, and growers who kindly carried out trials for us.

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Proc. 5th Br. Insectic. Fungic, Conf. (1969)

IODOFENPHOS - A BROAD REVIEW OF A PROMISING NEW INSECTICIDE

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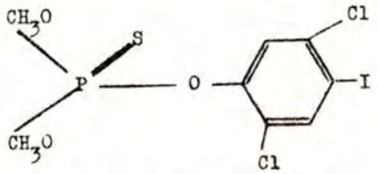
Summary: Iodofenphos is an organophosphorus insecticide of very low mammalian toxicity which is effective as a contact and stomach poison against a wide range of insect pests. Its low toxicity and long persistance make it a very useful insecticide in farm and public hygiene and in protection of stored products. A deposit of 1g a.i./m^2 of treated surface gave effective protection against flies for up to eight weeks on alkaline surfaces. On refuse tips the relatively low weekly dose of 0.3 g a.i./m^2 has given control of flies comparable to that of fenitrothion. Preliminary trials in grain stores and laboratory investigations have shown that iodofenphos is effective against a wide range of insect pests occurring in such buildings and appears to be more active than malathion. Work from the Continent indicates that iodophenphos also shows promise for protection of bulk grain. In crop protection iodofenphos has given good control of pre and post-blossom pests in top fruit, dipterous and lepidopterous larvae in Brussels sprout buttons and pollen beetle and seed weevil in oil seed rape.

INTRODUCTION

Iodofenphos is the common chemical name for O,O-dimethyl-O-2,5-dichloro-4-iodophenyl phosphorothionate and has the following structural formula:



Iodofenphos was first synthesised by CIBA Limited in Basle, Switzerland and introduced into the U.K. in 1966 with the code number C 9491. Since then it has been extensively tested as an insecticide for use in the crop protection and hygiene fields of use.

PHYSICAL AND CHEMICAL PROPERTIES

Molecular weight:	413
Physical state:	white crystals
Melting point:	75°C
Odour:	slight
Solubility:	soluble in acetone and xylene, slightly soluble in alcohol. Solubility in water less than 2 ppm.

Iodofenphos is available as a 20% e.c., 50% w.p. and as a 5% dust for use in the different fields of application.

TOXICITY

Table 1

Acute toxicity data

Animal	Sex	Method of Application	LD 50
Mouse	Female	Oral	>10,000 mg/kg
Rat	Both	Oral	2,100 mg/kg (1550-2910)
Rabbit	Male	Oral	ca. 2,000 mg/kg
Cat	Male	Oral	3,000 mg/kg
Dog	Both	Oral	3,000 mg/kg
Rat	Both	6 hr inhalation of 246 mg/m^3	Caused no effect
Rabbit	Both	Skin application of 0.5 g	Caused no irritation
Rabbit	Both	Injection of 0.1 g into conjunctival sack	Caused no irritation

Table 2

Sub-acute toxicity data

Animal	Sex	Method of Application	Symptoms
Rat	Both	242 mg/m^3 ($\pm$ 20) 19 x 6 hr daily inhalation	Slight - moderate cholinesterase inhibition
Rabbit	Both	0.3 g/kg applied 21 times to the skin	Slight cholinesterase inhibition

Ninty day feeding studies in rats and dogs showed no clinical symptoms when fed at the rate of 45 mg/kg. Histopathological examination of the central nervous system showed no evidence of neurotoxic damage.

Toxicological investigations with wild birds showed that iodofenphos presented no hazard to these animals but it has been found to be toxic to bees and fish.

MODE OF ACTION

Tests have shown that iodofenphos is a contact and stomach poison, which is effective through inhibition of the cholinesterase system, and which has a very long residual life as a spray deposit.

TRIAL RESULTS

Farm Hygiene

Since 1968 extensive trial work has been undertaken to examine the efficiency of iodofenphos in controlling Musca domestica and Fannia canicularis in various farmyard situations.

Materials and Methods

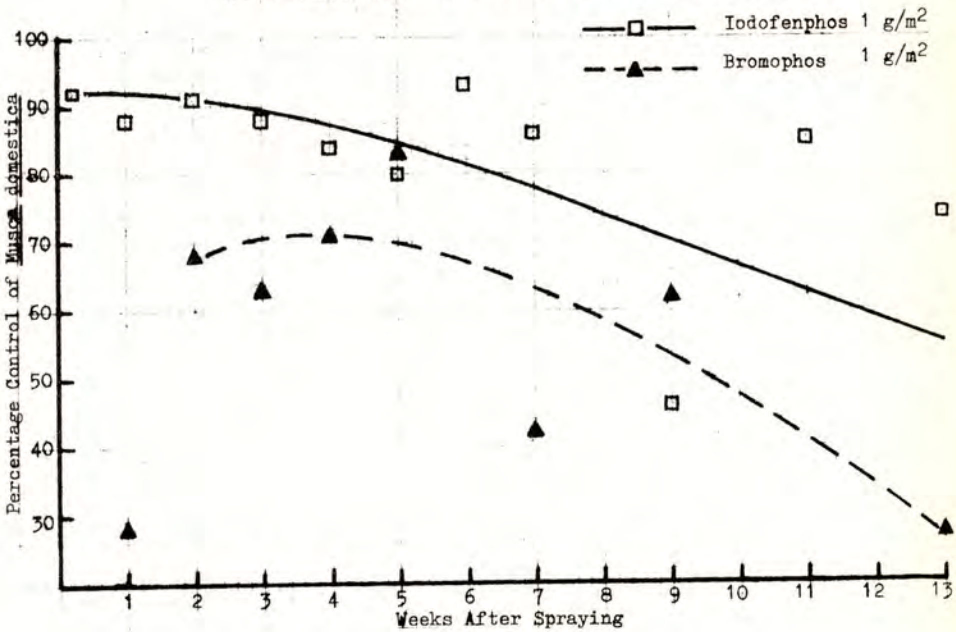
Iodofenphos	50% w/w w.p.
Bromophos	25% w/w w.p.

Work was carried out in piggeries, calf-rearing houses and a mink farm, and covered a wide range of surfaces including wood, corrugated iron, asbestos, concrete and brick walls which were either unpainted or whitewashed. The majority of surfaces treated were therefore of alkaline reaction. Wherever possible bromophos was used as a comparator under similar conditions to those in which iodofenphos was being tested. Applications were made to run off (100 ml/m^2) with a high pressure pumping unit and hand lance fitted with hollow cone nozzles.

Assessments were made by direct counts of flies on a given area at regular intervals following treatment of the calf-rearing houses and mink farm. Additional counts of flies resting on the calf suckling units were also made in the calf-rearing houses. In the piggeries the number of flies resting on the pigs were counted. Assessments were continued until fly numbers in treated and untreated sections approached each other or the population decreased to insignificant numbers. The results of these trials are summarised in Fig. 1.

Fig. 1

Mean Percentage Control of Musca domestica at Intervals After Spraying in Farm Hygiene Trials, 1968–1969.



Refuse Tips

During 1968 several trials were undertaken to assess the effectiveness of iodofenphos against flies infesting domestic refuse tips. The fly species present were mainly M. domestica and F. cinicularis.

Materials and Method

The tipping faces of domestic refuse tips were divided up into three areas. Two of these areas were treated with 30 g a.i./100m^2 of iodofenphos and 45 g a.i./ 100 m^2 of fenitrothion respectively, the third area being left untreated. Weekly applications for a period of eight weeks were made using a pumping unit and hand lance. The tip face and surrounding area were aprayed and counts of the numbers of flies resting on ten 1 ft^2 surfaces randomly selected within each area were made prior to spraying.

In addition to the plot trials, commercial user trials were also undertaken by a number of Local Authorities.

Refuse tip trial results

This work indicated that iodofenphos gave a control of flies comparable to fenitrothion, although in most instances the number of flies present on the treated areas remained high, due to incursion from untreated areas. However, in the Local Authority trials where the whole tipping face was treated, season long control of flies was obtained.

Grain Stores

Preliminary trials against insect pests of grain stores was begun during 1969.

Materials and Method

Iodofenphos	50% w/w w.p.
Iodofenphos	20% w/v e.c.
Malathion	60% w/v e.c.

A number of empty grain stores were treated with iodofenphos 50% w.p., iodofenphos 20% e.c. or malathion 60% e.c. Suitable control areas were left at each site.

Applications were made with a pumping unit and hand lance. In each case the range of surfaces assayed included metal, wood and brick.

A fortnight after treatment bio-assay tests were carried out using Sitophilus granarius, Oryzaephilus surinamensis and Cryptolestes ferrugineus. A number of these insects were enclosed in insect traps attached to the treated surfaces. Forty eight hours later the traps were removed and counts of beetles alive and dead were made.

Grain store results

Chemical And Formulation	Dose g a.i./m^2	% Control Of All Test Species
Iodofenphos 50% w.p.	1.0	100
Iodofenphos 20% e.c.	1.0	100
Malathion 60% e.c.	1.0	100

Brussels Sprouts

In recent years with the growth of acreage of Brussels sprouts for processing, the damage to sprout buttons caused by dipterous and lepididipterous larvae has become increasingly important. During 1968 two trials were undertaken to assess the effectiveness of iodofenphos against these pests.

Materials and Methods

Iodofenphos	20% w/v e.c.
Trichlorphon	80% w/w w.p.

Both trials were of randomised block design with 4 replicates. Single dose applications were made one month and a forthnight before harvest, with a knapsack sprayer using 50 gal water/ac in one trial and 100 gal water/ac in the other. Assessments were made on 66 buttons per plot collected from 11 plants and examined for superficial lepidopterous larvae damage and for dipterous larvae mines.

Trial 1

Pests: (i) Dipterous

Cabbage root fly larvae, *Erioischia brassicae*
Onion and bean seed fly larvae, *Delia antiqua* and *D. cilicruva*

(ii) Lepidopterous

Diamond back moth larvae, *Plutella maculipennis*

Compound	Dates Of Application	Dose In lb a.i./ac	% Damage By Lepidopterous Larvae	% Damage By Dipterous Larvae
Iodofenphos	6 & 19 AUG	0.5 + 0.5	14.6	2.5 a
Iodofenphos	6 AUG	1.0	12.1	4.0 a
Trichlorphon	6 & 19 AUG	1.0 + 1.0	12.6	2.5 a
Untreated			28.8	10.6 b
			NS	

Results followed by a common letter are not signifanctly different at P = 0.05 using Duncan's Multiple Range Test.

Trial 2

Compound	Dates Of Application	Dose In lb a.i./ac	% Damage By Lepidopterous Larvae	% Damage By Dipterous Larvae
Iodofenphos	9 & 19 AUG	0.5 + 0.5	9.1	3.0
Iodofenphos	9 AUG	1.0	14.1	3.5
Trichlorphon	6 & 19 AUG	1.0 + 1.0	4.5	5.0
Untreated			20.7	9.6
			NS	NS

DISCUSSION

The farm hygiene trials showed that a deposit of 1 g a.i./m^2 iodofenphos gives a good control of <u>M. domestica</u> for up to 8 weeks. According to Moyses (1968) iodofenphos can give effective control of flies for up to 3 months. Parkin and Forster (1967a) have also shown that there was no significant breakdown of iodofenphos deposits on filter paper up to 2 months after application. However, in the trials reported in this paper most of the surfaces treated were of alkaline reaction which, as has been shown by Parkin (1966) and Burkholder and Dicke (1966), can reduce the toxicity and severely reduce the persistence of many organophosphorus insecticides. Nevertheless, a 8 week effective control period is considerably longer than that obtained with materials such as malathion where respraying every three weeks is recommended.

The low toxicity of iodofenphos to mammals makes it particularly suitable for use in animal houses, and enables application by farm workers with comparative safety in enclosed areas. Iodofenphos has also been successfully used in trials as a sheep dip for control of ectoparasites.

In the trials on refuse tips, iodofenphos gave control of <u>M. domestica</u> and <u>F. canicularis</u> equivalent to that of fenitrothion. However, the control afforded by both compounds in the plot trials was poorer than would have been expected, due to incursion of flies from the untreated plots. Where tips were treated in commercial user trials, the control given by iodofenphos was excellent.

The bio-assays following treatment of grain stores showed that iodofenphos gave excellent control of <u>Sitophilus granarius</u>, <u>Oryzaephilus surinamensis</u> and <u>Cryptolestes ferrugineus</u>. In laboratory tests carried out by Parkin and Forster (1967b), iodofenphos was as good as or up to five times more effective than malathion in controlling <u>S. granarius</u>, <u>S. zeamais</u>, <u>Tribolium castaneum</u>, <u>Lassioderma serricorne</u>, <u>O. surinamensis</u> and <u>Rhyzopertha dominica</u>; against <u>Stegobium paniceum</u> iodofenphos was fifty times better than malathion.

In crop protection iodofenphos has been used on top fruit, Brussels sprouts and oil seed rape. In trials on top fruit, 0.025% iodofenphos, applied at 200 gal/ac., gave control of pre- and post-blossom pests comparable to that of azinphos-methyl at the recommended dose. Work on this use has been discontinued due to phytotoxicity.

In trials on oil seed rape, Gould (1967) obtained excellent control of Meligethes spp and Ceuthorynchus assimilis. Good control of dipterous and lepidopterous larvae in Brussels sprout buttons was obtained using 1 lb a.i./ac. iodofenphos applied either as a single spray one month before harvest or split into two applications applied one month and two weeks before harvest. Work is being continued on this use and residues are thought to be satisfactory after a harvest interval of three weeks.

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Proc. 5th Br. Insectic. Fungi. Conf. (1969)

C 8514, N-(2-METHYL-4-CHLOROPHENYL)-N',N'-DIMETHYLFORMAMIDINE A PROMISING NEW ACARICIDE

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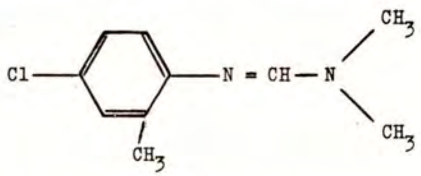
Summary C 8514, N-(2-methyl-4-chlorophenyl)-N',N'-dimethylformamidine, was first introduced into the U.K. in 1965. Field work is discussed and shows that concentrations of 0.05% a.i. control *Panonychus ulmi* on apples, *Tetranychus urticae* on strawberries and *T. cinnabarinus* on carnations without adverse effect on the crop. C 8514 is effective against adult and egg stages, penetrating the crop leaves and subsequently being redistributed and released from the leaves as a vapour. C 8514, having a novel chemical structure is effective against organophosphorus and tetradifon resistant mites. Residues were found to be acceptable in top fruit but in strawberries where only a short harvest interval occurred, they were high.

INTRODUCTION

N-(2-methyl-4-chlorophenyl)-N',N'-dimethylformamidine has the following structural formula:



This compound, of novel structure, was introduced into the U.K. in 1965 with the code number C 8514. Since then it has been extensively tested as an acaricide on apples, strawberries, and under glass on carnations, cucumbers, roses and chrysanthemums.

PHYSICAL AND CHEMICAL PROPERTIES

Molecular weight:	196.7
Specific gravity:	1.110 at 20°C (liquid)
Melting point:	17°C
Boiling point:	165-177°C at 13 mm Hg
Vapour pressure:	3.5×10^{-4} mm Hg at 20°C
Volatility:	saturation at 20°C 4mg/m^3 of air
Characteristics:	colourless crystals with an amine-like odour
Solubility:	250 ppm in water at 20°C; dissolves readily in all organic solvents; dissolves in aquous acids with formation of salts.

Hydrolysis: is hydrolysed in a neutral or basic medium to N-formyl-chlorotoluidine and then to 5-chlorotoluidine (= 2-amino-5-chlorotoluol); in an acid medium, hydrolysis is very slow.

C 8514 has been formulated as a 50% w.p. and a 50% e.c. The e.c. formulation, as Galecron, is now commercially available in many countries including Japan, Italy and America for use on top fruit.

TOXICOLOGY

Table 1

Acute toxicity of C 8514

Route	Animal	LD_{50} C 8514 Base	LD_{50} C 8514 Hydrochloride
Oral	rat	250 mg/kg	335 mg/kg
	mouse	290 mg/kg	-
	rabbit	625 mg/kg	-
Dermal	rat	-	4,000 mg/kg
	rabbit	-	>4,000 mg/kg

Short term feeding studies in rats have shown that 80 mg/kg daily did not cause any toxic effects after 28 days but 100 mg/kg daily retarded growth rate without causing pathological changes. Long term feeding studies are still in progress and no adverse effects have been noted after the first year. Toxicological investigations have shown that C 8514 is unlikely to present a serious hazard to birds and bees.

MODE OF ACTION

Initial laboratory experiments carried out by CIBA Limited, Basle, showed that C 8514 had a distinct vapour phase important in the control of mite eggs. (Dittrich 1966, 1967) By means of autoradiographs following treatment with C^{14} labelled C 8514, penetration and subsequent distribution within a leaf was also demonstrated. This distribution appeared to be adversely affected by lower temperatures. (Nayak 1969)

Dittrich (1966) showed that organophosphorus resistant strains of Tetranychus telarius and sensitive strains of T. urticae were equally susceptible to C 8514. Mortality checks during this work indicated a slow mechanism of poisoning. Further work by Dittrich (1969) has shown that organophosphorus resistant strains of T. urticae are in fact more susceptible to C 8514.

FIELD TRIALS - APPLES

Since 1967 extensive trial work has been undertaken to examine the efficiency of C 8514 against the fruit tree red spider mite, Panonychus ulmi, on apples in comparison with standard acaricides. This work involved assessment of the effects of differing doses, volumes and times of application.

Materials

C 8514	50% w/v e.c.
Tetradifon	8% w/v e.c.
Tetrasul	20% w/w w.p.
Binapacryl	50% w/w w.p.

Methods

These varied somewhat from trial to trial depending on the particular aspect under investigation (i.e. different volumes or times of application.)

High volume applications of approximately 200 gal/ac were made using a hand lance from a high pressure pumping unit at 300 p.s.i., while low volume applications at 50 gal/ac were made using a motorised knapsack sprayer giving a fine droplet spectrum.

Counts were made before treatment and at regular intervals following application. Both the imprint method for evaluating adult and egg population, and direct visual counts of motile stages were used, depending on the trial.

Trials 1 - 3, 1967

In these initial trials a range of application dates were covered:

Trial 1

Variety: Emneth Early
Application technique: High volume
Temperature at spraying: 23.5°C
Application date: 7th June
Assessment technique: Visual counts on 40 leaves/tree

Number of days after treatment	Percentage control of *P. ulmi* adults		Adults/ 160 leaves	Percentage control of *P. ulmi* eggs		Eggs/ 160 leaves
	C 8514 0.08% a.i.	Tetradifon 0.0125% a.i.	Untreated	C 8514 0.08% a.i.	Tetradifon 0.0125% a.i.	Untreated
5	34	62	244	-	-	-
12	47	48	688	-	-	-
22	59	80	222	65	75	1,699

Trial 2

Variety: Cox O.P.
Temperature at spraying: 17°C
Application date: 14th June
Assessment technique: Visual counts on 40 leaves/tree

Number of days after treatment	Percentage control of *P. ulmi* adults		Adults/ 160 leaves	Percentage control of *P. ulmi* eggs		Eggs/ 160 leaves
	C 8514 0.08% a.i.	Tetradifon 0.0125% a.i.	Untreated	C 8514 0.08% a.i.	Tetradifon 0.0125% a.i.	Untreated
5	69	44	259	-	-	-
12	69	68	119	68	64	1,873
21	91	90	165	45	66	1,652

Trial 3

Varieties:	Ellisons, Bramleys, Laxtons uperb, Lambourne.	Application date:	28th June
		Application volume:	High volume
		Assessment technique:	Visual counts on 80 leaves/tree
Conditions at spraying:	Warm and close		

Number of days after treatment	Percentage control of *P. ulmi* adults			Adults/ 240 leaves
	C 8514 0.08% a.i.	C 8514 0.04% a.i.	Tetradifon 0.0125% a.i.	Untreated
7	90	73	78	1,199
20	21	9	4	2,541

Trial 4 - 5, 1968

These trials were designed to evaluate the relative efficiencies of high and low volume applications. In both trials counts were made of motile stages on 20 leaves per tree at 1st assessment and in subsequent assessments adults and eggs were counted by the imprint method. The results from these trials were analysed using Duncan's Multiple Range Test and results followed by a common letter are not significantly different at P = 0.05 .

Trial 4

Variety:	Worcester	Application date:	28th May
Conditions at spraying:	Warm and sunny		

Compound and Appl$^{\underline{n}}$ method	Dose % a.i.	Percentage control at intervals in days after treatment						
		Adults				Eggs		
		3	14	21	30	14	21	30
C 8514 High volume	0.04	86 b	63 bc	74 a	29 b	68 a	80 a	73 abc
C 8514 Low volume	0.16	74 b	34 c	59 a	42 ab	65 a	75 a	71 abc
Tetrasul High volume	0.1	91 b	97 a	97 a	100 a	90 a	97 a	97 a
Untreated mean number/20 leaves		771 c	194 d	225 b	24 b	351 b	1286 b	784 e

Trial 5

Variety: Worcester — Application date: 10th June
Conditions at spraying: Fine and warm

Compound and Appl^{n} method	Dose % a.i.	Percentage control at intervals in days after treatment: Adults				Eggs		
		7	15	21	28	15	21	28
C 8514 High volume	0.04	66 a	46 ab	82 a	80 a	84 a	88 a	86 a
C 8514 Low volume	0.16	70 a	67 ab	92 a	86 a	78 a	81 a	89 a
Tetradifon High volume	0.0125	30 a	33 b	92 a	84 a	53 ab	79 a	73 a
Untreated mean numbers/20 leaves		96 b	30 c	198 b	294 b	533 c	609 b	191 b

FIELD TRIALS - STRAWBERRIES

During 1967 two trials were undertaken on strawberries to compare the efficiency of two doses of C 8514 compared with standard materials, for the control of *Tetranychus urticae*.

Materials

C 8514	50% w/v e.c.
Tetradifon	8% w/v e.c.
Dichlorvos	50% w/v e.c.

Method

A randomised block design with 15 - 20 yd^2 plots and three replicates was used for all trials. Applications were made with a knapsack sprayer using 150 gal water/ac. Counts were made before and at intervals after treatment on 5 - 40 trifoliate leaves per plot, depending on the mite population present.

Trial 1

Variety: Cambridge Vigour — Application date: 4th July
Assessment technique: Counts on 40 leaves/plot

Compound	Dose % a.i.	Percentage control of *T. urticae* adults at intervals in days after treatment: 6	13	22
C 8514	0.08	40	59	63
Tetradifon	0.0125	40	62	70
Untreated mean numbers/40 leaves		1,916	3,800	11,323

Trial 2

Variety: Cambridge Favourite   Application date: 22nd June
Assessment technqiue: Counts on 10 leaves/plot

Compound	Dose % a.i.	Percentage control of *T. urticae* adults at intervals in days after treatment		
		7	20	31
C 8514	0.05	93	72	58
Dichlorvos	0.1	54	39	42
Untreated mean number/40 leaves		483	438	1,943

FIELD TRIALS - CARNATIONS

Since 1967 a number of trials have been carried out to determine the optimum dose rate for efficient control of *Tetranychus cinnabarinus* on glasshouse carnations.

Material

C 8514	50% w/v e.c.
Tetradifon	8% w/v e.c.
Dicofol	20% w/v e.c.

Methods

Treatments were applied to run-off using a knapsack sprayer on plots of $2yd^2$ area. Assessments were made before spraying and at intervals after spraying. The method of assessment varied according to the mite population present.

Trial 1 - 1967

Variety: Caravelle Tangerine.   Application date: 4th August
Assessment technique: Counts of adults on 10 leaves/plot   Temperature at spraying: 23°C

Compound	Dose % a.i.	Percentage control of *T. cinnabarinus* adults at intervals in days after treatment		
		6	14	20
C 8514	0.08	79	94	90
C 8514	0.04	79	84	93
Untreated mean numbers/10 leaves		60	220	298

Trial 2 - 1968

Variety: White Sim
Assessment technique: Counts of eggs and adults on 20 leaves/plot

Application date: 30th July
Conditions at spraying: Hot and sunny

Chemical	Dose % a.i.	Percentage control of *T. cinnabarinus* at intervals in days after treatment			
		Adults		Eggs	
		3	10	3	10
C 8514	0.05	67 a	47 ab	0	5
C 8514	0.1	77 a	89 a	0	57
Tetradifon	0.0125	72 a	87 a	0	59
Dicofol	0.05	75 a	78 a	33	59
Untreated mean numbers/20 leaves		88 b	89 b	594	1,617

Percentage controls followed by common letters are not significantly different at P = 0.05 as indicated using Duncan's Multiple Range Test.

Trial 3 - 1969

Variety: White Sim, Pink Laddy, Moonglow
Assessment technique: Counts of adults on 10 leaves and 5 heads/plot

Application date: 24th July
Temperatures at treatment: 31°C

Chemical	Dose % a.i.	Percentage control of adults at intervals in days after treatment		
		6	14	27
C 8514	0.1	53	66	67
C 8514	0.05	60	73	54
Dicofol	0.05	0	15	18
Untreated mean number/10 leaves & 5 heads		308	216	174

PHYTOTOXICITY TRIALS UNDER GLASS

Between 1965 and 1968 a number of phytotoxicity trials have been carried out to assess the safety of C 8514 on a range of crops under glass. Treatments included conventional wet sprays and vapourisation from heat sources.

These trials indicated that a range of varieties of cucumbers, tomatoes, chrysanthemums and roses were damaged at wet spray concentrations of 0.04 and 0.08% a.i. Damage on cucumbers, tomatoes and chrysanthemums took the form of marginal chlorosis of leaves, particularly at the growing point. Symptoms of damage on roses were chlorosis and drop of older leaves. On house plants, Coleus, Cyclamen and Impatiens were damaged, whilst Tradescantias showed leaf-blotch.

Vapour concentrations of 10 mg/m^3 C 8514 have proved safe on chrysanthemums, cucumbers, tomatoes and roses, although observations have indicated a somewhat variable control of mites at this rate. A dose of 22 mg/m^3 caused chlorosis and drop of older leaves of the rose cultivars Tiara and Carina.

DISCUSSION

The trials reported show that C 8514 when applied to apples, strawberries and carnations, effectively controls both motile and egg stages of various species of spider mite. Dittrich (1966) states that control of both stages of the mites is achieved by direct contact action on application and is then increased on the release of the vapour phase following penetration and distribution within the leaf.

The top fruit trials results indicate that spray volume has little effect on the activity of C 8514. The influence of temperature in the field work is inconclusive, but Nayak (1969) suggests that effectiveness may be increased by high temperatures. 1969 work, as yet unreported, suggests that sprays of 0.05% a.i. C 8514 applied high volume to apples at 80% egg hatch (late May to early June) or later, and repeated after an interval of 2 - 3 weeks give an effective control of P. ulmi. Residue levels in apples are thought be be satisfactory providing a withholding period of approximately six weeks is observed.

Although T. urticae was effectively controlled on strawberries at similar concentrations, residue levels were high due to the short interval between treatment and harvesting. Under glasshouse conditions with generally higher ambient temperatures, effective control of T. cinnabarinus on carnations was achieved using concentrations of 0.04 to 0.1% a.i.

Most other commercially important glasshouse crops show signs of damage following treatment with sprays of C 8514. However, use of heat sources to produce the vapour phase merits further investigation.

Acknowledgements

We gratefully acknowledge assistance given by growers and other collaborators, which has enabled work to be done on this interesting new acaricide. Thanks are also due to the staff and students of U.C.N.W., Bangor for their contributions to this work.

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Proc. 5th Br. Insectic. Fungic. Conf. (1969)

PIRIMICARB: A NEW SPECIFIC APHICIDE FOR USE IN INTEGRATED CONTROL PROGRAMMES

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Summary The development of integrated control programmes will be facilitated by the introduction of specific insecticides to control a pest without disturbing the biological equilibrium regulating the numbers of other species. Pirimicarb is an aphicide of such specificity that its use for aphid control should not directly eliminate some of the more important predators concerned with the regulating of aphid numbers. It should also permit the conservation of predators and parasites of other pest species, allowing aphids to be taken out of an insect complex without affecting the numbers of other species of economic importance.

INTRODUCTION

The object of integrated control programmes is to allow full scope for the activity of all available controlling agents, and clearly an important aspect of this is the conservation of insect and other arthropod predators and parasites. Unfortunately recourse must frequently be had to broad-spectrum insecticides, often of considerable persistence, which destroy many or all of the beneficial arthropods. When the effects of the insecticide on the pest species have disappeared, no natural enemies may remain in the environment to restrain the rapid resurgence of the pest. The most dangerous situation develops when the pest eventually becomes resistant to the established insecticide. By this time few natural controlling agents may have survived over a wide area and even these will be destroyed by repeated applications of the insecticide at increasing dosage rates. This sequence can be expected to occur most readily in extensive monocultures where large areas are sprayed and opportunities for recolonisation of predators and parasites from outside are minimal. It has occurred in the Po valley of Italy and the Rhone valley in France, where it is difficult to find any predators or parasites of the peach aphid (*Myzus persicae*) and where the peach aphid itself has become resistant to established aphicides.

THE DEVELOPMENT OF PIRIMICARB

Pirimicarb is a pyrimidine carbamate (5,6-Dimethyl-2-dimethylamino-4-pyrimidinyl dimethylcarbamate) which has a very high degree of aphicidal activity. Table 1 gives the contact toxicity to normal organophosphorus susceptible peach aphids when 0·0016 ml/cm^2 was applied to aphids on mustard leaves using a Potter Tower.

Table 1

Contact toxicity to peach aphid

Chemicals	% kill after 24 hours 0·005% a.i.	0·0025% a.i.	0·0012% a.i.
Pirimicarb	100	99·7	90
Demeton-S-methyl	98	95	76
Malathion	83	75	17
Propoxur	68	52	52

In view of the problem in Spain, Italy and France resulting from the increasingly poor control of peach aphid given by organophosphorus insecticides great importance may be attached to the activity of pirimicarb against local strains from these areas. Table 2 shows the results obtained when pirimicarb was compared to oxydemeton methyl on resistant strains of the peach aphid collected from three different sites. Fully grown apterae were placed on glass plates treated 30 minutes previously with aqueous dilutions of the insecticides at the rate of 0·0015 ml/cm^2.

Table 2.

Contact toxicity to resistant peach aphids

Origin of aphid	% kill after 6 hours					
	Oxydemeton methyl				Pirimicarb	
	% a.i.				% a.i.	
	0·05	0·03	0·015	0·005	0·005	0·0025
Ferrara (Italy)	100	50	20	3	100	74
Ravenna (Italy)	99	-	-	0	100	-
Mallemort (France)	99·2	-	-	3·5	100	-

The high activity of pirimicarb against organophosphorus resistant peach aphid has been confirmed in a series of field trials over several seasons and the product has now been introduced commercially in Europe for application on peaches. It is also highly effective against organophosphorus resistant aphids on chrysanthemums and other ornamentals under glass, and is being introduced for this purpose in England and overseas.

THE SPECIFICITY OF PIRIMICARB

During the initial screening of pirimicarb it was noted that its activity was confined to the aphids and the Diptera. This indicated the possibility of its being sufficiently selective to be used against aphids without destroying the important Coccinellid and Chrysopid predators or Hymenopterous parasites of the aphids.

Parasites and predators are difficult to find in the peach orchards of France and Italy, but at Lerida in Spain in May 1966 reasonable numbers were found on 4 year old peaches heavily infested by aphids and the opportunity was taken to compare pirimicarb with dimethoate, the most widely used aphicide in the region. Dimethoate 0·04% a.i. and pirimicarb 0·025% a.i. were applied to run off using a knapsack sprayer, and 24 hours later dead insects were counted on large plastic sheets placed underneath three treated trees. Results are given in Table 3.

Table 3.

Effects of pirimicarb and dimethoate on predators of peach aphid

Treatment	Aphid % control	Number of dead predators counted			
		Coccinella larvae	Coccinella adults	Anthocoris spp.	Chrysopa spp.
Dimethoate	16	29	9	8	3
Pirimicarb	98	2	0	0	0

Visual assessment showed there to be as many predators remaining on the pirimicarb-treated trees as on 3 unsprayed controls. No live predators could be found on the dimethoate treated trees.

For laboratory investigations Aphidius matricariae was selected as an example of an efficient parasite of peach aphid. When aphids containing the pupae of this parasite were sprayed with pirimicarb at 0·0375% a.i. the adults emerged unscathed, and after exposure to a fresh deposit of pirimicarb at this concentration on mustard leaves only 15% of adults of A. matricariae were killed in 24 hours.

Laboratory work also confirmed the low degree of activity of pirimicarb on Coccinellidae (Coccinella septempunctata and Adalia sp.), Anthocoris sp. and Chrysopa sp.

The only important predators of aphids found so far to be susceptible to pirimicarb are the Syrphids, adults and larvae of which are rapidly killed by pirimicarb at 0·037% a.i. This, of course, may be expected as a result of pirimicarb having some general activity against the Diptera.

One of the more encouraging developments in the biological control of glasshouse pests in recent years has been the introduction of the predatory mite Phytoseiulus riegeli for the control of red spider. This advance may provide a solution to the impasse resulting from the widespread development of resistance to the common acaricides by spider mites. When organophosphorus insecticides are applied to control aphid on chrysanthemums or other glasshouse crops, the predatory mite is eliminated. The lack of toxicity of pirimicarb to Phytoseiulus therefore removes one obstacle to this development of integrated control methods. Table 4 gives results of pirimicarb sprays applied to French bean plants carrying a population of Phytoseiulus predating on red spider.

Table 4

Effect of pirimicarb on Phytoseiulus riegeli

Treatment	% kill 1 day	% kill 3 days
Pirimicarb 0·05% a.i.	5	5
" 0·025% a.i.	6	6
" 0·0125% a.i.	0	0
Untreated	0	0

Pirimicarb has also been found to be reasonably safe on bees, concentrations up to 0·025% applied through a Potter tower having no effect, and 0·2% (four times the normal highest recommended rate) killing only 56%.

DISCUSSION

In field practice complete control of aphid infestations is rarely achieved. A very few survivors usually remain, and in the absence of natural enemies rapidly increase. It is these survivors, furthermore, that are likely to pass on to their progeny characteristics that reduce their susceptibility to the insecticide applied and they may thus be the founders of a resistant strain. The conservation of the natural enemies of aphids is therefore valuable not only in the short term, in that they may obviate the need for retreatment, but also in the long term in that if an undisturbed population of predators cleans up the less-susceptible survivors of an insecticide treatment, the emergence of resistant strains may be delayed.

Specificity in an aphicide will also serve to preserve the natural enemies of other species which potentially may be more serious than the aphids. The possibility of conserving predatory mites when pirimicarb is applied against aphids in the glasshouse, thereby avoiding an upsurge of red spider, has been mentioned. Similarly *Phytoseiulus* and other predatory mites are being employed for the control of *Panonychus ulmi* and *Bryobia rubioculus* on apple in the Lebanon and Israel, and a selective aphicide holds promise for eliminating *Aphis pomi* without killing them. Less familiar in a temperate setting is the situation in cotton, where Coccinellids and Chrysopids are important egg predators of the bollworms *Heliothis armigera* and *H. virescens*. Their destruction by broad-spectrum aphicides applied to control early season attacks by *Aphis gossypii* may result in more severe bollworm attack later on, and selective control of *A. gossypii* by stem application of systemics has been shown to increase markedly the importance of *Chrysopa carnea* as a controlling agent for bollworm (Ridgeway and Jones, 1968).

The great limitation to the widespread use of the more selective insecticides now available is, of course, their limited spectrum of activity. This is to say that their main virtue is also their principal limitation, for while specificity serves to conserve beneficial insects it also limits the range of pests against which they may be employed. The fundamental obstacle to the rapid integration of insecticides with other means of control is that there is normally a complex of pests on a crop and in the absence of an armoury of specific insecticides, broad-spectrum materials must frequently be used. One application of such a compound may frustrate the potential advantages of a specific insecticide applied at another time. However the discovery and development of pirimicarb will strengthen the armoury of the integrated control specialist and must surely indicate the direction of advance in the future.

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Proc. 5th Br. Insectic. Fungic. Conf. (1969)

CONTROL OF COTTON LEAF-WORM IN EGYPT

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Summary O-2,5-dichloro-4-bromophenyl-O-methylphenylthiophosphonate ('Phosvel') is intensely active against Lepidoptera. It has passed successfully through successive stages of experiment and development in Egypt, and is now used commercially in that country for control of the cotton leaf-worm Prodenia litura.

For this purpose 'Phosvel' is usefully formulated as emulsifiable concentrate 300 g a.i./litre to be applied at 2 - 2¼ litres per feddan or alternatively as wettable powder 50 % active applied at 1.5 kg/feddan, in either case at dilution 200 litres water/feddan (1 feddan = 4200 m^2). Its effects against larvae of 2nd to 4th instar provide nearly 100 % control during the first period of 48 hours, 96 to 98 % control up to 6 days, and 92 % control or better up to 10 days following application.

In another emulsifiable concentrate 'Phosvel' 150 g a.i. is combined with endrin 200 g a.i./litre. At 2 litres/feddan this formulation also provides good control of 2nd instar larvae during 10 days.

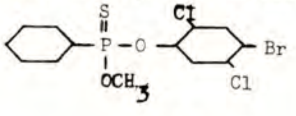
Data are presented concerning susceptibility of larval Prodenia litura as observed during the summer of 1969 at Giza.

INTRODUCTION

In the lower valley andddelta of the Nile the most serious of all pests which attack cotton is the cotton leaf-worm Prodenia litura, also known as Spodoptora littoralis; not only does this pest appear every year, but it has a remarkable ability quickly to acquire resistance towards many insecticides. This arises partly from the circumstance that the cotton growing areas are surrounded by desert where there is no other vegetation upon which the leaf-worm can live. Thus, if the cotton leaf-worm of one locality have been decimated by a particular insecticide the survivors tend to re-generate mainly a more resistant strain. For example, if endosulfan or parathion-methyl are employed in the same locality for several successive seasons cotton leaf worm may acquire significant resistance towards them. Instead it is a good policy for each locality to practise rotation of insecticides and to introduce new ones from time to time.

For obvious reasons the tendency to breed strains resistant towards a particular new insecticide which may be on trial out-of-doors must be weakened so long as that trial remains on a relatively small scale. Accordingly, it is acknowledged that successive generations may continue for a long time to be highly susceptible to a new insecticide which is applied only on a small experimental scale; it is only after an insecticide has been used on a commercial scale in the same neighbourhood for several years that it becomes remarkable if cotton leaf-worm remains susceptible to it.

O-2,5-dichloro-4-bromophenyl-O-methylphenylthiophosphonate



is intensely active against Lepidoptera. The acute oral toxicity of this compound to albino rats is about LD_{50} 90 mg/kg, and its five known metabolites are each less toxic. The acute dermal toxicity of the compound in the albino rabbit LD_{50} is numerically greater than 800 mg/kg. Many other toxicological data are available.

Its formulations, known by the trade marks 'Phosvel' and 'Abar' also by the Velsicol code VCS-506, have been employed on cotton in the lower valley and delta of the Nile and on a large scale at Giza during three consecutive seasons. There appears to be no evidence that Prodenia litura has yet acquired any significant resistance to 'Phosvel'.

The purpose of this paper is to record data concerning susceptibility during the summer of 1969.

MATERIALS AND METHOD

These tests were made at the Experimental Farm of the Faculty of Agriculture, University of Cairo, U.A.R. The farm is situated at Giza where the soil is a medium clay-loam. The trials to be described were laid out over an area of 4 feddans divided into 64 plots. (1 feddan = 4200 m^2). The area is one on which numerous large scale trials of 'Phosvel' have been made in each of three consecutive years.

The experiments were on cotton of the variety Ashmouni whose seed was previously dressed with 'Anthio' 80 % emulsifiable concentrate at the rate 1 litre per kg seed. On 30 March 1969 thirty kg dressed seed were planted per feddan, and during the growing season the area was irrigated six times in accordance with local practice.

Insecticide formulations and rates of application were as follows:

(1) 'Phosvel' emulsifiable concentrate
300 g a.i./litre
applied at each of two rates
2.25 litres/feddan
2.00 litres/feddan

(2) 'Phosvel' wettable powder
50 % a.i.
applied at 1.5 kg/feddan

(3) 'Phosvel' in association with endrin as emulsifiable concentrate
'Phosvel' a.i. 150 g) per litre
endrin a.i. 200 g)
applied at 2.00 litres/feddan

Some other insecticides were included in this trial, and there were also untreated control plots. Each treatment and the control were replicated four times on 28 June 1969. Inside treated plots only one row in seven rows of cotton was sprayed while six were left as guard rows to prevent drift of insecticide outside. Each formulation was sprayed at a dilution of 200 litres water per feddan and at a nozzle pressure of 6 kg/cm^2. By this technique every leaf in the row to be treated could be wetted thoroughly without risk of contaminating adjacent plots.

Samples of the treated leaves were collected just after treatment, also on the 2nd, 4th, 6th, 8th and 10th days following the day of treatment. At each collection there were placed into each of 10 glass jars (capacity 1 litre) some leaves from each of 4 replicates of each of the 4 different treatments, and similarly for the controls. Immediately after each collection ten larvae of the second instar Prodenia litura were placed in 5 jars of each kind of leaves and ten larvae of the fourth instar in the other 5 jars of the same kind. Each jar was covered with muslin and the larvae were left to feed on the leaves.

After 24 hours the numbers of dead and live larvae were counted, and the percentage mortality was calculated after correction according to Abbot's formula.

RESULTS

Table 1 summarises the observations and calculations.

Table 1

Mortality of second and fourth instar larvae of Prodenia litura when fed on cotton leaves selected at various periods after spraying formulations of 'Phosvel'

Insecticide	Rate g a.i./ feddan	Time of collecting leaves for test Days after spraying	% Mortality of larvae after 24 hours feeding on leaves Second instar	Fourth instar
'Phosvel' e.c.	675	0	100	100
		2	100	100
		4	98	96
		6	100	96
		8	98	92
		10	100	92
'Phosvel' e.c.	600	0	100	100
		2	100	100
		4	98	96
		6	98	98
		8	96.	92
		10	100	92
'Phosvel' w.p.	750	0	100	100
		2	98	94
		4	98	96
		6	98	96
		8	98	92
		10	100	92
'Phosvel'/ Endrin e.c.	'Phosvel" 300 + Endrin 400	0	100	100
		2	96	76
		4	90	72
		6	86	38
		8	88	48
		10	88	32

DISCUSSION

Initial effects

At the respective rates tried in these experiments, viz.

'Phosvel'	emulsifiable concentrate	600 to 675 g a.i./feddan
'Phosvel'	wettable powder	750 g a.i./feddan
'Phosvel'/Endrin	emulsifiable concentrate	'Phosvel' 300 g a.i.) Endrin 400 g a.i.) per feddan

the toxicities of all formulations are sufficient to cause 100 % mortality of the 2nd and 4th instar larvae of the cotton leaf-worm within the period of 24 hours following application.

Persistence

The persistence of 'Phosvel', after application either as aqueous emulsion at 600 to 675 g a.i./feddan or as wettable powder at 750 g a.i./feddan suffices to cause 96 to 100 % mortality of the 2nd instar larvae of the cotton leaf-worm even on the tenth day following application. At the same rates 'Phosvel' is not quite sufficiently toxic and persistent to kill all 4th instar larvae during so long a period, although at least 96 % mortality was recorded on the sixth day and 92 % on the tenth day following application.

The formulation of 'Phosvel' and endrin which provides 'Phosvel' 300 g a.i. + endrin 400 g a.i./feddan causes 88 % mortality of 2nd instar larvae of cotton leaf-worm on the tenth day following its application to cotton, whilst against 4th instar larvae the same treatment causes about 70 % mortality after the fourth day.

In view of its high toxicity and long persistence, 'Phosvel' both as emulsifiable concentrate and as wettable powder, is considered valuable for the control of cotton leaf-worm <u>Prodenia Litura</u> on Egyptian cotton. Separate observations suggest that 'Phosvel' favourably affects the cotton plant in consequence of which a high yield may be expected. Data concerning yield are not ready for submission in this report, but will be made available as soon as possible.

Proc. 5th Br. Insectic. Fungic. Conf. (1969)

A NEW INSECTICIDE 5-BENZYL 3-FURYLMETHYL D-TRANS ETHANOCHRYSANTHEMATE

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Summary 5-benzyl 3-furylmethyl d-trans ethanochrysanthemate (RU-11.679) which has a good stability to light and no KD effect (like NRDC 107) is so far the pyrethrinoid showing the highest insecticidal action on :

Musca domestica
Blatella germanica
Tribolium confusum
Oryzaephilus surinamensis L.
Sitophilus granarius L.

while it seems necessary to carry out further research we feel confident that RU-11.679 will prove to be valuable to control not only household but also phytophagous insects.

INTRODUCTION

Quite recently, L. VELLUZ, J. MARTEL and G. NOMINE (1969) described a new insecticide of the synthetic pyrethrinoid group : namely : the 5-benzyl 3-furylmethyl d-trans 3,3-dimethyl 2-cyclopentylidenemethyl cyclopropanecarboxylate, also mentioned as 5-benzyl 3-furylmethyl d-trans ethanochrysanthemate (RU-11.679).

J. LHOSTE and F. RAUCH (1969) have shown, specially concerning Musca domestica L. that RU-11.679 associated to 8 parts of piperonyl butoxide, has a better insecticidal action than the synthetic pyrethrinoids which are known up to now, as reported in the following table :

Table 1.

Lethal effect of some pyrethrinoids synergized with piperonyl butoxide on Musca domestica

Pyrethrinoids	Relative toxicities
dl-allethrolonyl d-trans chrysanthemate	80
5-benzyl 3-furylmethyl d-trans chrysanthemate (NRDC 107)	570
dl-allethrolonyl dl-trans ethanochrysanthemate	68
5-benzyl 3-furylmethyl d-trans ethanochrysanthemate (RU-11.679)	760
Natural pyrethrins	100

Under testing conditions, i.e. spraying in Potter Tower (ref. J. LHOSTE *et al.*, 1967), each product beeing synergized with the same ratio of piperonyl butoxide, RU-11.679 is 7,6 times more active than natural pyrethrins and 1,3 time more toxic to *Musca domestica* than NRDC 107. Tests were also carried out with the following insects species : *Blatella germanica* L., *Sitophilus granarius* L., *Tribolium confusum* L., *Oryzaephilus surinamensis* L.

RESULTS

Tests on *Blatella germanica*

The first results have already been reported (J. LHOSTE and F. RAUCH, 1969). It was obvious that RU-11.679 had a higher insecticidal activity than the other tested pyrethrinoids. Further experiments were carried out to assess the lethal doses 50 for RU-11.679 and NRDC 107. These LD 50 have been obtained by applying on metasternum a microdrop of an acetonic solution of pyrethrinoid. Results are shown in table 2.

Table 2.

LD 50 of RU-11.679 and NRDC 107 synergized with piperonyl butoxide on male *Blatella germanica*

Chemical	LD 50	Relative toxicities
RU-11.679	58 (1)	435
NRDC 107	250 (1)	100

(1) in nanogramme per insect.

RU-11.679 is therefore four times more active than NRDC 107 to *Blatella germanica*.

Tests on *Sitophilus granarius, Tribolium confusum, Oryzaephilus surinamensis*

The conditions used in the test were close to that of the COULON method (1963). With a glass sprayer, 1 ml of a solution containing water, acetone and pyrethrinoids, is sprayed on 50 g of wheat, the pyrethrinoid beeing associated to piperonyl butoxide in the ratio of 1:10. During the spraying work, the wheat is mechanically stirred in order to get a uniform distribution of the active ingredient. Controls are carried out on insects which have been left for 7 days with treated wheat. Results are reported in table 3.

RU-11.679 was more toxic to all the undermentioned species than NRDC 107 taken as control.

S. granarius was found the most susceptible species to both products and *T. confusum* was the most resistant.

Table 3.

Lethal concentrations of RU-11.679 and NRDC 107 on three insects (Coleoptera species)

Pyrethrinoids	Insect species	Lethal concentrations in ppm of active ingredient with regard to wheat	
		LC 50	LC 75
RU-11.679	S. granarius	0.39	0.50
	T. confusum	1.25	-
	O. surinamensis	-	0.19
NRDC 107	S. granarius	0.65	0.80
	T. confusum	(1)	-
	O. surinamensis	-	0.47

(1) No mortality was observed with 1.25 ppm.

DISCUSSION

The action of RU-11.679

Both NRDC 107 and RU-11.679 have no KD effect. But, on the other hand, they have a remarkable lethal action. This type of biological activity would be in relationship with the radical 5-benzyl 3-furylmethyl. Such an alcoholic part would result, as regards chrysanthemates and other cyclopropenecarboxylates with similar structural features, in a nearly exclusive lethal activity (ref. J. MARTEL et al., 1969). Thus, it is obvious that the higher insecticidal activity of RU-11.679 compared with NRDC 107 is due to the d-trans éthanochrysanthemic acid part of the molecule.

RU-11.679 and NRDC 107 have a further activity in common, at least concerning Musca domestica, Tribolium confusum and Oryzaephilus surinamensis L. : they have a very low synergistic effect when associated with piperonyl butoxide in the ratio of 1:10. For these three species, a synergistic factor inferior to 2 was found. On the contrary, on S. granarius, this factor was higher than 8.

RU-11.679 has an additional property : it is relatively stable to light. This property was determined according to the following method. 2 ml of acetone solution are distributed over the bottom of a Petri dish so as to obtain a deposit of 1.6 microgram of pyrethrinoids and 16 micrograms of piperonyl butoxide per sq. meter. The dishes are exposed to daylight at 20°C temperature. Every week, the insects are left during 60 minutes on the toxic film and put again under normal conditions of life. The percentages of kill are checked 5 days after treatment. Results are reported in table 4.

This table shows that RU-11.679 keeps its full efficiency during two weeks on T. confusum, three weeks on S. granarius and four weeks on O. surinamensis, whilst NRDC 104 losses its effectiveness very quickly towards these three species, under the testing conditions.

Table 4.

Percentages of kill obtained after various spaces of time

Chemical	Species	Number of weeks after treatment 0	1	2	3	4
RU-11.679	S. granarius	100	100	100	100	80
	T. confusum	100	100	100	66	83
	O. surinamensis	100	100	100	100	100
NRDC 104	S. granarius	100	100	92	96	21
	T. confusum	93	38	0	0	0
	O. surinamensis	100	100	98	70	13

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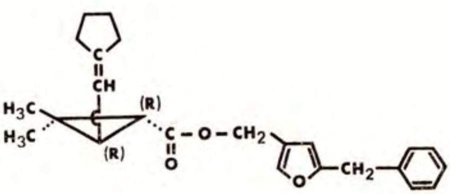

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Proc. 5th Br. Insectic. Fungic. Conf. (1969)

THE USE OF CYCLOHEXIMIDE FOR THE CONTROL OF DIDYMASCELLA THUJINA ON WESTERN RED CEDAR (THUJA PLICATA)

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Summary Didymascella thujina causes a serious needle blight in nursery stock of Western Red Cedar. The use of cycloheximide to control this disease was reported in 1962. Experiments since that time have been directed towards determining the optimum times and rates of application of this fungicide.

It has been concluded that cycloheximide should be applied at a concentration of 85 ppm of active ingredient and at a rate of 100 gal/ac. Two applications, one at the end of March and the other at the end of April give excellent control except in nurseries on the west side of the country where a third application in mid June may be necessary.

INTRODUCTION

Western red cedar is a valuable hedge plant and ornamental tree and is planted on a limited scale in British forests. Didymascella thujina causes a serious needle blight on this species in the nursery. The fungus attacks and kills individual small leaves on the fronds of the nursery plants. If the attack is severe, whole fronds die back and such plants cannot be successfully transplanted for use outside the nursery.

Pawsey (1962, 1964) used various derivatives of cycloheximide to control this disease. He reported that two applications of cycloheximide at the end of March and at the end of April gave excellent control. On some occasions one spray at either of these times gave good control. All applications were made at the rather high rate of 200-250 gal/ac. The optimum concentration of cycloheximide was 85 ppm (a.i.). As this method of control became more widely used in Forestry Commission nurseries, several cases of a breakdown in control were reported.

A number of trials were laid down to test various rates of application and also to test various spray programmes. Two of these trials are described below.

MATERIALS AND METHODS

The first experiment was laid out in a nursery in south east England. There were six main treatments (plus a control) replicated four times and each main plot was split for two spray programmes.

Details of the treatments can be seen in Fig. 1. The main treatments included various rates of application (in the range 50 - 200 gal/ac) combined with various concentrations of cycloheximide (85 - 340 ppm a.i.). The two sub plot treatments were spraying in March only and spraying in March and April.

The level of infection in each sub plot was assessed by taking ten fronds of western red cedar at random and counting the total number of infected leaves on each frond. Infected leaves were identified by the presence of apothecia of D. thujina. In general one apothecium is produced on each infected leaf.

A second experiment was laid down in a nursery in South Wales where in the previous season, one application of cycloheximide had failed to control an outbreak of D. thujina. There were four treatments incorporating four spray programmes (plus a control) each replicated four times. Times of spray applications were:

1. End of March
2. End of March and end of April
3. End of March, end of April and mid June.
4. End of March, end of April, mid June and end of July.

Several different methods were used to assess this experiment. In one method, an eye score was made of the percentage of foliage killed in each plot as a result of infection by D. thujina. In a second method, all the plants in each plot were cut off at soil level and the total fresh weight of the stem and leaves was measured. The results obtained by these two methods of assessment are shown respectively in Figs. 2 and 3.

RESULTS

In the first experiment (see Fig. 1.) there were no significant differences between the various combinations of rates of application and concentrations of cycloheximide or between the two different spray programmes. There was a slight indication that two spray applications may be better than a single application.

In the second experiment, when assessed by scoring the percentage death of foliage (see Fig. 2), spraying on three occasions was very significantly better than spraying once or twice. Four spray applications were very significantly better than three, though from a practical point of view the difference was of no importance. When assessed by taking the fresh weight of stem and foliage (see Fig. 3) all treatments up to and including three sprays were significantly different from one another and from control.

CONCLUSIONS

From the results of the first experiment it is clear that the rate of application of cycloheximide can be reduced to 50 gal/ac and good control be achieved. A concentration of 85 ppm of cycloheximide (a.i.) gave good control at this rate of application.

From the results of the second experiment (see Figs. 2 and 3) which was laid down in South Wales, it can be seen that three spray applications were necessary to achieve good control. As a rule of thumb, nurserymen are advised to reject any plants which have more than one third of their foliage killed. The results shown in Fig. 2 (see 'Critical level') allow the experimental results to be translated into practical terms.

As breakdown of control after one or two spray applications have only occurred on the west side of the country current recommendations are that two sprays (at the end of March and at the end of April) should be applied in all nurseries except those on the west side of the country where three sprays should be applied (as above plus mid June).

Although good control has been achieved experimentally when sprays are applied at the rate of 50 gal/ac, it is currently recommended that a rate of 100 gal/ac should be used in nursery practice to ensure adequate cover of foliage.

References

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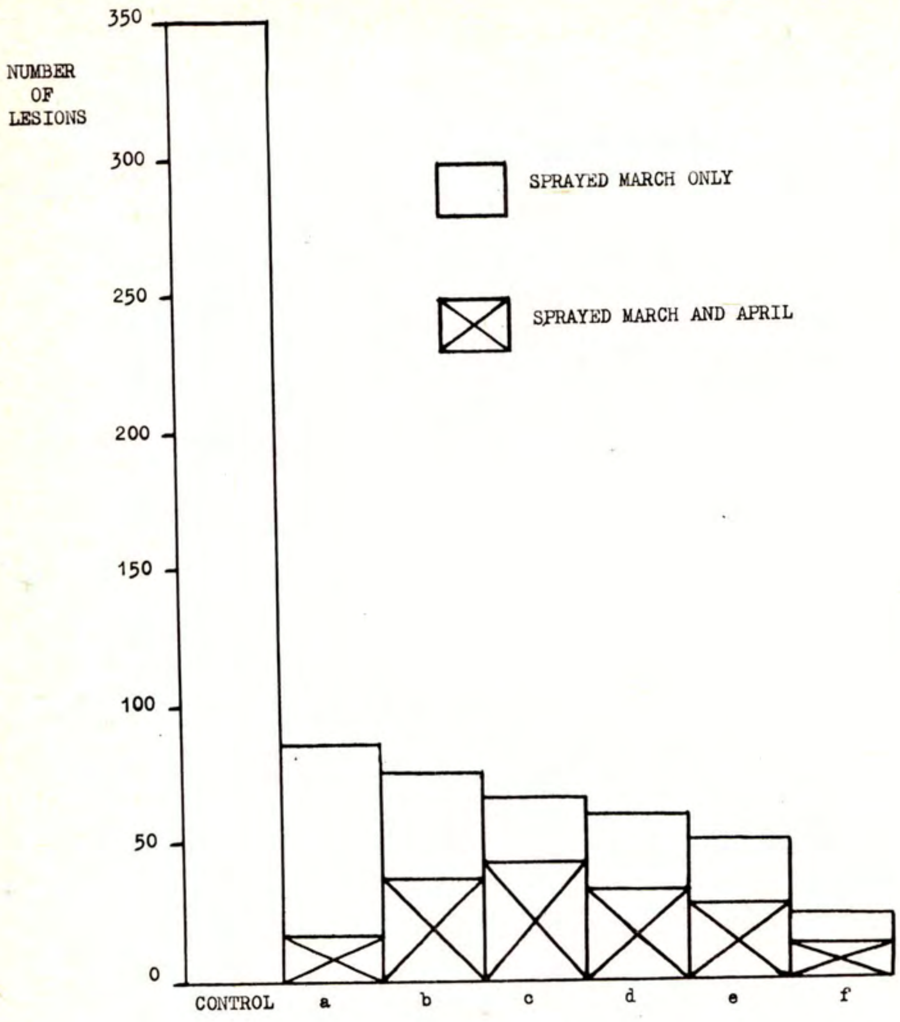

a 227 ppm/75 gal.
b 85 ppm/50 gal.
c 85 ppm/100 gal.
d 85 ppm/200 gal.
e 170 ppm/100 gal.
f 340 ppm/50 gal.

Fig. 1 Cycloheximide trial - South East England.

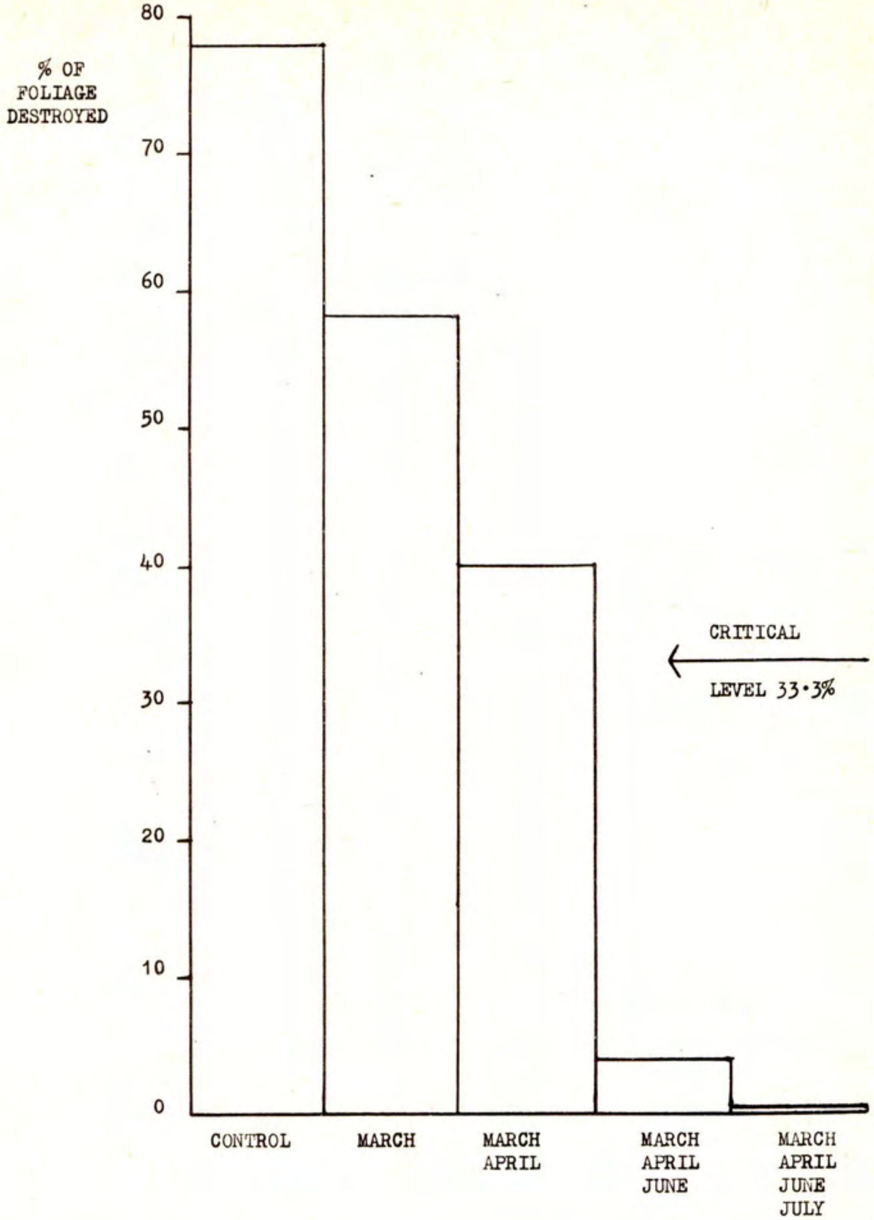

Fig. 2 Cycloheximide trial - South Wales

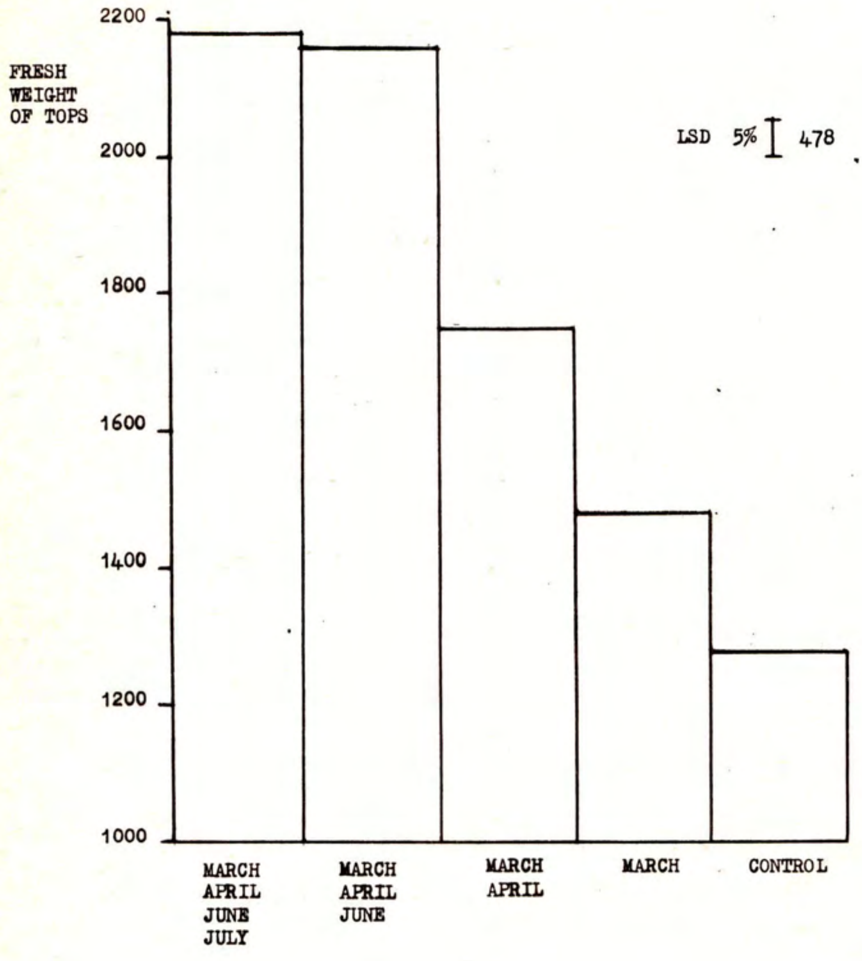

Fig. 3 Cycloheximide trial - South Wales

Proc. 5th Br. Insectic. Fungic. Conf. (1969)

MEBENIL (BAS 3050 F), A NEW COMPOUND WITH SPECIFIC ACTION AGAINST SOME BASIDIOMYCETES

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Summary A number of substituted benzoic acid anilides have a specific activity against Basidiomycetes fungi. Out of all tested compounds so far the 2-methylbenzoic acid anilide (o-toluanilide or 2-methylbenzanilide; common name mebenil)can be considered to be a promising fungicide for the control of rust fungi on cereals. The experience, which we have made with mebenil (code number BAS 3050 F) in our field experiments will be presented in this report.

INTRODUCTION

For more than 40 years attempts at developing a fungicide to control rusts in cereals have been made, and fortunately remarkable progress has been achieved in this direction in recent years with the introduction of fungicides on the zineb-nickel and maneb-nickel basis as well as the oxathiin derivatives. For the plant breeder the development of economically acceptable control measures can have a positive effect inasmuch that qualitatively high valued varieties that are not fully rust resistant can be kept healthy by chemical means. Furthermore the problem of the appearance of new rust strains which is feared not only by breeders would lose its significance.

In our search for fungicides with an appropriate spectrum of activity we found several in the 2 position substituted benzoic acid anilides that generally are only active against Basidiomycetes.

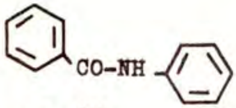
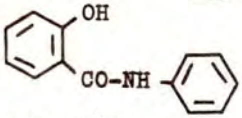
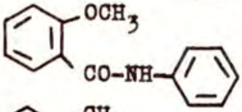
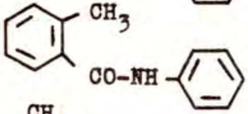
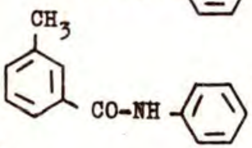
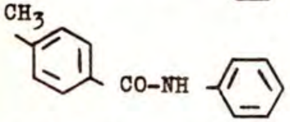
TRIALS AND RESULTS

Laboratory and greenhouse trials

Of the numerous benzoic acid anilides that have been tested in laboratory and greenhouse trials, the dependancy of the fungicidal activity on the chemical constitution is to be shown with the aid of a few examples.

In table 1 we have compiled the fungicidal activities of the benzoic acid- and 2-hydroxybenzoic acid anilide (salicylanilide) against those of 2-, 3- and 4-methylbenzoic acid anilides.

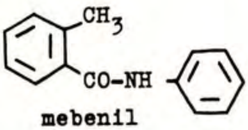
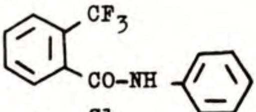
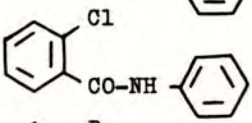
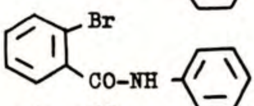
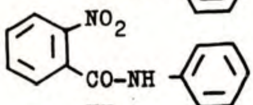
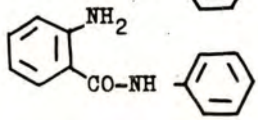
Table 1.

	Basidiomycetes		Ascomycetes		Phycomycetes	
	Rhizoct. solani	crown rust (oat)	Asper-gillus	mildew (barley)	Plasmopara viticola	Pythium spec.
	-	-	-	-	-	-
	-	-	+	-	+	-
	-	-	-	-	-	-
	+	+	-	-	-	-
	±	-	-	-	-	-
	-	-	-	-	±	-

+ effective ± less effective -insufficiently or not effective

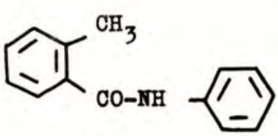
Whilst the unsubstituted benzoic acid anilide is completely inactive the well known fungicide salicylanilide is very active against fungi of the classes Ascomycetes and Phycomycetes, but not or insufficiently against Basidiomycetes. With the methylbenzoic acid anilides it is striking, that only the 2-methylbenzoic acid anilide shows a wide activity against the Basidiomycetes, whilst in comparison the 3- and 4-methylbenzoic acid anilides are not sufficiently effective. The introduction of the substitutent in the 2-position is apparently of pronounced importance in bringing about the specific fungicidal property, whereby not every substitutent, as the examples of 2-hydroxy- and 2-methoxybenzoic acid anilides show, must be suitable. In table 2 substituted effective benzoic acid anilides in 2-position that have been tested in laboratory and greenhouse trials are presented.

Table 2.

	Basidiomycetes		Ascomycetes		Phycomycetes	
	Rhizoc. solani	crown rust (oat)	Asper-gillus	mildew (barley)	Plasmopara viticola	Pythium spec.
 mebenil	+	+	-	-	-	-
	+	+	-	-	-	-
	+	+	-	-	-	-
	+	+	-	-	-	-
	+	±	-	-	-	-
	±	+	-	-	±	-

+ effective   ± less effective   - insufficiently or not effective

Of the numerous compounds that have been tested in laboratory and greenhouse trials the 2-methylbenzoic acid anilide (mebenil)



(o-toluanilide or 2-methylbenz-anilide; mebenil)

has been, because of the favourable results, undergoing further tests against several, economically important harmful fungi of the class Basidiomycetes since 1968.

The results that have already been obtained point to the fact that mebenil is particularly suitable for combatting rusts in cereals.
The following are some of the experimental results.
Mebenil is tested as a w.p. with 75 % a.i. under the code number BAS 3050 F.

Field trials

In the Federal Republic of Germany the damage in cereals due to yellow rust infection during 1968 was only slight. Only relatively late date did it come to a visible infection in several places. In winter wheat trials in which 10 susceptible varieties or strains were included, a two applications (27.4. and 24.5.1968) of mebenil, in each ease at a concentration of 2,25 kg/ha, showed a clear activity.

Table 3.

Effectiveness against Puccinia striiformis (yellow rust) on winter wheat

	Degree of yellow rust infection (1 to 9) 4 weeks after the last treatment (24.5.68) 10 susceptible varieties and strains
Untreated	6,2
Mebenil 2,25 kg/ha (treated twice)	2,3

The first treatment on 27.4.68 was evidently too early since by carrying out a planned treatment against a susceptible breeding strain with 2,25 kg/ha mebenil at the beginning of June the yellow rust could be halted for more than 3 weeks.

Table 4.

Effectiveness against Puccinia striiformis (yellow rust) on winter wheat

planned treatment after commencement of infection (1 trial)

	Degree of yellow rust infection (1 to 9) Prior to treatment	2 weeks after treatment	3 weeks after treatment
Untreated	3	6,5	6
Mebenil 2,25 kg/ha		1,0	2,5

In spring barley the relationship is similar. During the year 1968 the weather conditions in northern Germany were not favourable for a spraying against yellow rust towards the end of the tillering stage to the beginning of jointing. A treatment approximately two weeks later i.e. during jointing, however prior to earformation produced distinct results. In table 5 the average values of a trial embracing 17 susceptible varieties are reproduced.

Table 5.

Effectiveness against Puccinia striiformis (yellow rust) in spring barley
(17 susceptible varieties)

Degree of yellow rust infection after ...	Degree of infection (1 to 9) Mebenil 2,25 kg/ha	Untreated
2 to 3 weeks	2,1	3,3
4 to 5 weeks	3,8	6,4
	Yield kg/ha	
	4290	3960
rel.	108	100

In trials that were conducted in Switzerland, noteworthy increased yields were gained on spraying mebenil once during jointing (Table 6). Where mebenil was applied either once or twice in comparable trials, the double treatment resulted in a higher yield increase than the single treatment (Table 7).

Table 6.

Effectiveness against yellow rust in winter wheat
(3 trials)

One treatment during jointing	Green leaf surface as a % 1. leaf under the ear	2.	3.	Yield kg/ha	rel.
Mebenil 1,87 kg/ha	44	14	0	3750	117
Comparable compound/ 1,87 kg/ha	62	21	3	3550	111
Untreated	28	9	0,3	3210	100

Table 7.

Effectiveness against yellow rust in winter wheat
(2 trials)

One and two treatments a) during jointing b) 10 - 12 days later	Green leaf surface as a % 1. leaf under the ear	2.	3.	Yield kg/ha	rel.
a) Mebenil 1,87 kg/ha	39	21	0	3650	110
a+b) Mebenil 1,87 kg/ha	56	23	2	3980	120
a) Comparable comp./1,87 kg/ha	51	22	5	3480	105
a+b) Comparable comp./1,87 kg/ha	75	29	4	4040	122
Untreated	30	14	1	3320	100

DISCUSSION

The results achieved with mebenil show that it is possible to control yellow rust in cereals with this compound and that strikingly increased yields can be attained. However, the choice of the correct time of application is still a problem. In contrast to the mildews in cereals the rust is also found on the upper stalk and on the ear (glumes and awns), which means that a late infection can also cause considerable damage. The trials represented in table 5 clarify this; they show, that with one mebenil treatment at the start of an infection, a relatively long period of protection is realized against on aggressive rust infection, which is clearly suppressed. In the ease of yellow rust in wheat a planned treatment at the start of an infection also markedly reduces the rust infection (Table 4). Since according to general German experience the yellow rust appears prominently shortly before or during earformation, an early treatment should be followed up by a second spraying if there is an early infection:
Time a) during jointing and time b) shortly before or during earformation. The data of trials given in table 6 clarify this, particularly if one compares the yields.

Acknowledgements

We thank the company Maag, Dielsdorf, Switzerland, for premission to reproduce the results quoted in tables 6 and 7.

Proc. 5th Br. Insectic. Fungic. Conf. (1969)

A new systemic, CELA W 524 [N,N'-bis-(1-formamide-2,2,2-trichloroethyl)-piperazine] with action against powdery mildew, rust and apple scab.

P. Schicke and K.H. Veen

C.H. Boehringer Sohn and Cela G.m.b.H.,
Ingelheim, Germany

Summary

CELA W 524 [N,N'-bis-(1-formamide-2,2,2-trichloroethyl)-piperazine] is a new systemic. Up till now it has shown activity against powdery mildew on cereals, apples, cucurbits and ornamental plants and against rust on cereals and ornamental plants and against apple scab.

W 524 can be applied as a seed dressing for cereals and as an emulsifiable concentrate or wettable powder.

Introduction

CELA W 524 [N,N'-bis-(1-formamide-2,2,2-trichloroethyl)-piperazine] was first synthesised at C.H. Boehringer Sohn, Ingelheim/ Germany, Chemische Fabrik in 1967. It is active as a foliage fungicide, particularly against powdery mildew, rust and scab but can be applied too as a seed dressing for cereals in which case it has systemic action.

W 524 is a white, crystalline solid without odour, almost insoluble in water, acids and bases. It is very slightly soluble in chloroform and aromatic hydrocarbons and more so in ketones (1% approx.) and methanol (1% approx.). Its solubility is good in dimethylformamid (30% approx.) and n-methyl-pyrrolidone (40% approx.)

Formulations

W 524 has been formulated as emulsifiable concentrates (5 - 50%), wettable powders, granulates and seed dressings. For application as a foliar fungicide the emulsifiable concentrates seem to be more active.

Toxicity

The following values have been determined:

Acute oral toxicity to mammals:

Species	LD_{50}
Mouse	>6000 mg/kg
Rat	>6000 mg/kg

Toxicity to fish (Lebistes reticulatus):

no toxicity with 50 ppm in the water

Toxicity to bees:

no toxicity with 1000 ppm in the food

Biological activity

The activity of W 524 was found by testing the substance against *Erysiphe graminis* on the rye variety "Pettkuser Winterroggen" in the primary screening. 5 ml of an 250 ppm emulsion of the compound in water were poured on the sand in which the rye plants were growing (approx. 0,014 mg a.i./ml sand). The so treated plants remained free from powdery mildew after inoculation as distinct from the control plants which became heavily infected. It appeared that root treatment protected the whole plant, which indicates systemic activity (table 1).

In one experiment 10 mg W 524 was put into closed dialysis tubes which were then put into 100 ml of a nutrient solution in which rye plants were grown. The plants growing in this solution remained free of powdery mildew after inoculation, control plants became heavily infected. Apparently W 524 can be active in the water phase even though it is only very slightly soluble (about 4 ppm) in water.

Table 1

Greenhouse experiments with W 524 against powdery mildew of rye.

Root treatment.

Root treatment	A.i. in ppm	No of pustules	% Leaf infection
W 524 (e.c. 5%)	40	0	0
" "	10	0	0
" "	2,5	19	1,8
" "	0,6	408	40,4
" "	0,15	547	54,1
Control	-	1011	100

In this context another experiment should be described. Wet and dry rye plants were sprayed with an emulsion in water containing 20 ppm W 524. These plants were divided into three groups, the first being transferred directly into a dry atmospere, the second after three hours exposure to and the third after 6 hours exposure to 100% relative humidity. Every group of plants was subdivided into two, one of which got 20 mm of artificial rain 24 hours after foliar treatment. All plants were inoculated with *Erysiphe graminis*. The results of this experiment are given in table 2.

Table 2

Foliar treatment of rye leaves. Plants were kept afterwards 0,3 and 6 hours at 100% relative humidity and than one group wetted by artificial rain, the other not.

Condition of the leaves prior to spraying	Artificial rain after 24 hours	Time plants were kept at 100% relative humidity		
		0 hours	3 hours	6 hours
dry	none	5,7 %	0 %	0 %
wet		38,3 %	6,5 %	1,0 %
dry	20 mm	22,1 %	2,1 %	0 %
wet		32,7 %	7,0 %	0 %

Percentages indicate infection as compared with the untreated control plants.

Of course a spray put on wet leaves is diluted and this results in higher infection. However plants kept in a wet environment after foliar treatment show less infection than those remaining in dry conditions. This also seems to indicate that following foliar treatment W 524 needs the water phase to act.

W 524 does not act systemically after leaf-treatment. The substance however does not influence the germination of the conidia as was shown by in vivo experiments but acts in a later phase of the infection process. This will be reported on more extensively elsewhere. With *Erysiphe graminis* W 524 seems to need the living plant to become effective.

Thus foliar treatment only protects the treated surfaces. Results of such an experiment are given in table 3.
There were no signs of phytotoxicity.

Table 3
Greenhouse experiments with W 524 against powdery mildew of rye.
Foliar treatment.

Foliar treatment	A.i. in ppm	No of pustules	% Leaf infection
W 524 (e.c. 5%)	250	14	0,8
" "	64	25	1,3
" "	16	99	5,0
" "	4	973	51,9
" "	1	1537	82,0
Control	-	1874	100

Rust of cereals.
Table 4
Greenhouse experiments with W 524 against wheat leaf rust.
Root treatment.

Root treatment	A.i. in ppm	No of pustules	% Leaf infection
W 524 (e.c. 5%)	50	0	0
" "	16,7	0	0
" "	5,6	10	2,1
" "	1,9	388	81,1
Control	-	478	100

W 524 shows considerable activity against wheat leaf rust, caused by *Puccinia recondita* and other rusts on wheat. The following gives results of experiments with *Puccinia recondita* using the wheat variety "Pfeuffers Schernauer".

Table 5
Greenhouse experiments with W 524 against wheat leaf rust.
Foliar treatment.

Foliar treatment	A.i. in ppm	No of pustules	% Leaf infection
W 524 (e.c. 10%)	250	0	0
" "	83,3	198	15,8
" "	27,8	519	41,5
" "	9,3	1078	86,0
Control	-	1253	100

Field experiments with powdery mildew of gherkins.
W 524 was tested in the greenhouse against powdery mildew on gherkins. As these trials turned out very positive, field experiments were initiated. Here the results of such an experiment are given. The plants were sprayed twice with a 2 weeks interval between treatment. The first treatment was given when powdery mildew pustules started to appear.

Table 6
Foliar treatment of outdoor gherkins with W 524 against powdery mildew.

Treatment	Formulation	A.i. in ppm	% Leaves infected
W 524	10 % e.c.	250	2,5 %
"	"	125	3,8 %
"	50 % w.p.	250	15,0 %
Dinocap	25 % "	125	13,5 %
Control	-	-	51,3 %

Field experiments with scab and powdery mildew on apples.

The action of W 524 has been investigated in small-scale field experiments against apple scab, (*Venturia inaequalis*) and powdery mildew, (*Podosphaera leucotricha*). For these experiments the final formulation has not yet been determined, so these results only give a preliminary impression of the activity of W 524.

The apple leaves did not show any sign of phytotoxicity after they had been sprayed 6 times during the season. Results of these experiments are given in table 7 and 8.

Table 7
Foliar treatment of apple trees against scab with W 524 in an outdoors experiment using the variety "Lodi"

Treatment	Formulation	A.i. in ppm	% Leaves infected	% Fruit without scab
W 524	10 % e.c.	125	1,8	70
"	10 % "	250	1,5	97
Metiram	80 % w.p.	1600-1200*	2,0	48
Control	-	-	18,0	6

*) Before flowering 1600 ppm were sprayed, afterwards 1200ppm

Table 8
Foliar treatment of apple trees against powdery mildew with W 524 in an outdoors experiment on variety "Weißer Klar"

Treatment	Formulation	A.i. in ppm	% Leaves infected
W 524	10 % e.c.	125	7,5
"	"	250	3,3
Dinocap	25 % w.p.	250	3,5
Control	-	-	25,0

Field experiments with powdery mildew and rust on roses.

W 524 is particularly effective against powdery mildew (Sphaerotheca pannosa) and rust (Phragmidium mucronatum) on roses. It has been tested for compatibility on different varieties of roses in the greenhouse.

When W 524 emulsifiable concentrates were applied at 175 ppm a.i. to the very sensitive variety "Baccara", no phytotoxicity was be observed even at high temperatures. Despite these facts W 524 emulsifiable concentrates should not be sprayed on opened flowers. Results of these experiments are given in tables 9 and 10.

Table 9
Foliar treatment of outdoor roses, variety "Lys Assia", with W 524 against powdery mildew.

Treatment	Formulation	A.i. in ppm	% Leaves infected Term 1	Term 2
W 524	5% e.c.	250	4 %	5 %
"	"	125	10 %	6 %
Dinocap	25% w.p.	187	10 %	19 %
Control	-	-	24 %	87 %

Table 10
Foliar treatment of outdoor roses, variety "Frau K. Druschki" with W 524 against rust.

Treatment	Formulation	A.i. in ppm	% Leaves infected
W 524	10% e.c.	250	2 %
"	"	125	3 %
Cyclomorph	28% "	840	4 %
Dodine	5,8% "	174	
Control	-	-	71 %

W 524 has shown activity against powdery mildew on chrysanthemums and Kalanchoe sp. and rust on geraniums.

Actually orientating experiments with W 524 against Cercospora beticola on sugar beets seem to indicate that W 524 is active against this disease.

Proc. 5th. Br. Insectic. Fungic. Conf. 1969

CHARACTERISTICS AND FIELD PERFORMANCE OF A NEW, BROAD-SPECTRUM SYSTEMIC FUNGICIDE

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Summary

EL-273 (α-(2,4-dichlorophenyl)-α-phenyl-5-pyrimidinemethanol) has been widely tested during the past two years in the United States, United Kingdom, Europe and South Africa. It was effective against a number of economically important phytopathogenic fungi at very low application rates. The acute oral LD_{50} of technical material in rats is 500 mg/kg. Three months feeding studies have shown the compound to be safe to mammals.

The compound has provided effective control of scab on apple (*Venturia inaequalis*) and pear (*V. pyrina*) at application rates of 30 to 40 ppm, and of apple powdery mildew (*Podosphaera leucotricha*) at 40 to 50 ppm. On the other hand, effective control of powdery mildew of grape (*Oidium*), cucurbits (*Erysiphe cichoracearum*) and rose (*Sphaerotheca pannosa* var. *rosae*) has been obtained by application rates of 15 to 20 ppm, while 30 to 40 ppm has provided excellent control of stripe rust (*Puccinia striiformis*), powdery mildew (*Erysiphe graminis*) on wheat, American gooseberry mildew (*Sphaerotheca mors-uvae*) on blackcurrant and cherry leaf spot (*Coccomyces hiemalis*).

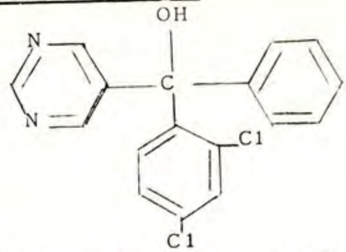
All of the above rates of application are given as active ingredient and were applied in spray volumes of 1500 to 2000 l/ha.

INTRODUCTION

A new class of broad-spectrum, locally systemic fungicides has been discovered and is currently being developed by Eli Lilly and Company. Greenhouse experiments have demonstrated the systemic activity of these compounds in cucurbits, beans, grapes and apple seedlings. Field Research has shown some of these compounds to be effective and locally systemic at very low application rates. Of these, α-(2,4-dichlorophenyl)-α-phenyl-5-pyrimidine-methanol has been widely tested not only in the United States, but also in the United Kingdom, Europe, the Middle East and South Africa. This new fungicide has been evaluated under the research code number, EL-273.

It is the purpose of this paper to summarize its physical and chemical characteristics and its fungitoxic properties under field conditions in the UK-European Area during the past two years

Physical and Chemical Properties



$C_{17}H_{12}Cl_2N_2O$ M.W. 331.2

α-(2,4-dichlorophenyl)-α-phenyl-5-pyrimidinemethanol

The pure compound is a white crystalline solid which is stable at room temperature. It melts at 96-97°C and is readily soluble in most organic solvents but not in petroleum ether or hexane. Water solubility is very low.

Toxicology

The acute oral LD_{50} of technical material in rats is approximately 500 mg/kg. (1) Laboratory results have shown the compound to be relatively non-hazardous, and that rats are somewhat more sensitive than dogs. (2) Three month feeding studies indicate the safe feeding level in rats and dogs to be 400 and 800 ppm, respectively. Application of 100 mg/kg, as a 10% suspension, to rabbit's skin caused only a slight initial reddening which cleared in 72 hours. Placement of 0.1 ml of a 5% suspension in the rabbit's eye was not irritating.

RESULTS AND DISCUSSION.

During 1968, trials were established in Great Britain and Germany, utilizing different dosages and spray schedules. Data on both powdery mildew and apple scab were obtained from two experiments in Germany, and the apple scab data are summarized in Table 1.

Table 1

Percent control of apple scab on apple leaves after five applications

Treatment	Dosage ppm	a/ g/h	Mean Percent Control
EL-273	10	20	64.8
EL-273	20	40	82.9
EL-273	40	80	94.0
Propineb	150	300	89.0
Control	-		(63.4)b/

a/ Applied on 0 + 14 + 28 + 28 + 28 day spray schedule with first spray at green tip.

b/ Mean percent of leaf surface infected with apple scab on non-treated trees.

The above observations were made on older leaves which had been treated. There was little control on the young terminals, developed since the last spray. However, the older leaves were virtually free of scab lesions from new infections, 45 days after the last spray. Trees receiving treatment with propineb had small lesions appearing on the corresponding leaves. The superiority of EL-273 is considered to be an expression of good local systemic movement within sprayed leaves.

An early infection of powdery mildew occurred in these same experiments. EL-273, at 20 ppm and above, gave commercially acceptable control of this pathogen during the period when application was on a 14-day spray schedule. However, extending the spray schedule to 28-day intervals resulted in commercially unacceptable control of powdery mildew. This indicates that the systemic activity of EL-273 was probably not sufficient to cause movement from the treated leaves to newly developed untreated branches. No phytotoxicity was noted at any of the above rates of application.

Data presented in Tables 2, 3 and 4, were obtained from experiments conducted in South Africa during 1968/69. Tables 2 and 3 present scab data on Red Delicious and Golden Delicious apples, respectively. Although control with EL-273 was equal or better than reference compounds, it is felt that the unplanned 21-day spray interval due to sprayer breakdown between the first and second spray did not permit a valid assessment of the efficacy of EL-273 in relation to the reference standard in these experiments.

Data are presented in Table 4 where the efficacy of EL-273 against powdery mildew was evaluated in South Africa. It can be noted that the graduated (7-14-21 day) spray schedule was more effective than the 14-day schedule. Excellent protection was noted on older treated leaves, with newly developed terminals being infected. This again is an indication of local systemic movement within leaves.

Table 2

Percent control of foliar scab on Red Delicious apples in South Africa

Treatment	Dosage ppm	Mean Percent Control Nov. 9	Dec. 10
EL273	16 a/	75.6	56.4
"	24 a/	68.4	59.6
"	48 a/	81.1	82.8
Mancozeb + Dinocap	b/ c/	70.2	78.3
Control	-	(21.1) d/	(11.8) d/

a/ Spray Schedule 0 + 21 + 14 + 14 + 14 + 14 days
b/ Spray Schedule 0 + 7 + 14 + 7 + 7 + 14 + 21 days
c/ Mancozeb 24 oz/100 Imp gallons plus
Dinocap 12 oz/100 " "
d/ Mean percent of leaf surface infected on non-treated trees.

Table 3

Percent control foliar scab on Golden Delicious apples in South Africa

Treatment	Dosage ppm		Mean Percent Control Nov. 9	Dec. 10	Jan. 6
EL-273	16 a/		83.6	89.2	92.7
EL-273	24 a/		80.8	92.2	94.7
EL-273	48 a/		85.2	92.5	92.9
Dodine + Dinocap	b/	c/	90.8	91.0	95.4
Control	-		(36.4)d/	(29.1)d/	(35.9)d/

a/ Spray Schedule 0 + 21 + 14 + 14 + 14 + 14 days
b/ Spray Schedule 0 + 7 + 14 + 7 + 7 + 14 + 21 days
c/ Dodine 8 oz/100 Imp gallons plus
Dinocap 12 oz/100 " "
d/ Mean percent of leaf surface infected on non-treated trees.

Table 4

Percent control of powdery mildew on Rome Beauty apples in South Africa

Treatment	Dosage ppm			Mean Percent Control Nov.15	Dec. 12	Jan. 1
EL-273	16	a/		61.6	62.7	59.5
EL-273	24	a/		67.9	69.7	73.8
EL-273	48	a/		95.0	79.7	82.6
Dodine + Binapacryl		a/		73.2	73.9	62.9
EL-273	16	b/		76.9	49.5	60.2
EL-273	24	b/		64.2	67.4	82.5
EL-273	48	b/		68.4	76.3	76.3
Dodine + Binapacryl		b/	c/	70.9	71.1	70.4
Control	-			(5.5)d/	(36.4)d/	(38.7)d/

a/ Spray Schedule 0 + 7 + 7 + 7 + 7 + 14 + 21 + 21 days
b/ Spray Schedule 0 + 14 + 14 + 14 + 14 days
c/ Dodine + Binapacryl each at 8 oz/100 Imp gallons
d/ Mean percent of leaf surface infected on non-treated trees.

Results from 1969 trials conducted in Italy indicated that application rates as low as 16 to 20 ppm were sufficient to provide excellent control of apple scab on foliage of Rome Beauty apples (Table 5). However, at least 30 ppm was required to provide satisfactory control of pear scab on fruit (Table 6). Unfortunately, an application rate of 40 ppm was not included, but 50 ppm provided excellent control of fruit scab. No phytotoxicity was noted in either experiment at rates of 80 ppm. Disease incidence in both of the above experiments was extremely high.

Table 5

Control of apple scab on foliage of Rome Beauty in Italy.

Treatment	Dosage (ppm AI)	Percent Control a/
EL-273	16	92.7
EL-273	20	92.9
EL-273	24	93.5
EL-273	32	97.0
EL-273	50	98.3
EL-273	80	98.9
Captan 50W	1000	94.0
Control	0	0(87.4) b/

a/ Mean of four replications and ten ratings per plot after six sprays on May 29, 1969

b/ Mean percent of leaf surface infected on non-treated trees

Table 6

Control of pear scab on fruit of Morotini variety in Italy.

Treatment	Dosage (ppm AI)	Scab Lesions per Fruit a/
EL-273	16	11.4
EL-273	20	10.4
EL-273	24	8.4
EL-273	30	3.3
EL-273	50	1.8
EL-273	80	0.7
Captan 50W	1000	2.7
Control	0	35.6

a/ Mean of five replications with thirty fruit counted per plot after five sprays May 19, 1969.

A large number of experiments were conducted throughout France in 1969. The data presented in Tables 7 and 8 are from representative experiments conducted in Southern France. In the experiment reported in Table 7, applications were made on 10 and 15-day spray schedules at the following rates: 30 ppm, 40 ppm and 20 ppm until petal fall followed by 40 ppm thereafter. Captan was included as a reference fungicide. It can be readily noted that EL-273 at all rates and dates of application provided essentially complete control of apple scab. In the same experiment, Captan provided equally good control on a 10-day spray schedule, but was inferior to EL-273 on a 15-day schedule.

Table 7

Control of apple scab on foliage and fruit of Golden Delicious in France.

Treatment	Dosage (ppm AI)		Apple Scab Leaves c/	Apple Scab Fruits d/
EL-273	30	a/	0	0
EL-273	40	a/	0.9	0
EL-273	20+40	a/	0.04	0
Captan	1000	a/	0.08	0
EL-273	30	b/	0	0
EL-273	40	b/	0.27	0
Captan	1000	b/	12.0	0.33
Control	-		285.0	27.75

Table 7 (continued)

a/ 10-day spray schedule.
b/ 15-day spray schedule.
c/ Mean number of lesions/100 leaves.
d/ Mean number of lesions/50 fruits.

Identical application rates were evaluated against powdery mildew in the experiment reported in Table 8, but only on a 10-day spray schedule. Here, as well as in other experiments, 40 ppm EL-273 throughout the season provided slightly better control of powdery mildew than did either 30 ppm throughout or 20 ppm through petal fall followed by 40 ppm. All EL-273 treatments provided control of powdery mildew superior to Dinocap.

Table 8

Control of apple powdery mildew on foliage of Golden Delicious in France

Treatment	Dosage (ppm AI) a/	Percent Leaves in each class b/ 0	1	2	3
EL-273	30	87.0	11.0	0.9	0.0
EL-273	40	92.0	6.0	0.4	0.0
EL-273	20 + 40	88.0	10.0	0.7	0.2
Captan + Dinocap	1000 + 500	77.0	18.0	3.0	0.2
Control	-	33.0	17.0	27.0	23.0

a/ 10-day spray schedule.
b/ Infection classes: 0 = no lesions; 1 = 1-2 lesions; 2 = 3 lesions to half leaf infected; 3 = more than half leaf infected.

In Table 9 are presented data obtained on Bramley apples in Kent, England. In this experiment, as in many others, split applications of EL-273 were evaluated. These treatments were included primarily for two reasons: (1) Apple scab infection usually occurs early in the season and can be controlled by lower dosages of EL-273 than apple powdery mildew which generally does not appear until mid-season in many areas; (2) Effective use of split dosage levels provides lowest product cost, fruit residue levels and maximum crop safety

It is evident that all treatments provided effective control of the powdery mildew pathogen. The level of control provided by 20 ppm of EL-273 was equal to that obtained with 500 ppm of Dinocap, the reference compound. However, it appears that the 30 + 60 ppm EL-273 treatment provided slightly better control than all other treatments. The 60 ppm rate was applied during the period when powdery mildew infection occurred.

Table 9

Control of powdery mildew on Bramley seedling in Kent.

Treatment	Dosage (ppm AI)		Total Number Infected Shoots c/
EL-273	20	b/	871
EL-273	30	b/	504
EL-273	40	b/	558
EL-273	20+40	b/	660
EL-273	30+60	b/	436
Captan + Dinocap	a/	b/	976
Control	-		2483
Control	-		2562

a/ Captan + Dinocap at recommended spray dosages.
b/ 0 + 10 + 10 +(10-14)+(10-14)+(10-14)+(10-14) day schedule.
c/ Total from 10 extension shoots per tree and six replicates.

Data in Table 10 were also obtained in England on Cox's Orange Pippins apples. It can be readily seen that EL-273 provided excellent control of apple scab, with no significant difference between rates or between EL-273 and Captan. All applications were made at 14-day intervals.

Table 10

Control of apple scab on fruit of Cox's Orange Pippins, Burrough Green Suffolk.

Treatment	Dosage (ppm) Prepetal fall	Postpetal fall	Mean Assessment
EL-273	15	30	1.17
EL-273	20	20	0.67
EL-273	20	40	0.17
EL-273	30	30	1.67
Captan + Dinocap/EL-273	1000+500	30	1.33
Captan + Dinocap/EL-273	1000+500	40	0
Captan + Dinocap	1000+500	1000+500	0.17
Control			10.17
Control			11.50
L.S.D. (5%)			4.27
L.S.D. (0.1%)			8.99

The data presented in Table 11 were obtained from an experiment in West Germany. The Golden Delicious trees were quite small (4 years old) at the time of application. Similar experiments were also conducted in Switzerland on similar age trees. Under these conditions, it was noted that 20 ppm, applied on an 8-12 day spray schedule provided excellent control of apple scab and powdery mildew. This may have been due to the fact that Golden Delicious is considered to be somewhat less susceptible to scab than other varieties.

Table 11

Control of apple scab on Golden Delicious in West Germany.

Treatment	Dosage (ppm AI)		Mean Percent Leaves	Infection Fruit
EL-273	20		1.4	0.0
EL-273	30		1.6	0.5
EL-273	40	a/	1.1	0.0
EL-273	20 + 40	b/	1.4	0.0
Captan + EL-273		c/	2.5	0.5
Mancozeb + Dinocap		a/	1.9	0.9
Control			(85.3)d/	(64.8)d/

a/ Five sprays at 8-10 days, then 5 sprays at 12-14 days
b/ First three sprays at 20 ppm on 8-10 day schedule followed by 40 ppm on 12-14 day schedule.
c/ Captan for first three sprays on 8-10 day schedule followed by EL-273 at 40 ppm on 12-14 day schedule.
d/ Mean percent scab infection in control trees.

In South Africa EL-273 at 25 ppm provided excellent control of Oidium on grape (Table 12). Additional experiments conducted in France have shown that equally satisfactory control of Oidium can be obtained with 15 to 20 ppm when applied to grape in combination with compounds which control *Plasmopora viticola*.

In experiments not presented here, it has also been demonstrated that 15 to 20 ppm is sufficient to provide excellent control of powdery mildew on roses, cucurbits and cereals. In addition, rates of 30 to 40 ppm have given excellent control of stripe-rust on wheat and American gooseberry mildew on blackcurrant.

Table 12 .

Efficacy of compounds for powdery mildew control on Muskadel grapes in South Africa.

Treatment	Dosage	Percent Oidium Control on grape bunches
		January 15 a/
EL-273	25 ppm AI	99.0
Sulphur 80% WP	3 lbs/100 Imp gal	73.9
Dusting Sulphur 90%	20 lbs/acre	86.0
Control		0(30.7) b/

a/ Rating based on 20 bunches/plot
b/ Mean percent of leaf surface infected on non-treated vines.

References

1. Anonymous. Technical Report on EL-273. Elanco Products Company Indianapolis, Indiana. March, 1969.

2. Worth, H.M. and R.C. Anderson. Plant Fungicide EL-273. Summary of Acute and Subacute Toxicology Studies. The Lilly Toxicology Laboratories, Greenfield, Indiana. July 1969. Unpublished.

Acknowledgements.

The authors wish to acknowledge the assistance of Drs. B. Anastasiadis, H. Brandes, C. S. James and S. Masri, and Messrs. A. Balty, K. Blumel and D. M. Farrant for their valuable assistance in obtaining many of the results presented in this paper. In addition, many of the data presented were obtained from experiments conducted by research personnel of the following organisations:

Boots Pure Drug Co. Ltd., Nottingham, England.
La Quinoleine, Paris, France.
Dr. R. Maag Co. Ltd., Zurich, Switzerland.
The Murphy Chemical Co. Ltd., Wheathampstead, Herts, England.

Proc. 5th Br. Insectic. Fungic. Conf. (1969)

AZEPINES, A NEW CLASS OF FUNGICIDES

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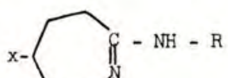
Conclusion: Field tests covering a wide range of crop diseases in various countries have shown the azepines to be primarily suitable for the control of foliar diseases of apples and pears. The data presented illustrate the unique preventive broad spectrum action against both major pathogens scab and powdery mildew. There are primary indications that also summer diseases are controlled well. Furthermore, the azepines possess excellent curative activity against scab, even under severe conditions of infection. These properties offer greater flexibility in spray programs, especially in timing. The azepines are well tolerated by the principal pip fruit varieties under a wide range of growing conditions; varietal differences have, however, been observed.

INTRODUCTION

The azepines which are dealt with in this paper are characterized by the general formula

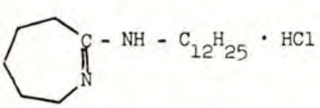


x = H or alkyl
R = alkyl

Already in the primary biological tests, they showed a quite surprising and up to then unknown feature: they controlled leaf spot pathogens as well as powdery mildews and rust fungi. This unique combination of fungicidal actions induced a field test program which started in 1964. It has been continued on a larger scale in the last couple of years mainly with two representatives of the group, i.e. GS 14449 and GS 16306, which had turned out to be the most promising ones.

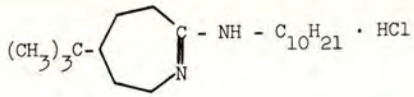
CHEMICAL AND PHYSICAL PROPERTIES

GS 14449 (7-dodecylamino-3,4,5,6-tetrahydro-2H-isoazepine-chlorohydrate) has the following structural formula



The substance is a white crystalline odourless solid with a molecular weight of 316,95 and a melting point of 144 to 145°C. It is soluble to 0,16% in water (25°C), slightly soluble in organic solvents, thermostable (100°C) and stable in aqueous solutions.

GS 16306 (=4-tert.butyl-7-decylamino-3,4,5,6-tetrahydro-2H-azepine-chlorohydrate) has the following structural formula



The substance is a white crystalline odourless solid with a molecular weight of 345,0 and a melting point of 191 to 194°C. It is soluble to 0,1% in water (25°C), slightly soluble in organic solvents, thermostable (100°C) and stable in aqueous solutions.

GS 14449 and GS 16306 have been formulated and tested as wettable powders of 25 and 50% a.i., respectively.

TOXICOLOGY

Acute oral LD 50 of GS 14449 in rat is 1110 mg/kg, that of GS 16306 is 1100 mg/kg. One month feeding studies have shown that 150 mg/kg/day of GS 16306 have no detectable effect on rats; the corresponding figure for dogs is 15 mg/kg/day. Further toxicological studies, especially medium- and long-term feeding studies, are underway.

In laboratory feeding experiments GS 16306 did not show any toxic effect to honey bees in concentrations up to 500 γ.

The investigation of the metabolism of azepines in animals and plants has been initiated.

THE BIOLOGICAL ACTIVITY OF GS 14449 AND GS 16306

1. Fungicidal performance in greenhouse and small scale field trials

Having shown an excellent action in residual greenhouse screening tests, the compounds were further tested in eradicant and systemic trials. The results of these experiments indicated the azepines to possess an eradicant activity against Alternaria solani (treatments up to 24 h after infection), Uromyces phaseoli (up to 48 h after infection) and Erysiphe cichoracearum (up to more than 72 h), as shown in figure 1. A systemic effect (GS 16306) could be demonstrated against powdery mildew of cucumber after application to nutrient solution. In correspondent trials with soil application this activity was completely lost.

Primary field trials confirmed the broad spectrum of protectant activity, as shown in table 1.

Figure 1 Part 1

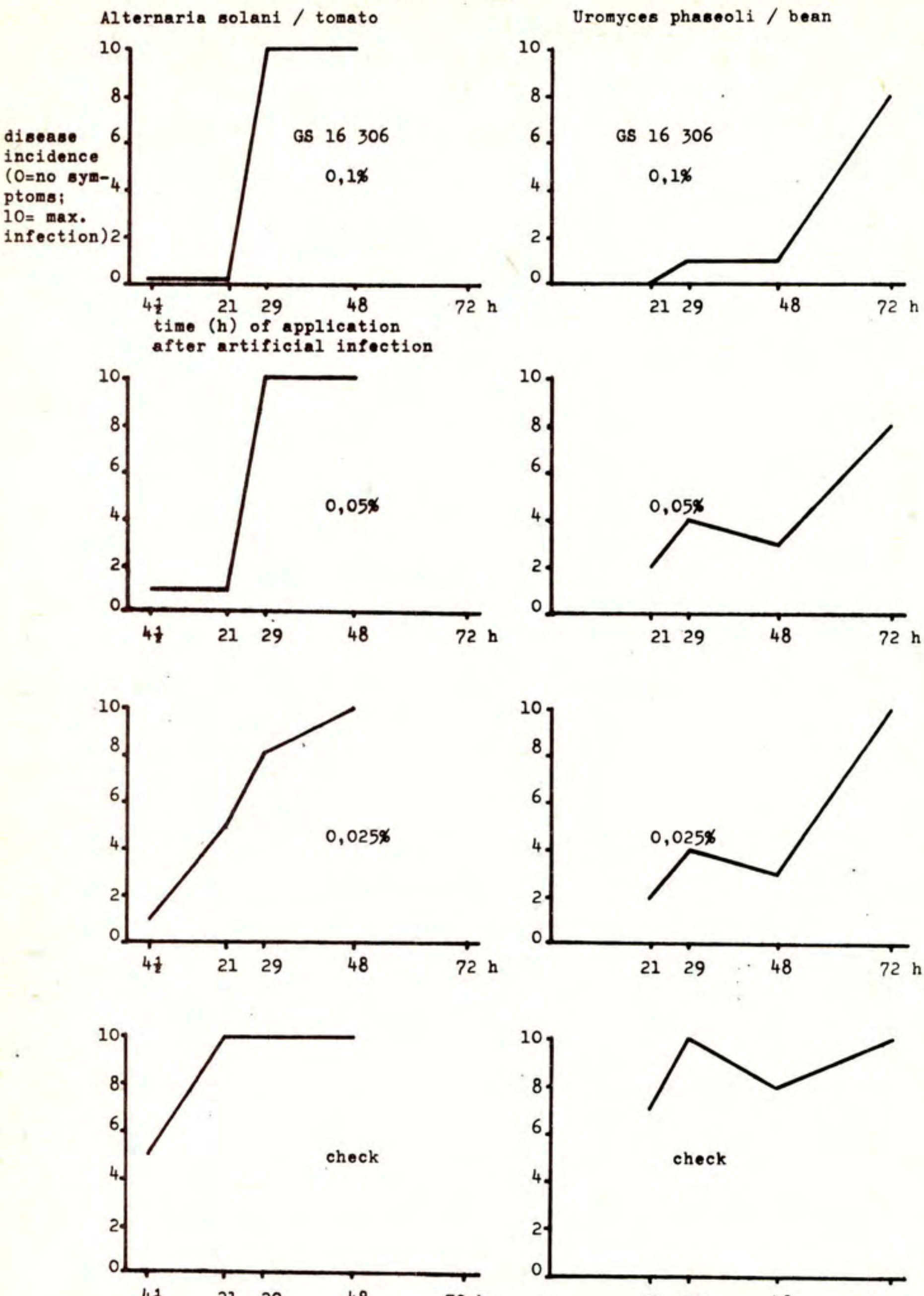


Figure 1 Part 2

Erysiphe cichoracearum / cucumber

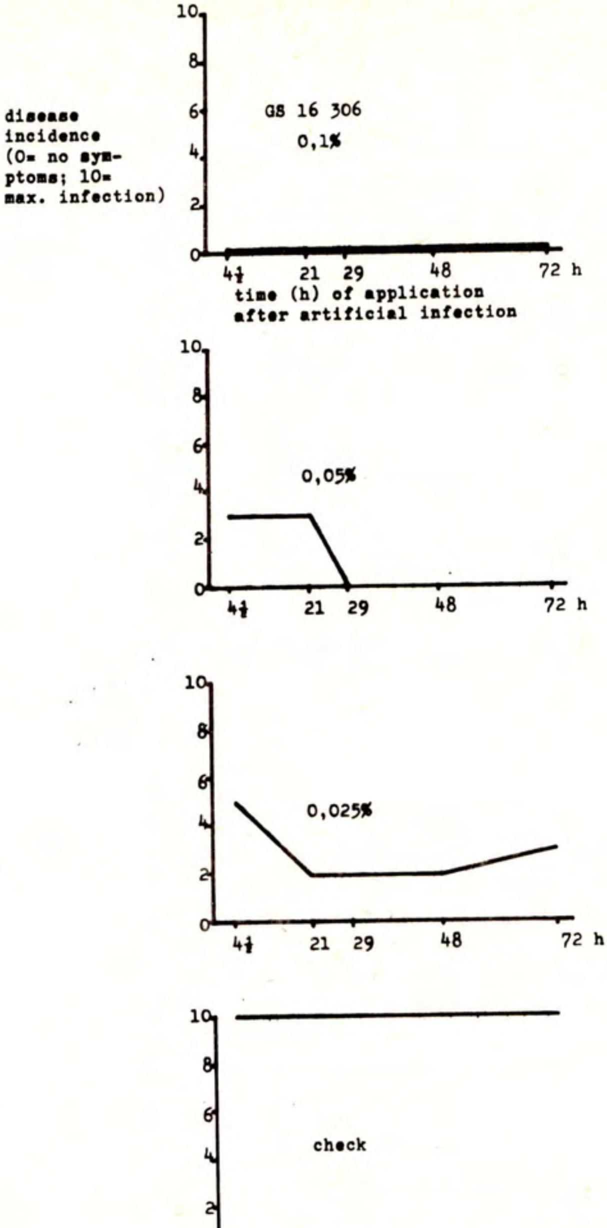


Table 1

Performance of representative azepines in primary preventive field tests

Pathogen/host plant	Fungicidal action of GS 14449	GS 16306	adverse side-effects to host plants
Alternaria solani/tomato	0	0	medium to strong
Cercospora beticola/sugar beet	2 to 4	1 to 3	slight
Diplocarpon rosae/rose	2 to 3	0 to 4	none
Septoria apicola/celery	0 to 2	0	slight
Venturia inaequalis/apple	0 to 2	0 to 2	none
Erysiphe cichoracearum/cucumber	0	0	strong
Podosphaera leucotricha/apple	2 to 3	2 to 3	none
Cronartium ribicola/black currant	0	1	slight
Spharotheca pannosa/rose	0 to 1	0 to 1	none
Uromyces phaseoli/bean	0 to 2	0	strong

dosage rate: 100 g a.i./100 l water
Rating system: 0 = maximal disease control, no disease symptoms
10 = no disease control

As both compounds caused significant phytotoxic damages to most of the vegetable and field crops and also certain varieties of roses, the further work was concentrated mainly to apple and pear trees and their major disease problems. In the following some typical examples of the results obtained are presented.

2. Activity against apple scab

2.1. Performance in preventive spray trials

The residual action against foliar and fruit scab is evident from the following two tables which summarize trials carried out under climatic conditions which provided medium to extremely strong scab infection.

Table 2

Effect of azepine treatments against scab in protectant spray programs (var. "Golden Delicious" and "Imperatore", respectively; 3 to 4 replicates per treatment; evaluation after 6 treatments; spray schedule of 6 to 8 days).

Treatment	Trial 1 (Rhone valley, Switzerland)		Trial 2 (Ferrara, Italy)	
	Rate g a.i./100 l	% leaves infected	Rate g a.i./100 l	% leaves infected
GS 14449	-	-	50	2,7
	-	-	100	1,3
GS 16306	37,5	5,3	-	-
	50	0,2	50	5,0
	75	0,5	-	-
	-	-	100	2,3
Dodine	-	-	50	0,5
Orthocide	100	4,6	-	-
Check	-	60,3	-	50,0

Table 3

Effect of GS 14449 and GS 16306 against fruit scab (Assessment of 6 treatments; 200 fruits per treatment. Trial in region of Ferrara, Italy).

Treatment	Rate g a.i./100 l	Percentage attacked surface					Total of attacked fruits (%)
		5	10	25	50	75	
GS 14449	50	6,5	3,5	2,5	0	0	12,5
	100	4,5	1,5	0	0	0	6,0
GS 16306	50	5,0	2,0	0	0	0	7,0
Dodine	50	4,0	2,0	2,5	0	0	8,5
Check	-	15,5	14,5	12,5	34,5	17,0	94,0

The results show that both of the azepines provide excellent protection of foliage and fruit. From these and many similar trials it can be stated that dosage rates of 37,5 to 50 g a.i./100 l provide good to complete disease control. Microscopic examination of single scab lesions developed on treated leaves showed that about 95% of them were killed by the subsequent treatments. Therefore, the eradicant activity of the test compounds was studied in detail.

2.2. The eradicant activity in scab spray programs

The marked eradicant effect of azepines against scab is illustrated by the following greenhouse and field tests (tables 4 and 5).

Table 4

Eradicant action of azepines against scab
(Greenhouse trial on "Imperatore" apple trees; evaluation 13 days after infection)

Treatment	Rate g a.i./100 l	Application 48 h after artificial infection % of attacked leaves	Application 48 h after artificial infection number of lesions/100 leaves	Application 72 h after artificial infection % of attacked leaves	Application 72 h after artificial infection number of lesions/100 leaves
GS 14449	60	6,0	12,0	8,6	46,6
GS 16306	60	2,0	2,6	3,3	10,6
check	-	30,6	81,3	dead	dead

Table 5

Eradicant action of GS 16306 against scab
(Field trial on "Golden Delicious" apple trees; treatments 72 h after Mills' period)

Treatment	Rate g a.i./100 l	% leaves infected May 15	% leaves infected June 24
GS 16306	50	0,2	0,3
	75	0,1	0,0
Benomyl	30	0,7	0,3
	60	0,5	0,65
Check		16,1	43,4

In both trials the azepine compounds revealed an excellent eradicant activity at rates of 50 to 75 g a.i./100 l. This feature offers more flexibility in the spray schedule and may reduce the total number of applications per season. Further work is in progress to obtain more precise information on eradicant activity.

Based on a large number of trials in various European countries it can be stated that scab of pears (Venturia pirina) is controlled similarly to apple scab at rates of 25 to 50 g a.i./100 l.

3. The fungicidal action of azepines against powdery mildew of apple trees

Results representative of those obtained in numerous trials against apple powdery mildew are given below in table 6.

Table 6

Action of azepines against powdery mildew of apple trees

(On "Jonathan" trees at Pfeffingen (trial 1) and Saillon, Switzerland (trial 2) respectively; Spray intervals 12/6 days, assessment after 6/8 treatments).

Treatment	Rate g a.i./100 l	% leaves infected trial 1	% leaves infected trial 2
GS 14449	100	16,1	-
	75	-	2,8
	50	-	17,6
GS 16306	100	10,0	-
	75	-	2,2
	50	-	8,3
Dinocap	120	8,2	-
	60	-	0,6
Check		33,0	78,8

From the results given in table 6 and corresponding results in other countries it can be concluded that a dosage rate of 50 g a.i./100 l for conditions providing a medium degree of attack and a rate of 75 g for severe infection conditions can be recommended for further trials.

4. The activity of azepines against summer diseases

Summer diseases, i.e. sooty blotch (Gloeodes pomigena), white rot (Botryosphaeria ribis), bitter rot (Glomerella cingulata), black rot (Physalospora obtusa), fly speck (Microthyriella rubi) and cedar apple rust (Gymnosporangium juniperi-virginianae) play an important role in certain fruit growing regions of the USA. Results of a number of tests carried out by co-operators of experimental stations indicate the effectiveness of GS 16306 against this type of disease, primarily against P. obtusa, B. ribis and G. juniperi-virginianae (for details see: Fungicide and nematicide tests 1968, Amer. Phytopath. Soc., 24, 1969).

PHYTOTOXICITY

Compounds of high biological activity carry, as a rule, the risk of damage to the host plant. In fruit trees the young growing fruit usually shows the greatest sensitivity to such side-effects. It is now well understood that weather conditions during the period of fruit formation are of major importance to the sensitivity of the fruit skin to chemicals. It has been clearly shown that cool weather with night temperatures around freezing point can cause heavy russetting of fruits which have never been treated with chemicals. On the other hand fungicides may increase the predisposition to or the degree of development of russetting.

The azepines are in general very well tolerated by apples. Sometimes, however, an increasedincidence of fruit russetting has been observed after their application under the climatic conditions described above. In such cases, the varieties "Golden Delicious" and "McIntosh" have proved particularly sensitive.

While damages by azepine treatments to the foliage of apple trees have only scarcely been reported and were in all cases economically acceptable, the leaves of the tested pear varieties showed quite different reactions. No phytotoxicity was observed after treatments with 50 to 100 g a.i./100 l of the varieties "Doyenne", "Triomphe de Vienne", "Durondeau", "Bunte Juli", "Packham's Triumph", "Williams' Favorite" and "Beurre Bosc". Medium to strong damages were reported from trials with the varieties "Conference" and "Gute Luise" at dosage rates of 50 g a.i./100 l. The variety "Passacrassana" turned out to be extremely sensitive to the azepines, even at low rates. Further trials with the sensitive varieties are not recommended.

QUALITY TESTS

No adverse influence of azepine treatments has been noted in taste tests with "Golden Delicious" apples selected from several field trials.

RESIDUES

20 to 30 days after a single treatment with GS 16306 at a range of 75 g a.i./100 l, residues in the range of 0,9 to 3,3 ppm were detected on the surface of apples. With increasing numbers of applications they reached 1,0 to 7,4 ppm. Further investigations on this subject are in progress.

SUMMARY

1. The azepines are a group of fungicides with unique features for the control of pip fruit tree diseases: they control scab of pear and apple tree as well as powdery mildew of apple at dosage rates of 37,5 to 50 and 37,5 to 75 g a.i./hl.

2. The azepines possess a particularly good eradicant effect against apple and pear scab. Applications up to 72 h after a Mills' period at rates of 37,5 to 50 g a.i./100 l provide an excellent control of the disease even in sites with heavy infection. Furthermore, already established and visible infections are regularly killed in protectant spray programs. These features offer greater flexibility in field application schedules.

3. Whilst the azepines cannot be applied to most vegetables, field crops and ornamentals due to strong phytotoxic reaction of these plants, they are well tolerated by the apple tree. Fruit russetting may occur on certain varieties under adverse climatic conditions after application during the period of fruit formation. Certain pear varieties are extremely sensitive to azepines, while most of the tested varieties tolerate them very well.

ACKNOWLEDGEMENTS

My sincere thanks are due to the GEIGY field research collegues in Switzerland, Italy and the United States who conducted several of the trials reported here, for their efforts and kind co-operation.

Proc. 5th Br. Insectic. Fungic. Conf. (1969)

A SYSTEMIC FUNGICIDE FOR RICE BLAST CONTROL

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Summary An organophosphorus compound, O,O-diisopropyl-S-benzyl phosphorothiolate (IBP), was tested for systemic fungicidal activity and found to act systemically against rice blast caused by Piricularia oryzae. In the greenhouse test, the leaf blast control was obtained by dipping root system of rice plants into dilute solution of IBP. A new product, IBP granule (17 per cent active ingredient), was tested by strewing onto irrigation water in pots and paddy field. IBP granule was applied before the initial outbreak of leaf blast and the heading of plants at the dosage of 5, 7.5 and 10 kg active ingredient per hectare. The application gave remarkable controlling effectiveness against leaf, neck and node blast. Fungicidal activity of the granule appears to be retained for at least 3 weeks. It was proved that the water application of IBP granule is an excellent method for the rice blast control in paddy field.

INTRDUCTION

Among the fungus diseases of rice, blast caused by Piricularia oryzae is of major economic importance. Some organomercuric compounds had been used for long years for the control of rice blast. Recentry, some antibiotics, organophosphorus compounds and pentachlorophenol derivatives in place of organomercuric compounds have been developed and used in Japan. Two organophosphorus compounds, O,O-diethyl -S-benzyl phosphorothiolate (EBP) and O,O-diisopropyl-S-benzyl phosphorothiolate (IBP), are the rice blast controlling agents developed by the research group of us (KADO et al. 1965 and 1968). Generally, all of the rice blast controlling agents in the world are used by two ways of foliar sprays and dusts.. IBP has also been used as a foliar fungicide in Japan and some countries. As universally known, organophosphorus compounds have generally been used for the control of various insects and their systemic action has often been discussed. Systemic pesticides have been used to control insects and weeds, but any systemic fungicide for the foliar diseases of rice plants has not been developed yet.

In our experiments on fungicidal mechanisms of IBP and EBP, their systemic action has been investigated with particular interest. The compounds were found to have the systemic action as one of excellent characters (YOSHINAGA et al. 1965 and 1969). It was proved that the root system application of IBP and EBP is an excellent method for the rice blast control in paddy field. A new product, IBP granule ("Kitazin-P" granule) has been formulated as a systemic fungicide. The results of our experiments carried out on IBP granule are mentioned in this paper.

METHOD AND MATERIALS

Organophosphorus fungicide, O,O-diisopropyl-S-benzyl phosphorotiolate (IBP), was tested for systemic fungicidal activity against rice blast. In these experiments, IBP emulsifiable concentrate (48 per cent active ingredient) and IBP granule (17 per cent active ingredient) were used. IBP granule is a new product formulated for the water application in paddy field.

In the greenhouse test, seedlings of rice (Oryza sativa) grown on the bed of moist sand until three leaf stage were transplanted to the nutrient solution in flasks wrapped with alminium foil. The seedlings were treated with 25 and 50 ppm solution of IBP by dipping the roots for 24 hours and 48 hours, and then removed to the nutrient solution. In the test, IBP emulsifiable concentrate diluted with water was used. Inoculation was made either before after the chemical treatments with spore suspension of Piricularia oryzae. The effectiveness against rice blast caused P. oryzae was assesed on the 7th day after inoculation. Per cent disease control was calculated as followed;

$$\left(1 - \frac{\text{Number of lesion on treated plants}}{\text{Number of lesion on untreated plants}} \right) \times 100$$

In the other experiment, rice plants were grown until the tillering stage in watered pots in the greenhouse. IBP granules were applied at the dosage of 10 kg a.i./ha into the pots beforehand, the treated plants were inoculated on the 4th, 10th and 21st day after the application of IBP granules with spore suspension of P. oryzae. Number of lesion on leaves were counted on the 10th day after inoculation, then converted to per cent disease control as above.

In order to investigate the rice blast control obtained by the water application of IBP granules in field, two varietes of rice plants, Norin No.29 and Aichiasahi, were cultivated in 1968. Norin No.29, sowed on 2nd May and transplanted on 14th June in paddy field, reached the heading stage on 21th August and was harvested on 11th October. In the other field, Aichiasahi, sowed on 2nd May and transplanted on 13th June in paddy field, reached the heading stage on 31th August and was harvested on 24th October. In order to discuss the best time and the dosage of application for the rice blast control, variety of Norin No.29 was treated with IBP granules by strewing them onto irrigation water with hands. Time of application was arranged before or after the initial outbreak of leaf blast and the heading stage of plant concerning with the outbreak of neck blast. The application of IBP granules was made at the dosage of 5, 7.5 or 10 kg a.i./ha. The effectiveness on leaf blast was assessed on 13th August, and one on neck blast and node blast on 1st October.

Variety of Aichiasahi in paddy field was used to discuss relations between the growth of plants and the water application of IBP granules. IBP granules were applied at the dosage of 5 and 10 kg a.i./ha on 2nd July and 28th August and the water suspension of IBP emulsifiable concentrate was sprayed at the same time. Height of plants was mesured on 19th September and then rice plants were harvested on 24th October.

RESULTS

Relations between the percentage of leaf blast control obtained by dipping roots into 25 or 50 ppm solution of O,O-diisopropyl-S-benzyl phosphorotiolate (IBP) and the time of inoculation are shown in Fig. 1. The treatments before inoculation were effectve in controlling leaf blast. The treatments of 48 hours after inoculation were also effective, whereas one of 24 hours failed to control leaf blast. One can speculate from these results that IBP is absorbed into rice seedlings through the root system and translocated to stalks and leaves. It appears that the treatment before inoculation is more effective than that after inoculation. The compound at a certain concentration in leaves is considered to have preventive action against visual lesion appearence. Foliar disease control was obtained by the root dipping method without causing injury to the plants.

The results of leaf blast control obtained by the water application of IBP granule in watered pots are shown in Fig. 2. IBP absorbed into rice plants through the root system effectively controlled the outbreak of blast on the leaves, whereas most of the leaves on the untreated plants were killed by blast. IBP granules gave 80 per cent disease control even when inoculated on the 4th day after the application, and 95 per cent control in the 10-day interval between the application and the inoculation and 70 per cent control in the 21-day interval. IBP applied in a flooded condition is considered to be readily translocated to leaves and accumulated to the maximum level at about the 10th day after the application. Activity of IBP granules in a paddy field condition was found to be retained for at least 3 weeks.

In the field test, IBP granules were applied to irrigation water at the dosage of 10 kg a.i./ha on 6th, 12th or 20th July and 13th August or September. Results of this field test are shown in Fig. 3. In this field, the initial outbreak of leaf blast was observed on 12th July. Each application of 6th, 12th or 20th July gave equal effectiveness against the leaf blast control. The best time of application for the leaf blast control could not be judged only from these results. For the neck and node blast control, two application on 13th August (8th day before the heading stage) and 3rd September (13th day after the heading stage) were more effective than others. With respect to yield, the application of 13th August gave 47 per cent increase in yield in comparison with the untreated, whereas one of 3rd September only 16 per cent increase. As the results of this field test, it was proved that the best time of application for the control of neck and node blast is on about 10th day before the heading stage of rice plants.

In the other field test, IBP granules were applied at the dosage of 5, 7.5 and 10 kg a.i./ha on 8th July (one week before the initial outbreak of leaf blast) and 13th August (8th day before the heading stage). To compare with the water application of IBP granules, the suspension of IBP emulsifiable concentrate in water was

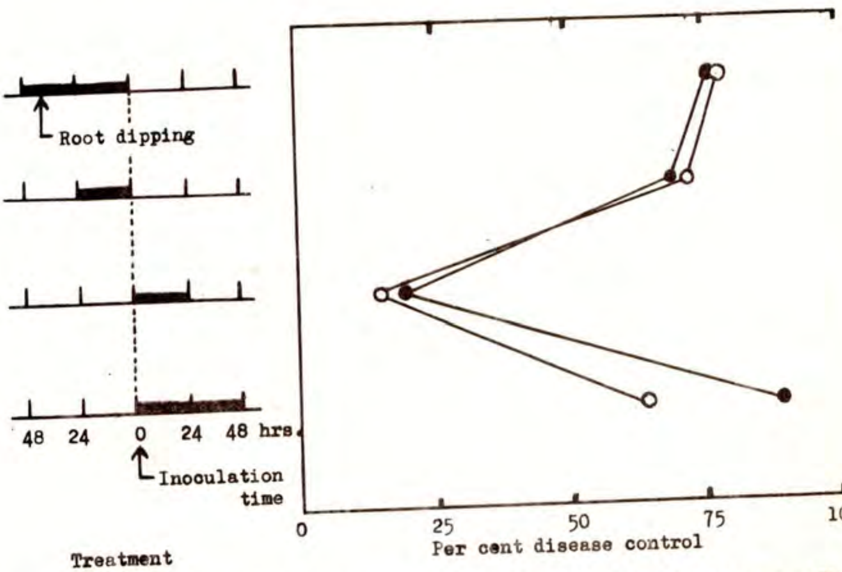

Fig. 1. Rice blast control obtained by the dipping of root system in IBP solution at 25 (O) or 50 (●) ppm.

sprayed at a concentration of 450 ppm on 12th July, 13th August and 3rd September. In this field, the outbreak of neck blast occured severely, while that of leaf blast was slight. As shown in Table 1, the applications of IBP granules gave excellent controlling effectiveness against leaf, node and neck blast. With respect to the relationship between the dosage and the control of disease, water applications of IBP granules at the dosage of 7.5 and 10 kg a.i./ha were extremely superior to foliar sprays of IBP emulsifiable concentrate. No great difference between the dosages of 7.5 and 10 kg a.i./ha was made. The dosage of 5 kg a.i./ha also was more effective than foliar sprays, but inferior to those of 7.5 and 10 kg.

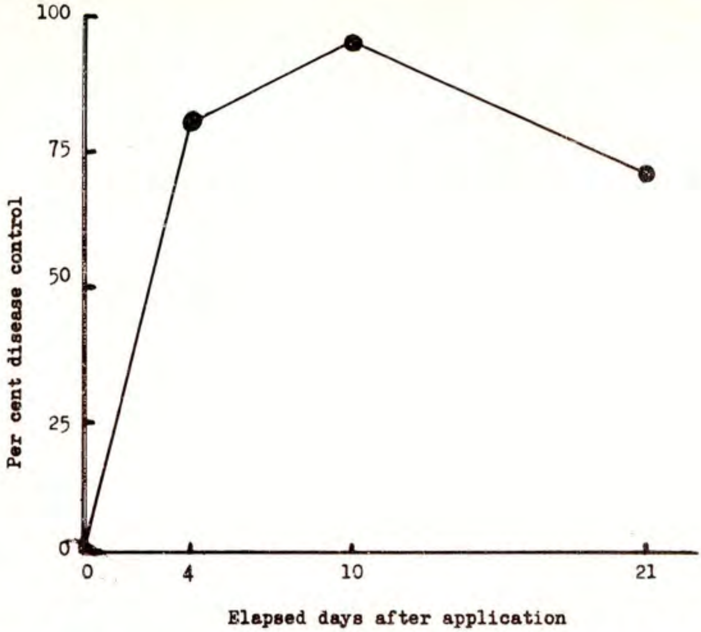

Fig. 2. Rice blast control obtained by water application of IBP at the dosage of 10 kg a.i./ha in pots.

In comparison with the yield of the untreated,IBP granules gave 87 per cent increase in yield at the dosage of 10 kg a.i./ha, and 71 per cent increase at 7.5 kg and 45 per cent increase at 5 kg. The results of this field test suggested that the water application of IBP granules is an excellent method for the rice blast control in paddy field.

In the field tests shown in Fig. 3 and Table 1, it was observed that the height of treated plants was shorter than the untreated. The same results obtained in other field test are shown in table 2. In this field, the outbreak of blast was too judged the effectiveness of IBP granules on blast. The height of plants treated with IBP granules on 2nd July (the tillering stage of plants) and 28th August (3rd day before the heading stage) was shorter than the untreated. The rice yield, however, was increased by the application of IBP granules. From the results of this test, it was provrd that the water application of IBP granules gives increase in yield, even when the outbreak of blast is very slight and the treated plants are made shorter in height.

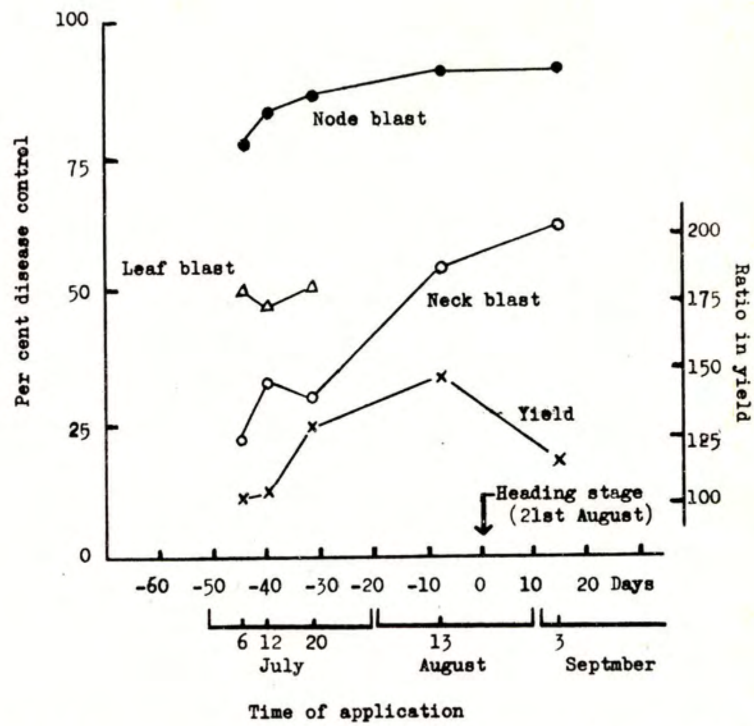

Fig. 3. Relations among rice blast control, rice yield and time of water application by IBP granule in paddy field.

Table 1.

Rice blast control and rice yield obtained by water application of IBP granule in paddy field

Fungicide	Application method	Dosage (a.i.)	Per cent disease stalk leaf blast	node blast	neck blast	Yield ($kg/3.3\ m_2$)
IBP granule	Water application	10 kg/ha	9.1	0.7	34.5	1.40
		7.5	9.5	0.5	38.4	1.28
		5	13.1	1.0	48.7	1.09
IBP emulsifiable concentrate	Foliar spray	480 ppm	13.4	7.8	52.5	0.92
Untreated	None	None	21.2	8.6	68.5	0.75

Table 2.

Growth and rice yield obtained by water application of IBP granule in paddy field

Fungicide	Application method	Dosage (a.i.)	Per cent disease stalk neck blast	Height of plant (cm)	Yield ($kg/3.3\ m_2$)
IBP granule	Water application	10 kg/ha	2.1	100.4	1.03
		5	2.6	101.4	0.95
IBP emulsifiable concentrate	Foliar spray	480 ppm	2.1	103.5	1.04
Untreated	None	None	4.9	104.9	0.92

DISCUSSION

The organophosphorus compound, O,O-diisopropyl-S-benzyl phosphorotiolate (IBP) was found to act systemically against rice blast caused by Piricularia oryzae. The compound is fairly water soluble (approximately 500ppm) and appears to be absorbed through the root system and readily translocated in the transpiration stream to the site of the pathogen. IBP in the plants has preventive action against visual lesion appearence, lesion enlargement and sporulation on lesion. I conclude that IBP is a new class of systemic fungicide which selectively control plant disease. Of particular interest is the fact that the fugicide gives remarkable controlling effectiveness on rice blast that is the most importance disease among those of rice.

IBP granule (17 per cent active ingredient, "Kitazin-P" granule) has been formulated for the water application in paddy field. Fungicidal activity of the granule is retained for at least 3 weeks in paddy field. That is, the fungicidal activity of the granules is two or three longer than that of foliar fungicide. Three to four applications of foliar fungicides are generally recommended for the control of leaf, neck and node blast in Japan. The effectiveness obtained by one or two applications of IBP granules against rice blast was found to be superior to three or four applications of foliar fungicide. Though the best time of the application for the control of leaf blast can not be judged in detail, the application on the 7th to 10th day before the initial outbreak of leaf blast will be recommended. The granules applied at one week before the heading stage give remarkable controlling effectiveness for neck and node blast and also help to increase the rice yield. With respect to the dosage, the water application at the dosage of 5 to 10 kg a.i./ha gives the higher effectiveness.

The remarkable increase in rice yield also is one of the excellent characters of IBP granules. The granules also have the effect of shortening stalks of rice plants, thus protecting them from being lodged by wind. No bad effect on rice yield is produced by making stalks shorter in height.

I was proved that the water application of IBP granules is an excellent method for the rice blast control in paddy field.

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Proc. 5th Br. Insectic. Fungic. Conf. (1969)

PYRIDINITRIL, A NEW ORGANIC FUNGICIDAL COMPOUND

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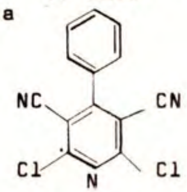
Summary In 1963 the new active ingredient pyridinitril* was discovered in the Research Laboratories of E. Merck AG, which distinguished itself by a remarkable fungicidal effect. In extensive laboratory and field trials and finally in practical applications as well pyridinitril*, also in combination with other compounds, has proven its suitability for the control of fungal diseases, particularly in fruit growing.

In addition to the broad spectrum of activity the persistency and the desirable side-effects of pyridinitril deserve special mention.

CHEMICAL-PHYSICAL AND TOXICOLOGICAL PROPERTIES OF PYRIDINITRIL

Chemical name 2,6-dichloro-4-phenylpyridinedicarbonitrile-(3,5)
Alternative name IT 3296
Structural formula



Molecular formula	$C_{13}H_5Cl_2N_3$
Molecular weight	274
Melting point	208 - 210°C
State	colourless, crystalline solid
Solubility	readily soluble in ethyl acetate, chloroform, benzene, acetone
Stability	at normal temperatures stable under acid and alkaline conditions
Toxicity	acute oral LD50 > 5000 mg/kg rat

BIOLOGICAL PROPERTIES OF PYRIDINITRIL

The mode of action of pyridinitril alone and in combination with other active substances has already been dealt with previously (Mohr, G., et al., 1968; Lust, S., 1969).

*accepted common name, formerly IT 3296

In table 1 the fungi are listed against which pyridinitril was found to be effective in the laboratory and field trials to date.

Table 1 : Fungi found to be susceptible to pyridinitril

Alternaria tenuis	Phacopsora ampelopsidis
Botrytis cinerea	Phytophthora infestans
Cladosporium fulvum	Plasmopara viticola
Diaporthe citri	Pseudoperonospora humuli
Glomerella cingulata	Sclerotinia fructicola
Penicillium expansum	Venturia inaequalis
Phaeoisariopsis vitis	Venturia pirina

This table demonstrates the broad spectrum effectiveness of pyridinitril. The tests are being continued.

EXPERIENCES WITH PYRIDINITRIL IN FIELD TRIALS

The initial trials carried out using a 75 % WP formulation were aimed primarily against Venturia inaequalis on apples and Plasmopara viticola on vines. The degree of control obtained against these two diseases entirely confirmed our expectations based on the positive experience of the laboratory tests.

Noteworthy in particular was the fact that this effectiveness is obtained at very low dosage rates. For example against apple-scab the standard recommendation is 50 g/100 l of water pre-blossom and 35 g/100 l post-blossom for the 75 % WP formulation. This represents 37.5 and 26.25 g of active ingredient per 100 l of water respectively.

These low rates of course are only possible because pyridinitril exhibits excellent stability even under adverse environmental conditions. This is enhanced by adequate formulation so that a satisfactory control can still be expected where the effectiveness of certain other fungicides becomes questionable.

Besides this reliability the persistency of pyridinitril is also responsible for the outstanding prevention of storage scab, i.e. scab on apples in storage where the infection took place on the tree. A pre-requisite for this type of protection is an extraordinarily long lasting fungicidal effect, which is thus demonstrated by pyridinitril once again.

Similarly favourable results were obtained with pyridinitril on vines against Plasmopara viticola, as well as ripe rot, Glomerella cingulata, and several other diseases.

When evaluating a fungicide the associated complex of side-effects is at least of the same importance as the fungicidal effect itself. In this respect pyridinitril again fully meets the demands of the fruit producer. The low toxicity to warm-blooded animals was already mentioned; the absent insecticidal effect permits pyridinitril to be used during blossom time, too. In addition beneficial predators and parasites are not adversely affected. Moreover, according to our experience up to date, pyridinitril has no direct physiological influence on red spider mites, and does not stimulate this pest.

The side-effect against powdery mildew on fruit and vines can justly be described as indifferent. In fact, trials have not only revealed the absence of a stimulation, instead a certain suppression has been observed. This question, however, needs further investigation.

Pyridinitril in general exhibits a favourable effect on the crop. The quality of treated fruit regarding finish as well as colour is good. The foliage distinguishes itself by its healthy firm texture and dark green colour.

COMBINATIONS OF PYRIDINITRIL WITH OTHER FUNGICIDES

The laboratory investigations up to date, which have already been mentioned in detail (Lust, S. 1969), lead to the recognition that captan is especially suited for use in combination with pyridinitril. A commercial product containing these compounds named Ciluan(R) is already being marketed.

This mixture of pyridinitril and captan combines the special advantages of these two active ingredients, i.e. the persistency of pyridinitril and the initial effect of captan. This results in a product of superior reliability. At the same time the fungicidal effect is enhanced, i.e.the effect of the combination is greater than the sum of the effect of the two components when used separately. Thus in comparison to the individual application of pyridinitril and captan, a reduction of the active ingredients is possible. The combination product mentioned above (formerly EMD 6037 F) contains 14.7 % pyridinitril and 25 % captan. It is recommended at a rate of 100 g of the formulated product per 100 l of water, i.e. 100 l of spray contain 14.7 g of pyridinitril plus 25 g of captan.

There is no need to emphasize that especially today, at a time in which increasing attention is being paid to the danger to the consumer due to pesticide residues such a decrease complies with this trend, especially since both pyridinitril and captan possess an extraordinarily low toxicity.

The combination of pyridinitril and captan further enlarges the fungicidal spectrum already determined for pyridinitril alone.

The fact that the favourable properties of the two components are maintained not only regarding the fungicidal effect itself but also as to the side-effects is particularly advantageous. This combination also exhibits an extraordinarily good plant safety. Foliage and fruit develop remarkably well. Regarding russeting, even on especially susceptible apple varieties, such as Golden Delicious, this combination is neutral, i.e. similar to captan when used alone.

Most of the information available on the use of the combination concerns the control of apple scab. The above mentioned properties result in an exceptionally reliable control which is particularly noticeable when weather conditions are favourable for the development of fungi. The duration of the fungicidal effect of pyridinitril results in good control of storage scab, whilst the combination prevents storage rots caused by Gloeosporium, Penicillium and other

(R) Registered trade-mark E. Merck AG

fungi.

The exceptionally broad spectrum of activity also makes this combination suitable for the control of many other fungus diseases of economic importance on fruits, vines, hops and vegetables. In this connection investigations are presently underway in several countries with the aim of establishing further possible fields of application under practical conditions. However, it has already become obvious that the combination product has a good potential against the diseases listed in table 2 .

Table 2 : Plant diseases which can be controlled by pyridinitril/ captan

Fungus	disease	crop
Venturia inaequalis	scab	apples
Venturia pirina	scab	pears
Glomerella cingulata	bitter rot/ ripe rot	apples/vine
Botryosphaeria dothidea	bot.-rot	apples
Gloeodes pomigena	sooty blotch	apples
Mycrothyriella rubi	fly speck	apples
Penicillium sp.	green rot	apples
Monilia laxa	blossom blight	apricots
Taphrina deformans	leaf curl	peaches
Plasmopara viticola	downy mildew	vine
Phomopsis viticola	dead arm disease	vine
Pseudopeziza tracheiphila	Rotbrenner	vine
Pseudoperonospora humuli	downy mildew	hops
Peronospora sparsa	downy mildew	roses
Bremia lactucae	downy mildew	lettuce
Peronospora schleideni	downy mildew	onions
Albugo trapogon	white rust	salsify

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