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BENOMYL - A BROAD SPECTRUM FUNGICIDE

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Summary Benomyl ("Benlate"*) has been in trials in the United Kingdom on a wide range of crops for the last 3 years. The material has been applied as a foliage spray, as a seed dressing and by soil application. Results indicate a high toxicity against many pathogenic fungi with a high level of plant safety. Good residual protectant activity has been demonstrated and local systemic activity within leaf tissues gives an eradicant action. Systemic disease control has been demonstrated by soil and by seed dressing applications.

INTRODUCTION

In field tests carried out in the United States in 1966 and 1967, benomyl was shown to be active against a wide range of plant pathogens. Protectant and eradicant activity were demonstrated against apple scab (Venturia inaequalis) and powdery mildew (Podosphaera leucotricha). Botrytis cinerea was controlled in a number of crop plants including strawberries and blueberries. A number of seed-borne diseases, including Ustilago nuda, were controlled in cereals. Some important diseases of vegetable crops were controlled by applications of benomyl. Rhizoctonia solani on a number of crops was controlled systemically by in-furrow treatments. Erysiphe cichoracearum was controlled in cucurbits by both foliage and soil applications. Septoria apii-graveolentis in celery was controlled by foliage application. Powdery mildews were controlled on a number of ornamentals including roses where black spot (Diplocarpon rosae) was also effectively controlled by foliage application.

The spectrum of activity and mode of action indicated that benomyl could be particularly suitable as a significant improvement for the control of many diseases of top fruit, soft fruit, vegetable crops and ornamentals in the United Kingdom. Trials have therefore progressed during the last 3 years in many locations in the United Kingdom and Eire in many of these crops to determine method and rate of application.

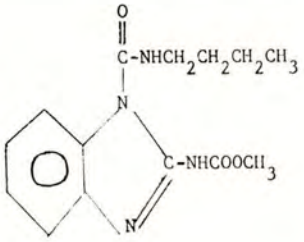
* "Benlate" is the Trademark of E.I. Du Pont de Nemours & Co. (Inc.)

PHYSICAL & CHEMICAL PROPERTIES

a. **Chemical Properties**

Chemical Name : Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate

Structural Formula:



Empirical Formula : $C_{14}H_{18}N_4O_3$

Molecular Weight : 290.3

b. **Physical Properties**

Appearance	White crystalline solid
Odour	Faintly acrid
Vapour pressure	Non volatile
Solubility	Essentially insoluble in water and oil

c. **Toxicological Information**

The approximate lethal dose for benomyl by oral administration to male rats is greater than 9590 mg/kg, the maximum feasible dose. 90-day feeding studies in male and female rats fed dietary levels of 0, 100, 500 and 2,500 p.p.m. without any nutritional, clinical, hematological, urinary, biochemical or pathologic evidence of toxicity. Longer term studies are in progress. Dermal tests with guinea pigs have shown that the compound is not a skin irritant, nor does it pose a dermatitis hazard. Eye irritation studies with rabbits have shown only mild, transitory effects.

BIOLOGICAL PROPERTIES

Benomyl has shown a high degree of activity against a wide range of Ascomycetes and Fungi Imperfecti, variable results with the Basidiomycetes and little or no control of Phycomycetes.

In vitro tests with benomyl have generally provided a reliable indication of the disease control to be expected.

Benomyl has been applied in varying ways to a wide range of crops and a high level of plant safety has been indicated.

Top Fruit

Benomyl was shown to be active against apple scab (Venturia inaequalis), powdery mildew (Podosphaera leucotricha) and spider mites when evaluated on apple seedlings in the greenhouse. Both protectant and eradicant activity were demonstrated at 100 p.p.m. against the two diseases, and it was found that spider mites feeding on treated plants did not multiply because their eggs failed to hatch. Under some circumstances control of some diseases was further improved by the addition of surfactants to the spray suspension.

Laboratory studies demonstrated that apple scab and apple mildew could be controlled in apple seedlings by soil drenches. Further investigations, involving spot treatments to individual leaves, showed that benomyl was absorbed into the leaf and translocated distally.

Subsequent field trials in the United States confirmed the activity against both apple scab and powdery mildew and the suppressant activity against spider mites. A rate of 4 oz a.i. plus 4 oz of Surfactant F per 100 U.S. gallons sprayed every 10 to 14 days was indicated for both diseases.

The disease control provided in the trials was outstanding over two years, and no deleterious effect on the trees occurred after having received treatments for the second year.

The eradicant effect against apple scab was further investigated by extended schedule spraying and successful control was achieved with half the number of normal sprays. It was further demonstrated (Connor and Heuberger, 1969) that a single late season application of benomyl at 8 oz a.i./100 gal completely inhibited perithecial development by Venturia inaequalis.

In field studies other diseases were prevalent and several fruit spots such as black rot (Physalospora obtusa), flyspeck (Microthyriella rubi) and sooty blotch (Gloeodes pomigena) were prevented by benomyl application. Cedar apple rust (Gymnosporangium juniperi-virginianae) on the other hand was not controlled.

Preliminary trials in the late summer of 1967 in the U.K. indicated that 4 oz a.i./100 Imp. gal was active against both apple scab and powdery mildew.

Full season trials in 1968 were carried out to investigate the optimum rate of application for benomyl against powdery mildew on Cox. Benomyl was applied at a number of sites at the rate of 5, 7.5 and 10 oz active per acre on a 10 to 14 day schedule; treatments were applied in 60 gal/acre for the first five applications and in 120 gal/acre for the second five applications. Surfactant F was added to the spray suspension for all applications at 4 fl oz/100 gal. Results from a trial carried out in Kent are as follows :-

Table 1.

Apple disease control on Cox in Kent - 1968.

Treatment	Rate oz a.i./ac	Primary mildew control. % infection cover	Secondary mildew control			Storage rot Control. % infected	Fruit % cracked
			Per cent control				
		Truss and Shoot	Infected Leaves. July	Infected Leaves. August	Score/ 100 leaves	Gloeosporium	
Benomyl	5 oz	7.1	71.4	41.7	55.6	20	19.3
Benomyl	7.5 oz	7.4	74.5	49.1	63.5	24	14.6
Benomyl	10 oz	6.6	81.6	58.3	71.0	10	26.4
Untreated	-	82.8	0.0	0.0	0.0	42	22.9
Infection Level	-	-	(44.8)	(50.4)	(88.8)	-	-

In this trial, which is typical of the number carried out, primary mildew infections of both the truss and the shoot were significantly dried up by all rates of benomyl. Secondary mildew as expressed as numbers of infected leaves and percentage cover, by scoring the leaves on a 0 to 5 geometric scale, showed that progressive improvement in mildew control was achieved by increasing the dosage. The level of control achieved was more marked when assessed during the spraying season than after completion of the schedule, and the degree of control as assessed by percentage leaf cover was more marked than by simple leaf infection assessment indicating the eradicant nature of the material.

Storage rots, assessed after barn storage during the winter, were effectively decreased by the scab and mildew programme of benomyl. No effects on fruit size or skin quality were detected but there was an indication that at the highest rate the amount of cracked fruit was increased marginally.

Trials continued in 1969 to confirm the rate of application, the value of a Surfactant and the degree of control to be expected with extended schedule spraying. In addition, a number of ½ to 2 acre blocks of orchard were treated to estimate the effectiveness of a commercial application of benomyl.

The replicated trials work carried out on single Cox tree plots indicated that for secondary powdery mildew control benomyl was superior to both dinocap and binapacryl, the 8 oz a.i./acre rate was marginally superior to the 6 oz a.i./acre rate, a 14 day schedule was significantly better than a 28 day schedule, and the effects of surfactants were minimal. Apple scab control was superior to Dodine Acetate, Captan and Dithianon, the 8 oz a.i./acre rate was superior to the 6 oz a.i./acre rate, a 14 day schedule was only marginally superior to a 28 day schedule, and the addition of surfactants had no advantage. The results obtained from a trial in Worcestershire were as follows :-

Table 2.

Disease control on Cox in Worcestershire - 1969.

Treatment	Secondary Mildew Per Cent leaves infected		Scab Mean Per Cent leaf infection	
	Mid season	Late season	Mid season	Late season
Benomyl 8 oz a.i./acre	22.0	19.8	0.044	0.038
Benomyl 6 oz a.i./acre	24.0	17.0	0.125	0.100
Benomyl 8 oz a.i./acre Spreadite to flowering	34.0	27.8	0.034	0.031
Benomyl 8 oz a.i./acre (every 28 days- (Spreadite to flowering)	44.5	53.4	0.125	0.046
Benomyl 8 oz a.i./acre Spreadite throughout	23.0	24.3	0.029	0.034
Binapacryl	42.8	31.0	-	-
Dinocap	41.8	34.8	-	-
Captan	-	-	0.150	0.150

The degree of control achieved from semi-commercial applications on blocks of apples of up to 2 acres has been more marked especially with respect to mildew control.

Table 3.

Mildew control on semi-commercial sites - 1969.

Treatment	Per cent active primary mildewed clusters	Per cent extension leaves infected	
		June assessment	July assessment
Benomyl 8 oz a.i./acre + wetter and spreader up to flowering	4.6	4.2	9.8
Standards (Dinocap, Binapacryl or Sulphur)	6.1	14.0	22.8

N.B. Results means from thirteen sites.

At all of these sites standard anti-scab materials were used in the standard programme and in all cases scab control was a 100% in both the standard and the benomyl blocks. Where mite infestations were heavy, which was common in 1969, benomyl had only a marginal effect.

The control of mildew in these larger scale grower blocks was superior to that achieved in the smaller scale replicated trials. It is possible that the eradicant properties of benomyl are resulting in a lowering of the inoculum level progressively in the orchard.

Stone Fruit

Field trials in 1966 and 1967 in the United States demonstrated that benomyl was active against a number of diseases of stone fruit (Delp, 1968).

In peaches, both weekly and fortnightly applications at 4 oz active/100 U.S. gallons + Surfactant F at 4 oz/100 U.S. gallons gave 100 per cent control of peach scab (Cladosporium carpophilum). Peach leaf curl (Taphrina deformans) was not controlled by dormant season spraying. Both pre-harvest sprays and post-harvest dips at 4 oz active/100 U.S. gallons gave excellent control of brown rot (Monilinia fructicola).

Benomyl was found to be active against cherry leaf spot (Coccomyces hiemalis) and powdery mildew (Podosphaera oxyacanthae). 3 oz active/100 U.S. gallons + Surfactant F at 4 oz/100 U.S. gallons gave excellent control of both diseases with residual control of leaf spot for up to 10 weeks after the last application. Brown rot blossom blight (Monilinia laxa and Monilinia fructicola) was controlled by blossom time applications of 4 oz active + 4 oz Surfactant/100 U.S. gallons.

Soft Fruit

Strawberry grey mould (Botrytis cinerea) was controlled in trials in both the United States and Canada in 1966 and 1967 by application of benomyl applied at flowering and subsequently. Rates varied from 1 lb active/100 U.S. gallons at 125 U.S. gallons per acre to 4 oz active/100 Imp. gallons at 100 Imp. gallons per acre. Powdery mildew (Sphaerotheca macularis) was effectively controlled by post-harvest applications of 4 oz active/acre (Freeman & Pepin, 1968). Verticillium wilt (Verticillium dahliae) was completely controlled by 4 lb active/12,000 ft of row applied in the planting hole at planting time.

In raspberries grey mould (Botrytis cinerea) was controlled (Freeman & Pepin, 1967) by benomyl applied three times from flowering at 4 oz active per acre in 180 gallons of water per acre.

Benomyl applied at 4 oz/100 U.S. gallons gave excellent control of grape powdery mildew (Uncinula necator), no activity was demonstrated against downy mildew (Plasmopura viticola).

Benomyl was tested initially in the United Kingdom on blackcurrants in 1967 for its activity against American gooseberry mildew (Sphaerotheca mors-uvae) and black-currant leaf spot (Pseudopeziza ribis).

For mildew control a 14 day schedule of 4 oz active/100 Imp. gallons gave excellent control superior to a 2 oz active rate and to a 4 oz active extended schedule rate. In the case of leaf spot control the 4 oz active/100 Imp. gallons on a 14 day schedule again gave excellent control but was only marginally superior to the 2 oz active rate and the 4 oz active extended schedule treatment.

Trials in 1968 and 1969 have confirmed the activity of benomyl against both powdery mildew and leaf spot and indicated a rate of 4 oz active per 100 Imp. gallons. For mildew control sprays every 14 days as necessary are indicated, but for leaf spot three post-blossom, pre-picking sprays and a single post-picking spray are indicated.

Strawberry grey mould has been controlled effectively in the United Kingdom and Eire by benomyl applied three times from the early flower stage at a rate of 8 oz active per acre. Lower rates of 4 oz active per acre have also given control equivalent to commercial treatments, but at lower levels of disease incidence. Control was also achieved with applications of less than three, but the control was not as complete as with the full number of applications. Applications both to strawberries under cloches and in the open have had a beneficial effect on berry size.

In the case of raspberries, Botrytis cinerea has been controlled by three applications of benomyl from early flowering at a rate of 4 oz a.i./acre. Control, although not complete, was superior to existing commercial treatments and the effect on berry size was beneficial.

Citrus Fruit

Initial tests in the United States (Delp, 1968) demonstrated that citrus post-harvest green mould (Penicillium digitatum) was eradicated from pre-inoculated oranges by a 50 ppm benomyl dip treatment.

Subsequent tests (Harding, 1968 and Gutter, 1969) have shown that citrus fruits dipped in 500 ppm or coated with wax containing 500 ppm of benomyl are effectively protected from post-harvest decay caused by Penicillium digitatum.

It was also shown that a 500 ppm pre-harvest spray applied 1 week before harvest reduced decay in oranges and tangerines caused by Penicillium digitatum, Diplodia natalensis, Phomopsis citri and Colletotrichum gloeosporioides. Geotrichum candidum was not controlled. (Brown, 1968).

Field and cereal crops

In the drier, hotter sugar beet growing areas of the world, leaf spot (Cercospora beticola) can cause severe defoliation. Benomyl applied at 1 oz a.i./100 U.S. gallons on a 14 day schedule in Delaware, United States in 1967 gave 99% control (Delp, 1968).

Preliminary greenhouse tests demonstrated the systemic activity of benomyl in cotton when mixed in the soil at 20 to 100 ppm. Fusarium spp., Rhizoctonia solani, and Verticillium albo-atrum were all controlled. A soil drench of 80 ppm eradicated Verticillium from cotton plants previously inoculated and showing wilt symptoms. (Delp, 1968.). Soil pot incorporation at 100 ppm gave persistent control for up to 4 months against Verticillium albo-atrum and soil drenches of 25 mg/pot gave control in 5-week-old plants (Erwin, Mee and Sims, 1968).

Initial tests against rice blast (Piricularia oryzae) indicated that weekly sprays of benomyl at 3 oz active per 100 U.S. gallons provided 99% control of this important disease of rice (Delp, 1968).

Tuber dips with benomyl at 0.5 lb a.i./5 U.S. gallons for 3 minutes controlled a light tuber infection of black scurf (Rhizoctonia solani) in potatoes, but not a heavy soil infection. Some effect against Verticillium albo-atrum was detected (Biehn, 1969).

In cereals, flag smut (Urocystis agropyri) was partially controlled in wheat by a seed dressing of benomyl of 3.6 oz a.i./100 lb of seed and by a soil incorporation of 1 lb a.i./12,000 ft row. The seed dressing completely controlled a 6% incidence of loose smut (Ustilago nuda) (Metcalfe and Brown, 1969).

In the United Kingdom, skin spot (Oospora pustulans) of potatoes has been controlled by a 10% dust of benomyl applied at the rate of 10 lb/ton. Both the infection in the mother and daughter tubers was controlled. Benomyl applied as 10% dust also achieved some effect against gangrene (Phoma exigua) in the mother tubers, but a 1.0% dip treatment for 5 minutes gave an improved control.

Many cereal diseases have been controlled by foliage and seed dressing applications of benomyl in the United Kingdom.

Loose smut (Ustilago nuda) has been controlled effectively in both Winter wheat and Spring barley at rates of 2 oz a.i. to 4 oz a.i. per cwt.

Benomyl applied to seed at 2 oz a.i./cwt achieved control superior to organomercurials of Fusarium nivale in rye, Septoria tritici in wheat and Tilletia caries in wheat. No effect was demonstrated against Pyrenophora avenae in oats or Pyrenophora graminea in barley.

Benomyl achieved control of powdery mildew (Erysiphe graminis) in wheat, barley and oats with a programme of sprays at 4 oz a.i./acre. Single foliage applications of 4 oz a.i./acre in 1968 at the "ligule of last leaf just visible" stage gave a significant control of mildew and a corresponding increase in yield. In 1969, benomyl applied at 1 lb a.i./acre as a seed dressing or as a foliage spray at late tillering or at the "ligule of last leaf just visible" stage all gave significant mildew control. The persistence of mildew control achieved by the seed dressing and the early foliage applications was not, however, sufficient to result in a yield response in 1969. The late application did, however, produce a yield response in 1969 in those areas where mildew was prevalent.

Foliage applications of benomyl have been shown to have a marked effect against glume blotch of wheat (Septoria nodorum). Tillering stage foliage applications have resulted in significant control of eye spot in wheat (Cercosporella herpotrichoides). Foliage applications have indicated some effect against leaf blotch of barley (Rhyncosporium secalis) and a marginal effect against brown rust of barley (Puccinia hordei). A possible effect against black mould (Cladosporium herbarum) and black point (Alternaria spp.) was also detected by foliage applications.

Vegetable Crops

Preliminary trials in the United States (Delp, 1968) demonstrated that benomyl was very active against powdery mildew (Erysiphe cichoracearum) of cucurbits. Foliage sprays of 3 oz a.i./100 U.S. gallons on a 14 day schedule resulted in excellent control. Greenhouse studies have shown that cucumber seed treated with

3 to 4 oz a.i./100 lb resulted in protection from powdery mildew and two-spotted mites for periods up to 5 to 6 weeks. Soil drench treatments of potted cucumber seedlings with benomyl at 7 mg a.i. per 5-inch pot (equivalent approximately to 5 lb per acre) resulted in eradication of powdery mildew with prevention of re-infection and suppression of mite damage for 5 weeks. Rates eight times higher gave more prolonged pesticidal effects with only a slight marginal leaf chlorosis. Further work (Schroeder and Provvidenti, 1968 & 1969) has indicated systemic activity against Sphaerotheca fuliginea in cucurbits with a soil drench at rates of 1.5 to 150 mg per pot with persistence ranging from 2 to 4 weeks. A form of the fungus resistant to benomyl was also reported.

In tomatoes some effect was detected against anthracnose (Colletotrichum phomoides) and a reduction in wilt symptoms caused by Fusarium oxysporum f. sp. lycopersici was obtained by sub-irrigation to inoculated plants of 15.5 ppm of benomyl (Biehn and Dimond, 1969).

Trials in Florida (Delp, 1968) showed that benomyl applied at 4 oz a.i. plus Surfactant F at 4 oz/100 U.S. gallons on a 7 day schedule gave excellent control of early blight (Cercospora apii) and late blight (Septoria apii-graveolentis). Extended schedule spraying on a 14 day schedule at 8 oz a.i. also gave good control of both diseases.

In the United Kingdom the activity of benomyl against powdery mildew of cucumbers has been confirmed both as a foliage spray and as a soil drench. At Levington, Suffolk, benomyl at 2 oz and 4 oz a.i./100 Imp. gallons as a spray was compared with benomyl applied as a soil drench at 100 mg a.i.per plant and Dinocap applied as a spray at 1 lb a.i./100 Imp. gallons. All treatments were applied 12 days after inoculation with mildew. Dinocap was re-applied 17 days later and all the treatments were applied a further 12 days later.

Table 4.

Control of cucumber powdery mildew

Treatment	Rate	Mildew Score			Weight of fruit in lb
		after 13 days	after 29 days	after 13 days	
Benomyl spray	2 oz active/100 gal	1.0	2.2	2.8	72.5
Benomyl spray	4 oz active/100 gal	0.8	2.0	2.5	67.6
Benomyl soil drench	100 mg active/plant	2.5	2.2	2.0	91.9
Dinocap spray	16 oz active/100 gal	5.2	7.3	8.5	48.1
Control	-	8.0	9.2	9.0	35.8

N.B. Mildew score on a 0 to 10 scale (0 = no mildew, 10 = 100% leaf area infected). In this trial, the effect of benomyl applied both as a spray and as a soil drench persisted for 4 weeks, indicating in the case of the spray that benomyl was being absorbed by the fleshy stems and subsequently translocated to new growth.

Further trials in the United Kingdom have indicated marked control of black root rot (Phomopsis sclerotioides) with soil drenches of 100 mg active per plant.

Trials with tomatoes in the United Kingdom with benomyl have given indications of good verticillium wilt (Verticillium albo-atrum) control with soil drenches of 100 mg a.i. per plant. Activity was demonstrated both with pre- and post-infection applications.

Work is continuing against the difficult problem of grey mould (Botrytis cinerea) control in tomatoes and activity is indicated at 4 oz a.i./100 Imp. gal.

Trials in the United Kingdom and Eire on celery have indicated that benomyl at 4 oz a.i./100 Imp. gallons sprayed on a 14 day schedule results in good control of Septoria apii-graveolentis.

In recent years Botrytis cinerea has been a problem in french beans, especially with regard to blemishes on pods for processing. Benomyl applied at flowering time at 4 oz a.i./100 Imp. gallons has given indications of excellent control.

Ornamentals

Initial trials in the United States showed that benomyl applied at 2 oz and 4 oz a.i./100 U.S. gallons, together with high levels of surfactant gave good control of powdery mildew (Sphaerotheca pannosa) and black spot (Diplocarpon rosae), (Delp, 1968).

Control of Fusarium oxysporum in gladiolus corms was achieved with a 1 : 400 water dip of benomyl (Forsberg, 1969). Blight of geranium cuttings caused by Botrytis cinerea was controlled by sprays of 4 oz to 8 oz active plus 4 oz Surfactant F per 100 U.S. gallons (Manning and Glickman, 1969). Cylindrocladium saporium and Cylindrocladium floridanum were controlled in azalea by soil drenches of 8 oz a.i./100 U.S. gallons applied at 1 pint/ft^2 (Horst & Hoitink, 1968).

Benomyl has proved in the United States to be particularly active against a wide range of turf diseases. Dollar spot (Sclerotinia homoeocarpa) and brown patch (Corticium fuciforme) were controlled by benomyl applied at 0.25 oz to 2.0 oz a.i. per 1,000 square feet every 2 weeks, (Massie & Cole, 1969). Stripe smut (Ustilago strifinnis) in Kentucky Bluegrass was controlled by benomyl applied once at 12 oz a.i./1,000 ft^2 (Halisky, Funk and Babinski, 1969). Leaf spot (Helminthosporium vagans) was controlled on Kentucky Bluegrass by benomyl applied at 3 oz a.i./100 ft^2 (Cole, Duich, Taylor and Brown, 1969).

In the United Kingdom trials on roses have confirmed the activity of benomyl against powdery mildew and black spot. A rate of 4 oz a.i./100 Imp. gallons, and the need for a double strength of surfactant, is indicated.

Systemic activity in bulbs against Botrytis tulipae has been indicated with benomyl, as has systemic activity in carnations against Verticillium cinerescens. The activity against Cylindrocladium spp. in roses has been confirmed with the use of a soil drench.

Activity against turf diseases at a rate of 3 oz a.i./1,000 ft^2 has been confirmed with regard to the control of Fusarium nivale and Corticium fuciforme.

DISCUSSION

Benomyl has exhibited a very wide spectrum of activity, with a particularly high degree of activity against a wide range of Ascomycetes and Fungi Imperfecti. Some pathogens from the Basidiomycetes have been controlled but no control has been achieved against any pathogens from the Phycomycetes. It is of interest to note that a very wide range of powdery mildews have been effectively controlled, and this is not frequently the case with powdery mildewicides. This broad spectrum of activity imparts the immediate advantage of multiple action. In top fruit, powdery mildew and scab have been effectively controlled by the same programme of sprays, and there are indications of further advantages of storage rot, red spider mite and blossom wilt control. A programme of sprays applied to blackcurrants has controlled both American gooseberry mildew and leaf spot and there are indications of Botrytis control. Soil drenches to cucumber plants have resulted in control of powdery mildew and black root rot; an effect has also been noted on mite infestation. Both powdery mildew and black spot of roses have been controlled by an identical schedule of benomyl.

The systemic activity of benomyl has been demonstrated conclusively in a number of crops. It is apparent that the material is absorbed by root, stem and leaf, and translocated distally. Translocation in a reverse direction has not been demonstrated and hence growth subsequent to foliage application is not protected systemically. The true systemic effect with uptake from the roots has been demonstrated to give significant control of diseases of cucumbers, tomatoes, cereals, potatoes, cotton and some ornamentals. Uptake and translocation has also been indicated from the fleshy stems of cucumbers.

With foliage spray applications the systemic effect is not so marked but does have a profound effect. Foliage is penetrated by the fungicide and hence eradication is achieved by the killing of surface and sub-leaf surface mycelium. This eradicatory effect has been most marked in the control of powdery mildew in apple orchards. Where semi-commercial blocks have been treated, the accumulatory effect of a decrease in disease inoculum has resulted in an improved control as compared with standard non-systemic mildewicides.

The possibility of reduced schedule spraying has been investigated with benomyl and it has been found that there are distinct possibilities for lengthening the schedule when one considers pathogens that have a fairly long life history. It must be remembered that growth subsequent to application is not protected. It has been found that the control of a disease such as powdery mildew with a short life-cycle does not respond nearly so well to extended schedule spraying as does the control of apple scab which has a longer life-cycle. Further diseases, such as blackcurrant leaf spot and celery leaf spot, are being investigated for the possibility of extended schedule spraying.

Where final crop yield is more important than crop quality, and economic pressures indicate limited application, as is the case with cereals, the use of a systemic fungicide could be significant. Single applications of benomyl to cereals have delayed the development of powdery mildew epidemics sufficiently to produce an increase in yield. Insufficient information on cereal mildew is available at present to determine the exact timing of such a spray, but 1969 data indicates a spray late in the growth season.

Benomyl is the first systemic fungicide to be used really extensively on a wide range of crop plants and against a wide range of plant pathogens in the United Kingdom. The use of such a material is resulting in fresh thinking on the methods of application of fungicides and on the potential for the control of diseases that have not formerly been controlled by the use of fungicides.

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CONTROL OF POTATO TUBER DISEASES WITH SYSTEMIC FUNGICIDES

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Summary Fungus and bacterial diseases have increasingly become causes for complaint about the health of potato seed tubers. The pathogens are not effectively controlled by contemporary fungicides. Tubers formed by rooted stem cuttings are usually initially healthy but fungicides are required to maintain their health through the years of multiplication to commercial use.

Fungicides are easiest to apply to dormant tubers and should be active in store and during growth of the crop against the widest possible range of pathogens. Two benzimidazoles were selected as the most promising materials for further testing.

INTRODUCTION

At the last of these Conferences we summarised evidence on the prevalence and importance of fungus diseases of potato tubers (Hide, 1967; Hirst, 1967). Since 1967 we have concentrated on possible methods of control, including systemic fungicides.

Fungus and bacterial pathogens are now the subject of most complaints about the health of seed potatoes (Hay, 1969). The most notorious have been skin spot (Oospora pustulans) and gangrene (Phoma exigua), but silver scurf (Helminthosporium solani), black scurf (Rhizoctonia solani), Verticillum wilt (Verticillum dahliae), powdery scab (Spongospora subterranea) among the fungi, the actinomycete Streptomyces scabies that causes common scab, and the complex of blackleg/soft rot bacteria are all troublesome. It is improbable that any single control measure will be effective against so many pathogens, ranging from soil inhabitants to others transmitted only by the seed tubers, but it is obviously an advantage to have one effective against as many as possible. Control of a wide range of pathogens is characteristic of chemical sterilants and physical methods such as heat treatment. However, although hot water or hot air can kill many fungi on potato tubers, there is too little tolerance between effective control and severe damage to the tubers. Further, disinfection of seed tubers, either physical or with chemicals such as the organo-mercurial fungicides, seldom completely eradicates the pathogens; it may also be inconvenient and hazardous to use and is ineffective during crop growth against surviving inoculum, whether derived from the tubers or the soil. By contrast, systemic materials able to penetrate stored seed tubers and survive at least the early part of growth, should combat diseases during storage and attack from soil-borne inocula during the growth of the crop.

The best commercial seed stocks now carry much more potential inoculum of the pathogens that cause such diseases as skin spot, gangrene, silver scurf and blackleg, than are ever likely to be encountered in the soils of fields where potatoes are grown only once in 4 to 7 years. Hence, such diseases are effectively tuber-borne; other diseases, such as black scurf, common and powdery scab and verticillium wilt may as often, or even more often, arise from soil-borne inoculum as that carried on tubers. Increasing the amount of inoculum usually makes control more difficult, requires more fungicide and raises problems of cost and residues. Because organo-mercurial fungicides did not eradicate skin spot from commercial seed stocks, we suggested an initial cleaning by propagating from rooted stem cuttings (a process now

practised by the Department of Agriculture and Fisheries for Scotland). Our experience proved two things, first, that healthy stocks acquired most pathogens from soils that had previously grown potatoes, and second, that the organo-mercurials delayed this process but never prevented it.

Present needs can be summarised as follows:-

1. To restore the confidence of ware growers in seed-potato suppliers, the prevalence of fungus and bacterial diseases in tubers must be decreased.

2. Different pathogens reside in different sources, but the soil becomes relatively a much more important source when healthy seed is used.

3. Present fungicides are only partially or temporarily effective, and there is no effective bactericide.

4. So long as any infection remains on seed, pathogens need to be killed not only on stored tubers but also when they attack growing plants - disinfection is not enough.

5. Effective materials must control many pathogens, must have little toxicity to plants or mammals and must be easily applied at a convenient date.

RESULTS AND DISCUSSION

Alternative fungicides were first tested in 1967 but 1968 was the first year when systemic fungicides were included. They were applied to infected King Edward tubers, as dusts (active ingredients diluted with kaolin and the mixtures applied at 10 lb/ton of tubers) or dips (1 min. for organo-mercurials, 5 min. for all other materials).

Seed treated, immediately after lifting, with the chlorinated phenols (Nardisol and Aretanol) and one organo-mercurial (Agallol), yielded 1-2 ton/acre less than untreated seed. One benzimidazole (Thiabendazole), in its best formulation, significantly increased yield by 2 tons/acre. Most treatments increased the proportion of seed-sized tubers. Mechanical damage to sprouts during dusting of well-chitted seed immediately before planting delayed emergence and on average decreased yield by 2 tons per acre. Despite this, tubers dusted with another benzimidazole (Benomyl) at planting yielded as much as untreated, undamaged seed.

Disinfectant materials controlled O.pustulans well on tubers during storage, but by July the fungus was well established on the buds of growing tubers and, after lifting crops from treated seed, only Thiabendazole and Benomyl had still decreased its incidence considerably. These systemic materials also decreased R.solani and H.solani. The oxathiins (Vitavax and Plantvax) were, as expected, more active against the basidiomycete R.solani, which was also controlled well by F849 and the chlorinated phenols. Several materials decreased the incidence of H.solani but Benomyl, Thiabendazole and Trametan were most effective. (Table 1)

Seed used in the 1968 experiment had too little gangrene to test the value of the fungicides against Phoma exigua. The treatments did not compare rates and times of applying Thiabendazole and Benomyl, the materials that seemed active against most fungi and were further tested in 1969.

Because the 1968 field experiment failed to provide any test of activity against gangrene, two small experiments were made on stored tubers. Table 2 shows the percentage of tubers of four varieties, all the produce of gangrene infected seed, that developed gangrene during 3 months' storage at 5°C after they were washed, treated, dried and each given four uniform crush wounds in December.

Table 1.

Chemicals and tuber diseases: Rothamsted, 1968

	Disease incidence (% eye-plugs infected[1])					Yield (total, ton/acre)
	Preplanting[2] (March)	In growth[2] (July)	After lifting (October)			
	Oospora	Oospora	Oospora	Rhiz.	Helmin.[3]	
Autumn Treated			± 6.8	± 5.2	± 0.59	
Untreated	48	56	78	28	37	18.5
Agallol (dip,0.5%)	0	34	54	35	17	16.9
Aardisan (dip,0.5%)	0	44	62	15	11	19.3
Aardisol (dip,2%)	1	46	58	4	22	17.3
Aretanol (dip,2%)	3	40	66	1	24	16.7
Trametan (dust,50%)	13	39	66	10	5	19.8
Polyram (dust,7%)	46	42	69	24	11	20.0
Thiabendazole (dip,0.1% lactate)	1	13	36	15	9	20.6
Thiabendazole (dip,0.01% lactate)	1	41	57	24	24	20.2
Thiabendazole[4] (dip,0.1% wettable)	4	22	35	33	8	18.3
Vitavax (dip,0.2%)	20	54	75	4	41	18.6
Plantvax (dip,0.2%)	35	46	68	16	31	19.6
Spring Treated						
Vitavax (dust,10%)	-	32	67	7	20	16.2
Plantvax (dust,10%)	-	39	65	4	20	14.6
F.849 (Uniroyal) (dust,10%)	-	38	52	0	23	16.1
Benomyl (dust,10%)	-	3	14	4	0	18.4

1 see Hide, Hirst and Salt (1968)
2 March 26, July 22.
3 Oospora pustulans, Rhizoctonia solani, Helminthosporium solani.
4 A veterinary formulation (Thibenzole) containing < 10 ppm cobalt

Table 2.

Fungicides and gangrene on stored tubers

	% tubers with gangrene			
	King Edward	Red Craigs Royal	Pentland Crown	Majestic
Unwashed[1]	90	74	92	74
Washed only	74	72	56	56
Hypochlorite (dip, 2.5%, 3 min.)	18	17	6	13
Agallol (dip, 0.5%, 45 sec.)	0	2	0	5
Thiabendazole (lactate) (dip, 0.1%, 5 min.)	2	9	0	2
Benomyl (dip, 0.1%, 5 min.)	25	16	6	13

1 Each treatment of 3 replicates of 20 tubers/variety

Table 3.

Gangrene infection and eye damage on stored tubers (April)

	King Edward		Red Craigs Royal	
	% Gangrene	% < 3 live eyes	% Gangrene	% < 3 live eyes
Untreated[1]	45	5	11	10
Agallol (dip, 0.5%, 45 sec.)	8	5	13	20
Thiabendazole (lactate)				
(dip,[2] 0.1%)	0	3	0	3
(dip,[2] 1.0%)	0	29	0	80
(dust,[3] 1.0%)	8	10	5	10
(dust,[3] 10.0%)	0	10	3	8
Benomyl				
(dip,[2] 0.1%)	15	3	8	0
(dip,[2] 1.0%)	0	8	3	5
(dust,[3] 1.0%)	13	3	5	15
(dust,[3] 10.0%)	10	10	0	8

1 Each treatment 3 replicates of 20 tubers/variety
2 5 min. dip
3 Active ingredient diluted with Kaolin and applied at the rate of 10 lb/ton (average 6.7g retained/20 tuber sample)

Table 3 shows the percentage of unwashed tubers that developed gangrene after each received four crush wounds, four months after lifting. Fungicides were applied later the same day and after drying, the tubers were stored at 5°C for 12 weeks for gangrene lesions to develop. The King Edward tubers were the produce of seed with gangrene lesions, but the seed for the Red Craigs Royal were not visibly infected. Both varieties suffered some eye damage but most with Red Craigs Royal and Thiabendazole as a 1.0% dip. The figures in Table 3 are the percentage of tubers with no more than 2 live eyes in April.

Benomyl is also active against Verticillium albo-atrum on cotton (Erwin, Mee and Sims, 1968) and in our experiments on stored potatoes prevented verticillate conidiophores developing (probably Verticillium tricorpus) on tissues of dormant tubers. To test its activity during growth, King Edward plants were grown in pots with soil and chopped haulm from a crop infected with Verticillium dahliae. Seed tubers were dusted with Benomyl (10% dust at 10 lb/ton of tubers) before planting, or the material was applied as a soil drench (100 ml of 80 ppm a.i./5 in. pot) after planting. Both treatments prevented the blackening of haulm by the microsclerotia of V.dahliae that formed profusely on untreated plants. Benomyl as a drench 3 or 7 weeks after planting also prevented infection. However, in 1969, dusting tubers before planting did not prevent infection of plants in a field naturally infested with V.dahliae, although it slightly delayed death of the haulm.

The results of these experiments and observations on samples from the 1969 experiments suggest that systemic fungicides may prove useful in decreasing the incidence of several tuber-borne fungus pathogens, although evidence is scanty, especially for the control of gangrene. We intended seed for the 1968 experiment should carry much inoculum of most diseases so that the fungicides would be tested in difficult conditions. Later experiments are testing different amounts of fungicide and using cleaner stocks, so control may be more effective or be effective with less fungicide.

To be fully effective, organo-mercurial fungicides should be used within a week of lifting tubers (Edie & Boyd, 1966), which is very inconvenient for farmers. Treating chitted tubers just before planting damages sprouts and cannot control diseases during storage. We are testing treatments applied while tubers are dormant, preferably as part of the seed-dressing procedure. The efficacy and possibilities of damage by applying materials to tubers as sprays, dust or dips at various times is to be compared on a range of varieties, and the compatibility of these treatments with others, such as control of nematodes and bacteria or inspections will be investigated. These problems must be solved and the lack of harmful residues demonstrated before the materials could be approved for practical use.

However, should we find ideal fungicides and produce potatoes devoid of pathogens, we may still be far from an agricultural success. It seems that tuber formation is at present partly restricted by root pathogens, for improving health increases the number of tubers/plant and usually decreases their size. With some varieties, e.g. Pentland Dell, this may be an advantage and often it does not matter, but varieties such as King Edward may produce up to 60 tubers per plant from healthy seed, most of a size useful only for canning or seed. Unless we can select other clones, or modify shoot number or plant spacing to change the tuber size, our major achievement may be to show that diseases can sometimes be beneficial !

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BENOMYL APPLIED TO SOIL FOR THE CONTROL OF SOME PATHOGENS OF TOMATO AND CUCUMBER

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Summary Benomyl applied at four rates as a soil drench, to tomato plants potted after roots were artificially inoculated with Verticillium albo-atrum, controlled aerial wilt symptoms. Slight marginal chlorosis occurred on leaves of plants treated with 0.4 g and 0.2 g a.i. per plant, and yields averaged 79% and 83% of untreated healthy controls, compared to 25% from untreated inoculated plants. At rates 0.1 g and 0.05 g a.i. per plant, leaf chlorosis was negligible and yields were 89% and 92% respectively of healthy controls. V. albo-atrum could not be isolated from stems cut down 16 weeks after treatment with benomyl at 0.4 g and 0.2 g a.i., but slight xylem browning was seen and the fungus was isolated from some plants treated at 0.1 g and 0.05 g a.i. per plant.

Cucumbers planted into manure beds and straw bales on plots infested and non-infested with Phomopsis sclerotioides the causal pathogen of black root rot, and treated with drenches of benomyl 0.1 g a.i. per plant at planting and seven weeks later, resisted attack by powdery mildew, Sphaerotheca fuliginea. Untreated plants in the same plots became heavily infected, and yields were decreased. Root rot was largely checked when benomyl was applied to plants in infested plots, yields being 91% of benomyl treated plants in non-infested plots. Where no benomyl was applied yields, considerably decreased by the effects of powdery mildew alone, decreased even more in plots where root rotting also occurred.

The direct application of benomyl to soil as a means of controlling plant pathogens has been described in the U.S.A. with reference to cotton wilt caused by Verticillium albo-atrum (Erwin, D.C. et al. 1968), cucumber powdery mildew caused by Sphaerotheca fuliginea (Schroeder & Provvidenti, 1968) and seedling diseases of cotton and pea (Al-Beldawi & Pinckard, 1968; Harper, 1968). The success in controlling root infecting wilt pathogens or leaf infecting powdery mildew has been mainly in pot experiments on young plants and little information is available on cropping plants. The use of benomyl in protecting seedlings from attack by, for example, Rhizoctonia, may be crucial in establishing a direct sown field crop, but in the glasshouse industry in the U.K. interest in a new fungicide is likely to be centred on its use for disease control on mature cropping plants. In these trials benomyl was used on pot grown tomatoes artificially inoculated with V. albo-atrum and grown on for a 6-8 week crop, and on cucumbers growing on traditional manure beds or straw bales within an experiment on the control of black root rot on a naturally infested soil.

In a series of three pot experiments (see Table 1) benomyl was applied at different rates as a soil drench in 0.5 l water per pot to 5-week-old tomato plants immediately after these had been inoculated by dipping root balls into a mycelial and spore suspension of V. albo-atrum and repotted into 10 inch pots (9 l) of steam-

sterilised compost. Two sets of untreated control plants were used, Verticillium-inoculated plants, and healthy non-inoculated plants; replicates of the latter were also treated with benomyl in the absence of wilt fungus. Wilt symptoms, flagging or leaf yellowing, developed from 12-21 days after potting, at which stage benomyl was applied to half of the replicates of the wilting control plants, to see whether symptoms could be arrested or controlled at this stage.

Table 1

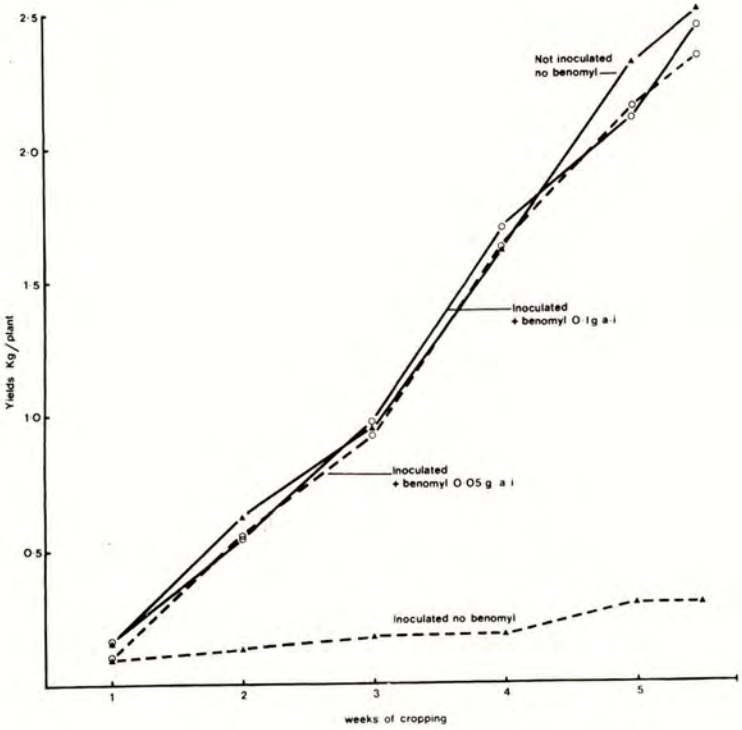
Details of pot experiments on control of wilt (V. albo-atrum) in tomatoes using benomyl drenches

	Inoculated with Verticillium	Benomyl g a.i./ plant	Wilt symptoms	Final yield as % of control	Max.height xylem browning 16 wks.after potting	Verti-cillium isolated
I 12/9 - 31/12/68	+	0.4	-	79	-	-
	+	0.2	-	75	-	-
	+	0.0	✓	45	3 feet	✓
	-	0.0	-	100	-	-
II 9/10/68 - 28/1/69	+	0.2	-	90	Base	-
	+	0.1	-	81	Base	-
	+	0.0	✓	5	To top	✓
	-	0.0	-	100	-	-
III 14/3 - 1/7/69	+	0.1	-	97	3 feet	✓
	+	0.05	-	93	3 feet	✓
	+	0.0	✓	12	Top	✓
	-	0.1	-	96	-	-
	-	0.05	-	92	-	-
	-	0.0	-	100	-	-

Where benomyl was applied at 0.4 g and 0.2 g a.i./plant some marginal chlorosis appeared on leaves 11 days later, and this occasionally developed a restricted necrotic margin. No differences were seen in growth in height, and plants did not wilt or flag. When main stems were cut down and examined 16 weeks after treatment xylem browning was rarely seen and only at the stem base and the pathogen was not isolated from stem sections. Inoculated untreated plants flagged and wilted and growth was retarded although most recovered sufficiently to survive 16 weeks, when xylem browning extended to the top of the plant and Verticillium was readily isolated from all stem levels examined. Applying benomyl as a soil drench to plants two days after shoot symptoms were first seen appeared to control the fungus and xylem discoloration was confined to the stem base, whereas when applied seven days after shoot symptoms appeared, xylem discoloration was seen and the pathogen was isolated from 18 inches up the stem.

At the lower rates of application, 0.1 and 0.05 g a.i./plant, the slight marginal chlorosis observed did not develop into necrosis. Again no wilting was observed and the final yield from inoculated and treated plants was over 90% of control plants.

Fig. 1 Effect of benomyl drench on yields from potted tomato plants artificially inoculated with Verticillium albo-atrum.



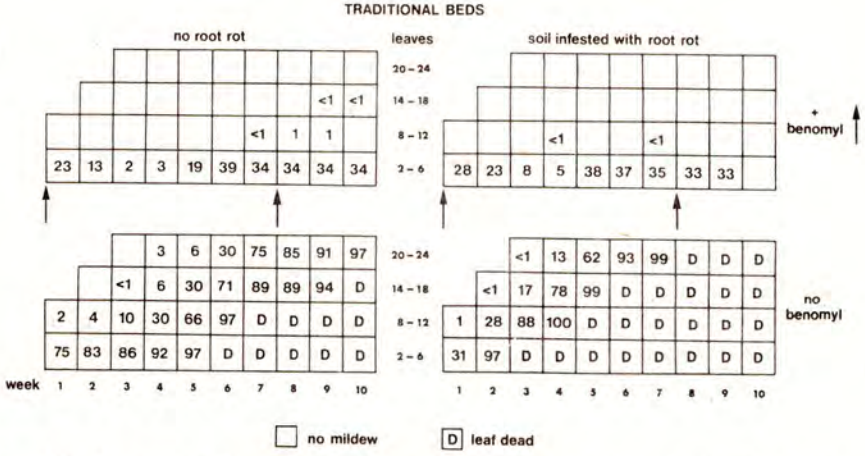
However, 16 weeks after treatment xylem was brown and Verticillium was reisolated from some replicates treated with 0.1 g a.i./plant and from all replicates treated at 0.05 g a.i./plant. Benomyl, applied to half the inoculated control replicates 14 days after potting but before shoot symptoms had appeared, prevented wilting, yields from these plants being 90% of those of uninoculated untreated controls, although again the pathogen was later isolated from stem sections.

In pot experiments, benomyl used as a soil drench may have some fungicidal action in the infested soil around the roots, but when applied to plants already infected and showing wilt symptoms, its effect must be due to preventing further growth of the pathogen within the plant. Although some soil fungicidal action may have occurred at higher rates in these trials, preventing heavy infection, the systemic activity at lower rates was still adequate to control fungal growth internally and so prevent wilt symptoms for a considerable period. In the glasshouse a soil fungicidal effect would be ineffective against Verticillium wilt because of the depths at which the fungus survives and the roots are infected. Where uptake of benomyl by plants

inhibits fungal growth within the aerial parts of the plant, disease control is achieved as with resistant varieties where roots are infected but the fungus does not grow into the stems. The one application of benomyl in these limited trials was effective in the first experiment for at least 7½ weeks, when healthy untreated plants were attacked by leaf mould Cladosporium fulvum, 7 - 20% of the leaf surface of 8 recorded leaves/plant being infected, while benomyl treated plants remained completely free.

An experiment on the control of black root rot of cucumber in a soil naturally infested with Phomopsis sclerotioides using methyl bromide as a soil fumigant was planted up in January 1969 on replicated 4 plant plots of traditional manure beds and straw bales. In March two plants were removed for root samples from all plots in two of the six blocks and these gaps were replanted with pot plants already slightly infected with powdery mildew. A suspension of benomyl (50% w.p.) 0.1 g a.i. per plant in 0.5 l water was watered around the base of plants in half of the replanted sub-plots, the other replanted sub-plots being left untreated. Mildew was recorded on leaves 2, 4, 6 ... 24 as % leaf covered, 10 days after planting and thereafter at weekly intervals. The results are shown in Figure 2 as mean % mildew per three consecutive recorded leaves; leaves which were classed as 'dead' as a result of mildew attack were counted as 100% infested when calculating means. Three weeks after benomyl was applied mildew on the lower 3 recorded leaves had actually decreased, the later increase at this level being almost entirely caused by high levels of mildew on leaf 2. Seven and a half weeks after the benomyl treatment mildew had reappeared above the 6th leaf and a second drench of benomyl 0.1 g a.i. per plant was applied.

Fig. 2 Incidence of powdery mildew on cucumbers, grown on traditional beds, treated with benomyl 0.1g a.i./plant or untreated. (mildew as mean %/3 recorded leaves).

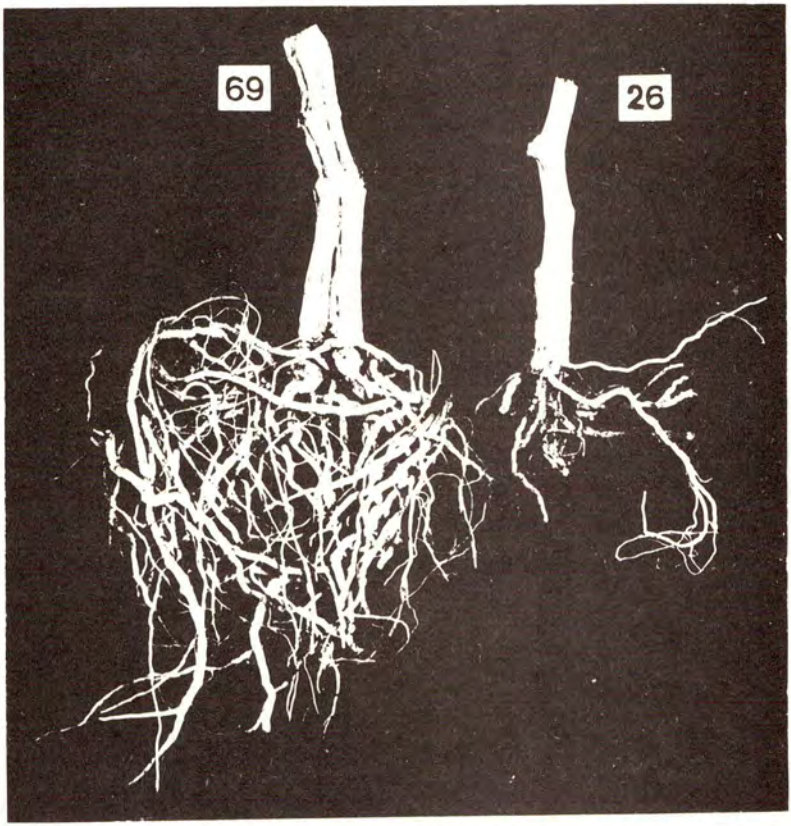


The effectiveness of benomyl in controlling mildew is seen in Figure 2 which shows the mildew levels developing on plants in root rot infested and non-infested soil in traditional beds. However, there was some indication that the second drench applied to c.8 week old plants was less effective on plants on straw bales than on

those grown in traditional beds, and it seems possible that this may reflect the amount of benomyl taken up by the plants. When the first drench was applied, the functional roots were confined to the relatively small region of the propagating soil ball, the fungicide would have readily penetrated the plant. When treating older plants, possibly spray treatments or larger quantities of benomyl drench may be required for effective uptake to occur.

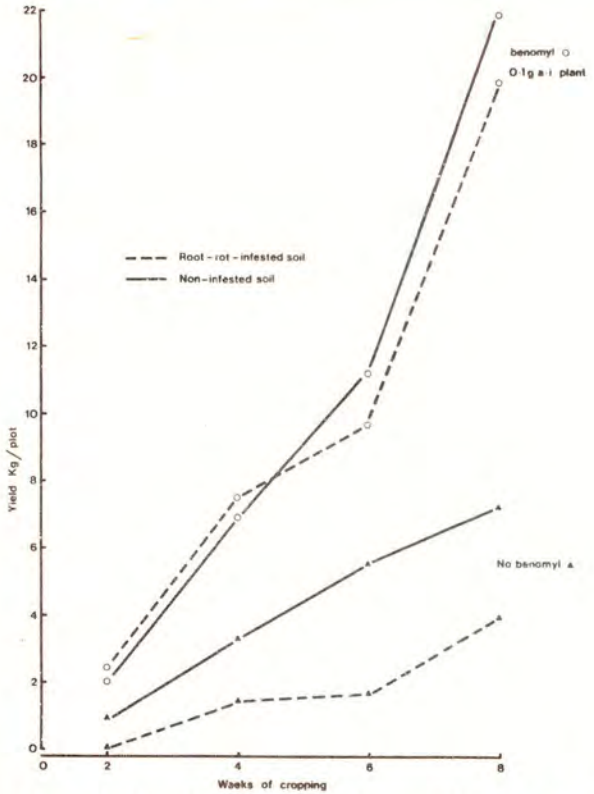
Apart from the outstanding effect in controlling mildew, it was observed that plants treated with benomyl appeared more vigorous than expected on the plots infested with P. sclerotiicidas, and at the end of the crop when roots of all plants were dug and examined, root rot on these treated plants was found to be much decreased.

Fig. 3 Cucumber roots (3 month old plants) from soil infested with black root rot. 69, treated benomyl 0.1g a.i./plant; 26, untreated.



The mean yields from plants on root rot infested and non-infested plots with and without treatment with benomyl are shown in Figure 4.

Fig. 4 Effect of benomyl drench on yields from cucumbers naturally infected with powdery mildew (S. fuliginea) on soils infested, and non-infested, with black root rot (F. sclerotioides).



The decrease in final yield between plants treated and not treated with benomyl on fumigated plots was 66% and can be attributed to the effects of mildew. On plots infested with root rot yields from plants treated with benomyl were 91% of those of treated plants in non-infested plots. The low yields from untreated plants in infested plots reflect the combined effects of a root and leaf pathogen, but the evaluation of the part played by the two diseases is complicated by an indication from the mildew data that whereas on straw bales mildew attack was approximately the same on plants not treated with benomyl, whether growing on root-rot-infested or non-infested plots, the decrease in yield attributable to root rot being 28%, on traditional beds mildew on plants not treated with benomyl was more severe in infested plots, the decrease in yield attributable to root rot and increased mildew attack being 66%. Further trials are in progress in which mildew is being controlled by Morestan sprays on all plants with and without benomyl drenches which should give more information on the efficacy of the fungicide in preventing root rot.

Phomopsis sclerotioides spreads relatively quickly on a cucumber root system and the appearance of roots from infested soil suggests that either soil fungicidal activity persists for some time after application or that the roots which actually absorb the benomyl remain resistant to fungal attack although this systemic effect does not pass into roots formed later.

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FATE OF CARBOXIN IN SOIL, PLANTS AND ANIMALS

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Summary The systemic fungicide carboxin (D735) was found to be oxidized to its sulfoxide (F831) in water, soil, plants (barley, wheat, cotton) and animals (dogs), but hydrolysis has not been detected. Further oxidation to the sulfone (F461) was not found in plants or animals. No residue was present in wheat, barley or cotton seed harvested from ^{14}C treated seed. Dogs did not accumulate D735 in their bodies. Excretion in the feces and urine occurred as both D735 and F831.

INTRODUCTION

In a systematic search for systemic fungicides, using a bean rust bioassay, the systemicity of carboxin was reported by von Schmeling "et al" (1966). Its main use is seed treatment of grains, cotton and peanuts. Carboxin proved to be particularly effective against basidiomycete type fungi, Edgington "et al" (1966), and showed unique activity against the systemic loose smut disease of barley and wheat (Ustilago nuda and U. tritici, respectively), von Schmeling "et al" (1966), Hansing (1967), Kiesling (1966). A study of its fate in soils, plants and animals was made to allow its safe use on food crops.

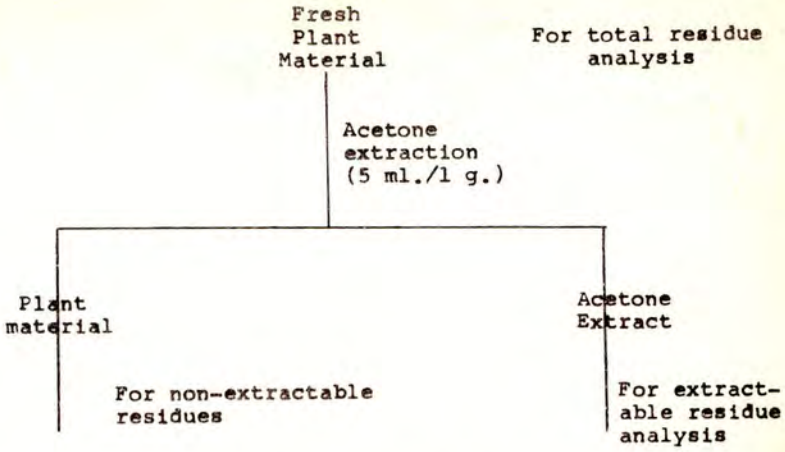
METHOD AND MATERIALS

Thin layer chromatographic techniques were developed to characterize the following materials: carboxin (D735) 5,6-Dihydro-2-methyl-1,4 oxathiin-3-carboxanilide; F831 3,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide-4-oxide; oxycarboxin (F461) 5,6-dihydro-2-methyl-1,4 oxathiin-3-carboxanilide-4,4-dioxide.

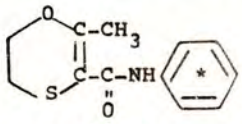
The chemicals were subjected to buffer solutions of pH 2, 4, 6, 8 and 10 and stored at room temperature to check their stability to hydrolysis. Extractions with $CHCl_3$ were made at different time intervals and analyzed by TLC.

A soil-sand mixture with 20 ppm D735 was prepared to measure chemical stability using TLC as the chemical assay tool and bean rust (Uromyces phaseoli) for bioassay. Bean seeds were planted in this soil in time intervals and the inoculation with rust spores was kept constant at 10 days after planting.

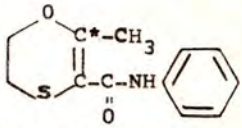
Barley seed treatments at 4 ounces D735-75 W.P./100 lbs seed were planted in the greenhouse. The subsequent top growth was analyzed colorimetrically 1, 3, 5 and 7 weeks after planting. Samples of plant material were analyzed directly for total residues. Acetone extractable residues and unextractable residues were determined separately by the same method, Lane (1965). The overall scheme for the residue analysis can be summarized in the following diagram:



In radiotracer studies wheat, barley and cotton were treated with ^{14}C carboxin at rates equivalent to field treatment. Plants were grown to maturity under greenhouse and field conditions. Two ^{14}C tagged Vitavax samples were prepared with labeling as shown



carboxin (Uniform Aniline Ring Tag)



carboxin (Hetero Tag)

The translocation pattern was determined by radioautograms of plants at 2, 4 and 8 weeks. Quantitative data on ^{14}C in plant parts was obtained by wet oxidation and scintillation counting, (Mahin, 1966). The sensitivity of this method is 0.5 ppm per mg which is equivalent to 0.05 ppm at the specific activity used (1 mc/mM).

The fate of D735 in animals was studied by analyzing organs and excreta from dogs used in a two year feeding study (feeding rates 100 and 600 ppm in the total diet). The analyses were made by the colorimetric and TLC techniques previously described.

RESULTS

The observations on stability to hydrolysis were made over a period of 27 weeks. It was found that D735 was quite stable at pH's 6, 8 and 10. At the pH 2 D735 was oxidized mainly to F831 which was detectable beginning at the four week time interval. A trace of F461 was detected after ten weeks at pH2. No hydrolysis products were found. The results are summarized in Table I.

Table 1. pH influence on D735 in water

Weeks	pH Range	Composition Change
1	2-10	None
4	2	Trace F831
	4-10	None
10	2	F831, trace F461
	4-10	None
27	4	Trace F831-F461
	6-8	Trace F831
	10	None

The fate of D735 in soil was studied by TLC and control of bean rust as shown in Table 2.

Table 2. Fate of D735 in greenhouse soil

Planting No. Days After Soil Treatment	% Bean Rust Control	% D735 Left by TLC*	% F831 Produced by TLC*
0	99	100	0
7	40	50	50
14	0	0	100

*Estimates

In a separate experiment oxidation of D735 to F831 in soil was shown to take place under sterilized conditions.

Interesting results were obtained in following the fate of D735 in barley plants. The data showed that the ppm residues decreased rapidly, particularly after the third week. The results are summarized in Table 3.

Table 3. Residues in barley plants

Sampling Weeks	Total Residue PPM	% Acetone Extractable	% Unextractable
1	20	98	10.0
3	13	85	7.3
5	7.9	76	8.3
7	2.9	73	23.0

The extractable residues decreased with time due to conversion to a non-extractable form.

The identification of the extractable residues was made by microcolorimetric gas chromatography (MCGC) and by TLC. The results of this analysis are shown in Table 4.

Table 4. Extractable residues and their identification

		PPM Residues after Weeks				
Residue Type	Method	1	2	4	6	Mature*
Total Extractable	Color	28.3	17.3	6.3	1.3	0.0
D735	MCGC	3.4	1.2	0.3	0.0	0.0
F831	TLC	19.0	15.0	6.6	0.5	0.0

*15 weeks

It was interesting to note that even after the first week there was considerably less D735 (sulfide) than F831 (sulfoxide). No D735 at all could be detected six weeks after emergence. The disappearance of D735 could be due to its conversion either to F831 or to a non-extractable form.

Attempts were made to identify the non-extractable residue. Indications obtained in the experiments strongly point toward the possibility that F831 is bound to lignin by complex formation. This is believed to be the possible mechanism of detoxification through immobilization.

As a backup of the residue analysis work previously described, the fate of D735 was also studied using ^{14}C tagged chemical. Two types of radiolabelled D735 were studied: aniline tagged and heterocyclic ring tagged. This work allowed an insight into the translocation pattern of D735 and the ^{14}C distribution in plants grown from treated seed.

It was found that the translocation pattern of ^{14}C D735 is similar in wheat, barley and cotton for both ^{14}C tagged positions. The heaviest concentration is in the lower part of the stem and the first leaves, as well as along the vascular bundles. The radioactivity moves with the transpiration stream to the tips of the first leaves. No redistribution of ^{14}C to the newest growth or roots was observed. The translocation pattern also appears to confirm previous work showing that the molecule travels intact as there was no difference between the two tagged positions. None of the harvested seed of wheat, barley or cotton showed any ^{14}C residues at the sensitivity of the method of 0.05 ppm. No evidence of any metabolites derived from the oxathiin moiety could be found in the seed.

The fate of D735 in dogs

Organs and excreta from twelve of the dogs (and from additional control animals) used in the chronic feeding studies were obtained for this work. Six animals (3 males and 3 females) each from the groups fed 100 ppm and 600 ppm D735 in their total diets for two years were involved. The results are summarized in Table 5.

Table 5. Analysis of tissue and excreta from D735 treated dogs after two-year feeding trials.

Material Analysed	Total Residue in PPM		PPM TLC Detection of	
	600*	100*	D735	F831
Fat	N.D.**	N.D.	--	--
Muscle	N.D.	N.D.	--	--
Kidney	Trace	N.D.	--	--
Liver	5.0	1.0	1.7	0.9
Urine	10	---	1.3	2.2
Feces	26	---	15.3	9.6

*PPM D735 fed in daily diet

**Not detectable

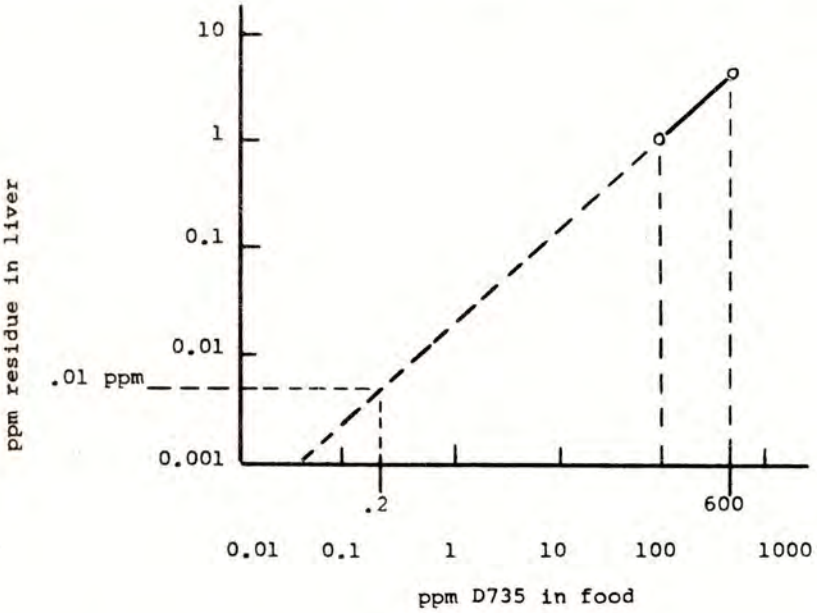
The data in Table 5 show the largest residue to occur in the feces with smaller amounts in the urine and liver. No significant residues occurred in the fat, muscle or kidney.

In the feces the residues were both D735 and F831, indicating that D735 is oxidized in the digestive tract even before being absorbed.

The higher ratio of F831 to D735 in the urine compared to the feces indicates that further oxidation of D735 to F831 took place after absorption.

The liver was the only organ where significant residues were detected. However, considering the small quantities found at the two feeding levels, it is seen (Fig. 1) that virtually no residue would be expected if the dosage is reduced to a negligible tolerance level of 0.2 ppm.

Fig. 1. Relation Between D735 in food and residues in liver



DISCUSSION

The experiments with D735 in water, soil, plants and animals have shown that the main chemical change is oxidation to the sulfoxide. In water the oxidation rate is retarded by higher pH with no chemical change occurring at pH 10. Hydrolysis was not detected at any pH. The oxathiin D735 is translocated in plants intact to the site of the pathogen. The translocation system was found to be the xylem, as indicated with radiotracer studies. Virtually no phloem (downward) translocation was observed.

Interesting results were obtained, coupling bioassay (bean rust control) and TLC analysis in D735 soil treatments. Similar results were obtained with D735 seed treatments and subsequent bean rust control, as reported by von Schmeling "et al" (1966). The relatively rapid loss of activity through oxidation of D735 to the sulfoxide F831, not only in water and soil, but also in the plant, was confirmed by bioassay. The value of D735 as a chemical control is therefore greater as a therapeutant than as a protectant. Oxycarboxin (F461), the sulfone, on the other hand, is considerably more stable

and therefore a more effective protectant than D735, the sulfide. The fungicidal activity of the sulfoxide F831 *in vitro* was found to be at least 5000 times lower than D735 against wheat leaf rust (unpublished data). This explains the loss of activity of D735 through oxidation to F831. Of interest also is the fact that the further oxidation step from the sulfoxide to the sulfone does not take place under the biological conditions tested. Apparently the energy requirement for this oxidation is not available under natural conditions in either soil, plants or animals.

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SEED TREATMENTS WITH CARBOXIN FOR THE CONTROL OF LOOSE SMUT OF WHEAT AND BARLEY

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Summary Field trials of carboxin seed dressing and seed soak treatments of barley and wheat seed infected with loose smut were carried out during 1967 - 1969. Seed dressings with 'Vitavax' and 'Murganic R.P.B.' eliminated Ustilago nuda from barley without adversely affecting germination and yield. Ustilago tritici on winter wheat was not completely controlled by seed dressings but was eradicated by soaking infected seeds in 0.2% aqueous 'Vitavax' for 6 h at 30°C.

INTRODUCTION

In 1966 Von Schmeling and Kulka reported on the systemic activity of a new fungicide, carboxin, (5,6 - dihydro - 2 - methyl - 1,4 - oxathiin - 3 - carboxanilide) and demonstrated that surface dressings of infected barley seeds with the compound gave control of infection caused by Ustilago nuda. Similar results have since been obtained by other workers (Reinbergs, E. et al 1966, Moseman, 1968). In the course of experiments by the authors, during 1967, on the effect of the thiram soak method of seed treatment upon the control of loose smut of spring barley a preliminary trial of carboxin seed treatments was made (Maude and Shuring, 1968). As a high degree of disease control was obtained with carboxin seed dressings and seed soak treatments, further tests were carried out against loose smut of both wheat and barley in 1968 (Maude and Shuring, 1969) and in 1969. The results of this work are reported here.

MATERIALS AND METHODS

Three stocks of diseased seed were used (1) a hybrid barley with 3.8% embryo infection (1967 only) (2) Impala spring barley - 0.75% infected (1968 & 1969) and (3) Capelle Desprez winter wheat - 0.75% infected (1968).

In the 1967 experiment, 6000 seeds per treatment were sown in four field plots of 1500 seeds each. In all other trials 5 lb of seed per treatment was drilled into four randomised field plots, each 52½ x 6 ft, with an Oyjord 10-row batch seeding drill. Emergence counts were made on two occasions on random one foot lengths from each of the ten rows in a plot. The numbers of smutted plants were counted twice and the crops were combine harvested, when mature, to determine yields.

Two commercial formulations of carboxin were mainly used (a) 10% 'Vitavax' dust and (b) 75% 'Vitavax' wettable powder. (a) and (b) were used as seed dressings and (b) was also used as a seed soak. 'Murganic R.P.B.', a mixture of 55% W/W carboxin with an organo-mercury compound, was used as a seed dressing in one field trial.

Seed dressings were applied to 5 lb amounts of wheat and barley seeds in a hand rotated drum. Seeds were soak treated with 'Vitavax' by placing them in a 0.2% suspension of the fungicide (75% W.P.) which was circulated at 30°C for the appropriate length of time. Afterwards the seeds were dried back to their original weight in an airstream at 25°C. Seeds were also soaked in 0.2% suspensions of thiram for 24 h at 30°C, and hot water treatment of grain was carried out at 42.7°C for 2 h.

FIELD TRIAL - 1967

Application of carboxin to spring barley

Four seed treatments were applied namely, 'Vitavax' soak (0.2% a.i. for 24 h at 30°C), 10% 'Vitavax' dust (applied to excess), hot water (42.7°C for 2 h) and

'Ceresan' dust (1 oz/28 lb seed). Approximately 6000 seeds per treatment were sown on 12 May. Germination counts were made on 2 and 9 June from which the number of plants per plot was estimated and the percentage emergence calculated. Smut counts were made on 6 and 11 July (table 1).

Table 1.

The effect of various seed treatments upon plant emergence and control of loose smut

Seed treatment	Estimated No. plants per treatment	% emergence	Smutted plants No.	Smutted plants %
Thiram soak	5419	90.3	0	0
'Vitavax' soak	4488	74.7	0	0
Hot water	5181	86.4	3	0.06
'Vitavax' dust	5625	93.8	8	0.14
'Ceresan' dust	5613	93.5	186	3.31
Nil	5625	93.8	194	3.44

Both carboxin treatments had a considerable effect on disease, the 'Vitavax' soak giving 100% control of loose smut and 'Vitavax' dust giving disease reduction almost equivalent to that achieved by hot water. Some reduction of emergence, however, occurred where seeds had been soaked for 24 h in 'Vitavax' suspension.

FIELD TRIALS - 1968

In these trials to reduce the chances of germination depression the duration of the 'Vitavax' soak was reduced to periods of 1 and 6 h, and both 10% and 75% 'Vitavax' dressings were used at definite rates i.e. 4 or 8 oz/100 lb seed. These and other treatments were applied to spring barley and winter wheat seeds infected with U. nuda and U. tritici respectively.

Application of carboxin to winter wheat

The seed was sown on 31 January, each treatment being applied to 5 lb seed (approx. 55000 seeds). The dusted seed was sown last to avoid contamination of the drill. Seedling emergence was recorded on 22 March and 5 April; the numbers of smutted plants were counted on 26 June and 1 July and the plots were combine harvested on 10 September to determine yields. The results obtained are given in table 2.

Table 2.

Effect of 'Vitavax' treatments of winter wheat on loose smut incidence and plant performance

Seed treatment	Mean No. plants emerged per foot of row	Total No. smutted plants /55,000 seeds	Total yield in kg
75% 'Vitavax' (4 oz/100 lb)	21.98	15	37.28
75% 'Vitavax' (8 oz/100 lb)	23.68	8	35.90
10% 'Vitavax' (4 oz/100 lb)	22.28	66	34.85
10% 'Vitavax' (8 oz/100 lb)	24.98	72	35.62
0.2% 'Vitavax' soak (1 h at 30°C)	22.30	23	35.37
0.2% 'Vitavax' soak (6 h at 30°C)	21.17	0	31.27
Nil	21.65	250	34.39
L.S.D. (P = 0.05)	3.66	-	5.12

The 6 h soak in 'Vitavax' (0.2% at 30°C) completely eliminated loose smut from wheat embryos without adversely affecting emergence. There was a slight,but not significant, reduction in grain yield with this treatment. No other carboxin treatment was as effective although 75% 'Vitavax' applied to seeds at the 8 oz rate gave considerable disease reduction.

Application of carboxin to spring barley

Apart from the addition of two further treatments (a seed soak in 0.2% thiram for 24 h at 30°C and treatment of seeds in water at 42.7°C for 2 h) the trial was carried out in the same way as the wheat trial. The seeds were drilled on 15 March; emergence counts were made on 8 and 26 April and the numbers of smutted plants were recorded on 17 June and 1 July. The experiment was combine harvested on 27 August and yields were determined (table 3).

Table 3.

Effect of carboxin, thiram soak and hot water treatments of spring barley on loose smut incidence and plant performance

Seed treatment	Mean No. plants emerged per foot of row	Total No. smutted plants /55,000 seeds	Total yield in kg
75% 'Vitavax' (4 oz/100 lb seed)	21.65	0	45.24
75% 'Vitavax' (8 oz/100 lb seed)	21.38	0	39.21
10% 'Vitavax' (4 oz/100 lb seed)	21.12	3	44.16
10% 'Vitavax' (8 oz/100 lb seed)	22.23	1	46.38
0.2% 'Vitavax' (1 h at 30°C)	23.08	0	40.36
0.2% 'Vitavax' (6 h at 30°C)	21.02	0	40.01
0.2% thiram (24 h at 30°C)	21.45	0	43.74
Hot water (42.7°C for 2 h)	22.35	1	43.78
Nil	23.96	293	42.74
L.S.D. (P = 0.05)	2.74	-	4.05

All treatments with the exception of the two 10% 'Vitavax' seed dressings and hot water gave complete elimination of the disease. Some treatments caused a slight depression of emergence but none significantly affected grain yields.

Although embryo infection levels were similar in both wheat and barley it is apparent that carboxin seed dressings and soaks much more readily controlled U. nuda than U. tritici. Similar results have been reported in America (Jones and Barnett, 1968). The reasons for this are not certain but the following possibilities exist (1) that wheat seed tissues were less readily penetrated than those of barley seeds (2) that U. tritici mycelium in the embryo was more resistant to treatment than U. nuda.

FIELD TRIALS - 1969

Application of carboxin to spring barley

Since both of the 75% 'Vitavax' seed dressing application rates gave control of U. nuda on spring barley it was considered that lower doses might be effective. 5 lb weights of infected seeds were therefore treated with 1, 2 and 4 oz fungicide per 100 lb seed each. The experiment was drilled on 27 March and germination counts (15 April and 24 April), infection assessments (19 June) and yield records were made (table 4).

Table 4.

Effect of carboxin seed dressings of spring barley on loose smut incidence and plant performance

Seed treatment	Percent field emergence	Total plants infected	Yield in kg
75% 'Vitavax' (1 oz/100 lb seed)	66.4	2	74.28
75% 'Vitavax' (2 oz/100 lb seed)	66.1	0	75.90
75% 'Vitavax' (4 oz/100 lb seed)	65.7	0	74.50
Nil	74.1	187	75.08
Nil	72.0	181	76.76
L.S.D. (P = 0.05)	12.2	-	5.32

Thus the 2 oz dressing (i.e. 1 g per bushel) of 75% 'Vitavax' was the lowest application rate at which 100% control of U. nuda on barley was obtained.

In the foregoing experiments (except for 1967) each treatment occupied an area of 1/35 of an acre. To test a commercially available carboxin seed dressing on a farm scale at the 2 oz/100 lb seed rate, ½ bushel of infected barley was dressed with 1 oz of 'Murganic R.P.B.' (55% W/W carboxin). This was drilled on ¼ acre of land with a Nordsten seed drill. A similar area was sown with untreated seeds. Emergence (2 May) infection (7 July) and yield records were made on random plots marked out within the total area. The results are given in table 5.

Table 5.

Effect of commercial carboxin seed dressing on loose smut incidence and plant performance

Seed treatment	Mean No. plants per foot row per plot	Total infected plants/4 plots	Mean yield kg/ plot
'Murganic R.P.B.' (2 oz/bushel)	25.9	0	38.72
Nil	27.0	182	38.12
L.S.D. (P = 0.05)	5.2	-	3.28

A disease survey of the whole area planted with carboxin treated seeds failed to reveal any loose smut infected plants, whereas infection occurred in the area raised from untreated seeds. Treated and untreated seeds gave comparable emergence and yield results.

DISCUSSION

The results of the field trials carried out over the three years 1967-1969 clearly indicate the effectiveness of carboxin seed dressings for the eradication of U. nuda from the embryos of barley grains. In this respect they agree with those of many others (Moseman, 1968) and indicate that carboxin application rates of 1 oz/bushel (75% 'Vitavax') or 2 oz/bushel ('Murganic R.P.B.') may be used for this purpose. Similar results, however, were not obtained against U. tritici on winter wheat seed and complete disease control was only achieved by soaking infected seeds for 6 h in a 0.2% suspension of 'Vitavax' at 30°C. It is presumed that by this method of application the fungicide was made more accessible to the internal seed tissues although it is also possible that the temperature of the soak water (i.e. 30°C) may have had some eradicant effect on the disease.

Although a considerable use for carboxin seed dressings can be envisaged for the treatment of bulk quantities of infected barley seed such treatments would require that the fungicide should be available at an economic price. If the price is too high for the material to be used in this way it might presumably be used for stock seed production.

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PYRIMIDINE FUNGICIDES

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Summary Systemic and fungitoxic properties have recently been discovered in a group of pyrimidines. Certain of these compounds are very specific. Dimethirimol, 5-n-butyl-2-dimethylamino-4-hydroxy-6-methylpyrimidine, is being developed for the control of powdery mildews on cucumbers, melons and some ornamentals and it is being tested against powdery mildew on hops, strawberries, peas and other crops. Ethirimol, 5-n-butyl-2-ethylamino-4-hydroxy-6-methylpyrimidine, is being developed for the control of powdery mildew on cereals.

These compounds are taken up by roots, permeate throughout the plant, eradicate established fungal infections and protect the plant for long periods against subsequent attack. Dimethirimol and ethirimol are loosely adsorbed onto soil particles, movement through the soil is slow and they do not appear to be broken down rapidly in soil. Therefore, the soil functions as a reservoir releasing the active ingredient gradually to protect the crop over a considerable part of its life.

Dimethirimol and ethirimol have very low mammalian toxicities and no special precautions are necessary when using them. They have no significant insecticidal properties and preliminary experiments suggest that they have little effect on the soil fauna, such as earthworms, or on predators of red spider, an important consideration in glasshouse crops.

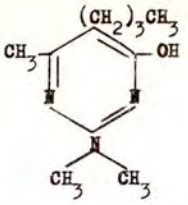
INTRODUCTION

Recently at Jealott's Hill Research Station remarkable fungicidal activity has been discovered in a group of pyrimidine compounds, which are taken up by plant roots, permeate throughout the plants, and eradicate established infections of certain fungi and protect the plants for long periods against subsequent attacks.

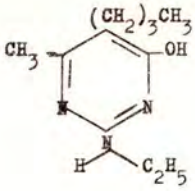
These compounds are loosely absorbed onto soil particles, movement through the soil is very slow, and they do not appear to be broken down rapidly in soil. Therefore, the soil functions as a reservoir releasing the active ingredient gradually to protect the crop over a considerable part of its life.

When applied to a leaf they are moved towards the tip and across the leaf and from the upper to the lower surface, but since they are carried in the transpiration stream they will not move back into the petiole and up to new growth. In other words when applied to the roots they are fully systemic but when used as foliage sprays their movement is limited.

Two of these compounds are being developed by Plant Protection Limited; dimethirimol, 5-n-butyl-2-dimethylamino-4-hydroxy-6-methylpyrimidine, for the control of powdery mildews on cucumbers, melons and some ornamentals and ethirimol, 5-n-butyl-2-ethylamino-4-hydroxy-6-methylpyrimidine, for control of cereal mildews.



Dimethirimol



Ethirimol

Figure 1.

Chemically these compounds are closely related but they are very specific in their action. Only very low concentrations are required to give absolute control of powdery mildews.

DIMETHIRIMOL (PP 675)

This compound is in an advanced stage of development. In 1968 it was sold in England for the control of powdery mildew on cucumbers in greenhouses and this year it was also available in Belgium, Germany and Holland.

With glasshouse cucumbers, an application of 0·25g of dimethirimol watered around the base of the plant when the first spots of mildew appear in the crop, protects the plant for six to eight weeks even if the crop is being bombarded continuously by spores from outside. One application may be sufficient to keep the crop free of infection throughout its life, but under the worst conditions where there is a continuous source of infection two or at the most three applications will give a disease-free crop.

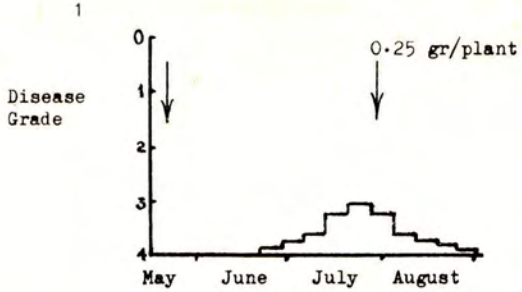
Figures 2 and 3 illustrate the eradicant properties of dimethirimol and the long lasting protection it gives to plants, compared with 10 sprays of a standard foliage fungicide (figure 4).

In the first trial (figure 2) dimethirimol was applied when a few spots of mildew appeared on the plants and this eradicated the infection and kept the plant free of disease for 6 - 7 weeks.

In the second trial (figure 3) mildew was allowed to establish itself before the plants were treated with dimethirimol, and following treatment the amount of disease in the crop declined over a period of 8 - 10 weeks.

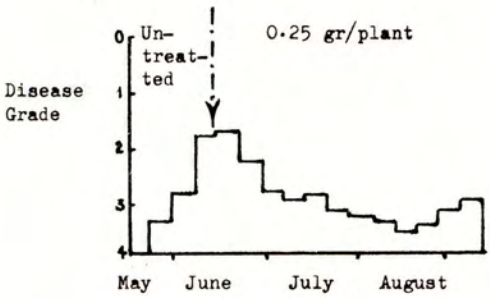
From figures 2 and 3 it is obvious that 10 sprays of the standard fungicide, dinocap, were far less effective than two applications of dimethirimol.

Fig. 2 Eradicant and protective effects of dimethirimol on cucumber powdery mildew



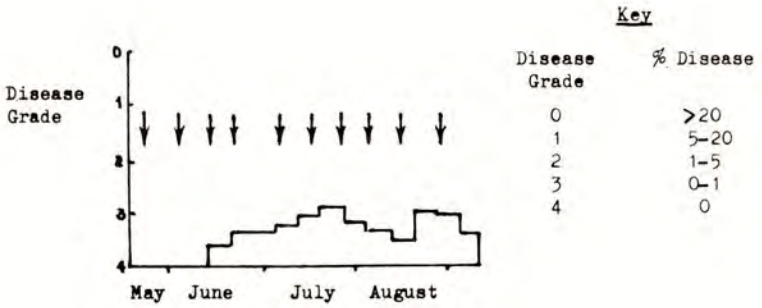
Arrows indicate the dates when dimethirimol was applied around the base of the stem.

Fig. 3 Eradicant effect of dimethirimol on cucumber powdery mildew.



Arrows indicate the dates when dimethirimol was applied around the base of the stem.

Fig. 4. Effect of dinocap sprays on development of cucumber powdery mildew



Arrows indicate spraying dates

Losses in yield due to powdery mildews are not often appreciated and in cucurbits they can be quite large. Both the numbers of fruit and quality are reduced by mildew. In a trial on commercial glasshouse cucumbers in the U.K. the yield over a period of four months was increased by 20 fruits per plant when mildew was controlled by dimethirimol.

For field crops of melons, cucumbers and gherkins, experiments are in progress to determine the best position for the placement of dimethirimol in the soil. Because it is absorbed on to soil particles, and its movement through the soil is slow, dimethirimol must be placed near to the absorbing roots, the position of which changes during the growing season and is dependent on the irrigation system used.

It is also being tested against powdery mildews on tobacco, hops, strawberries and on certain ornamentals e.g. cinerarias, sweet pea, etc.

ETHIRIMOL (PP 149)

Surveys carried out by the Ministry of Agriculture in 1967 and 1968 showed that powdery mildew (Erysiphe graminis D.C.) is by far the most important disease of spring barley in Great Britain; the annual loss in yield is probably more than 15%. There is also evidence from many other European countries that mildew causes substantial losses in yield on barley. Less is known about the damage caused by the disease on winter wheat, but the incidence of the disease has increased in recent years. In Hungary Dr. Podhradszky showed that mildew causes appreciable losses on both wheat and barley.

Although non-systemic fungicides e.g. drazoxolon, are available for the control of mildew on cereals, the problem is how to apply them to the crop to achieve sufficient persistence. The most convenient time to apply a spray is with the hormone weedkiller. Only foliage present at the time of spraying is protected and thus a build up of the disease epidemic can only be delayed, not prevented. Treatment with a systemic compound persisting for the whole or a major part of the life of the crop will clearly be more effective. Therefore the discovery of ethirimol, a xylem systemic compound very active against powdery mildew of cereals and grasses, represents a major break through.

Since ethirimol is translocated in the xylem, but not in the phloem of cereal plants, best results are obtained when it is applied in the root zone. Application to the root zone can be effected by dressing the seed, by combine-drilling special granules or by application of a dust formulation in the drill box. Broadcasting the chemical on to the soil surface is less effective.

After small scale trials in 1967, the first large scale field trials were carried out in England in 1968 and repeated in a modified form in 1969. In both years trials were also carried out in all the major cereal growing countries in Europe. The main treatments compared were seed dressing, combine-drilled granules, and spray with the hormone weedkiller. For granule application ordinary compound fertilizer pellets were used as the support, and the fungicide was added as a coating to these. The granules were drilled using the normal fertilizer box of a combine drill. The plot size used in 1968 was 1 acre. Various plot sizes were used in 1969.

Disease assessments were made at two weekly intervals by removing 50 tillers from each plot and estimating the percentage area infected on the lowest two living leaves.

In 1968, the disease appeared in most of the U.K. trials in early May and built up rapidly until late June, when the spread was checked by heavy rain (Figure 5). As was to be expected the spray application at 1 lb/acre gave only moderate disease control: nevertheless this compared favourably with standard fungicides and was better than had been achieved with the spray in very small plots (2 x 10m.) in 1967; if very large areas were treated, quite good control should be achieved. Application as a seed dressing or as granules at 1 lb/acre gave almost complete disease control for the whole season; at 0·5 lb/acre the two soil applications were still superior to the spray at 1 lb/acre.

In 1969, the incidence of mildew in the U.K. and in most parts of Europe was very low but marked differences in the amount of infection in treated and untreated plots have been recorded in some trials. All the results from the 1969 trials have not yet been collated.

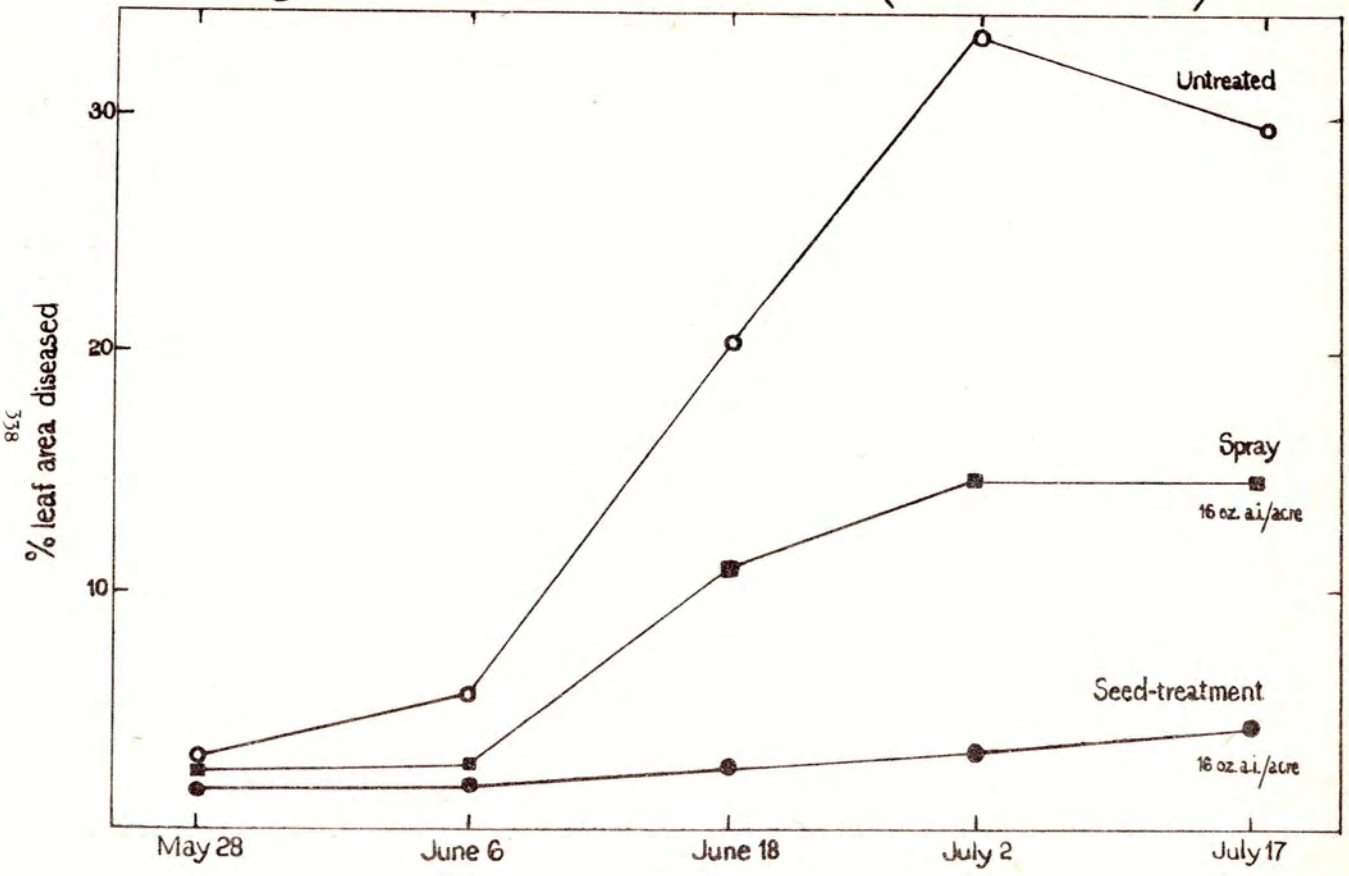
In 1968, it was extremely difficult to assess yields because of very severe lodging caused by the heavy storms. The treated plots were more severely damaged than the untreated because where mildew was controlled the plants grew taller and leaves were more lush. Nevertheless the best treatment (granule at 16 oz a.i./acre) gave an average increase for all the trials of 10·4%. In several trials carried out by research workers in Germany, Holland and England yield increases of over 20% were recorded from application as a seed dressing.

Detailed assessments on some trials showed that mildew reduces yield by affecting both the number of tillers reaching maturity and the size of the ears. The disease must be controlled for the whole season if the full yield is to be obtained, but disease control for the first part of the season will result in some yield increase because it is at this stage that the tillers are formed.

Numerous research workers in Europe had extremely promising results with ethirimol in 1968; the chemical was equally effective on all varieties of barley and against all of the mildew races in the European collection. It is active in a wide variety of soil types, but there are indications that it may be partially inactivated in soils containing very high amounts of organic matter ($>50\%$), particularly at low pH.

More trials to provide accurate disease incidence/yield reduction correlations are needed to determine the optimum rate of application. Present indications are that in England 16 oz. a.i./acre is higher than is necessary. More experiments will also be carried out to investigate different methods of application of the chemical.

Figure 5. PP149 Trials 1968 – (Mean of 8 Trials)



Seed dressings are the most attractive method and appropriate formulations are being developed so that they can be applied successfully through commercial seed dressing machinery.

Almost all of the results obtained so far have been on spring barley. The chemical is extremely active against mildew of winter wheat, though slightly higher concentrations may be necessary to give equivalent control. Further it is not known whether soil application is persistent enough on an autumn sown crop, or whether a spray application in the spring would be preferable.

RESIDUES

These compounds are not very stable in plant tissues. Residues in cucumber fruits, melons and cereal grains have been below detection level (0·1 p.p.m.).

TOXICITY

Although full toxicological tests and metabolism studies are still in progress it is quite clear from available evidence that ethirimol and dimethirimol have low systemic and cutaneous toxicity and no special precautions are necessary when using them.

The acute toxicities of both compounds have been determined in a number of animal species and the results are tabulated below:-

	Oral LD50 (mg/kg)	
Animal species	Dimethirimol	Ethirimol
Rat (female)	2350	4000
Mouse (female)	2000	4000 (approx)
Rabbit (male)	-	1000-2000
Guinea pig (female)	500	500-1000
Cat (female)	500-1000	1000
Hen (adult)	4000	4000

The acute oral toxic dose to the rat is at least ten times the interperitoneal LD50 (200 to 400 mg/kg) indicating that the compounds are poorly absorbed from the gut. They have shown no irritant effects on either skin or eyes (rabbit).

A survey of wild life has been made on fields drilled with cereal and seed dressed with ethirimol and no adverse effects have been observed on birds or other animals.

Ethirimol and dimethirimol have no significant insecticidal properties, and preliminary experiments suggest that they have little effect on soil fauna, such as earthworms, or on predators of red spider, an important consideration in glasshouse crops.

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

THIABENDAZOLE, A NEW SYSTEMIC FUNGICIDE

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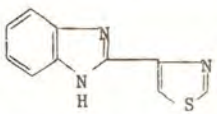
Summary Thiabendazole is the generic name of a new fungicide being actively developed by Merck & Co., Inc. It is quite stable as a solid or in aqueous suspension, and is relatively insoluble in most organic solvents. Acute oral toxicity studies of Thiabendazole indicate it has a low order of toxicity. Chronic administration of Thiabendazole is well tolerated by rats, dogs, sheep, and swine. Thiabendazole is systemic in growing plants and can be readily detected by either radioactive or bioassay techniques. Uptake occurs through either the leaves or roots. The United States Government has approved Thiabendazole for use on bananas for control of the stem-end rot complex (Thielaviopsis, Gloeosporium, Verticillium, Fusarium, Deightonella), on citrus for control of blue and green mold (Penicillium) and stem-end rot (Diplodia), and on ornamental bulbs and corms for control of Fusarium rot and blue mold (Penicillium). Thiabendazole is marketed as 'Mertect®' and 'Tecto®' 2-(4-thiazolyl)benzimidazole . Registrations for the control of sugar beet, turf, and apple diseases are anticipated in the near future.

INTRODUCTION

Thiabendazole has been known in medical circles since 1961 as an anthelmintic. At the present time, it is widely used in animals and humans. In 1964, Staron, Th., et al. and Robinson, H. J., et al. reported that Thiabendazole possessed a broad spectrum of antifungal activity. In 1966, Staron, Th., et al. established that Thiabendazole was translocated in plants. Thiabendazole is regarded as a breakthrough in the development of urgently needed systemic fungicides for use in control of plant diseases. It possesses both curative and protectant action against plant pathogens. Merck & Co., Inc. has been extensively evaluating Thiabendazole against plant diseases in the laboratory and the field during the last 5 years.

CHEMICAL AND PHYSICAL PROPERTIES

Chemically, Thiabendazole is 2-(4-thiazolyl)benzimidazole having the following structure:



The compound has a molecular weight of 201.25, a melting point of 304-305°C, and a sublimation point of 250°C. Thiabendazole is quite stable as a solid or in aqueous suspension. No change in UV absorption was found in samples stored for 8 days at 100°C. At pH 2.0, it is stable at room temperature for at least 2 years. Autoclaving at 121.5°C for 20 minutes in various microbiological culture media (pH 5.6 to 7.0) had no apparent effect on its antifungal potency. Heating at 220°C at atmospheric pressure had no apparent adverse effect of its antifungal properties. Thiabendazole is soluble in dimethyl sulfoxide, dimethyl acetamide, dimethyl formamide; slightly soluble in alcohols, esters, chlorinated hydrocarbons; and essentially insoluble in water. The solubility of Thiabendazole in water at 25°C varies depending on the pH. In the pH range 5 to 12, it is less than 0.05mg/ml. Below pH 5 the solubility increases, particularly in the 2 to 4 range. The solubility of Thiabendazole at pH 3 to 5 is approximately 0.25 mg/ml, whereas at pH 2.0 it is near 10.0 mg/ml.

TOXICOLOGY

Today more emphasis is being placed on the safety of a pesticide by the consumer and producer alike. Thiabendazole was subjected specifically to extensive acute and chronic toxicity studies in a variety of animal species in the early 1960's. It was shown that Thiabendazole is well tolerated, even when administered orally, over a two-year period. Repeated daily doses of 100 mg/kg in rats produced no outward evidence of toxicity. The results of acute oral toxicity studies in mice, rats, and rabbits are as follows:

Table 1

Acute oral toxicity, LD_{50} in mice, rats and rabbits

Animal	Acute oral toxicity, LD_{50}
Mice	3.81 g/kg
Rats	3.33 g/kg
Rabbits	3.85 g/kg

In addition to those listed above, single oral doses were administered to dogs, sheep, goats, and swine. These animals tolerated large quantities of Thiabendazole. The results of a subacute inhalation study demonstrated that Thiabendazole has a very low order of subacute toxicity. Chronic inhalation at a rate of 70 mg/m^3 Thiabendazole led to no observable clinical effects. From the studies on fish and wildlife, it was concluded that Thiabendazole would appear to present a low order of hazard to fish and wildlife.

SPECTRUM OF THIABENDAZOLE FUNGICIDAL ACTIVITY

Over the years, through the efforts of research workers throughout the world, the number of fungi found to be sensitive to Thiabendazole has steadily increased. To demonstrate the large spectrum of activity of Thiabendazole, the most sensitive plant pathogens are as follows:

Inhibited microorganism

Ascochyta pisi	Endothia parasitica	Phymatotricum spp
Alternaria tenius	Fusarium spp	Pullularia pullulans
Aspergillus spp	Gloeosporium musarum	Rhizoctonia solani
Botrytis cinerea	Helminthosporium spp	Rhizoctonia violacea
Ceratocystis spp	Lenzites trabea	Sclerotinia spp
Cercospora spp	Monilinia spp	Sclerotium rolfsii
Chaetomium globosum	Neurospora sitophila	Stachybotrys spp
Cladosporium spp	Oidium spp	Trichoderma viride
Claviceps purpurea	Oospora citri-auranti	Thielaviopsis paradoxa
Colletotrichum spp	Penicillium spp	Trichothecium roseum
Dactylium dendroides	Phacidiopycnus furfuracea	Ustilago zeae
Deightoniella torusalum	Phoma betae	Venturia pirina
Diplodia viticola	Piricularia oryzae	Venturia inaequalis
		Verticillium spp

Thiabendazole is not active on Mucor fumosus, Oospora citri-auranti, Phoma betae, Phytophtora spp, Pythium ultimum and Rhizopus spp.

UPTAKE AND TRANSLOCATION OF THIABENDAZOLE IN PLANTS

Many research workers have demonstrated by bioassays, chromatography, and radio-autography, that Thiabendazole is systemic in many plants. Our most recent information clearly proves Thiabendazole to be translocated in tomato and cotton plants

in addition to barley, bean, wheat, apple (fruit tissue), artichoke, lettuce, cotton, and sugar beet (Staron and Allard, Staron et al., Erwin et al., Solel, Stallknecht and Crane, Stipes, and Lauber and Greenwald). Tests initiated early in 1969, at the laboratories of Merck & Co., Inc., established the movement of the chemical in tomato plants. Chemical assays revealed the movement of Thiabendazole from the root to the leaves and from the leaves to the root.

THIABENDAZOLE DETERMINATION IN TOMATO PLANTS

The systematicity of Thiabendazole as a soil treatment was evaluated on 6 to 8 week-old tomato plants (8 to 10 inches high). They were uprooted, washed free of soil and individually transplanted into 4 1/2 inch pots containing soil treated by incorporation with Thiabendazole at 10 and 20 lb a.i./ac respectively. Eight plants were used for each treatment and the untreated controls. Seven days after transplanting, the plants were uprooted, washed free of soil, and immediately frozen. The frozen plants were divided into three parts: stem, root, and leaves. Individual samples were chemically analyzed for Thiabendazole, utilizing the spectrophotofluorometric method developed by Merck & Co., Inc. Results of this assay are shown in Table 2.

Table 2

Thiabendazole soil treatment for determination of translocation to foliar portions of tomato plants

Thiabendazole lb/ac	Assay results in ppm Root	Stem	Leaf
10	--	4.8	48.0
20	107.5	33.5	160.2

A 60% Thiabendazole w.p. formulation was used for application to the foliage of tomato plants. The compound was sprayed to run off. Only the foliar portions of the mature (just prior to blossom stage) tomato plants were treated. Rates of 500 ppm and 2000 ppm were utilized on each of 8 plants. Seven days after treatment, the plants were removed from the soil, the root systems washed free of soil, and immediately frozen. Eight untreated plants were also processed for reference purposes. The method for analysis of the samples was the same as mentioned previously.

Table 3

Thiabendazole foliar treatment for determination of translocation to root portions of tomato plants

Application rate in ppm	Assay results in ppm Root	Stem	Leaf
500	2.83	3.87	46.75
2000	6.33	8.05	131.75

THIABENDAZOLE DETERMINATION IN COTTON PLANTS

Tests were conducted on cotton plants by D. C. Erwin in California. The plants were grown in pots containing 100 ppm of Thiabendazole incorporated into the soil. Three treatment levels were used: control - 0 ppm, 0 spores; Thiabendazole - 100 ppm, 0 spores; and Thiabendazole - 100 ppm, 5 x 10^3 spores (inoculated). The cotton plants were divided into upper leaves, lower leaves, upper stems, lower stems, and roots. The samples were analyzed spectrophotofluorometrically by Merck & Co., Inc.

and the results proved the translocation of Thiabendazole from the roots to the upper parts of the plants.

Table 4

The translocation of Thiabendazole in cotton plants as determined by chemical assay

	ppm Thiabendazole in soil	Spore Conc.	ppm Thiabendazole	ppm Thiabendazole in soil	Spore Conc.	ppm Thiabendazole
upper leaves	0	0	0.67	100	0	9.3
lower leaves	0	0	0.31	100	0	8.0
upper stem	0	0	0.29	100	0	8.7
lower stem	0	0	0.8	100	0	21.7
root	0	0	0.13	100	0	163.0

REGISTERED APPLICATIONS

The U.S. Government has approved Thiabendazole for use on bananas for control of the stem-end rot complex, on citrus for control of blue and green mold as well as stem-end rot, and on ornamental bulbs and corms for control of Fusarium rot and blue mold. Other areas for application of this systemic fungicide are being investigated. Recently a petition was filed in the U. S. for the use of Thiabendazole for control of Cercospora leaf spot of sugar beets. The highlights of control for the fore-mentioned diseases are summarized below:

Bananas - The evaluation of Thiabendazole for control of the plant pathogens which cause banana rot began in 1964. The major fungi involved are Fusarium roseum, Gloeosporium musarum, Verticillium theobromae, Thieloviopsis paradoxa and Deightonella torusolum. All five pathogenic organisms were controlled by 50 ppm of Thiabendazole in in vitro tests. The results from semi-commercial trials demonstrated a great reduction of banana rot when Thiabendazole was used. Thiabendazole at 200 ppm will control crown rot better than current control measures based on both natural and artificial infestations. This data is shown in Table 5.

Table 5

Crown rot control of bananas with Thiabendazole

Treatment	Conc. in ppm	Innoc. method	Crown rot rating ++ Degree of severity in % N	T	L	M	S
Thiabendazole	200	natural	8.0	81	11	0	0
Commercial standard*	Undisclosed	natural	0	2.1	63	24	11
Check	-	natural	0	4.5	56	22	17.5

++ N = none, T = trace, L = light, M = medium, S = severe

* Data provided by the United Fruit Company. Identity of fungicide and rate undisclosed

Citrus fruits - Losses due to decay are an important economic factor in the marketing of fresh citrus fruits. In the U. S. there are four species of citrus decay fungi: Diplodia natalensis and Phomopsis citri which cause stem rot, Penicillium digitatum

causing green mold, and Penicillium italicum causing blue mold. A commercial post-harvest fungicide treatment must control both primary decay and fungus sporulation to avoid losses during shipping. Table 6 illustrates the activity against Penicillium digitatum on oranges.

Table 6

Comparison of decay control between commercial and experimental citrus fruit fungicides 1/

Number	Treatment	Dip time in min.	No. of experiments	Av. % decay 2/
1	Check	-	58	13.5
2	Thiabendazole 500 ppm 3/	2	54	3.7
3	Dowicide A-hexamine 2%	2	44	6.3
4	Diphenyl pads, 2 per 4/5 bushel carton	0	37	6.6
5	Dowicide A-hexamine, 2% + 2 diphenyl pads	0	29	4.5
6	2-AB 1% 4/	2	54	4.1

1/ A. A. McCornack and G. Eldon Brown, University of Florida, Lake Alfred, Florida

2/ Two weeks after harvest, storage temperature of 70°F

3/ Thiabendazole (experimental)

4/ 2-aminobutane in carbonated or phosphated form (experimental)

Ornamentals - Basal rot is a serious world-wide disease problem in field and storage on ornamental bulbs and corms. Fusarium oxysporum seriously reduces commercial yields when no control measures are applied. Tests have been conducted over the recent years with Thiabendazole as a preplant dip treatment of corms. The increase in flower production as well as healthy corms is demonstrated in Table 7.

Table 7

Preplant dip treatment of Van Zantens Glory corms with Morsodren, Phaltan, and Thiabendazole for control of Fusarium corm rot *

Treatment	Rate, ppm (active)	Flower Index 11/28 - 12/12/66	Healthy Corms 3/15/67	
			No.	Wt in oz
Control	0	59	67	149
Morsodren	47 (Hg)	83	77	194
Phaltan	6000	85	71	191
Thiabendazole	500	144	98	210

* Data of Dr. R. O. Magie, University of Florida, Bradenton, Florida

Sugar beets - In areas of the U.S.A. where Cercospora leaf spot disease is favored by climatic conditions, lack of disease control can result in severe loss of crop and

sugar yield. In the last three years, extensive field tests have been conducted by Merck & Co., Inc. in the major sugar beet growing areas of the U.S.A. The results obtained in two of these experiments are shown in Table 8.

Table 8

The effect of Thiabendazole on Cercospora leaf spot of sugar beets

Treatment	No. of appl.	Yield/ac Tr.	Yield/ac Untr.	% increase	% sugar Tr.	% sugar Untr.	% increase	% sugar increase due to Thiabendazole
Experiment 1								
3.3 oz a.i. (5.5 oz 60% w.p.)/ac	2	30.1	27.6	9.0	15.0	14.4	4.2	13.8
6.6 oz a.i. (11.0 oz 60% w.p.)/ac	2	30.4	28.0	8.5	14.7	14.0	5.0	14.0
Experiment 2								
3 oz a.i. (5 oz 60% w.p.)/ac	3	23.8	22.2	7.1	15.2	14.3	5.5	13.5
12 oz a.i. (20 oz 60% w.p.)/ac	3	23.3	21.6	7.8	15.5	14.2	9.1	17.5

FORMULATION AND EXPERIMENTAL RATES

Thiabendazole is presently being marketed as a 60% w.p. Samples of this formulation are available for experimental evaluation. Thiabendazole will readily sublime when heated. Ninety percent a.i. tablets are available which contain 8.0 gm of Thiabendazole. They are readily sublimed when heated on an alcohol burner or other suitable heating unit. Experimental 5 and 10% dusts are available for trials. The following application recommendations as a guide to the experimental evaluation of Thiabendazole are suggested:

foliar	0.75 and 1.5 lb 60 w.p./100 gal
soil	0.5 and 1.0 lb 60 w.p./1000 ft^2
fumigation	1 and 2 tablets/4000 ft^3
seed treatment	0.25 and 0.5 lb 60 w.p./100 lb

Two and four week treatment intervals are recommended when more than one application is made.

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

SOME RESULTS CONCERNING THE SYSTEMIC ACTION OF TRIDEMORPH

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Summary The experience gained with tridemorph (N-tridecyl-2,6-dimethylmorpholine) in trials over several years is that this compound has shown itself to have both eradicative and systemic activity in the control of Erysiphe graminis in barley. A report on laboratory and greenhouse trials, in which both the chemical and biological evidence that tridemorph is taken up by the roots as well as the leaves and is translocated in the barley plant, is given. At the same time it is shown that as a systemically active fungicide tridemorph possesses eradicative qualities against Erysiphe graminis.

INTRODUCTION

To protect plants against fungal attack most fungicides must be applied, as is commonly known, prophylactically, since many fungi penetrate deeply into the plant tissue and can thus not be controlled by a compound whose activity is limited to the plant surface. In the case of the downy mildews, that to a large extent develop on leaf surface, control by applying an effectively eradicant fungicide is possible, however, whether these fungicides are used prophylactically or eradicantly, they only protect those portions of a plant that are covered by the spray.

It is thus not surprising, that Plant Pathologists have for years being trying -here we only need to quote the classical work of Müller "The inner therapy of plants"- to circumvent the disadvantages of the normally used compounds by developing fungicides that are active internally. The progress achieved in this sphere of systemic fungicides over the last few years has given the fungicide research new impulses.

In our trials with the compound tridemorph (N-tridecyl-2,6-dimethylmorpholine) we had already observed earlier that this product is curatively active against Erysiphe graminis in barley. Thereupon we established that tridemorph is taken up by barley and translocated in the plant. The following is a report on some of the trials and observations on the question of the systemic activity of tridemorph.

TRIALS AND RESULTS

To gain an idea of how much tridemorph is taken up, the absorption via root and leaf was examined in two trials. Because we did not have radioactively marked tridemorph, the active ingredient analysis were conducted by the following methods*: In previously refined plant extracts, tridemorph forms a chloroform soluble salt on addition of methyl orange (at a pH of 3). This salt is then

* N. Drescher, S. Otto; laboratory method

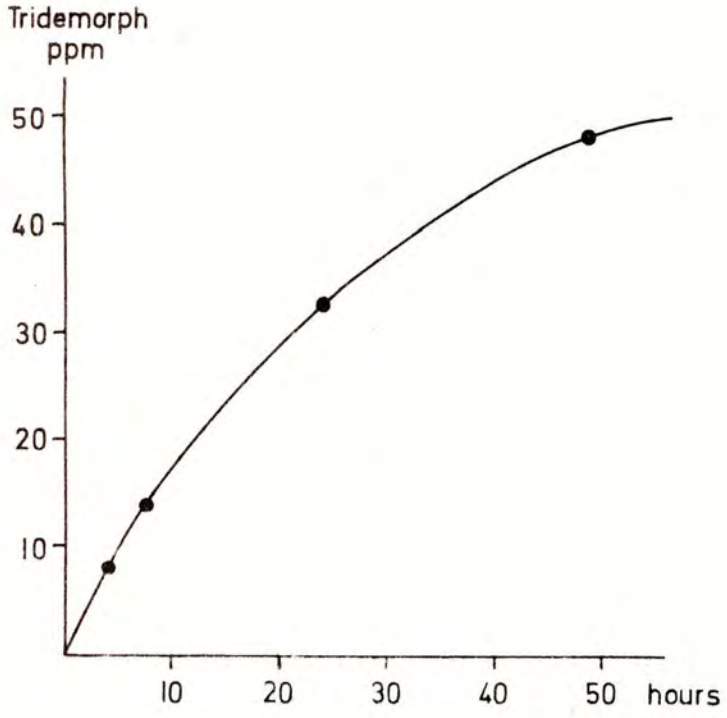
extracted with chloroform and split by the addition of hydrochloric acid; the tridemorph equivalent amount of colouring is then read photometrically. The lower detection limit of this method is approximately 0,05 ppm.

Root-uptake

Barley plants of the variety "Firlbecks-Union" which were in the 2 leaf stage were transferred to a nutritive solution after Knop, to which had been added 50 ppm of tridemorph. After 4, 8, 24 and 48 hours, samples for testing the increase in concentration of tridemorph in the leaves by analysis were taken. In figure 1 the progressive uptake of the compound is presented.

Figure 1.

Root-uptake of tridemorph



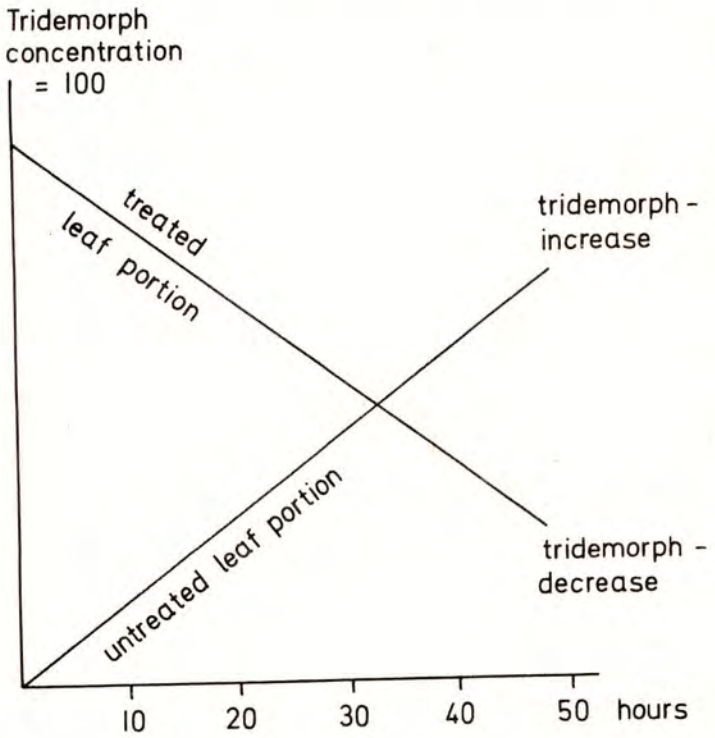
Already after 4 hours 7,2 ppm of tridemorph could be detected in the leaf extracts. The further course of absorption shows that an equilibrium is reached after approximately 48 hours. In further trials it was determined that even after a longer period no increased concentration of the compound could be determined in the barley leaves.

Leaf-uptake

To determine the uptake of the compound via the leaf, a 0,1 % watery solution of tridemorph was applied to the lower halves of fully developed barley of the variety "Firlbecks Union". The uptake and transport of the compound to the upper untreated portion of the leaf was determined by sampling and analysis after 8, 24 and 48 hours. Similarly we analysed the lower, treated portion of the leaf to determine the reduction in concentration. The result of these trials is given in figure 2.

Figure 2.

Leaf-uptake and transport of tridemorph



Compared to the uptake of tridemorph by the roots the absorption by the leaves was obviously slower. If the applied concentration of the compound was kept constant then the amount of translocated tridemorph to the untreated leaf halves was approximately proportional to the duration of the trial.

Greenhouse trials

In an earlier trial (Kradel et al, 1969) we were able to demonstrate that an applying tridemorph to the soil it was absorbed by barley roots and then translocated to the leaves (Table 1).

Table 1.

Application of tridemorph (50 ml/250 g soil)
Greenhouse trial with spring barley

Compound	.. % Active ingredient	Mildew infection (1 to 9) Days after treatment 8	16
Tridemorph	0,01	1	1
	0,005	1	3
Dinocap	0,012	4	8
Untreated		9	9

The artificial infection of the barley with Erysiphe graminis was performed immediately after soil treatment. On applying a 0,01 % solution of tridemorph a mildew infection during the 16 days observation period was definitely prevented.

In further trials to clarify the point of particular interest to us namely the leaf absorption of tridemorph, we treated the first fully developed barley leaves of the variety "Firlbecks Union" at the base only, of either the morphological upper or lower surface. The application of 0,025, 0,05 and 0,1 % tridemorph solution was applied with a brush in such a way that 0,5, 1 and 2 cm long portions of leaves were treated. With an average leafwidth of 7 to 8 mm, approximately 2 mg active ingredient was applied to a 2 cm length of leaf i.e. on applying a solution containing 0,1 % a.i. approximately 1,5 to 1,8 (0,0015 to 0,0018 mg) tridemorph was applied. To determine the eradicant effect of tridemorph, the barley plants were artificially infected 4, 3, 2 and 1 days prior to as well as on the day of application. An analysis was in each case made 10 days after the artificial infection. Out of the results of these trials only two are reproduced here (Figs. 3 and 4). The 0,25 % treatment of tridemorph on a 0,5 cm leaflength lies on the lower effective threshold. The absorption and rate of translocation of tridemorph were just sufficient to prevent a mildew attack of a day-old infection. With two and three day-old infections only the leaf portions immediately above the treated zone were protected and the already commenced infection stopped. The translocation of tridemorph was only upwards; a movement in the direction of the roots has hitherto not been established. The trial illustrated in fig. 4 clarifies the curative effect of tridemorph. Whilst, as already mentioned, it was impossible to stop by way of leaf absorption a four day-old infection by applying a 0,025 % tridemorph solution to a 0,5 cm length of leaf, it was

Figure 3.

Leaf treatment with a 0,025 % tridemorph solution
... days after mildew infection

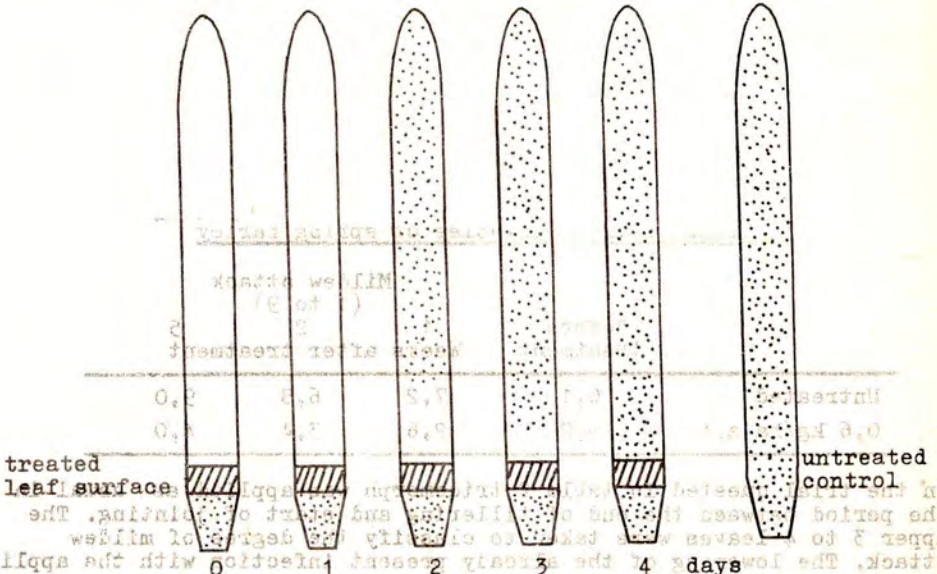
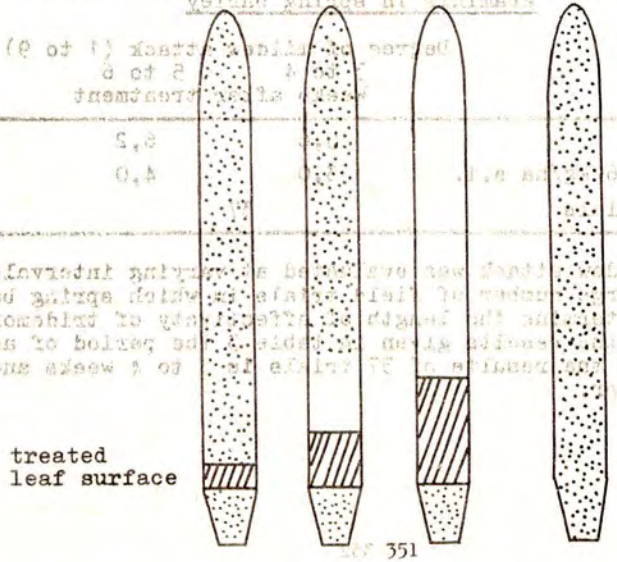


Figure 4.

Leaf treatment with a 0,025 % tridemorph solution
4 days after mildew infection



possible to eliminate the already started mildew attack by treating a 2 cm of leaf length.

Field trials

With leaf fungicides it is difficult to determine the eradicative as well as the systemic effectiveness under field conditions. The following results which are supplemantary to those the laboratory and greenhouse, should be seen in this light (Table 2 and 3), as they give evidence of the eradicative activity of tridemorph and to a certain degree on its systemic effectiveness.

Table 2.

Eradicative effect of tridemorph against Erysiphe graminis in 6 varieties of spring barley

	Mildew attack (1 to 9)			
	Before treatment	1 Weeks after treatment	2 Weeks after treatment	5 Weeks after treatment
Untreated	6,1	7,2	6,8	9,0
0,6 kg/ha a.i.	6,0	2,6	3,4	4,0

In the trial quested in table 2 tridemorph was applied as usual in the period between the end of tillering and start of jointing. The upper 3 to 4 leaves were taken to classify the degree of mildew attack. The lowering of the already present infection with the application of tridemorph is obvious, particularly if the mildew is evaluated a week after treatment.

Table 3.

Effective period of tridemorph against Erysiphe graminis in spring barley

	Degree of mildew attack (1 to 9)	
	3 to 4 Weeks after treatment	5 to 6 Weeks after treatment
Untreated	5,6	6,2
0,4 to 0,6 kg/ha a.i.	3,0	4,0
No. of trials	37	

The degree of mildew attack was evaluated at varying intervals after treatment in a large number of field trials in which spring barley was treated to determine the length of effectivety of tridemorph. As can be seen from the results given in table 3 the period of activity, was determined by the results of 37 trials is 3 to 4 weeks and declines thereafter.

DISCUSSION

Since tridemorph shows a direct fungicidal activity against various phytopathogenic fungi in agar-agar tests, it can be accepted that tridemorph is probably taken up as an intact molecule by the barley plant. According to the investigations of Brian (1966) the molecular weights of systemically active plant protection compounds are in the region of 200 to 600; tridemorph with a molecular weight of 297,5 would, according to this classification be in the lower region. We are of the opinion that the rapid uptake over the roots is due to the fact that the cells of the root epidermis as well as those of the root hairs are relatively thin and hydrophillic and this offer little resistance to water and dissolved substances. Whether the uptake by the leaves is via the stomata and/or the epidermis with its resistant cuticula cannot be answered as yet. It can however, be established that the penetration of tridemorph into the leaf tissues, is very much slower when compared to the root uptake. After penetration, however, a relatively rapid distribution takes place. It reaches, as was shown in leaf trials, in which 4 day-old mildew infections had been established into the epidermis cells. Thereby it appears that a minimal amount of the substance is sufficient to achieve a fungicidal effect. Since for reasons of economic work rationalisation spraying against barley mildew is carried out towards the end of the tillering stage and the beginning of jointing the systemic effect of tridemorph is thus of particular value because due to the uptake of the compound by the leaf and its distribution in the plant, the new growth is protected effectively over a longer period, without the danger of the fungicide being washed off.

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PROBLEMS OF ASSESSING CHANGES IN WILD BIRD POPULATIONS

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Summary The efficiency and attendant problems of various current methods of assessing wild bird populations are discussed and compared with those suggested in Working Document 4. All techniques suffer from the problem that to be fully understood, the results must be viewed in comparison with naturally occurring fluctuations in numbers and their subsequent effects. All are open to biases caused by varying behaviour patterns and the effects of time and weather. It is suggested that the straight-count surveys of living birds in the breeding season, recommended in Working Document 4, be developed towards the much more reliable territory mapping technique, which requires little additional effort and which would immediately be comparable with the picture of natural fluctuations now being obtained on a national scale. Similarly, it is thought that this important check on potentially toxic new materials, which serves as a final safeguard, should itself be field tested, under carefully regulated conditions, using a known toxic material.

INTRODUCTION

Working Document 4 of the Pesticide Safety Precautions Scheme suggests that field surveys be used to complement laboratory avian toxicity trials to assess the short-term risks of new pesticide materials. Mr. Terry discusses the operation of these tests in the field, my task is to examine the various available methods of investigating changes in bird populations and to discuss their relative merits. The British Trust for Ornithology has carried out a variety of censuses and surveys of wild birds since 1932, and its members are currently responsible for the Common Birds Census, an annual census generating population indices for a variety of common farmland and woodland birds. Against this background, survey and census methods will be examined.

For some years now the necessity of possessing adequate background numerical information has been apparent to ornithologists, for any purpose - biological, ecological or conservational. The evaluation of population change must be against the background of actual figures rather than the all too easily evoked subjective observations on increase or decrease.

THE METHODS

Winter counts Because of the ready long-distance mobility of bird flocks in winter, the effects of environmental contamination on a population become almost impossible to assess unless there is considerable immediate mortality, when body counts can be performed. For many species, especially small passerines, the natural hazards are also high, often highest, at this time. Ambient conditions are most severe, day-length (and thus feeding time) is short, and food for almost all, especially insectivores, is scarce. Severe weather, either coming suddenly, or with ground-covering snowfall, or if unusually extended, will increase 'natural' mortality and could well mask that due to other causes. Additionally, it seems highly likely that in such periods of stress, all fat reserves may be mobilised rapidly, on occasion releasing unusually large concentrations of stored toxic materials.

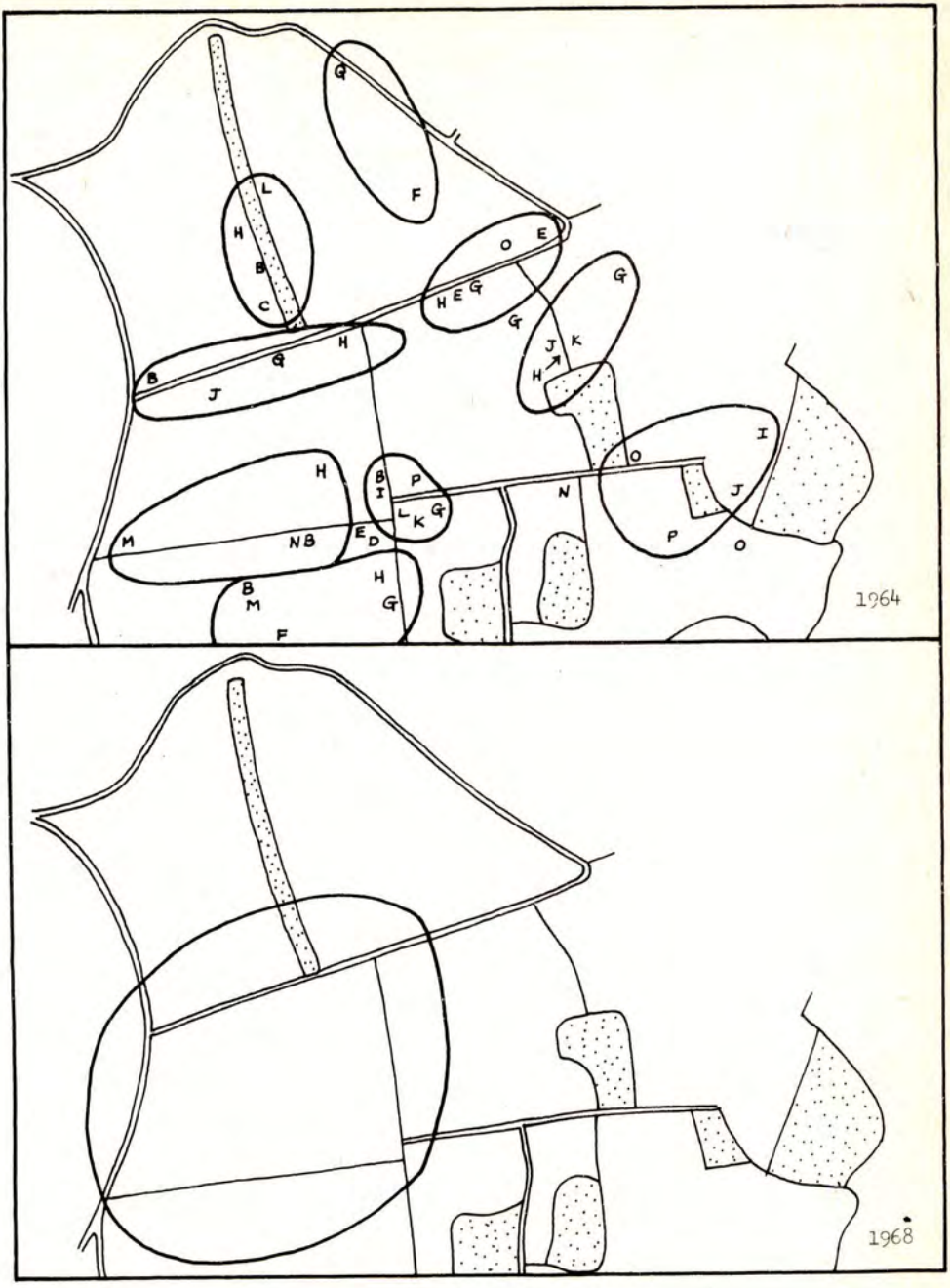

Fig. 1. PARTRIDGE TERRITORIES SUFFOLK FARMLAND

Because of the restriction on movement that holding territory and breeding imposes (other than for species with huge feeding range such as the Golden Eagle (Aquila chrysaetos) censuses during the breeding season, although by no means without faults, are more likely to be successful than winter counts.

Direct counts Direct counts of conspicuous, often colonially nesting, species, are by their very nature restricted to a few species, but given adequate coverage the results obtained can be of very high accuracy for almost any area. The sort of species for which this technique has value are the Rook (Corvus frugilegus) - common on most agricultural land, and the Heron (Ardea cinerea), a largely piscivorous bird. The Heron, a species subject both to exceptional mortality in severe winters and to the accumulation of high toxic chemical residues, has been the subject of annual census for many years, and it is possible to give almost precise figures for most areas. Nevertheless, the time of year that a heronry count is taken can make a considerable (10 to 20 per cent) difference to the figure, and counting of even such conspicuous objects as herons' nests can be made difficult by, for example, increasing leaf cover on the nesting trees in early May. With its very restricted range of species, this technique lacks flexibility and will provide only long term indications of population trends unless a large-scale disaster occurs. Such a disaster would be inexplicable unless bodies were found, and even then supplementary tests would be needed to determine their bodily condition, parasitological and toxicological status.

Presence/Absence surveys This technique is rapidly increasing in popularity for the recording of biological distribution data. As a technique for monitoring population changes it lacks sophistication, as only distributional rather than density changes can be recorded and the subtlety in detection of these depends on the dimensions of the grid chosen. For distributional purposes this system does possess flexibility - a 2 km square grid for county scale investigations, a 10 km square for national and a 50 km for international. At the moment ornithological data are being gathered by the British Trust for Ornithology for the whole of the British Isles on a 10 km grid, and for several counties with reasonable observer density, on a 2 km grid. Currently 'presence' is categorised into three grades: present; present, thought to be breeding; and present, breeding proved. Of course the parameters for these categories will vary from species to species, but for most common species they can be simply and reliably defined.

With suitably designed recording, this method has the advantage of lending itself readily to automatic data processing and automatic map production, but it seems highly doubtful that the grid size could be reduced sufficiently for the technique to be usefully modified to serve as a small scale check. The background information provided could be very useful in assessing long term population shifts, possibly connected with changing agricultural practice, in, for example, fruit, cereal or beet growing, areas or in prime grassland dairy farming regions.

Line transect techniques In these, the observer walks once, or more commonly, several t imes during the season, along a defined route through the survey area, recording all birds which he encounters within a given distance on each side of his path (see Yapp, 1956). This presents problems, some associated with the behaviour patterns of birds, some with the relation of the transect results to the population, and some with the slightly complex mathematics inherent in the technique. Even its most ardent supporters would only claim value in comparative studies, and as many of the biological problems can be eliminated or greatly lessened by the mapping technique described below, I will not discuss it further.

Territory mapping Following Enemar's (1959) review of the then available census techniques, the British Trust for Ornithology have since 1962 used a system of territory mapping to derive an annual index of population for a variety of common

farmland and woodland birds. The method is described in Williamson and Homes (1964) and the statistical aspects are examined, with favourable results, in Taylor (1965) and Snow (1965). Population changes on a given area from one year to the next are combined with the changes in the same species from other, similar, sample areas to derive an annual index of population change. Currently just under a hundred suitable farmland plots are in use, with an average size of about 70 h, scattered over the country. This scatter does allow some regional comparisons to be made but the method is essentially one designed to produce a baseline, established over many years, against which annual changes may ultimately be measured. As yet our picture of these possibly cyclic fluctuations is still emerging, and we have no idea of their 'wavelength'. Using other sources of data, this 'wavelength' has been estimated for some predatory species such as those dependent on small rodents (Southern 1954, Snow 1968); and, using game-book data, for some non-agricultural game species such as the Black Grouse (Lyrurus tetrix). The fluctuations of the indices for a number of common farmland species are shown in Table 1, which gives some idea of the degree of, in this context, 'natural' variation. Most species have an overlying tendency of improvement since the severe winter of 1962/63.

Table 1.

An index of population levels for selected species on farmland

	1962	1963	1964	1965	1966	1967	1968
Partridge (Perdix perdix)	194	158	131	137	100	99	88
Lapwing (Vanellus vanellus)	196	87	90	103	100	116	139
Skylark (Alauda arvensis)	88	72	92	102	100	109	111
Swallow (Hirundo rustica)	96	95	106	94	100	107	119
Carrion Crow (Corvus corone)	76	77	85	94	100	107	113
Great Tit (Parus major)	60	62	87	101	100	93	96
Blue Tit (Parus caeruleus)	66	65	97	107	100	103	110
Wren (Troglodytes troglodytes)	140	31	47	73	100	156	170
Mistle Thrush (Turdus viscivorus)	130	32	56	95	100	113	138
Song Thrush (Turdus philomelos)	112	48	81	100	100	119	125
Blackbird (Turdus merula)	69	57	90	101	100	102	107
Robin (Erithacus rubecula)	60	53	78	91	100	107	111
Whitethroat (Sylvia communis)	85	80	84	84	100	86	109
Dunnock (Prunella modularis)	59	56	78	99	100	100	102
Pied Wagtail (Motacilla alba)	160	57	69	98	100	100	91
Goldfinch (Carduelis carduelis)	68	61	84	67	100	103	103
Linnet (Acanthis cannabina)	88	60	86	96	100	103	89
Bullfinch (Pyrrhula pyrrhula)	44	51	75	118	100	98	78
Chaffinch (Fringilla coelebs)	69	76	90	100	100	98	96

The method is relatively simple, demanding an ability to map read with accuracy and a sound knowledge of bird identification, song and behaviour. This last does imply that some basic ornithological biology is necessary, especially if the difficulties mentioned later are to be overcome, rather than just the ability to tell one bird from the next. The whole area to be censused is covered as fully as possible on a number of occasions (preferably more than 8) at different times of day and encounters with any male birds are accurately plotted on a large scale (6 in or 25 in) map. For most species, males holding territory and thus probably, though not inevitably, breeding, tend to keep within it, and will have a variety of song posts, or may react aggressively with a neighbour of the same species. Although not all males will be seen on every visit, if visit days are designated by letters of the alphabet, the map ultimately will show clusters of

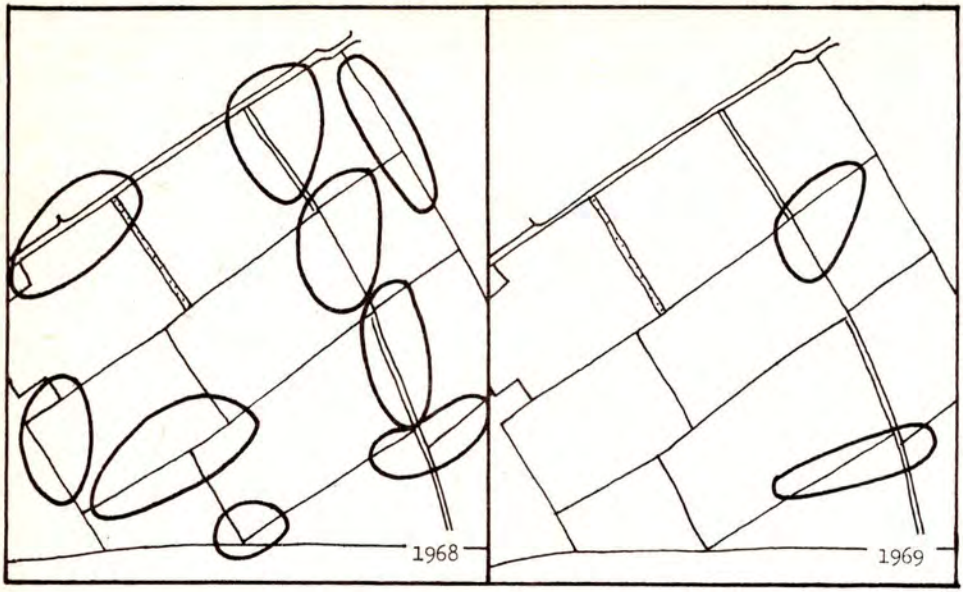

Fig. 2 WHITETHROAT TERRITORIES PENDLEY FARM, HERTS.

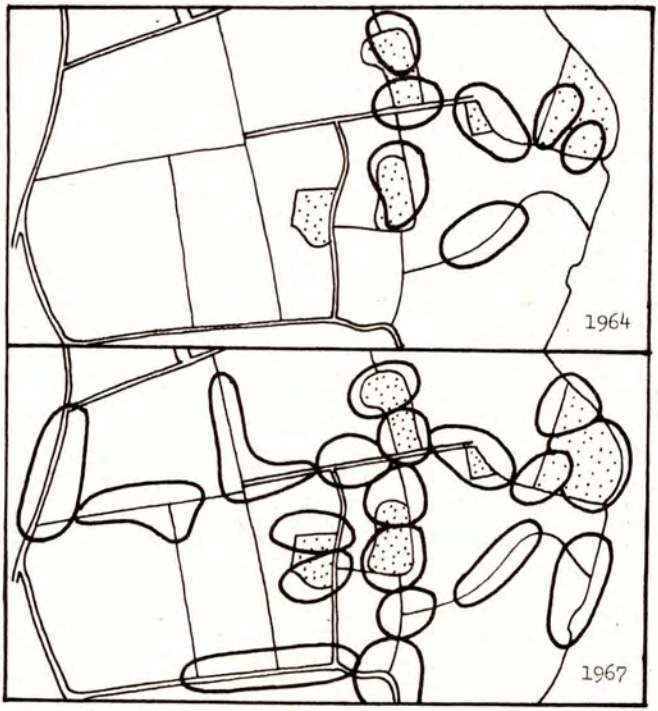

Fig. 3 WREN TERRITORIES SUFFORK FARMLAND.

records each representing the presence of a territorial bird (Fig. 1). The number of clusters will represent the number of pairs in the area. All territories may not be similarly clearly demarcated, and a variety of analytical techniques are being developed to cope with the various species. Examples of territory maps showing changes in recent years are given in Figs. 1, 2 and 3. If pursued with vigor, the technique can yield very accurate assessments of the population on a given plot and is thus well suited to assessment following the trial usage of pesticides or herbicides.

Obviously, as in all surveys not based on an absolute nest count, a number of factors contribute to the degree of error involved. The volume of song differs with season, with time of day, and greatly with weather - different species having very different reactions. The season is of course extended to cover one brood for some species, two to four broods in others, and in the latter case the territories held are often not the same for the first and subsequent broods. The conspicuousness of species varies - hence the need for a sound basic knowledge of bird behaviour patterns. The type and density of the habitat may not only make contact with the bird difficult, but also accurate plotting - in general terms farmland is moderately easy to work, woodland more difficult. Edge-effect is also of great importance - the margin between two habitat types is attractive to many species from each, and consequently the smaller the plot, or the longer its perimeter, the greater the effect due to inclusion of birds from 'outside'.

Nest counts Besides counts of conspicuously-nesting colonial species, small census plots can be searched for the nests of all species to yield, ideally, the true population. Regrettably, and predictably, there are difficulties with this technique besides those of season. While many species have fairly readily found nests e.g. thrushes (Turdidae), some do not, e.g. Robin (Erithacus rubecula), Wren (Troglodytes troglodytes), so that searching time increases, or should do. Others nest in situations such as bramble or nettles where a serious risk exists of destroying the nest accidentally while trying to find it, e.g. Whitethroat (Sylvia communis), Dunnock (Prunella modularis). Before such a method is used to estimate a population, a knowledge is necessary of the length of the breeding season and the number of broods commonly raised. Consequently for the technique to be successful, prerequisite is a detailed knowledge of the breeding biology of the species concerned. If possible changes are to be assessed during a season, the problem becomes more complex still, as losses of nests, eggs or young are varied in cause and in season, due to weather (causing food shortage, perhaps), the chance activities of predators such as Magpie (Pica pica) and Jay (Garrulus glandarius) and man, removing eggs or young. With such factors involved, it is difficult to see how an adequately controlled experimental plot could be set up. Again, a background knowledge, extended in time, of clutch size, hatching and fledging success (in short, the productivity) and variation with season before valid assessment could be made of the changes occurring during one season.

Working Document 4 This suggests that a series of counts be made at the trial site, both before and after the application of the chemical under investigation, on each occasion a total count being taken with identification to species where possible. These survey counts are to be supplemented by searches for bodies. Mr. Terry describes the performance of these surveys in the field - I will comment on the general principals behind them.

Some chemicals will not be applied, and thus should not be tested, during the breeding season. For these it is difficult to see how direct counts and searches for bodies can be bettered, but as already mentioned, it must be borne in mind that at times of the year other than the breeding season a varying amount of flocking behaviour is shown by different species, and considerable movement may occur between feeding grounds with very little provocation. Thus, if the chemical is likely to give an immediate knock-down, a search for affected birds is probably

the best means of assessing population change. If not, then counts over the treated area merely serve as a safety precaution, revealing any mortality, rather than as a monitor of population levels.

The survey area suggested, approximately 8 h with constituent sub-plots of 2 h or more, is unlikely to be large enough on farmland to produce an adequate number of birds for reasonable assessment of changes and will be very vulnerable to edge effect.

For chemicals applied during the breeding season, the same cautions apply as to the other techniques discussed as far as weather, time, season etc. are concerned. In the breeding season, on an 8 h plot, the fledging of only three or four nests, each containing four or five young could lead to a marked observed rise in population. Conversly, the natural death of a similar number of juveniles, quite possible in adverse weather conditions, could indicate a dramatic and alarming fall in the surveyed population. By the same token, any bodies found should be examined for cause of death, and the post-mortem in itself is not necessarily an easy or certain matter. A major difficulty in searching for bodies is that a search may come before any ill-effects are apparent, but if searching is too long delayed, the bodies will have been removed by one or other of the natural scavenging processes.

DISCUSSION

Perhaps the most important common feature to emerge from this consideration of methods of assessment is that any suspected population change must be viewed against a well-established, long-term, baseline indicating the naturally occurring fluctuations and their magnitude, and also subsequent population response. The examination of changes in one season in one locality cannot be undertaken without the biological and statistical safeguards of an adequate knowledge of population performance over the region as a whole. With the British Trust for Ornithology's Common Birds Census we are some way towards establishing a suitable baseline, but we are currently a long way from a knowledge of how general such population changes may be, or of how material marked local changes are to the population as a whole.

Whether those species most easily observed or censused are the ideal ones to reflect the effects of changes in the ecosystem is doubtful, as numerically common species may well be more adaptable in behaviour, food selection or physiology. This same criticism can undoubtedly be applied to the selection of laboratory test species, and certainly we need more detailed information on the reactions of a wide variety of species to environmental pollution. The ability to use relatively few indicator species would greatly simplify the techniques involved, but the current uncritical selection of a few species, often exotic, may only be misleading.

The available methods all have their disadvantages rooted in the apparent magnitude of what may be only local, or easily overcome, effects. Outside the breeding season feeding movements, weather movements or migration can remove or introduce birds to an area, and high mortality can be the result of sudden unfavourable weather conditions. In the breeding season the population is less mobile, but numbers can be dramatically increased by a series of fledging broods. Again, weather may have very marked effects on hatching and fledging success, and on the onset or finish of the breeding season. Perhaps the most important weather effects are on the behaviour patterns and consequent conspicuousness of birds: regrettably for the censuser these effects vary widely from species to species. Unlike many, the Blackbird (Turdus merula)will sing in the rain. Thus, inevitably, a series of visits is demanded, at different times of day and on differing days.

It should also be apparent that any attempt to assess population change must

consider the affected ecosystem as a whole, as the material effects of change or pollution may be indirect and possibly long-delayed. In this context weedkillers merit the attention commonly directed to pesticides, as they may remove a large, and possibly vital, element of a species' food supply - either directly, in seed-eaters, or indirectly, in eliminating the host plant of an insect upon which a bird species may largely depend. The problem of pollutant concentration through a food chain is no new one, nor are the dangers unknown of eliminating one element of such a chain. The mode of action, and possibly the means of transport of the material in the environment must also be considered: for example, waterways of all sizes may move toxic materials about, they may (through food chains) concentrate them, and they may later make them available to an unexpected victim.

Probably it is appropriate to stress at this stage the staffing problems associated with such exercises. At the British Trust for Ornithology we have the tremendous advantage of a skilled, but unpaid, network of amateur observers to provide the baseline information, a task probably otherwise impossible. Once this baseline has been achieved, and can be maintained, other studies will have far more readily interpretable results. It seems necessary that trained biologists be employed in field assessments, but the finding of nests appears to come under the heading of Art, rather than Science, and therein lies its major failing.

At the moment, the surveys recommended in Working Document 4 suffer from the failing of all census techniques in that interpretation is difficult, if not impossible, without site and species history. Prediction of long-term effects is similarly impossible. Working Document 4 surveys do form an admirable starting point, however, and given that preliminary laboratory tests efficiently screen out potentially troublesome materials, they could serve until more background information is available. With the suggested series of visits, the effects of spring - and summer - applied materials would better be assessed by a territory mapping technique than by straight counts. Although the final analysis is slightly more complex, field work is little different, and the removal of several numerical biases would be a considerable improvement.

A count of bodies would seem the only practical method available of assessing any immediate kill, but it has the attendant problems of finding the bodies before nature disposes of them, and of positively identifying the cause of the mortality. To test the efficiency of both survey and mortality assessments, it seems essential that a known toxic material is applied in the field, under strictly controlled conditions and with full safeguards. Until this is done, we are placing blind faith in an untried last-ditch defence mechanism.

Acknowledgments

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Experience in using Working Document No. 4 of the Pesticide Safety Precaution Scheme

by

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SUMMARY

Working Document No. 4, issued by the Scientific Sub-Committee on Poisonous Substances used in Agriculture gives guidance on methods to assess the short term risk to birds from pesticides under practical conditions of use. This paper describes three surveys (carried out between 1964 and 1968) following closely the suggestions laid down in the Working Document. One survey dealt with the risk to wild life of a herbicide (ioxynil) applied to cereals in the Spring and the others investigated the effect on birds feeding on grain treated with an insecticide (ethion) in the Autumn and Winter.

The limitations of the methods advocated in the Working Document are discussed but it is concluded that in each case the surveys were large enough to observe a significantly representative sample of birds and that there was unlikely to be any gross short term risk to wild life by the pesticides under test.

It is advocated that occasional surveys of the type described would be acceptable in the course of the development of a new pesticide but that any long term assessments of risks should be the subject of collaborative investigation by Government Departments or Agencies, Naturalist Organisations and manufacturers.

INTRODUCTION

Mr. D.S. Papworth in his paper in Session VI has already described the requirements for residue data in wild life investigations. He has explained the basis of Working Document No. 4 and the circumstances under which manufacturers are asked to provide data according to this protocol. Information on the toxicity and residues is obtained in the normal course of development of a new product and very often this data is sufficient on which to assess the risk to wild life. However, where there is any doubt the Scientific Sub-Committee asks the manufacturer of the potential new product to carry out surveys according to Working Document No. 4. This paper describes the operation and findings of three such surveys.

MATERIALS AND METHODS

Herbicides Survey 1964

Apart from the work described under "additional work" ioxynil was used throughout the experiment as a formulation (NPH 1300) containing ioxynil 20% and MCPA 30% (both as sodium salt) in aqueous solution. The rate of application was 1½ pints of the 50% formulation per acre (equivalent to 6 ozs. ioxynil and 9 ozs. MCPA per acre). The surveys were carried out on three pieces of woodland and adjacent fields of cereals in the area of Ongar, Essex. Two of the woodlands were about 300 yards long and 350 yards wide and third was an approximate square of 100 yards wide, of 2-3 acres in area. Approximately 10 acres of the crop surrounding each site was sprayed with the herbicide under test.

At each site two types of observations were employed :-

(a) Dawn Watches. At each site, two dawn observations were carried out, before spraying and two after. On each occasion, three or four observers, each an amateur ornithologist, started at dawn to patrol the borders of the woodland and to record the bird song by species and location. Where possible observers split into two parties on opposite sides of the woodland and then compared their observations at the end of the search.

(b) Nest surveys. At each site thorough surveys were made of the woodlands and adjacent hedgerows for new and occupied nests. The positions and development of these, i.e. the building, number of eggs, number of fledglings etc. was recorded and progress noted during the subsequent surveys. Where possible surveys were carried out two days before spraying and again, immediately after spraying. At each site after spraying thorough searches were made of the woodland surveyed area and a 50 yards strip surrounding the sprayed area for dead birds or mammals. The first search was made one to three days after spraying and except in the case of site A a further search was carried out about 10 days later.

A field of grass and clover was sprayed with a formulation of 40% ioxynil at a rate of 1½ pints in 15 gallons of water per acre equivalent to 12 ozs. of ioxynil per acre. 1½ hours after spraying, thirty three-week old pheasant chicks were put on the grass to feed. The cages were moved onto the fresh grass daily for a total of 21 days. At the end of this period the chicks were returned to their original establishment and their size and vigour compared visually with other birds reared there.

Insecticides Survey 1966 and 1967 - 68.

In both years, 'Embathion' seed dressing (67% Ethion) was used to dress Cappelle winter wheat at a rate of 2 ozs. per bushel, equivalent to 0.15% active ingredient w/w of seed. Most of this was dressed in a commercial Plantector dressing machine although 1 site of oats in the 1967 work was sown with seed treated in a simple rotary drum dresser.

In 1966 the three winter wheat sites and one winter oats site were of from 4 - 15 acres and were all in the area of Ongar, Essex. They were all in woodland areas and were spaced sufficiently apart from each other and from other ethion dressed cereal sites to avoid mixing of the feeding flocks of birds.

Work in 1967 - 68 was planned primarily to give information on the effects of wild life on birds feeding in the late winter or early spring when no alternative food sources were available. Three sites of Cappelle winter wheat dressed with ethion were selected in the Ongar area for detailed observation of birds feeding on the grain. At another 10 sites in Essex, Cambridgeshire and Suffolk and Northampton, farmers were asked to drill seed treated with ethion. These sites were visited once or twice during the early part of the year. The farmers were asked to fill in a sample pro-forma to record any observed effects on wild life in the area of the treated fields. These sites were also visited by regional pest officers of the N.A.A.S.

In both seasons the roosting areas of wood-pigeons feeding in the vicinity of the potential sites were located before drilling commenced. Jute sacking hides were constructed at the sites selected for detailed observation and placed in a position to enable observers to view the feeding habits of birds on the treated areas.

After drilling, the treated areas were observed from the hides twice a day until feeding ceased. Species and numbers of all birds visiting sites during the observation periods and when possible the peck rates and any changes in the general feeding behaviour of any birds were recorded. Searches were carried out at intervals of the treated areas and of the woodlands where pigeons and other

birds were known to roost. All corpses found were labelled and submitted for chemical analysis.

RESULTS

A considerable amount of data was obtained on all three surveys and this has already been submitted to support the clearance of the products in question. Restriction on space enables only a very small proportion of the data to be presented but it is hoped that there is sufficient to illustrate the field application of Working Document No. 4.

Herbicides Survey, 1964

The success of this type of survey depends largely upon the time actually spent in the field carrying out observations. Table 1 shows the time spent on the different types of observations at each of the three sites.

Table 1. Herbicide survey 1964

Method	Number and times of observations (in days related to spraying) (-0 means immediately preceding spraying) Site A Winter wheat	Site B Winter oats/wheat	Site C Spring barley
Dawn watch	4 (-3, -1, +3, +5)	4 (-3, -1, +1, +3)	4 (-2, -0, +2, +4)
Nest survey	3 (-0, +3, +4)	4 (-2, -0, +4, +11)	2 (+2, +13)
Search	1 (+3)	3 (+1, +4, +11)	2 (+2, +13)
Total time spent (man hours)	50	87	32

A schedule of observations was kept for each site as well as the number of each avian species, development of nests etc. Examples of the records of each type of observation for one site (Mill Lane, High Ongar) are given in Table 2. During dawn watches, a total of 20 species of song bird were recorded, besides a number of partridges, pheasants and mallard. Although in many cases, it was not possible to locate individual territories, just over 80 such territories were located at the three sites. Although the proportion of species varied from watch to watch the average number of birds per watch recorded after spraying was slightly higher than that recorded before spraying (table 3).

There was considerable difference in the number of nests discovered and their subsequent development, between the three sites. At one site , 12 nests located before spraying were all developing normally 4 days after. One of these, a blackbird's nest, had actually been covered by the ioxynil spray but developed normally. Nine more nests located after spraying also developed naturally. At the second site, not recorded here, 14 nests were located before spraying. After spraying half of these were found to be empty and it was obvious that these had been plundered. On the third site, only two nests could be observed before and after spraying; at least one was plundered, but the other developed normally after

spraying. Extensive searches of the woodlands, sprayed crops and surround areas of each site revealed only one dead blackbird and a dead woodpigeon from the total of the three sites. The pigeon had been shot and the blackbird was diseased.

In the feeding experiment, the young pheasants fed normally on the sprayed grass for three weeks and at the end of this time their development compared favourably with control birds.

Table 2 : Example of Schedule of Observations

Site A - Mill Lane, High Ongar, Map Ref. TL 56 030

Date	Time	Observation	No. of Observers	Man hours
4. 5. 64	0520-0600	Dawn watch (1st pre-spray)	3	2
6. 5. 64	0500-0545	Dawn watch (2nd pre-spray)	3	2¼
6. 5. 64	1000-0545	Search of woodland for nests.	5	11¼
7. 5. 64	A.M.	Adjacent crop sprayed with NPH 1300 at rate of 1⅓ pints in 20 gals water per acre = 6 oz. ioxynil and 9 oz. MCPA per acre		
10. 5. 64	0445-0615	Dawn watch (1st post-spray)	3	4⅓
10. 5. 64	1000-1215	Search of sprayed area, woodland, and hedgerows.	7	15¾
11. 5. 64	1400-1700	Examination of known nests (including extension of area of search).	2	6
12. 5. 64	0445-0615	Dawn watch (2nd post-spray)	5*	7⅓
				49¼

* Including Mr. D.D.B. Summers of M.A.F.F. and Mr. J.D. Norris of M.A.F.F.

Table 3, Herbicide Survey, Total number of birds observed at observation on three sites

	A	B	C	Mean
1st pre spray	23	35	28	Before spraying 33
2nd pre spray	37	44	31	
1st post spray	31	43	30	After spraying 35
2nd post spray	38	44	25	

Insecticide surveys

Both surveys were concerned with assessing the effect on wild life of a cereal seed dressing containing ethion. This dressing was developed primarily for control of wheat bulb fly. Farmers with this problem usually drill in early Autumn, whether they have treated the seed with a specific insecticide or not. Hence in 1966, our observations were carried out in October and November. Any feeding on treated seed that did occur was generally light and intermittent,

mainly because pigeons and other birds preferred alternative and more easily obtainable sources of food at this time of year, e.g. acorns, weed-seeds, and spilt grain on stubble. The fact that there is normally little feeding at this time was perhaps sufficient to show that the risk to wild life from eating treated seed under these conditions was negligible. However, it was considered that the case was "non-proven" and that the product should be submitted to a stronger challenge at the time when there was less alternative food available. A further larger scale trial was therefore set up in 1967 - 68 on a number of sites where ethion treated seed had been drilled in December and January, at the time when the birds would more likely be forced to feed on the treated seed. The comparison of the feeding times of wood pigeons for the two years (Table 4) shows that the feeding in the Autumn/Winter of 1967 - 68 was considerably greater.

One object of the observation at feeding sites was to see if there was any immediate change in behaviour of birds or mammals feeding on the treated grain. One way of obtaining the quantitative information on such changes is to study the peck rate of birds. Peck rate was usually constant for species throughout the whole of the survey for each year. Peck rates of wood pigeon in 1967 - 68 was generally higher than in 1966. This was probably because they confined their feeding to the treated fields and as the supply of grain in the centre of the field was exhausted, they tended to move outwards to the edges where bad drilling had exposed seed at the end of the rows.

Table 4 : Insecticide surveys

Comparison of feeding times of wood pigeons

in two seasons

(Bird minutes x 10^{-1})

Nov. 1967					Jan-Mar. 1968			
Days after drilling	Site No. 1	2	3	4	Days after drilling	Site No. 1	2	3
1	0	0	0	2	1	0	0	0
3	3	0	0	2	2	0	0	56
5	8	0	7	0	3	1	35	242
7	3	20	46	0	4	169	56	641
9	4	0	3	0	5	35	282	803
11	0	2	0	0	6	0	108	2052
13	8	784	0	0	7	5	191	856
15	16	1013	0	0	8	0	240	741
17	1	0	0	0	9	0	0	1990
19	38	32	0	0	10	0	0	0
21	46	0	0	0	-	-	-	-

		1967	1968
Total time spent on observation at each site. (Man hours)	Site 1	75	20
	2	100	23
	3	45	50
	4	30	-

Very few corpses were found as a result of searches in either year. In 1966 one wood pigeon was found partly buried under leaves and another mauled by a dog, but post mortem examination showed that neither had been feeding recently on the treated grain. In the following year there were more sites, including a

detailed observation site and farm sites further afield but in all, only five birds were found. The occurrence of dead birds is recorded in Table 5, and of these it was concluded that two were shot and three died of causes other than those related to the feeding of treated seed. In all cases the corpses were submitted for chemical analysis which revealed that none had levels of ethion in their bodies which could have contributed to the death.

Table 5 : Insecticide survey 1968, Record of searches

Specimen	Site No.	Days after drilling	Comments
Woodpigeon	1	20	
"	1	20	Shot
Woodmouse	2	1	Predator damage
Woodpigeon	2	4	Caught by dog near site.
Pheasant	4	16	Old, pre-drilling corpse
Woodpigeon	5	11	
"	5	31	Shot
"	5	31	
"	6	3	
"	6	3	
"	8	7	

DISCUSSION

In all three surveys we have attempted to follow the advice given in Working Document No. 4, but it should be made quite clear that we received considerable assistance in the interpretation of the document by members of the Ministry, particularly Dr. Murton, Mr. E.N. Wright and members of the Infestation Control Laboratory.

Organisation. The success of this type of work depends largely on the amount of time that can be spent on observations at the sites. Autumn and winter observations on the insecticide trials were generally easier to carry out because this was a time when there were less urgent calls on the Trials staff. The herbicide survey, however, was done in the middle of a busy spraying season when many trials were in progress on other aspects of the activity of the product under test. It was therefore necessary to work out a careful schedule of priorities and in fact the three sites were treated approximately at weekly intervals so that our technical manpower resources could be spread over a number of projects at the same time. Birdwatching and nest surveying requires some specialist knowledge and apart from having the advice of Mr. E.N. Wright and other Ministry ornithologists we were lucky enough to have two or three amateur ornithologists on our own staff.

The amateurs were involved in all the surveys, assisted by other members of the staff, and it was interesting to see how quickly members previously unfamiliar

with bird calls and visual identification of species began to recognise the species in the locality of the sites. There were no particular hardships involved and apart from the early rising for the dawn watches (although even this has its compensations on a May morning) the projects did not involve any more labour or skill than could normally be expected of an Agricultural research unit.

Significance of results. In the herbicides survey an average of over 30 birds was observed at each watch which was considered normal population for the type of territory studied at the particular time of the year. The average number recorded after spraying in adjacent fields was slightly more than before spraying showing that there was no significant change in populations over the period of the survey.

The significance of the nest surveys is rather more difficult to establish. The work was interesting in that it revealed the extent of hazards to which nests and broods are subjected in the natural state. On one site all nests examined before spraying were developing normally four days after, whereas plundering or destruction of nests on other sites seriously reduced the numbers and species on which pre- and post-spray observations could be made. On one site a blackbird's nest actually received the direct spray while the female was sitting on the nest. This apparently did not affect the bird and the eggs and fledglings developed quite normally. Although all the nests could not be observed throughout the whole period of trial, on two sites there were sufficient nests which were not disturbed and it can be concluded that no deleterious affect on the nests, adults, eggs or young could be related to the spraying of the surrounding crops.

These subjective results should also be considered in the light of actual residues of herbicides present in the crop during the period of the survey. In separate residue studies it has been shown that the levels of ioxynil fall to within 1 p.p.m. within 15 days of spraying at the normal dose of 6 - 9 ozs. ai/acre. The sprayed crop is unlikely to affect many birds but certain species including pheasants and pigeons may actually graze on the crop and it is possible that non-grazing birds could take in significant quantities of herbicide if they drank contaminated rain or dew in the crop.

One would not expect that these levels of residue would be toxic to birds and the fact that virtually no dead birds were found in the searches tends to confirm this view.

One of the most interesting revelations of the insecticides study was evidence on the extent of feeding by grain eating species in the two periods of the surveys, autumn and mid-winter. The comparison of the feeding times in tables 4 clearly shows that the birds are more likely to feed on drilled grain in winter when there were fewer sources of other food available.

The study of the peck rates indicated that there was apparently no change in the metabolism of birds feeding on the sites during the period of the surveys. Although some shot pigeons were found to have treated grain in their crops there was nothing to indicate that similar birds which were found dead in the neighbourhood of the trials had in fact died as a direct result of the insecticide dressing.

Limitations of the work. The surveys have shown that by following the suggestions laid down in Working Document No. 4, it is possible to study a representative sample of fauna in a limited area and the samples studied can be large enough on which to assess possible effects of the pesticide. Inevitably, with this type of survey, the results tend to be negative in that the object of them is to demonstrate "no effect". The preliminary routine toxicological, animal metabolism and residue work on any new pesticide can be used to establish the probable risk to wild life in practical use. The results of surveys should be considered in the light of these laboratory investigations and obviously, if laboratory work suggests any serious risk, it is likely that there would be parallel hazards to consumers

and users of the product and there would be doubt whether the material would be developed at all. Such surveys carried out by Manufacturers can only serve to demonstrate the risk to wild life in a limited area, and further long term studies should be more the responsibility of Government agencies, e.g. The Nature Conservancy or N.A.A.S., although working closely in co-operation with the manufacturers.

ACKNOWLEDGEMENTS

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EVALUATING RISKS OF PESTICIDES TO FISH

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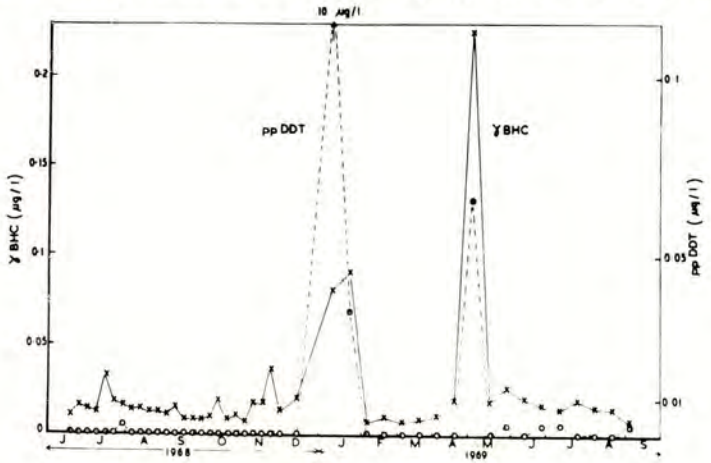
Summary General requirements for measuring the toxicity of pesticides to fish are outlined, together with the standard test developed by the Ministry of Agriculture, Fisheries and Food. Tests are discussed in relation to their usefulness for screening purposes and for predicting the effect of pesticides on fisheries under field conditions. Experiments on the uptake of organo-chlorine pesticides by fish are described.

INTRODUCTION

The most obvious risks to fish from pesticides arise from their misuse in agriculture and in river management; careless disposal and accidental spillage into fishing waters of surplus material and washings from spraying equipment and also direct spraying of water, including that arising from spray drift, all tend to produce sudden relatively high concentrations of pesticide in the water, sometimes capable of killing fish rapidly. The further contamination that may arise from surface run-off, sub-surface land drainage and even rainfall itself, would tend to maintain very much lower concentrations for relatively long periods. For some pesticides, however, more important sources of continuous low-level pollution of rivers would be domestic sewage effluents and, in some areas, industrial and trade wastes from processes that manufactured or used pesticides and discharged either direct to rivers or to sewage-treatment works. We must therefore envisage a situation in many of our rivers where concentrations of pesticides would be similar to those illustrated in Fig. 1 which shows concentrations of γ-BHC and DDT found in a river recently studied by the Water Pollution Research Laboratory; superimposed on low background levels would be small peaks at times of spraying and presumably much larger increases when accidents occurred, though the actual levels found would depend upon the properties of the pesticide concerned, its usage, and the characteristics of the catchment area. It has also to be borne in mind that many rivers carry other pollutants, the adverse effects of which, even at sub-lethal concentrations, are often likely to be at least additive (Brown, 1968).

Fig. 1 Concentrations of some organo-chlorine pesticides in the R. Roding, Essex



The potential risk to fisheries from such a situation has to be evaluated from a combination of field and laboratory experiments and observations. Laboratory tests can fairly easily define levels at which fish are likely to be killed under given conditions relevant to those in a natural aquatic environment, and this will determine what warnings should be issued to users and, in conjunction with chemical analyses of the water, will help diagnose the cause of fish kills. But such tests can demonstrate survival of fish only for the period they are continued, which for practical and economic reasons is relatively short compared with the life-span of a fish. Though the relation between time of survival and concentration of pesticide is often such that within a test period of a few days a threshold concentration is indicated below which fish are unlikely to be killed even after exposure for several months or even years, this is not always found. Furthermore a 'safe' concentration at which a fishery would thrive in a contaminated stream cannot be estimated unless additional allowance is made for sub-lethal, ecologically significant, adverse effects, such as reduced growth and fecundity of fish, and alterations in normal fish behaviour, including avoidance of low concentrations of pesticide, for which other tests may be necessary.

SHORT-TERM TOXICITY TESTS

General requirements

For screening purposes it is desirable to adopt a standard test giving reliable and consistent results relevant to effects on fish in the wild. Adequate sensitivity necessitates the use of a species such as the trout which is highly susceptible to poisons; reasonably high precision requires a sufficiently large number of fish; simplicity must be reconciled with the need to control environmental factors, such as temperature, water hardness, pH value, and dissolved oxygen, which can affect the toxicity of poisons to fish. However limitations attending standardization of the test conditions may call for other toxicity tests in which, for example, the dilution water and species of fish are appropriate to the river concerned, various temperatures are used, and the duration of the test is extended if necessary.

An arbitrary criterion of toxicity, such as the concentration of pesticide at which 50 per cent of the fish are killed at a given time (the median lethal concentration, or LC-50), can be adopted for the standard screening test, but the period chosen should be long enough to avoid anomalous results from the initial disturbance and diuresis of newly-handled fish and to include the time when toxicity changes least with change in exposure time, which for many poisons is within one or two days.

With these general considerations in mind the Ministry of Agriculture, Fisheries and Food has developed a standard method, capable of adaptation for some of the additional tests necessary for predicting concentrations of materials tolerable by fish in rivers (Alabaster and Abram, 1964).

Environment control during tests

In the simplest case, using fish in fixed volumes of a range of concentrations of poison, considerable changes in water quality can occur over a few days, or even hours. Respiration of the fish may reduce concentrations of dissolved oxygen to asphyxial levels and also increase concentrations of carbon dioxide sufficiently to affect the toxicity of other substances present, and in addition fish excrete ammonia which is itself toxic; on the other hand they may absorb a significant proportion of some poisons present, particularly those lethal at very low concentrations, and thus reduce the toxicity of the test solutions. Other changes may occur, including adsorption, precipitation, hydrolysis, oxidation, or biochemical degradation of the poisons and biochemical depletion of oxygen.

Some of these could be prevented by aerating the solutions, though the resultant loss of volatile material could cause an under-estimation of toxicity, especially

important for ponds and sluggish streams in which rates of aeration are likely to be much lower than in the tests. It is better to dispense with aeration and replace the test solution: a rate of replacement (containing dissolved oxygen) sufficient to meet the respiratory demands of the fish (assuming a reduction in concentration as it flows through the test tank of no more than 2 mg/l) is adequate to prevent not only loss of volatile materials but also a deleterious build-up of ammonia and carbon dioxide and a significant reduction of poison through absorption by the fish.

Choice of fish

Taking a respiratory demand of 0.16 mg O_2/g h for rainbow trout of an average weight of 20 g, the replacement of fresh solution for a batch of 10 fish would amount to about 768 l (168 gal) over two days, which seems far too high for a practical routine test. Using a smaller fish, like the harlequin (Rasbora heteromorpha) the respiratory need of which is about 0.46 mg O_2/g h for fish weighing about 0.14 g the volume is reduced to about 15.4 l (3.4 gal).

Harlequin fish have been chosen because they are not only small in size even when mature, but are also tolerant of summer temperatures in the UK and generally are more similar to the trout in their sensitivity to a number of poisons than many other similar-sized tropical species. Guppies (Lebistes reticulata), for example, were more resistant than harlequins to 9 out of 13 chemicals recently tested, their time of survival being up to tenfold longer and their 48-h LC-50 up to sixfold higher and on average twofold higher than that of harlequins.

Newly-hatched salmon and trout alevins have also been successfully used for some routine tests (Alabaster, 1969), and by the Water Pollution Research Laboratory, which has partly overcome the limited availability of small fish by importing the eggs of rainbow trout from New Zealand in the autumn. Further extension of the period when small trout can be used might be possible by holding fertilized eggs close to 0°C to delay hatching, provided it were then shown that such treatment did not markedly alter the resistance of the fish to poisons; the Ministry is pursuing this line of research.

The standard apparatus used by the Ministry of Agriculture, Fisheries and Food

In the Ministry test the fish are used in batches of 10 in 500-ml flasks, each of which is linked to a dosing unit which supplies freshly prepared test solution at the rate of 100 ml every 10 minutes. Though fairly complex, the compactness of the apparatus allows many units to be housed in a small space. Furthermore, because water requirements are small, large stocks of fish can be held easily in the laboratory (normally 20 fish/l), and the preparation of standard dilution water and the transport of natural river water to the laboratory for test or acclimation purposes is facilitated.

Full details of the apparatus and the test procedure, including the recipe for the standard dilution water, have been published in Working Document No. 5 of the Pesticides Safety Precautions Scheme published by the Ministry of Agriculture, Fisheries and Food.

Method of expressing results

In the Ministry test individual periods of fish survival are often observed in each test concentration, the median period of survival then estimated graphically or calculated, and the medians plotted against the concentrations tested, using logarithmic scales, as illustrated in Fig. 2. The best estimate of a lethal concentration at a particular time is given by interpolation on a curve fitted to the points, a line fitted by eye being satisfactory in practice. Simpler methods make use only of observations at the time for which the median is to be estimated, i.e. the percentage dead in several different concentrations, from which the concentration corresponding to 50 per cent kill can be estimated graphically either

by the probit method (Bliss, 1937) or by simply using arithmetic scales for percentage dead and concentration, and drawing either a sigmoid curve by eye through the points or merely a straight line between the two points bracketing 50 per cent kill.

The simpler the method used the more liable it is to be influenced by inherent variability in the resistance of the fish, as illustrated by an analysis of results obtained with 10 different samples in which batches of 10 fish were used in each test solution, and adjacent concentrations in a series for a given sample never differed by more than twofold. The 48-h LC-50, expressed as a percentage of the result obtained by the Ministry method was 103 ± 24, 105 ± 16, and 102 ± 47, using the probit method, fitting a sigmoid curve, and fitting a straight line respectively. Such variability could be reduced by increasing the number of fish used in each batch and by having the value of adjacent concentrations closer together.

Comparison of Ministry method with other tests

American methods The American Public Health Association standard methods (APHA, 1965) recommended a rate of replacement of test solution of at least 1 l/g fish d. Reviewing the literature on methods of toxicity testing Sprague (in press) concludes that solutions should be replaced at a rate adequate for the respiration of the fish, namely, for trout at 2-3 l/g d and for small tropical fish like the harlequin at perhaps 10 l/g d, the rate adopted in the Ministry method, and it seems that the forthcoming revision of the APHA methods will recommend rates on the basis of the respiration rate for trout. The time in which the flow of replacement solution should equal the volume of the test vessel is suggested as 3-5 h by Sprague (in press), will be recommended as a maximum of 6 h in the revised APHA methods, and is less than 1 h in the Ministry method.

Carter 'bottle test' Because the usual toxicity testing procedures require the use of both a large number of fish and fairly complicated apparatus, and also have to be continued for several days, attention has been given by the Ministry to a simpler procedure developed by Carter (1962), in which two brown trout are placed in 2-l bottles which are completely filled with dilutions of test solution and then sealed. Death of the fish results within a few hours from a combination of asphyxiation and poisoning; the greater the effect of the poison the less is the reduction of oxygen concentration in the bottle. The toxicity of the material is then gauged by the residual concentration of dissolved oxygen in each concentration.

Preliminary work showed that results with 3 harlequins placed in 50-ml bottles would be roughly comparable with those of the Carter test using trout, and therefore harlequins were later used both in 50-ml bottles and in the standard Ministry apparatus with samples of 16 pesticides. The concentrations of pesticide at which the reduction in dissolved oxygen concentration was 50, 80, and 98 per cent of that in the controls were calculated and compared with the 24- and 48-h median lethal concentrations. No constant relation was found between results from the two tests; thus, the concentration corresponding to a 98 per cent reduction in dissolved oxygen ranged from about one-third to over tenfold the 48-h median, suggesting that the sealed-bottle test is much less suitable than present alternative testing procedures.

Delayed lethal effects

Measurements of toxicity are generally carried out with fish kept continuously in the toxic solutions until they overturn and die. With some poisons, like cyanide, overturned fish recover quickly if transferred to clean water; with others, irreversible toxic effects may occur long before death, for example, exposure of the fish for 30 minutes to a concentration of a formulation containing diquat which would have killed them in about 8 hours continuous exposure resulted in their death a week later. Mortality occurred sooner with exposures for longer than 30 minutes, there being a fairly regular relation between survival time and exposure time at

this concentration. Similar effects have been found for other concentrations and for other poisons. It is important to know whether such effects are found with other pesticides, especially, for example, aquatic herbicides, for then precautions could be taken to prevent fish being exposed to very high concentrations even for short periods in waters being treated, and too much reliance would not be placed on short-term tests.

Effect of formulation

For the purposes of the Pesticides Safety Precautions Scheme clearance for a pesticide is given for the active ingredient, but toxicity is greatly influenced by both the type of formulation - wettable powders are often less toxic to fish than emulsifiable concentrates - and the nature of additives present, many of which are themselves toxic. For example, the 24-h LC-50 in soft water of two formulations containing the same amount of diquat was 430 and 64 mg/l respectively, and was not predictable from the toxicity of all constituents. The LC-50 in hard water was not predictable either, for in the first case it was increased to 840 mg/l and in the second reduced to 23 mg/l.

SUB-LETHAL AND LONG-TERM EFFECTS

Relation between laboratory results and effects on fisheries

At a site where effluent from a pesticide manufacturing plant discharged to an otherwise unpolluted stretch of river containing trout a field study was carried out to relate the results of a standard toxicity test to the survival of fish in the river. Samples of effluent were taken for testing over a period of a few months, and additional on-site checks were made using batches of trout kept in cages in undiluted effluent. Maximum concentrations of effluent in the river, based on the minimum dilution observed during the study, were calculated as a fraction of the 48-h LC-50 for harlequin fish and averaged 0.06 (range 0.02-0.16). Dissolved oxygen in the river was adequate to support fish, and trout were known to thrive above and below the point of discharge. Whether this fishery would still have flourished had the average toxicity in the river and the fluctuations in quality been higher than observed during the field survey is not known. Nevertheless the results provide a rough guide to the maximum fractions of the 48-h LC-50 for harlequins that are not inimical to a trout fishery.

The few other studies of this kind that have been carried out suggest that fisheries may be adversely affected if the fraction of the 48-h LC-50 is 0.1 to 0.4, depending on the particular pollutant, but some laboratory studies, for example that of Mount and Stephan (1967), suggest that at much lower fractions of the 48-h LC-50 sexual maturation and fecundity may be adversely affected. Whether these could be ecologically signifcant however is not yet known. Until the necessary field observations have been made a great deal of reliance will necessarily have to be placed on long-term laboratory studies.

Fish behaviour

One study suggests that avoidance reactions might determine whether or not fish are present in a river polluted by copper and zinc (Sprague et al, 1965). If this behaviour occurred with pesticides it seems likely, on the basis of laboratory tests with various poisons, that the fraction of the 48-h LC-50 at which avoidance occurred would vary with different materials (Sprague and Drury, 1968).

Effect on fish-food organisms

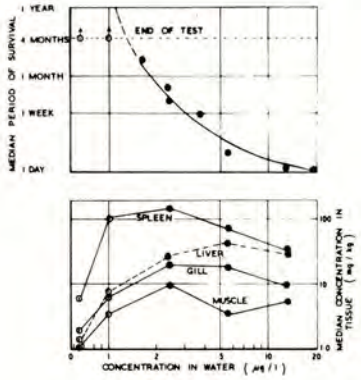
The main risk to fisheries from pesticides may derive mainly from direct effects on fish, but toxicity to fish-food organisms may also be important. The Ministry apparatus has been modified by dividing the test flask horizontally with a piece of

gauze to enable fish and invertebrates to be separated while being tested simultaneously in the same test solutions. Results obtained with several herbicides show that Gammarus, Asellus, Daphnia, and Tubifex are in general similar to harlequins in their sensitivity or somewhat more resistant (Alabaster, 1967). Fish-food organisms may prove more susceptible to insecticides; on the other hand natural populations of some species may be more resilient than those of fish and although, for example, sensitivie organisms like caddis flies may take several years to re-establish in streams sprayed with DDT, the survival of more resistant species could ensure an adequate food supply for fish. Poisoned invertebrates may contain sufficient residues of pesticide to affect their predators, but the little experimental evidence available suggests that fish are unlikely to take up as much pesticide from eating live poisoned food organisms than they are from the water itself to which the food organism had been exposed. Algae can concentrate some pesticides to a high degree; this might also lead to the uptake of residues by fish.

Long-term toxicity tests and accumulation of residues

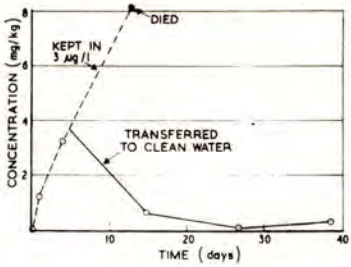
For some pesticides, for example fluoracetamide and organo-mercury and organo-chlorine compounds, it may be desirable to carry out long-term tests to make more precise estimates of threshold concentration for fish survival or to measure the uptake of residues by fish. To obtain sufficient material for chemical analysis large fish may have to be used, for which appropriately large volumes of solution are required. Apparatus designed for such tests (Abram, 1960) has been used successfully for several materials, including dieldrin, results for which with rainbow trout are shown in Fig. 2. Tests had to be continued for several months to indicate the median threshold concentration - approximately 1 µg/l. Chemical analyses of fish killed during the tests and of those surviving the test period show a significantly higher concentration of dieldrin in the tissue of fish at 1 µg/l and above than in the batch surviving at 0.65 µg/l. Furthermore, the reduced net uptake of dieldrin, particularly in the liver, gill, and muscle, at the two lowest concentrations tested, suggests either a reduced rate of uptake or, more probably, a mechanism for excreting or metabolizing the residues.

Fig. 2 Survival of rainbow trout and concentration of dieldrin in their tissues in solutions containing dieldrin



An experiment designed to examine the loading and unloading of dieldrin by fish under constant-flow conditions is illustrated in Fig. 3.

Fig. 3 Uptake and loss of dieldrin in the muscle of rainbow trout during exposure to a solution containing 3 μg/l and after transfer to clean water



Six batches of rainbow trout were analysed after exposure to a concentration of 3 μg/l dieldrin; one batch was removed and killed for analysis after 1 day, another after 4 days, and three more were transferred to clean water after 5 days, while the last was kept in the test solution until all fish had died. At intervals those transferred to clean water were also removed and killed for analysis. A rapid uptake and loss of dieldrin is evident and similar results were obtained for analyses of viscera and for lower concentrations of dieldrin.

Results such as these offer a basis, not only for defining threshold concentration for survival but for a tentative diagnosis of the cause of a fish kill based on tissue analysis and also for predicting the likely effect on fish survival of concentrations found in natural waters. In the case of the River Roding for example (Fig. 1), the concentration of both DDT and dieldrin was generally below 0.005 μg/l, but in one sample taken during January 1969 was 10 and 0.8 μg/l respectively. No fish kill was reported and therefore it seems likely that the peak concentration did not continue long enough to kill fish. Probably the interval since then has been long enough to allow residues of pesticide in the tissues to return close to values at equilibrium with normal background aqueous concentrations; a sample of fish taken in March had residue of DDT of approximately 0.2 ng/kg.

PRACTICAL EVALUATION OF RISKS

The practical evaluation of the risks to fish from pesticides must continue to be made from routine short-term standard toxicity tests such as that recommended in the Pesticides Safety Precautions Scheme, supplemented where special circumstances demand by tests under different environmental conditions, for longer test periods, with different species of fish, different stages in the life cycle, and also perhaps using invertebrates and plants.

Additionally cadavers from such tests could be analysed to provide information on the accumulation of residues that might be significant to the fish themselves and their predators, including man. It would be useful, however, to include other tests in which fish that had survived sub-lethal exposure were kept for a period in clean water to see whether or not they continued to survive and lost their body residues as well as any associated taint.

Some field observations, even of short-term duration, would be desirable to check that a method of pesticide usage judged to be safe for fish caused no mortality or lasting accumulation of toxic residues in fish tissues. This might be most easily achieved by relying on captive fish introduced to the field site either in tanks or in cages in the nearby natural waters, care being taken to run controls, acclimate the fish to the test condition beforehand (for, say, 24 hours), and maintain the dissolved oxygen concentration close to the air saturation value and temperature below 20°C.

When short-term toxicity tests fail to provide a clear indication of a median threshold concentration for survival, consideration should be given to the need for determining this parameter by longer term tests. By carrying out such tests over a breeding cycle with populations of small-sized fish species some indication of the possible effects on behaviour, growth, maturation, fecundity, and survival of eggs and fry can be obtained; however the results should be applied to other species with caution.

Further indications of possible adverse effects of pesticides on fish may be obtained in many other ways, for example by studying their effect on the toxicity of other poisons, on behaviour (for example, temperature selection), on learning ability and memory, on physiological functions (for example, respiration), on blood chemistry, on morphology and histology, and on in vitro enzyme activity and cell division. Many of the techniques used for such studies are attractive because they have the considerable advantage of being quick to carry out and are of value diagnostically, and also to indicate the direction of the most rewarding longer term direct experimental approaches using fish. On the other hand, the effects they reveal are often difficult to relate to the likely effects of pesticides on natural fish populations.

Ultimately long-term field observations on natural fish populations are essential for evaluating the hazards of pesticides to fish, and they are perhaps best undertaken by enlisting the aid of specialists in Government agencies, River Authorities, and universities.

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LABORATORY AND FIELD ASSESSMENT OF PESTICIDE POISONING OF HONEYBEES (APIS MELLIFERA)

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Summary The significance of laboratory tests of the toxicity of pesticides to honeybees, which are an essential first step in assessing possible hazards to bees, are discussed. When the pesticide will be used where the risk of killing bees is particularly great, for example when spraying a flowering crop, a more complicated field trial of the formulated material will be necessary and a method of doing this is suggested. The risk to honeybees of a particular pesticide may be greatly affected by the type of formulation used and the time and method of application.

INTRODUCTION

The honeybee (Apis mellifera L.) is beneficial as a pollinator and as a source of honey. Colonies are often taken to flowering crops to improve pollination and this may increase the risk of poisoning if pesticide application programmes are not well organised and properly timed. The risk will depend on variables which can be assessed only in the field, including the type of formulation of the pesticide, the method and time of application, whether the crop is flowering, and the weather; but an essential first step in assessing the potential hazard to honeybees of using a pesticide is to determine its toxicity in the laboratory. The methods used in the laboratory and field are summarised by Anderson and Atkins (1968).

LABORATORY TESTS

Acute Contact Toxicity

Contact toxicity in the laboratory can be determined by spraying anaesthetised bees (e.g. Glynne Jones and Connell, 1954; Palmer-Jones, 1965; Twinn, 1967), by subjecting caged honeybees to mist sprays (e.g. Johansen, 1961), by exposing the insects on a treated surface (e.g. Glynne Jones and Connell, 1954; Beran and Neururer, 1956; Palmer-Jones, 1965) or by applying measured drops of a solution of the pesticide to the insect surface (e.g. Beran and Neururer, 1956; Stevenson, 1968). Application of measured drops is preferred because it is the only method in which the precise dose received by the insect is known. Also spray apparatus in different laboratories may not be sufficiently standardised to give comparable results. The Ministry of Agriculture, Fisheries and Food therefore hopes that the measured drop procedure (Stevenson,1968) will become standard in this country for comparing contact toxicities. Results determined by this method for a number of pesticides are given in Table 1.

Table 1

Acute contact toxicity of some pesticides to worker honeybees determined in the laboratory during 1964 and 1965. The table gives mean median lethal doses (LD50) expressed as µg compound per insect, mean slopes of regression lines, the number of regression lines (n) used to obtain each mean, and LD90 values derived from these means (Stevenson, 1968) One µg/bee is equivalent to 10-15 mg/kg

	1964				1965			
	n	Mean LD50 µg	LD90 µg	Mean Slope	n	Mean LD50 µg	LD90 µg	Mean slope
Mevinphos	6	0.070	0.10	7.3				
"Bidrin"	1	0.076	0.10	9.6				
Dimethoate	3	0.12	0.17	8.4	9	0.11	0.14	11
Dieldrin	8	0.16	0.23	7.6	6	0.16	0.26	6.0
Diazinon	2	0.22	0.30	9.4				
Malathion	2	0.27	0.38	8.5	3	0.22	0.32	8.1
Pyrethrins	4	0.29	0.45	6.6	4	0.13	0.20	4.4
Phorate	3	0.32	0.42	11				
BHC	3	0.46	0.68	7.4	6	0.20	0.33	5.8
Demeton-methyl	3	0.74	0.90	15	1	0.41	0.52	12
Endrin					3	1.2	2.1	4.9
Carbaryl	2	1.3	7.4	1.7	1	1.1	1.5	0.96
Chlordane	3	1.4	1.9	10				
Allethrin	4	3.4	4.6	9.7				
DDT	3	3.9	6.2	6.4				
Disulfoton	3	4.1	5.9	8.1	4	4.3	8.0	4.7
Menazon	3	4.3	8.1	3.0				
Endosulfan	4	7.1	13	4.6				
Ethyl mercury chloride	2	22	43	4.4				
Standard deviation		27%		23%		21%		24%

Acute Oral Toxicity

Oral toxicity was determined by feeding individual worker honeybees with the pesticide dissolved in sugar syrup (Glynne Jones and Connell, 1954; Beran and Neururer, 1956) or by feeding the bees in groups (Stevenson, 1968; Twinn, 1967). Group feeding is preferred because it is quicker, and again the Ministry of Agriculture hopes that the method of Stevenson (1968) will become a standard procedure in this country.

Worker honeybees caged in groups of ten are given the insecticide dissolved or suspended in a solution of 20% sucrose and 5% acetone in water. It is assumed that they share the dose of 0.2 ml solution (i.e. 20 µl per bee) equally (Free, 1959; Nixon and Ribbands, 1953). Some results obtained with this method are given in Table 2.

Significance of Laboratory Tests

Simple tests of oral and contact toxicity of unformulated samples should be made for all pesticides. Median lethal doses (LD50) of 25 µg per bee or

Table 2

Acute oral toxicity of some pesticides to worker honeybees determined in the laboratory during 1964 and 1965. The table gives mean median lethal doses (LD50) expressed as µg compound per insect, mean slopes of regression lines, the number of regression lines (n) used to obtain each mean, and LD90 values derived from these means (Stevenson, 1968) One µg/bee is equivalent to 10-15 mg/kg

	1964				1965			
	n	Mean LD50 µg	LD90 µg	Mean slope	n	Mean LD50 µg	LD90 µg	Mean slope
Mevinphos	3	0.027	0.057	3.9				
"Bidrin"	3	0.068	0.20	2.7				
Dimethoate					8	0.15	0.31	4.0
Carbaryl	2	0.14	0.26	4.8	2	0.11	0.39	2.3
Pyrethrins	2	0.15	0.61	2.1	2	0.15	0.41	2.9
Diazinon	2	0.20	0.68	2.4				
Dieldrin	6	0.32	0.66	4.1	3	0.33	0.94	2.8
Malathion	3	0.38	0.88	3.5				
Phorate	4	0.44	0.90	4.1				
BHC	1	0.45	2.5	1.7	1	0.76	2.7	2.3
Menazon	2	0.46	0.77	5.7				
Demeton methyl	3	0.61	1.0	5.5	2	0.83	1.8	3.9
Endrin					2	1.4	2.8	4.2
DDT	5	3.7	7.5	4.2				
Endosulfan	3	6.9	43	1.6				
Allethrin	2	9.1	20	3.8	3	4.6	10	3.8
Phenyl mercury acetate	2	10	32	2.5				
Ethyl mercury chloride	1	13	18	9.2				
Disulfoton	2	16	38	3.4	1	23	41	5.0
Standard deviation		35%		55%		22%		33%

more, obtained, for example, for many herbicides and fungicides in the laboratory, implies that poisoning during, or after, use in the field is unlikely, and field trials would not be necessary.

If a non-persistent compound (e.g. malathion) has a high acute toxicity it could be applied to a crop a few days before flowering, when bees are not present, and again a field trial would not be necessary.

A non-persistent toxic compound could also be applied to a flowering crop attractive to bees, provided they are not foraging at the time of application, but this practice is best avoided. The pesticide might be applied early or late in the day, or in poor weather when the bees are not flying, or the beekeeper may keep his hive shut during the treatment. A field trial would be necessary to show that the procedure was really safe.

Persistent compounds with high or moderate acute toxicity might be more dangerous than highly toxic non-persistent compounds with similar toxicity, because bees might pick up the poison some time after application, and a pre-flowering treatment might persist into the flowering period when honeybees are visiting

the crop. For such compounds, a field trial of each proposed use, when bees are at risk, is necessary.

The systemic organophosphate insecticides present a special case. While they may not persist on the plant surface and can be applied before flowering, they may persist within the plant. When a systemic insecticide also has contact activity there is a danger to bees at the time of application and if it is persistent there will be danger after application and the arguments already put forward for persistent and non-persistent chemicals would apply. However when field beans are sprayed, the danger to honeybees seems to be almost entirely at the time of application, although the insecticide may persist as an aphicide for several weeks. There is another danger with systemic insecticides, that the compound or a metabolite may render nectar toxic and if laboratory tests show this to be so, careful observations must be made in the field.

The results in Tables 1 and 2 are probit calculations (Finney, 1952) of regression lines, obtained by treating duplicate lots of ten bees with each of five or six concentrations of poison. The standard errors of the estimated average LD50s depend on the variation between the results of the individual experiments. Because of the small number of degrees of freedom between experiments for each type of insecticide, a pooled within-treatment estimate of the percentage standard deviation (S.D.) was calculated for each year. Thus the percentage standard error for an individual LD50 or slope value in the table is given by $(S.D./\sqrt{n})\%$; e.g. for the contact LD50 for mevinphos in 1964, this standard error is $(27/\sqrt{6})\%$; i.e. 11%. Values for LD90 (the dose required to kill 90% of the insects) were calculated from the mean slope and LD50 values.

If less accuracy will suffice, fewer bees are required and the tests involve less work. For example, in screening tests one dose based on recommended rates for spraying crops may give the required information. For a crop where bees are at risk, such a simple test would show which of the compounds available to control a pest was the safest for bees.

FIELD TESTS

Field tests to assess the hazard of pesticide applications to honeybees are complicated and time consuming. They should therefore be made only if the proposed treatments put bees at risk. Field tests are also essential to find how altering the time or method of application, or formulation may affect the hazard to honeybees. For example, when the application to field beans is made during the flowering period, use of granular formulations of systemic aphicides kills far fewer honeybees than spraying (Free et al. 1967). The field tests should always be done with material formulated as proposed for practical use.

Tests with small colonies of bees confined in cages over a treated crop are of limited value because of the abnormal foraging behaviour of the caged bees and the limited amount of forage available, even with quite large cages. The following method is therefore suggested to assess the effect of pesticide applications to honeybees under field conditions, and is based on Free et al. (1967):-

General procedure

The treatments, applied when the bees are foraging, usually consist of the test formulation and a formulation known to be highly toxic to the bees, and an untreated control.

The effects on the colonies are assessed in as many ways as is practicable.

Location and size of plots The plots should be at least 7-10 acres, large enough to ensure that a high proportion of the foraging bees are actually working the treated area and not foraging in significant numbers on another plot or the untreated areas of the plots. A series of plots separated by 300-500 yards or more would be ideal. An arrangement which proved satisfactory was to use 7-10 acre plots in the four corners of a roughly square 100 acre field.

Siting and number of colonies An experienced beekeeper should be in charge of the honeybee colonies. They must be moved at least two miles to the site, otherwise the workers will probably return to their old foraging grounds. When bees are placed in or near an attractive crop they tend to forage first in the area near the hives; later they are more likely to fly some distance to other crops. Therefore they should be moved to the site not more than two days before insecticide application. Foragers from some colonies may ignore the test crop altogether, so that at least four or five colonies are needed at each site. The hives should be placed in, or on the edge of, the crop or very close to it with an unobstructed flight path. They should be as far away as possible from the other plots in the experiment. A corner of the plot is often the best position for the colonies. Treating all the test crop within some 200 yards from the hives would probably affect a sufficiently large proportion of the bees.

Weather and state of crop The bees must be at risk at the time of application and the flowering crop must therefore be really attractive, not, for example, in the later stages of petal fall. The pesticide application should be made in the middle of the day, between 10.00 and 16.00 hrs, and in fine weather. How fine depends on the previous few days; after a period of bad weather bees will forage in worse conditions than when the previous period had been exceptionally fine.

Controls and comparative treatments An unsprayed control treatment is useful, but it is equally important to include in any experiment an application of an insecticide known to kill bees, (e.g. dimethoate or demeton-methyl), to ensure that the bees are actually at risk. A negative result for the compound being tested would be suspect if there was also a negative result from the compound known to be toxic. The observations should be continued for a sufficient period to ensure that any delayed action of the insecticide will be revealed. A minimum of three weeks is suggested.

Assessment of results

Pesticide poisoning of honeybees should be considered in relation to whole colonies, not merely to individual worker bees. A healthy colony can stand the loss of two or three hundred workers without apparent effect, but a similar loss to a very small colony could deprive it of all foragers. Although a colony may recover from pesticide poisoning, and survive the following winter, all foragers may have been lost so that the farmer is deprived of valuable pollination when most needed, and the beekeeper loses all or part of a honey crop.

For this reason the following methods of assessing pesticide damage are suggested:-

Dead-bee traps This is probably the most useful method. The traps are square boxes, the width of the hive, having a fine wire netting bottom which will not permit the passage of bees, and a coarse galvanised wire (1 in. mesh) top. These are placed so that one of the top edges is immediately below the hive entrance and the coarse wire is extended over the entrance. A proportion of poisoned bees, which die in the hive and are thrown out by the other bees, appear in these traps. After poisoning by organophosphate insecticides, many of the affected bees return to the colony to die and well over half of these probably appear in the dead-bee traps. The effects of other types of insecticide is being investigated. These traps thus provide good evidence of poisoning, and bees can be collected from them

for some weeks after the application of insecticide to record delayed mortality.

Hive population Estimations of population before and after treatment, although not very accurate, will show catastrophic losses and whether bees have died away from the hive, and so have not appeared in the dead-bee trap.

The general state of the colonies can be assessed, if only subjectively. The area of brood developing, the rate at which the queen is laying eggs, and the condition of the colony is best assessed by an experienced beekeeper.

Honey crop and the general state of the colonies can be estimated by weighing the hives at intervals during the observation period. This should give an overall picture of the activity and strength of the colonies.

Activity at the hive entrance The frequency and direction of foraging is best recorded as the number of landings by foragers on return from flights. This, together with a pollen analysis, will indicate whether the test crop is being worked, and whether any decrease in activity is caused by the toxic or repellent effect of the pesticide.

Foraging activity can be estimated by observers walking slowly through the crop for a set distance in a set time and noting all foraging bees seen. For example, in one experiment a 220 yard strip, 6 feet wide was covered in 15 minutes. These counts are replicated and made simultaneously on all the treated fields, to overcome the very large variation in foraging due to fluctuations in cloud cover, wind and rain. Comparative figures obtained before and after treatment will then show if some of the foraging population have been killed or repelled by the pesticide.

Pollen traps are wire grids fitted to hive entrances through which the bees must crawl to enter their hives. In doing so, pollen loads are scraped off the legs of some of them into a collecting tray, and can be examined to show what proportion of the bees are working the test crop. As different types of pollen loads vary in size and ease of detachment from the legs, the method is not exact, but it is useful for comparisons between colonies, and to show changes in foraging after spraying.

Analysis of dead bees, honey and wax by biological and chemical assay will show the presence of insecticide or indicate whether poisoning is directly responsible for deaths.

All the observations may not be necessary for each experiment.

HAZARDS NOT ASSOCIATED WITH FLOWERING CROPS

Apart from application of pesticides to flowering crops, other hazards to honeybees should, if possible, be anticipated and suitable tests devised to assess the hazard. For example, bees may be killed in large numbers while foraging on flowering weeds when a crop, not in flower, is sprayed. Metcalfe (1967) reported that malathion sprayed on sugar cane to control canefly caused deaths of honeybees because the bees were foraging on the honeydew exuded by the infesting caneflies.

Other hazards to bees which should be watched for include wasp baits and the use of wood preservatives on bee hives.

BUMBLE BEES AND OTHER BENEFICIAL INSECTS

The acute contact toxicity test recommended above has been used against two species of bumblebees (Stevenson and Racey, 1967). As would probably be the case with other beneficial insects, the small number of bumblebees available made it

impossible to calculate regression lines or LD50 values. However it was possible to show the limits within which the LD50 values lie (Table 3) by applying a range of concentrations of each of four insecticides to groups of three to six bumblbees.

Table 3

Contact toxicity of four insecticides to bumblebees. Dose range (µg per bee) within which LD50 lies (from Stevenson and Racey, 1967). The number of tests for each compound and the total numbers of insects are also given. For comparison, see Table 1

	Demeton-methyl (µg)	Dimethoate (µg)	Disulfoton (µg)	Phorate (µg)	Total no.of insects used
Bombus lucorum					
Queens	6 - 24 (4)*	5 - 20 (4)	Over 40 (3)	6 - 23 (4)	99
Workers/drones	1 - 2 (5)	2 - 5 (4)	2 - 10 (5)	1 - 2 (5)	181
B. agrorum					
Queens	10 - 24 (3)	1 - 5 (3)	5 - 10 (3)	1 - 5 (2)	94
Workers/drones	1 - 3 (4)	0.5 - 2 (4)	1 - 4 (4)	1 - 2 (5)	283

*No. of tests given in parentheses

In field tests, foraging activity of bumblebees might be measured before and after pesticide application, but when I tried this with plots of 10-30 acres (Free et al., 1967) results were inconclusive, perhaps because bumblebees have larger foraging areas than honeybees and any killed are replaced by others from further afield (Free & Butler, 1959). If this is so, even larger plots may be necessary.

CONCLUSION

The determination of acute contact and oral toxicity of pesticides to honeybees is important to assess whether their use is likely to be a hazard to bees. When a proposed use of a pesticide will definitely put bees at risk, a field trial with the formulated material is essential to establish that bees are not affected.

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INSECT HORMONES AND ANALOGUES AND THEIR POTENTIAL CONTRIBUTION TO INSECT CONTROL

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Endocrine glands in insects are involved in the regulation of moulting and metamorphosis. The ultimate regulation is by the cerebral neurosecretory cells. These activate the prothoracic glands and the corpora allata, which release moulting hormone and juvenile hormone respectively. It is these hormones and their analogues that have a potential use in insect control.

Moulting hormones

All known moulting hormones in insects are steroids. The first to be isolated and crystallised was α-ecdysone from Bombyx pupae (Butenandt & Karlson, 1954). This is a steroid of empirical formula $C_{27}H_{44}O_6$, of structure 2, 3, 14, 22R, 25-pentahydroxy-Δ^7-5β-cholesten-6-one (see Highnam, 1969, for review). Karlson (1956) reported the isolation of small amounts of β-ecdysone from Bombyx. Both α and β-ecdysones have been extracted from other insects and another, ecdysterone, has been found in Bombyx, which is similar in structure to α-ecdysone but possesses an extra -OH group at C-20 (Hoffmeister & Grutzmacher, 1966). In Manduca two ecdysones are present; one in smaller amount is α-ecdysone; a more polar steroid present in greater amount is β-ecdysone. The greater polarity of β-ecdysone is due to an -OH group at C-20 (cf ecdysterone) (Kaplanis et al, 1966). It is of interest that a steroid with moult inducing activity has also been isolated from the crayfish Jasus, a 20-hydroxy-ecdysone called crustecdysone. This is identical with β-ecdysone from Antherea & Bombyx (Horn et al, 1966). Authors have reported a variety of differences in the biological activities of β-ecdysone, 20-hydroxyecdysone, crustecdysone and ecdysterone, but it now seems that these are in fact all the same compound (Hocks et al, 1967; Galbraith et al 1967; Highnam, 1969). Other more polar ecdysones have been extracted from insects and crustacea.

Surprisingly, plants are rich sources of steroids with moult inducing activity and some 15 phytoecdysones including α and β-ecdysone have been isolated. These possess similar chemistry, i.e. - OH groups at C-2 and C-3, = O at C-6, Δ^7 double bond in ring B and α-OH at C-14. However, it is difficult to decide on the present information whether phytoecdysones directly affect moulting or whether they are first converted to compounds similar or identical with the insect hormones.

The exact composition of the moulting hormone is disputed. In the majority of insects and crustacea examined β-ecdysone is the predominant ecdysone and there is evidence that α-ecdysone is converted to β-ecdysone (King & Siddall, 1969). In Calliphora α-ecdysone is not demonstrable (see also Galbraith et al 1969) and when α-ecdysone-23-24-H^3 is injected into pupae considerable amounts of labelled β-ecdysone are produced. This conversion of α to β-ecdysone occurs in isolated abdomens, i.e. peripheral to the prothoracic glands and suggests that moulting hormone may be produced elsewhere than in the prothoracic glands, as has also been proposed by Locke (1969). However, this evidence is as yet inconclusive. On the one hand the hydroxylation of α-ecdysone by epidermal or other tissues is not surprising since the prothoracic glands are after all only modified epidermal cells. On the other hand, Locke's evidence is based upon cytological observations and it is not enough to show that certain cells - the oenocytes - have characteristics of steroid-producing tissues, when it is well established that insects are unable to synthesise the steroid nucleus de novo.

Junvenile hormone

The first extracts of juvenile hormone were made from the abdomens of male Hyalophora (Williams, 1956). However, the analysis and characterisation of juvenile hormone has been made difficult by the large variety of compounds which mimic its activity. The terpenoid farnesol and its oxidation product farnesal extracted from Tenebrio faeces were shown to have high juvenile activity (Schmialek, 1961). Moreover, the methyl ether and diethylamine derivatives of farnesol are especially active in some insects (Wigglesworth 1961). Further evidence for farnesol or its derivatives being juvenile hormone was the isolation of farnesol from Hyalophora abdomens. However, farnesol was shown not to be the juvenile hormone since Hyalophora extracts were considerably more potent than farnesol (Schneiderman & Gilbert 1964; Gilbert & Goodfellow 1965). Removal of the corpora allata does not affect the farnesol concentration of cecropia oil but does abolish the juvenile hormone activity. Thus farnesol is not itself the juvenile hormone. This is now known to be methyl trans, trans, cis-10-epoxy-7-ethyl-3, 11-dimethyl-2-6 tridecadienoate, which has 16 possible stereoisomers. Its stereoisometric form is not yet resolved.

Synthesised dl hormone has the same activity as the natural hormone which may thus be a mixture, or, as seems more likely, the enantiomers may have similar biological activities (Roller & Dahm, 1968). A juvenile hormone of considerable biological activity in which the ethyl group at C-7 is replaced by a methyl group is reported in cecropia oil (Meyer et al, 1968). The biological activity of the all trans isomer is some 40% of the authentic hormone, thus the stereochemistry of the oxirane ring seems to be of secondary importance (Dahm et al, 1968). If Δ^2 & Δ^6 are changed to cis from their trans configuration, the biological activity is considerably reduced. The all cis isomer is relatively inactive. All the juvenile hormone activity is lost if the C-10, C-11 epoxy function is removed. Considerable work has been carried out on naturally occurring and synthetic terpenoid analogues of juvenile hormone (see Highnam, 1969). Plants also produce compounds with juvenile hormone activity (as well as producing moulting hormones). The best known of these is the paper factor of Slama & Williams (1965, 1966) present in balsam fir and now known as "juvabione" - the methyl ester of todomatuic acid (Bowers, et al, 1966). In addition dehydrojuvabione 4(2, 6-dimethyl-4-oxo-2-hexenyl)-1-cyclohexen-1-carboxylic acid methyl ester is present in balsam fir wood and shows the same selective action upon Pyrrhocoridae (Cerny et al, 1967).

Hormone release

Insect growth and metamorphosis is controlled by changes in the levels of brain neurosecretory hormone, ecdysones and juvenile hormone. In hemimetabolous insects the progressive development to the adult form is thought to be achieved by decreasing titres of juvenile hormone and differences in the time interval between the release of juvenile hormone and ecdysone (Wigglesworth, 1952). The larval stages of holometabola are maintained by continued high titres of juvenile hormone, which is reduced at the last larval moult (Gilbert & Schneiderman, 1961). In both groups the final moult to the adult occurs in the absence of juvenile hormone.

In Calliphora and Bombyx (Karlson & Shaaya, 1964; Shaaya & Karlson, 1965) the level of ecdysone increases before the pupal moult and falls markedly after ecdysis. However, in Oncopeltus the quantity of ecdysone increases before moulting but the titre in relation to the mass of the animal remains constant (Fier & Winkler, 1969). Its effect is early in the instar. In Sarcophaga ecdysone level is uniformly low before pupation, and the effect of the ecdysone upon development is due to the 'summation of covert effects', moreover, any injected ecdysone is quickly removed from the blood (Ohtaki, et al, 1968).

Juvenile hormone induces the formation of supernumerary larval stages and in some instances is able to reverse metamorphosis. Consistent results can only be obtained if the hormone is applied early enough in the instar (Williams & Slama,

1966; Sehnal, 1968; Sehnal & Meyer, 1968). Using pure hormones it has been shown that juvenile hormone will only exert its morphogenetic effects if it acts prior to ecdysone (Roller & Dahm, 1968). The disruption of normal growth by juvenile hormone is a result of the different sensitivities of cells, some remain sensitive to larval-adult or pupa-adult intermediates. Early administration of juvenile hormone in the instar either results in supernumerary larvae or perfect pupae (Sehnal & Meyer, 1968). The juvenile hormone initiates a larval programme of development, whereas in its absence a pupal or adult programme ensues. Once a non-larval programme is initiated, very high concentrations of hormone are needed to upset this programme. Regenerating cells may also reset their programme. The cells have a latent period before they respond to juvenile hormone and an early application is quickly metabolised so that normal development ensues (Sehnal & Meyer, 1968). The timing of the application of exogenous hormone or analogue designed to produce a lethal disruption in the development of the insect, obviously is of prime importance.

Hormones and analogues as pesticides

The success of hormones or their analogues as pesticides will depend upon the application of sufficient compound to the insect, either topically or orally, to provide the necessary amount at the correct time to cause an economically useful disruption in normal development.

The effects of ecdysones have normally been studied by injection, a method of no field use. Oral administration has varied effects and, in locusts, does not disrupt development (Carlisle & Ellis, 1968) although in other insects steroids supplied in the food do affect growth (Fourche, 1967; Robbins et al, 1968; Bowers, 1968; Mordue, 1968). Ecdysones can now be applied topically and when applied in methanol or in non-volatile solvents such as undeclyenic acid will penetrate the cuticle. A number of compounds synthesised from cholesterol, such as Δ^{7}-5β-cholestene - 2β, 3β, 14α-triol-6-one, when applied orally (at a dose of ppm) can lethally disrupt growth (Robbins et al, 1968). Moreover, this triol will also inhibit ovarian development in some insects. Other compounds which interfere with the normal functioning of ecdysone are diazosterols such as 20, 25-diazocholesterol, which interferes with the formation and metabolism of cholesterol in phytophagous insects. These diazo compounds can produce abnormal development (Svoboda & Robbins, 1967).

At present the use of ecdysones as pesticides seems limited, probably due to the lack of research concerned with topical or oral administration of these hormones. There is no question that uptake of excessive amounts of ecdysones cause lethal upsets in insect development. Moreover, ecdysones are relatively easy to obtain from plant sources and, since they can be applied topically, they may have a potential use as control agents.

The use of juvenile hormone and its analogues is less problematical since these can be applied topically. However, the problem of applying the necessary amount of hormone, at the appropriate time in the growth cycle, is critical. The rhythmic variations in ecdysone and juvenile hormone titres during normal development are not yet fully understood, nor indeed is the way in which the two hormones interact. Applications of a hormone at a suitable time will certainly kill the insect, but either hormone is also capable of accelerating growth and development if applied at other times. The resultant shortening of the maturation period and possible acceleration of oocyte production is, of course, quite the reverse of the desired effect.

Like ecdysone, juvenile hormone proper is not species-specific and therefore its potential use is perhaps restricted to such situations as, for example, stored products where all the insects are harmful and no selectivity is required. It is with the analogues of juvenile hormone that the greatest potential in hormonal pesticides is seen, since some of these are selective in their action and only cause

lethal disruptions in growth in particular Orders or even species of insects. The best documented of these are the effects of juvabione and its derivatives in disrupting growth in Pyrrhocoridae exclusively. Derivatives of farnesenic acid show quite different juvenilising effects when assayed on different insects. The methyl and ethyl esters of this acid show substantial juvenile activity in Tenebrio but only very low activity when tested on pyrrhocorids and pentatomids. However, the ethyl ester of trans-dihydrodichlorofarnesenic acid when compared with ethyl farnesoate is 10 times more active on Pyrrhocoris and 10 times on Dysdercus, while having very little effect upon Tenebrio. Further selectivity in the action of juvenile hormone analogues is demonstrated by the fact that juvabione and p-dimethylhexylbenzoic acid esters produce lethal effects only in pyrrhocorid and not pentatomid bugs (Slama et al, 1968; Slama et al, 1969). Selectivity is not necessarily restricted to chemical or biological action. Differences in behaviour and ecology between species may allow broad-spectrum pesticides to act selectively. The potential usefulness of hormonal compounds will depend upon their commercial competitivity, they must be active in quantities as cheap as, or cheaper than, conventional pesticides. Work so far has suggested that hormonal products will be able to compete in that respect with conventional pesticides. This is particularly true for derivatives of juvabione or of farnesenic acid, which seem to be particularly acceptable as pesticides (or give indications of the development of similar chemicals which may act in similar ways against insects which can be truly called pests), since they are active in small quantities (in some instances less than 1 µg) and are specific. Moreover, they can be applied to males, which pass on sufficient material when mating to sterilize the females. The hormonal sterilisation of females results from the fact that eggs brought into early contact with juvenile hormone analogues do not complete embryonic development (Slama & Williams, 1966). Contaminated females mated with untreated males can pass on sufficient material to sterilize another set of females when the males mate again (Masner et al, 1968). This process invites comparison with the irradiation-induced sterility technique used in screw worm eradication. However, hormonal sterilization of females by factors carried by the male, which do not impair the sexual activity of the male, could be a very useful method in the control of a wide range of insects. Obviously considerable work still needs to be done before these methods prove suitable for large scale field application, but Order-specific, if not species-specific materials are being developed, and this is particularly promising.

Under field conditions any pesticide must remain active for a sufficient length of time to affect the pest. Little is known of the half-life of the hormonal materials in the animal, let alone when exposed to the elements. Moreover, in a number of the laboratory experiments the hormones have been applied in solvents, such as acetone, which are of little use for field application. Considerable care will have to be exercised under field conditions to ensure that sufficient compound penetrates the pest's cuticle in the necessary amount and does so at a time when it can produce the desired effect, rather than acting synergistically with the insect's own hormones.

Toxicity and effects upon other organisms

Since hormones and their analogues are such potentially useful control agents it is surprising how little is known of the effects of these compounds upon other organisms (though this has not prevented a number of workers from suggesting that insect hormones have little if any effect upon other animals or, indeed, upon plants). Any application of ecdysones or their inhibitors against an insect pest may well affect other arthropods or even plants, since they are both known to possess similar steroids.

Little information is available concerning the possible effects of juvenile hormones on animals other than insects. Topical administration of a synthetic juvenile hormone is reported to protect mice against schistosomiasis (Mors et al,

1967). The possible effects of juvenile hormone upon embryonic development and metamorphosis in other invertebrates will have to be examined closely.

It has often been suggested that insects will not be able to develop resistance to hormones or their analogues, since the development of such resistance mechanisms would automatically prevent normal growth and development. However, enzyme systems certainly exist within the insect to breakdown these hormones. Ecdysone is known to have a very short in vivo half-life, so these mechanisms may be very efficient. The hormones themselves are not likely to be used as pesticides owing to their lack of specificity, but it appears possible that resistance to their analogues could develop from these breakdown mechanisms. It has been suggested by Williams (1967) that adaptation to changes in hormone systems and their relevant cellular receptor mechanisms may have already occurred as insects responded to changes in the composition of juvenile hormone in plants. Perhaps further adaptation could lead to resistance. It is also well established from comparative endocrinology, that it is the hormonal receptor mechanisms and the uses to which the hormones are put and not the hormones themselves which change during evolutionary processes. This change in the response of cells to hormones may possibly indicate pathways for the development of resistance to hormonal pesticides.

Conclusions

1. Moulting hormones, or compounds which interfere with their action, have a potential use as control agents, since they will affect development in all juvenile stages of insects.

2. The development and possible future elaboration of analogues of juvenile hormone, which specifically disrupt normal growth and metamorphosis in particular orders of insects, promise well for the use of these compounds as pesticides.

3. A number of these compounds are effective in µg and ng doses and are therefore potentially competitive, in this respect, with conventional pesticides.

4. The successful application of a hormonal pesticide in the field is a major problem yet to be solved. The timing of application to ensure a lethal disruption in development is critical. If certain stages of the pest, such as the last larval instar are to be treated, then a certain amount of damage done by the preceding instars will have to be tolerated. However, if the pesticide is to be present throughout the complete developmental period of the insect then persistent forms of pesticide will be needed, The correct time of application and dosage may be an impossibly delicate matter in species with overlapping generations.

5. There is a surprising paucity of information concerning the effects of insect hormones upon other organisms. Ecdysones, steroid 'anti-ecdysones' and juvenile hormone all have the potential to cause upsets in the development of both animals and plants. Evidence presently available suggests that these hormones may well affect development in other invertebrates. It cannot therefore be assumed that these compounds have the advantage over existing pesticides of causing no problems of environmental pollution.

6. If, as seems likely, juvenile hormones have little effect upon mammals then these hormonal compounds may be particularly suitable as control agents in Medical Entomology or Public Health work.

7. Whether or not insects will develop resistance to hormonal pesticides is controversial, but analyses of hormonal action and evolution of hormonal systems suggests that the development of such resistance does seem possible.

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in soil, to decrease biting by wireworms may be because wireworms have organs of taste that respond only to large concentrations of deterrent near the food. Mandibular pore canal organs and plate organs in the pre-oral cavity were described in Elaterid larvae by Zacharuk (1962), who thought they were organs of taste, responding to the presence of dissolved substances.

Our tests have used synthetic compounds known to affect the behaviour of many insects and have not been concerned with natural plant products as feeding deterrents or growth inhibitors of wireworms: several plants such as flax, peas, beans and vetch are reported to be relatively resistant to wireworm attack, and it would be worth using the methods described in this paper to examine these for components that might affect feeding of these soil pests.

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EVOLUTIONARY ASPECTS OF INSECTICIDE SELECTIVITY

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Summary Insecticide resistance is the result of evolution at the intra-specific level. Susceptibility differences between species of the same genus, i.e. at the intra-generic level, may be specific to particular toxicants. The mechanisms evolved appear to be similar to resistance mechanisms so that their study may enable us to predict future resistance problems. Many insects are able to tolerate plant toxins present in their normal food. When this is achieved by detoxication, it may be possible to inhibit the process with a synergist. Such synergists would provide selective chemical control agents lacking intrinsic toxicity. They may have been evolved already by plants. Hormone-mimetic substances produced by plants may lack intrinsic hormone activity but act as analogue synergists for hormones produced in insects. Plants have been evolving chemical mechanisms of insect control for many millions of years. A better understanding of these mechanisms might help us to devise more satisfactory methods of plant protection.

One of the most striking examples of rapid evolution in insects is the development of insecticide-resistant strains. Not all members of a species are affected, so this is evolution at the intra-specific level. The most frequent mechanisms of resistance are those involving enhanced detoxication (Perry, 1964). However, when the detoxication pathways in susceptible and resistant strains are compared, we often find they are similar. It is the greater efficiency of detoxication in the resistant strain which is important. The biochemical novelty which has evolved is thus often quantitative rather than qualitative. The insecticide susceptibility of species which lack resistant strains is partly determined by the degree to which they can detoxify toxicants. Many species which have not developed resistant strains can dehydrochlorinate DDT. These include the Mexican bean beetle (Epilachna varivestis), the red-banded leaf roller (Argyrotaenia velutiniana) the Indian meal moth (Plodia interpunctella) and the differential grasshopper (Melanoplus differentialis) (Sternberg and Kearns, 1952; Hoskins and Witt, 1958). The name usually given to the enzyme involved, DDT-dehydrochlorinase, is really a confession of ignorance. We do not know the natural substrate of this enzyme.

The consideration of the evolutionary aspects of insecticide susceptibility above the intra-specific level can be instructive. When we consider several species in the same genus we sometimes find notable differences in susceptibility to particular toxicants. Examples from four genera representing three of the families of beetles which infest stored foodstuffs are given in Table 1. These intra-generic differences are comparable to the intra-specific differences resulting from the evolution of resistant strains in several respects. They are often specific for particular toxicants. Thus, despite a ten fold difference in susceptibility to malathion, the hide beetles, Dermestes maculatus and D. lardarius are about equally susceptible to at least nine other organophosphorus insecticides (Lloyd and Dyte, 1965). Similarly, the two species of grain weevils (Sitophilus) which differ in their susceptibility to 'Gardona' (2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate), appear to have roughly similar susceptibilities to 15 other organophosphorus compounds, when allowance is made for their differing weights, viz S. oryzae 1.8 mg, S. granarius 3.2 mg (Table 1) (except for Trogoderma, LD50 values are expressed in µg/g to allow for weight differences). However, S. granarius is much more tolerant to 'Ethyl Gardona' (2-chloro-1-(2,4,5-trichlorophenyl)vinyl diethyl phosphate) than is S. oryzae (Strong and Sbur, 1965).

Table 1.

Contrasting susceptibilities to insecticides by species of the same genus

Topical application in cyclohexanone on adults of *Tribolium* and *Sitophilus*, or in 2-ethoxy-ethanol on larvae of *Dermestes*. Exposure to wheat sprayed with acetone solutions for larvae of *Trogoderma*. Data of Dyte *et al.* (1966), Strong *et al.* (1967), and Dyte, Daly and Rossiter (unpublished).

Insecticide	Genus	Species	LD50 (μg/g)	Factor of difference
DDT	*Tribolium*	*confusum*	20.2	X 913
		destructor	18440.0	
malathion	*Dermestes*	*lardarius*	0.40	X 10.5
		maculatus	4.20	
malathion	*Trogoderma*	*simplex*	5.12*	> X 10
		parabile	>50.0*	
'Gardona'	*Sitophilus*	*oryzae*	8.9	X 5
		granarius	43.7	

* LC50 (ppm on wheat) not LD50 (μg/g)

Intra-generic susceptibility differences are also comparable to insecticide resistance in that they are sometimes due to more effective detoxication in the less susceptible species. This is shown by the fact that the contrasting susceptibility differences between species can often be overcome or much reduced by synergists known to inhibit detoxication processes. The malathion-specific tolerance of *Dermestes maculatus* can be overcome by the synergist triphenyl phosphate, so that this species and *D. lardarius* have roughly similar susceptibilities to malathion-triphenyl phosphate mixtures (Dyte *et al.* 1966). This is comparable to the malathion-specific resistance of a strain of the red flour beetle (*Tribolium castaneum*) from Nigeria. The 400-fold resistance of this strain is also completely overcome by the same synergist. Triphenyl phosphate inhibits the carboxyesterases which enhance the degradation of malathion in these resistant flour beetles (Dyte and Rowlands, 1968) and it seems very probable that a comparable detoxication mechanism exists in *Dermestes maculatus* but not *D. lardarius*.

The pronounced tolerance of the dark flour beetle (*Tribolium destructor*) to DDT (Table 1) is considerably reduced by the synergist DMC (1,1-*bis*-*p*-chlorophenyl) ethanol) but this synergist fails to synergize DDT in the confused flour beetle (*T. confusum*). In this case the synergist does not overcome the susceptibility difference, but reduces it from x 913 to about x 38. DMC is a well known inhibitor of the dehydrochlorination of DDT in resistant houseflies, and it appears that dark flour beetles are able to detoxify DDT by a comparable process not found in confused flour beetles.

The apparent similarities between intra-specific and intra-generic susceptibility differences are not surprising since modern evolutionists regard the mechanisms of evolution above and below the species level as essentially similar (Rensch, 1966). However, intra-generic susceptibility differences are unlikely to be the result of selection by man-made toxicants, and they have probably evolved during a

period of many millions of years extending back long before the evolution of man himself.

The results with synergists indicate a similarity between the mechanisms producing susceptibility differences between species, and resistance. This suggests that the study of intra-generic susceptibility differences could have practical value. Such studies might indicate the type of resistance problem which may arise with new insecticides.

'Gardona' is a recently developed organophosphorus insecticide of potential value against some stored-product pests (Lemon, 1967), though, as yet, I do not know of its being used against them in practice. The 'Gardona'-specific tolerance of the grain weevil (Sitophilus granarius), which has been shown by laboratory studies in the U.S.A. and England (Strong and Sbur, 1965; Lemon, 1967), suggests that a Gardona-specific type of organophosphate resistance may well develop in some pest in the future. An understanding of the mechanism of the susceptibility difference in Sitophilus might enable us to anticipate the nature of this potential problem.

There appears to be little detailed information available about susceptibility differences above the generic level. Some insecticides may be more effective against particular orders e.g. Diptera or Lepidoptera, but the mechanisms are not well understood. One striking example at the sub-family level is the cyanide-tolerance of burnet moths (Sub-family Zygaeninae). This was first recorded as a sub-family character by Jordan (1907), and it has since been shown that immature and adult Zygaena spp. not only tolerate cyanide but also contain it, so that their tissues release hydrocyanic acid when crushed (Jones et al. 1962). The six-spot burnet moth (Z. filipendulae) feeds on bird's foot trefoil (Lotus corniculatus), some strains of which contain a cyanogenic glucoside (Dawson, 1941). This glucoside gives some protection to the plants against slugs and snails (Jones, 1966), but larvae of the six-spot burnet feed readily on cyanogenic strains (Jones et al. 1962). The moth is thus not only tolerant of cyanide, but uses this poison in its own tissues as a protection against predators.

The burnet moth is but one of many insects adapted to live with (and in this case utilize) potentially toxic substances in their food plants. Besides the plant toxins which have been utilized as insecticides, such as pyrethrum, rotenone, and nicotine, there are many others which are highly active pharmacologically. These include many alkaloids, glucosides and mustard oils. The natural function of many of these minor plant constituents is unknown, but it has been suggested that a number evolved as protectants against insects or other pathogens (Schreiber, 1958; Wood, 1965; Jones, 1966; Robinson, 1963). The host specificity of some plant-feeding insects may be due to their presence or absence (Fraenkel, 1959). Investigations of plant-host relationships have involved studies of the repellancy, attractiveness or toxicity to insects of secondary plant substances but little seems to be known about the mechanisms by which toxic substances in the normal host are tolerated. However, it has been shown that of seven insects feeding on tobacco plants, three are able to detoxify nicotine (Self et al. 1964).

It seems probable that some other plant feeding insects tolerate poisonous substances in their plant hosts because they have evolved detoxification mechanisms. If so, it should be possible to interfere with these mechanisms by the application of a synergist (Dyte, 1967). Such compounds would represent a new type of chemical control agent which need not be toxic because they would act by enhancing the effectiveness of naturally occurring plant protectant substances. They would be unlikely to affect predatory and pollinating insects and would probably be most useful in plants containing potentially toxic substances chiefly in those parts not eaten by man - for example, solanin in the leaves of potato plants.

In view of the long period during which the inter-relationships between plants and insects have evolved, it is possible that some secondary plant substances have

evolved as synergists to enhance the action of other naturally occurring protective substances. In this respect, it is of interest that over 300 compounds containing the methylenedioxyphenyl group are known to occur naturally in plants (Newman, 1962). Compounds of this type, both natural and synthetic are widely known as synergists of pyrethroids, carbamates, organophosphates and many other insecticides.

Of particular interest in relation to the reciprocal evolution between plants and insects are the insect hormone-like substances. Plants have evolved compounds which mimic the activity of the insect juvenile hormone and others mimicing the activity of the moulting hormone. Some plants are very rich sources of these substances. Karlson obtained 70 mg of the moulting hormone, ecdyson, from 1000 kg of silkworm pupae. The same activity could have been obtained from 70 gm of dried Taxus leaves (Staal, 1967). It seems likely that these substances evolved in plants as a protection against insects. If so, then hormone-mimetic substances are not "third-generation pesticides" as Williams (1967) has suggested, but very old-fashioned insecticides developed before man evolved.

Biochemical control mechanisms must be capable of being turned off as well as turned on. So to be useful a hormone must be rapidly removed when its job is done. This could be done by rapid excretion or rapid metabolism. Natural juvenile hormone is metabolized (Wigglesworth, 1969), so it should be possible to inhibit this metabolism with synergists. It seems quite possible that some of the hormone-mimetic substances are in fact synergists. When low levels of hormone are present, treatment with a hormone analogue in a bio-assay test may be comparable to treating an insect containing an endogenous sub-lethal dose of DDT with the analogue synergist IMC. The results of the IMC treatment might well be DDT poisoning. The results from the hormone analogue might well be enhanced hormone activity, but this could be because the substance was acting as an analogue synergist rather than a compound with intrinsic hormone activity. This interpretation is perhaps made more probable by the demonstration that some of the best known non-analogue insecticide synergists, the methylenedioxyphenyl compounds, have juvenile-hormone mimetic effects (Bowers, 1968).

If we accept the possibility that many minor plant constituents have evolved as toxicants against insect pests, and that others may have evolved as synergists, we shall be in a position to take advantage of the toxicological interaction between plants and insects that has evolved. Plants have been concerned with insect control for many millions of years before man became interested. We might well learn from their experience.

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SOME LABORATORY TESTS OF FEEDING DETERRENTS AND BAITS AGAINST WIREWORMS (AGRIOTES SPP.)

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Summary When filter paper discs soaked in nutrient solution were offered to wireworms, a mixture of 10% glucose : 1% peptone : 1.8% triolein caused more biting than did larger concentrations of glucose, peptone or triolein alone, and the omission of one component from the mixture could not be compensated for by doubling the amount of either of the others. Small discs dipped in a thick suspension of glucose, peptone and triolein and placed in tins of soil were found and bitten by wireworms, but the attractiveness of these discs declined in a few days. More work is needed to increase the persistence of food baits and to choose the insecticide they should contain, before tests can be done in the field.

Wireworms were deterred from biting nutrient discs treated with some commercially available insect repellents or feeding deterrents. Three of these materials, MGK 874 (2-(octylthio) ethanol), DEET (N,N-diethyl-m-toluamide) and Cyanamid 24055 (4'(Dimethyltriazeno) acetanilide) were also tried applied to soil at 100 ppm to weight of soil, but this method did not affect the wireworms. Applied to seed, MGK 874 and Cyanamid 24055 protected some germinating plants of Cappelle wheat from attack by wireworms during early but not during later plant growth.

INTRODUCTION

The use of organochlorine insecticides against soil pests was restricted after the publication of the Report by the Advisory Committee on Poisonous Substances used in Agriculture and Food Storage (1964). Considering the many materials tested, (Griffiths, 1968) progress in control of wireworms with non-organochlorine insecticides has been disappointing. Other possible methods of control are worth considering. This account describes preliminary tests on the practicability of using baits or feeding deterrents against wireworms.

I. REACTIONS OF WIREWORMS TO DIFFERENT FOOD MEDIA

The biting behaviour of wireworms as affected by different foods was studied by methods based on those of Thorpe, W.H. et al. (1947), Davis (1961) and Griffiths (1967). Larvae of Agriotes spp., known by means of a preliminary test to be in a biting phase, were confined individually in moist soil or vermiculite in 7.6 x 3.8 cm glass tubes. Each tube contained a filter paper disc cut to 3.5 cm diameter, one edge of which had been treated with 15 µl of a standard food medium made of 1g D-glucose, 1.8g glycerol trioleate (triolein) with 50 µl emulsifier-solvent containing "Agrilan A" 10% w/v in xylene, and 0.1g bacteriological peptone, made up to 10 ml with distilled water. The other edge of the disc was treated with the material under test. There were 20 to 40 tubes in each test. After 24 hours in the dark at 15°C, the holes the wireworms had caused by biting the treated areas were counted, using a colour-marking probe and portable counter (Arnold, 1969). Each

hole was called a bite, even though it did not completely penetrate the paper. The number of groups that made up the total number of bites was counted also, to help distinguish between materials which caused biting to start or to continue once started.

(1) Effects of concentration of food

Single foods were tested at different concentrations against the standard medium (Table 1). All concentrations of glucose were inferior to the standard medium with respect to number of groups, and numbers of bites in each group.

Table 1.

Different concentrations of single foods compared with standard medium (SM)

		SM	20% G	10% G	5% G
	No. groups	10	5.0	3.7	2.2
GLUCOSE	Mean bites/group	10	5.0	6.4	9.6
	Total bites	100	25	24	21

		SM	Undiluted T	50% T	25% T
	No. groups	10	1.0	4.9	2.1
TRIOLEIN	Mean bites/group	10	24.3	23.4	24.2
	Total bites	100	24+	115	51

		SM	30% P	20% P	10% P	5% P
	No. groups	10	9.5	10.9	7.7	3.1
PEPTONE	Mean bites/group	10	4.1	5.2	4.2	6.4
	Total bites	100	39	57	32	20

+ This small number bites may partly be due to the oily texture of the paper at this concentration of fat

Peptone by itself was also inferior to the standard medium, because although the larger concentrations of peptone had about as many groups as the standard medium there were fewer bites/group. Triolein by itself had numerous bites/group but very few groups. Except for 50% triolein, no single food, at any concentration, caused as much biting as did the mixture in the standard medium.

(2) Effects of different mixtures

Mixtures that lacked one of the components of the standard medium (Table 2) were bitten less often than the standard medium, even when the amounts of other components of the medium were doubled. Omitting the glucose would be expected to have a great effect on biting because much glucose is present in the standard medium, but omitting the small amounts of peptone or triolein from the standard medium also greatly decreased biting.

(3) Conclusions from tests of food media

Mixtures were bitten more frequently than large concentrations of glucose, peptone or triolein alone. There was no clear optimum for single food substances, but of the doses tested 50% triolein, or 20% glucose or peptone seemed best. All

foods seemed to have two effects: to cause biting to start (indicated by the number of groups) and to cause biting to continue (indicated by the number of bites/group). Peptone was particularly active in causing wireworms to start biting; triolein was particularly active in making them continue.

Table 2.

Effects of mixtures lacking one component, compared with standard medium (SM)

	SM ($G_1T_1P_1$)	G_1T_1	G_2T_1	G_1T_2	
No. groups	10	5.8	4.7	8.9	
Mean bites/group	10	6.9	7.1	8.4	PEPTONE MISSING
Total bites	100	40	33	75	
		G_1P_1	G_2P_1	G_1P_2	
No. groups	10	10.5	6.3	9.3	
Mean bites/group	10	7	4.5	6.4	TRIOLEIN MISSING
Total bites	100	74	28	59	
		P_1T_1	P_1T_2	P_2T_1	
No. groups	10	3.7	3.4	3.5	
Mean bites/group	10	7.2	11.8	6.7	GLUCOSE MISSING
Total bites	100	27	40	23	

G_1 = same concentration of glucose as in SM

G_2 = twice the concentration of glucose as in SM etc.

II. FOOD MEDIA AS BAITS

The results already described indicated that the standard medium would be improved by increasing the concentration of its components, especially the triolein and peptone. A new medium was made by mixing 2g of glucose and 2g of peptone with 4.6g of triolein and adding enough water, 3 ml, to give a reasonably homogeneous suspension. This improved medium (IM) was greatly superior to the standard medium as the following result shows:

	SM	IM
No. groups	10	29
Mean bites/group	10	16.3
Total bites	100	473

Separate tests were done to see whether wireworms could find small discs coated with the improved medium when placed in containers of soil, and whether discs so treated lost attractiveness after being in moist soil. These tests used tins, 23 cm diameter and 15 cm deep, containing 5 kg of moist John Innes no. 1 potting compost and 25 wireworms. This is equivalent to a field population of

5 to 7 million wireworms/ha (2 to 3 million wireworms/acre) based on calculation of surface area of soil. Small filter paper discs, 4 mm diameter, were soaked in the improved medium and allowed to dry. Some discs were stored in moist soil for 5 days, others were used fresh, and some discs were untreated. The discs were attached to the ends of split matchsticks and poked 2 to 3 cm into the soil. After 3 days in the dark at 15°C discs were removed and scored as bitten or not bitten (Table 3).

Table 3.

Numbers of small discs with improved medium found and bitten by wireworms during 3 days in soil

		Freshly treated discs	Treated discs stored in soil 5 days	Untreated discs
No. of discs	Bitten	35	13	1
	Not bitten	14	35	48
	Missing	1	2	1

Most of the freshly treated discs were found by these large populations of wireworms during three days. Discs that had been stored in moist soil had lost much of their attractiveness, thus limiting their potential usefulness as baits. In other tests, in which discs were stored in soil for two weeks, the filter papers themselves decayed.

Thorpe et al. (1947) suggested that a non-repellent contact poison would be the best type of insecticide to use in a poison bait. Griffiths (1967) showed that, when wireworms were confined in tubes of soil with nutrient discs treated with 100 µg insecticide, the mean numbers of bites/disc were: control (nutrient only) 85.7, aldrin 64.2, Bayer 38156 41.5, γ-BHC 22.7 and thionazin 4.1. In his tests wireworms could not escape contact with insecticide-treated discs and all died later, but in the field it is probable that baits containing insecticides such as aldrin and B 38156 would allow wireworms to have more intimate contact with the poison and so kill more wireworms than would baits containing γ-BHC or thionazin.

III. TESTS OF FEEDING DETERRENTS

By treating seeds with substances that inhibit wireworms from feeding, it might be possible to protect plants during their early, sensitive stages of growth. Crombie and Darrah (1947) showed that biting by wireworms of 2% glucose solutions was inhibited by lead acetate, quinine, allyl-iso-thiocyanate and common salt. Materials that prevent wireworm biting are worth more study, so several commercially available insect repellents and feeding deterrents were tested.

(1) Tests on filter papers

15 µl of standard medium was applied to opposite edges of 3.5 cm diameter filter paper discs and allowed to dry. Then 15 µl of a solution in acetone of the material under test was placed on one treated edge and 15 µl of acetone alone was placed on the opposite edge. The discs were placed in moist vermiculite in individual 7.6 x 3.8 cm glass tubes, each of which contained a single biting wireworm. After 24 hours in the dark at 15°C, the numbers of bites wireworms made on food medium + acetone, and on food medium + material under test were compared (Table 4). All compounds except MGK 326 had some effect on wireworm biting, but especially DEET and MGK 874, which as 10% solutions, equivalent to about 0.7 mg/cm^2 treated surface, almost completely eliminated biting.

Table 4.

Tests of feeding deterrents on filter paper discs

Material	Bites		Bites	
	SM + acetone	SM + 10% solution deterrent	SM + acetone	SM + 1% solution deterrent
DEET	100	0.1	100	17
MGK 874	100	0.7	100	25
INDALONE	100	4	100	84
DMP	100	8	100	93
RUTGERS 6-12	100	9	100	53
CYANAMID 24055[+]	100	45	100	36
MGK 326	100	127	100	137

[+] This material was not very soluble and was tested as a 2% solution

DEET	N,N-diethyl-m-toluamide
MGK 874	2-(octylthio) ethanol
INDALONE	Butyl 3,4-dihydro-2,2-dimethyl-4-oxo-2H-pyran-6-carboxylate
DMP	Dimethyl phthalate
RUTGERS 6-12	2-ethyl-1,3-hexanediol
CYANAMID 24055	4'(dimethyltriazeno) acetanilide
MGK 326	Di-n-propyl isocinchomeronate

(2) Tests in treated soil

Three of the compounds tested on filter paper discs were tried also as soil treatments to see whether they would poison wireworms or affect their sense organs to cause decreased biting. 1 kg batches of John Innes no. 1 potting compost were thoroughly mixed with 50 ml lots of hexane containing 0.1g DEET, MGK 874 or Cyanamid 24055, to give 100 ppm deterrent to weight of soil. Control soil was treated with hexane alone. The hexane was allowed to evaporate and the soils were moistened, split into 40g portions, and placed in 7.6 x 3.8 cm glass tubes, each of which contained a disc treated with standard food medium and a biting wireworm. The discs were replaced after 24 hours at 15°C and subsequent tests done during the next few days to compare the amount of biting by wireworms in treated and untreated soil (Table 5).

The soils tended to become dry during the course of the experiments and biting decreased in both control and treated soils. Treating the soil with DEET, MGK 874 or Cyanamid 24055 had very little effect.

(3) Tests on seeds

The two feeding deterrents that were most effective on filter paper discs, and Cyanamid 24055, reported to be systemic, were tested on seeds of Cappelle winter wheat. The liquid technical materials DEET and MGK 874 were first adsorbed onto

Table 5.

The effects of soil treatments with feeding deterrents on the biting behaviour of wireworms

		Days in treated soil		
		1	2	5
Control	Total bites	888	616	656
	No. biting wireworms	20	17	18
	No. dead wireworms	0	0	0
DEET 100 ppm	Total bites	1066	688	420
	No. biting wireworms	19	16	15
	No. dead wireworms	0	0	0
Control	Total bites	447	687	548
	No. biting wireworms	12	13	13
	No. dead wireworms	0	0	0
MGK 874 100 ppm	Total bites	790	880	552
	No. biting wireworms	17	15	12
	No. dead wireworms	0	0	1
Control	Total bites	1589	817	492
	No. biting wireworms	19	19	16
	No. dead wireworms	0	0	0
CYANAMID 24055 100 ppm	Total bites	1135	1012	421
	No. biting wireworms	20	18	11
	No. dead wireworms	0	0	0

siliceous earth to make 20% dusts. Weighed amounts of these dusts or of Cyanamid 24055 powder were stuck to seeds by stirring them with a glass rod in a beaker together with a few drops of a solution of 3% methyl cellulose in water as a sticker. The seeds were allowed to dry by spreading them on filter papers immediately after treatment. Preliminary germination tests in John Innes no. 1 potting compost showed that Cyanamid 24055 could safely be used at 2% a.i. to weight of seed, the largest dose tested, but that the greatest dose that gave good germination for DEET was 0.8 and for MGK 874 was 0.28% a.i. to weight of seeds.

Seeds treated with these amounts and control seeds (untreated) were sown in each of eight plastic boxes, 22 x 10 cm, as rows of seven seeds, with the position of the rows randomised in different boxes. Thirty wireworms were added to each box and damaged and healthy plants were counted at intervals (Table 6).

With these very large populations of wireworms (about 5 to 6 million/acre = 13 to 14 million/hectare), only a small proportion of the plants emerged. However, more seedlings emerged from seeds treated with Cyanamid 24055 or MGK 874 than from untreated seeds or seeds treated with DEET. In all treatments, the proportion of damaged plants increased during the course of the test and some plants decayed and could not be found in later counts. Twenty-five days after sowing nearly all the

plants had been attacked.

Table 6.

Protection of germinating plants by feeding deterrents on seeds

Material	Rate % a.i. to wt of seed	Healthy (H) and damaged (D) plants at indicated days after sowing							
		11 days		15 days		22 days		25 days	
		H	D	H	D	H	D	H	D
Control	-	17	2	18	2	6	13+1m	0	16+4m
CYANAMID 24055	2.0	25	2	24	5	7	15+7m	0	20+9m
DEET	0.8	12	1	10	4	2	6+6m	2	4+8m
MGK 874	0.28	26	1	26	3	8	15+6m	3	16+10m

m = missing plants

DISCUSSION

One of the greatest needs for an effective substitute for the organochlorines is to control wireworms that attack potatoes. If efficient baits could be developed, they might be used at planting to decrease the wireworm population before new tubers are formed. They could employ small amounts of relatively non-persistent insecticides and would be more selective than conventional soil treatments. Thorpe et al. (1947) showed that food materials, and various amides and dicarboxylic acids caused wireworms to accumulate in their presence. We chose to try food materials first because they have been the basis of most poison baits and because only food materials make wireworms bite. In our tests, mixtures of foods in large concentrations gave the best biting response. Such mixtures might attract more wireworms than the 2% sucrose solutions tested as baits by Thorpe et al. (1947), but a better carrier than filter paper is needed and the persistence of the food would have to be extended, perhaps by use of food preservatives. Insecticides, such as aldrin or Bayer 38156, that allow intimate contact with the poison would probably be best, but repellent insecticides might be used if they could be encapsulated inside the food. More laboratory work is necessary to obtain effective formulations before tests are done in the field.

Some feeding deterrents gave excellent results in filter-paper tests, and are worth considering as possible seed treatments. They have the advantage of being much less toxic to mammals than most insecticides and can be applied in fairly large amounts to seeds without affecting plant growth. When treated seeds were sown in plastic boxes, Cyanamid 24055 and MGK 874 protected some germinating plants but their effects seemed not to last long, either because they lacked persistence in soil or because they protected only the treated areas and failed to protect parts of the plants that had grown some distance from the seeds. However, the boxes contained exceptionally many wireworms and it would be reasonable to include the most effective deterrents as seed dressings in small scale trials in field conditions.

Feeding deterrents might be worth testing against other soil pests to see whether they protect seeds from direct attack, and those that are compatible with insecticides may be suitable as solvents in the formulation of seed dressings of more toxic materials. The failure of DEET, Cyanamid 24055 and MGK 874 at 100 ppm

in soil, to decrease biting by wireworms may be because wireworms have organs of taste that respond only to large concentrations of deterrent near the food. Mandibular pore canal organs and plate organs in the pre-oral cavity were described in Elaterid larvae by Zacharuk (1962), who thought they were organs of taste, responding to the presence of dissolved substances.

Our tests have used synthetic compounds known to affect the behaviour of many insects and have not been concerned with natural plant products as feeding deterrents or growth inhibitors of wireworms: several plants such as flax, peas, beans and vetch are reported to be relatively resistant to wireworm attack, and it would be worth using the methods described in this paper to examine these for components that might affect feeding of these soil pests.

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