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RESISTANCE OF FUNGAL PATHOGENS TO BENOMYL IN IRELAND AND RESULTS OF ALTERNATIVE SPRAY PROGRAMMES FOR DISEASE CONTROL

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Summary The presence of benomyl-resistant fungal pathogens defined as isolates which grew on PDA containing 100 µg/ml benomyl was surveyed on horticultural crops in Ireland. Of 26 tomato and 28 strawberry crops sampled 92% and 46% respectively had benomyl-resistant Botrytis cinerea present. The presence of benomyl-resistant Fulvia fulva on tomatoes is also reported. Alternative spray programmes for strawberry grey mould (B. cinerea) gave excellent control.

INTRODUCTION

Following early records by Schroeder and Provvidenti (1969), Bollen and Scholten (1971) and Bent et al (1971) of resistance in fungi to systemic fungicides many other reports have been made. From these it is clear that this phenomenon is widespread in a range of pathogens including those causing powdery mildew, Penicillum rot, grey mould, Verticillium wilt and apple scab. Since its first introduction into Ireland in 1968, benomyl and subsequently other benzimidazole derivatives have been extensively used on a wide range of horticultural crops. Up to 1975, there was no evidence of resistance in pathogens of commercial crops except for a suspected case in Pseudopeziza ribis causing leaf spot of black currant (O Riordain, 1973). Some reports of poor control of grey mould of tomato were investigated in 1975 and in view of the extensive use of systemic fungicides on this and other crops, a survey was initiated to determine the extent of resistance in pathogens infecting these crops. The findings from this survey are presented here. Alternative spray programmes on strawberries for grey mould (Botrytis cinerea) control designed to make best use of benzimidazole fungicides without allowing a build-up of a resistant population are also presented.

METHOD AND MATERIALS

Samples of Botrytis cinerea-infected tissue from black currant, strawberry, raspberry, tomato and lettuce crops were collected in the principal growing areas. B. cinerea was isolated from these samples on to PDA. Using a sterile cork borer 5 mm agar discs were taken from the edge of 4-day old colonies and transferred to agar plates containing various benomyl concentrations. Initially, five concentrations of benomyl ranging from 1 to 1,000 µg/ml were used but subsequently isolates which grew at a standard concentration of 100 µg/ml were considered resistant. Three replications were used in all tests and known susceptible and resistant strains were used for comparison as standards.

Field experiments for control of grey mould of strawberry using systemic fungicides were carried out from 1968 at the Soft Fruits Research Station, Agricultural Institute, Clonroche, Co. Wexford. The susceptible cultivar Cambridge Vigour was grown in 50-plant plots and the fungicide treatments were replicated four

times. Three applications of each fungicide were given during the flowering period using a high pressure, high volume sprayer. Though yields of diseased fruit were also taken, fungicide efficiency was judged chiefly on the increase in yield of healthy fruit.

RESULTS

Survey of resistance

Strawberry Although the incidence of grey mould of strawberry in 1975 was low, samples were collected from crops in four counties. Of the 28 crops sampled, thirteen (46%) had B. cinerea resistant to benomyl. In general, there was a good correlation between the use of benzimidazole fungicides and benomyl resistance. However, where in the case of unsprayed crops resistant strains were present these were close to crops which were frequently sprayed. There was no obvious correlation between disease incidence and the presence of resistance.

Black currant B. cinerea was isolated from five experimental plots of black currant at the Soft Fruit Research Station, Clonroche. Of these, four from, or adjacent to, plots sprayed with benomyl were resistant while the fifth, from a plot in a different part of the Station where mancozeb was used, was susceptible.

Raspberry Of five isolates of B. cinerea from raspberry crops four were benomyl resistant. The susceptible isolate was from an unsprayed crop.

Tomato Twenty six commercial tomato crops were surveyed in five counties. In all but two (92%) B. cinerea strains resistant to benomyl were found. In one of these cases no benzimidazole fungicides had been used while the other was in an isolated area and had only received a few sprays of carbendazol at irregular intervals. In most of the crops where resistance occurred, benzimidazole fungicides were used liberally over the years. In some of these, the growers had complained of failure to control grey mould.

Isolates of Fulvia fulva from four leaf mould-affected crops which were sprayed with benzimidazole fungicides grew readily on PDA containing 100 ug/ml benomyl.

Isolates of Didymella applanata from five crops affected with stem rot were tested for resistance to benomyl but results were all negative. Failure to control stem rot with benomyl was reported in some of these crops but it is probable that inadequate fungicide application was responsible.

Lettuce Isolates of B. cinerea from four glasshouse lettuce crops were tested. Two of these from crops which received applications of benomyl were resistant while the other two which were not sprayed with a benzimidazole fungicide were susceptible.

Alternative Spray Programmes for Strawberry Grey Mould

In 1972 and 1973 full programmes of dichlofluanid and of three systemic fungicides were compared with a combined dichlofluanid-benomyl programme for strawberry grey mould control at the Soft Fruit Research Station, Clonroche (Table 1).

In both seasons two applications of dichlofluanid followed by one of benomyl gave control equal to the best treatment and significantly better than the unsprayed treatment.

In 1975, these programmes were extended to include physical mixtures of dichlofluanid and benomyl each at half the normal recommended rates. Due to the exceptionally dry weather from flowering to harvest the incidence of grey mould was very low and significant differences between treatments did not emerge. However, the experiment showed at least that physical mixtures of these fungicides did not produce an adverse effect.

Table 1

Effect of fungicide programme on strawberry yields

Treatment	Yield of healthy fruit (t/ha) 1972	1973
Control	18.9	9.6
Benomyl	23.4*	18.2***
Carbendazim	22.6	17.6***
Dichlofluanid	21.7	17.1***
Thiophanate-methyl	25.0*	16.9***
Dichlofluanid & benomyl[1]	23.9*	18.9***

*, *** significantly different from control at 5% and 0.1% respectively.
[1] two applications of dichlofluanid followed by one of benomyl.

DISCUSSION

Although resistance to fungicides has been reported from many countries, this is the first record from Ireland. Since their first introduction, benzimidazole fungicides and particularly benomyl, have been widely and liberally used on glasshouse food crops in Ireland. For example many tomato growers apply these fungicides routinely at 14-day intervals regardless of the presence or absence of disease. This, no doubt, accounts for the high incidence of benomyl-resistant B. cinerea reported here from tomatoes. Strains of benomyl-resistant B. cinerea were less prevalent in soft fruit crops and this may be accounted for by the fact that these crops generally receive only two or three sprays each year. However, in both these crops there did not seem to be a good correlation between the presence of resistant strains as measured by our method and failure to control disease.

From the time it was first used in 1968 until 1974 inclusive, benomyl and subsequently carbendazim, thiophanate-methyl and carbendazol gave excellent control of grey mould of strawberries (Kavanagh and O'Callaghan 1968-1974, 1969, O Riordain et al 1971). This control was equal to that given by dichlofluanid which was used as a standard. In all but a few cases the increase in yield of healthy fruit from the sprayed plots was significantly higher than from the unsprayed areas. The exceptions were due to very unusual weather conditions e.g., 1975 when prolonged dry weather led to a virtual absence of grey mould from all plots whether sprayed or not. The average increase in yield of healthy fruit in a recorded area of ca 0.5 ha at the Soft Fruits Research Station sprayed with these five fungicides was 40% and 68% in 1973 and 1974 respectively. The fact that there were only slight differences between the effectiveness of the systemic fungicides and dichlofluanid indicates that if resistance was present it was having little, if any, effect on the efficacy of the former. Nevertheless, a combined dichlofluanid-benomyl programme would reduce the risk of poor disease control due to benomyl resistance.

In view of these results it is clear that the use of alternative spray programmes for diseases of other crops needs to be studied so that the valuable properties of benzimidazole fungicides may be utilised to the full.

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References

BENT, K.J., COLE, A.M., TURNER, J.A.W. and WOOLNER, M., (1971) Resistance of cucumber powdery mildew to dimethirimol. Proc. 6th Br. Insectic. Fungic. Conf. 274-282.

BOLLEN, G.J. and SCHOLTEN, G. (1971) Acquired resistance to benomyl and some other systemic fungicides in a strain of Botrytis cinerea in cyclamen. Neth. J. Pl. Path. 77, 83-90.

KAVANAGH, T. and O'CALLAGHAN, T.F., (1968-1974) Ann. Res. Reptr., Kinsealy Research Centre, Agricultural Institute, Ireland.

KAVANAGH, T. and O'CALLAGHAN, T.F., (1969) A comparison of fungicides for the control of Botrytis of outdoor and protected strawberries. Proc. 5th Br. Insectic. Fungic. Conf. 139-144.

O RIORDAIN, F., (1973) Effects of benzimidazole compounds and other fungicides on leaf spot of black currants. Proc. 7th Br. Insectic. Fungic. Conf. 477-481.

O RIORDAIN, F., KAVANAGH, T. and O'CALLAGHAN, T.F., (1971) Comparisons of systemic and protectant fungicides for disease control on four soft fruits. Proc. 6th Br. Insectic. Fungic. Conf. 324-331.

SCHROEDER, W.T. and PROVVIDENTI, R., (1969) Resistance to benomyl in powdery mildew of cucurbits Pl. Dis. Reptr. 53, 271-275.

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PERENNATION AND CONTROL OF BENOMYL - INSENSITIVE BOTRYTIS AFFECTING STRAWBERRIES

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Summary Benomyl-insensitive Botrytis cinerea was shown to overwinter on strawberry leaf debris and provide sufficient inoculum for flower infection in the spring. Considerable suppression of sporulation of the overwintering inoculum was obtained by treating debris with dichlofluanid (0.1% a.i.). Effective control of benomyl-insensitive Botrytis was achieved using a three-spray programme of either chlorothalonil, dichlofluanid, thiram, vinclozolin, or a benomyl/dichlofluanid tank mix. Alternating sprays of benomyl and dichlofluanid also gave satisfactory control, but the insensitive isolate was recovered from all fruit that became infected. The incidence of post-harvest fruit rot caused primarily by Rhizopus stolonifer was not significantly affected by any of the pre-harvest spray programmes.

Resume La perennation et la lutte contre le Botrytis benomyl-resistant des fraises.

On a montré que le Botrytis cinerea resistant au benomyl peut hiverner sur les debris de feuilles du fraisier et fournir assez d'inoculum pour infecter les fleurs au printemps. Une forte mesure de repression de la sporulation de l'inoculum hivernant a été effectuée par traitement des débris avec le dichlofluanid (0, 1% m.a.). La lutte contre le Botrytis non sensible au benomyl a réussi au moyen d'un programme de trois pulverisations de chlorothalonil, dichlofluanid, thiram, et vinclozolin, ou en utilisant un melange benomyl + dichlofluanid dans la citerne. Des pulvérisations alternees du benomyl et du dichlofluanid ont également montré une action antiparasitaire satisfaisante, mais l'isolat resistant a été rétrouve sur tous les fruits atteints de la maladie. Les cas de pourriture post-récolte des fruits dont le Rhizopus stolonifer était la cause premiére ne furent affectés d'une maniére significative par aucun des programmes de pulvérisation pré-récolte.

INTRODUCTION

Isolates of Botrytis cinerea (grey mould) insensitive to benomyl have recently been obtained from a number of crops, sprayed with the fungicide (Bollen & Scholten, 1971; Jarvis & Hargreaves, 1973; Miller & Fletcher, 1974). Since applications of benomyl to plants infected with benomyl-insensitive Botrytis are ineffective and may even result in an increase in disease (Bollen & Scholten, 1971; Jordan & Richmond, 1974) alternative spray programmes are urgently required both to prevent the increase of insensitive strains and to control Botrytis fruit rot of strawberry.

Previous work has shown that benomyl-insensitive strains of Botrytis were able to grow and compete with sensitive strains on strawberry (Jordan & Richmond, 1974). The present paper examines the perennating ability of an insensitive strain and evaluates several spray programmes for control of fruit rot.

METHOD AND MATERIALS

To establish whether sufficient benomyl-insensitive Botrytis can overwinter on strawberry plant debris to provide inoculum for flower infection the following year, the air flora 25 cm above strawberry plants, which had been inoculated with benomyl-insensitive isolates during the blossom period in 1973, was monitored using a 'Rotorod' air sampler (Perkins, 1957). The number of conidia caught was recorded twice weekly from November 1973 until flowering commenced in 1974.

Senescent strawberry leaves were collected from these plots at two-weekly intervals throughout this sampling period, and incubated in moist chambers in the laboratory. Sporing Botrytis lesions arising from the debris were checked for benomyl insensitivity by point inoculation on malt agar containing commercial benomyl (100 ppm a.i.).

In November 1973, further samples of senescent leaves from these plots and from another plot which had not been inoculated with benomyl-insensitive isolates, were dipped for 15 min. in dichlofluanid (0.1% a.i.) to test the effectiveness of the fungicide in reducing overwintering inoculum. Samples of treated and untreated leaves were then transferred to nylon mesh bags and placed on the ground in the open for natural weathering. At two-weekly intervals, one treated and one untreated sample from each plot were incubated and assessed for the presence of Botrytis lesions.

All spray trials were carried out in walk-through polyethylene tunnels. In September each year, the strawberry cv. Cambridge Vigour was planted as runners 30 cm apart in three to four randomised blocks of guarded plots each containing 4 plants, ready for spraying next spring.

The fungicides evaluated were:- A - thiram (0.32% a.i.), B - dichlofluanid (0.1% a.i.), C - didecyl dimethyl ammonium bromide (DDAB) (0.1% a.i.), G - chlorothalonil (0.1% a.i.), K - glyphosine (0.05% a.i.), L - poly /1,5-(hexamethylene) biguanide hydrochloride/ - PP 073 (0.1% a.i.), M - benomyl (0.025% a.i.), N - vinclozolin (0.05% a.i.), P - benomyl/thiram as Benlate-T, (a.i.: 0.05% benomyl, 0.05% thiram), Q - prothiocarb (0.15% a.i.), R - griseofulvin(0.01% a.i.), S - experimental fungicide N-87 (0.05% a.i.).

In addition the following mixtures and alternating programmes were evaluated at concentrations listed above, unless otherwise stated. D - benomyl/dichlofluanid/benomyl, E - dichlofluanid (0.05% a.i.)/benomyl (0.0125% a.i.) tank mix, F - dichlofluanid/dichlofluanid/benomyl, H - dichlofluanid/benomyl/dichlofluanid, J - benomyl/benomyl/dichlofluanid.

In all the trials a three-spray programme was used. The fungicides were applied by hand lance (high volume - 0.22 $1/m^2$) commencing at 'late white bud', with two further applications at 10 day intervals. In trials II and III, flowers were inoculated with a benomyl-insensitive isolate, obtained from infected strawberry fruit, by atomising flowers with a conidial suspension (50,000/ml) 6-8 h before each fungicide spray.

Ripe fruit was picked twice weekly and the number and weight of healthy and Botrytis-infected berries from each plant recorded. Botrytis was isolated from each infected fruit and tested for benomyl-insensitivity as before.

For observations on post-harvest infection, a random sample of 20 symptomless undamaged berries was taken from each treatment at each pick, and carefully transferred to sealed moist chambers. The berries were assessed for rots caused by Botrytis, Mucor spp. or Rhizopus spp. after incubation for 3 days.

RESULTS

Botrytis conidia were identified on the 'Rotorod' sampling surfaces on each occasion throughout the 1973/1974 sampling period, the largest numbers being recorded following moist periods with temperatures above 14°C.

Botrytis lesions were present on all samples of untreated strawberry leaf debris after incubation; 72 per cent of isolates from these lesions were benomyl-insensitive. Lesions were not however, found on dichlofluanid-treated debris until 14 weeks after dipping.

Trial 1.

The effectiveness of dichlofluanid in suppressing flower infection from overwintered benomyl-insensitive Botrytis was demonstrated in 1974 by applying it to two-year-old plants overwintered from a previous trial (Jordan & Richmond, 1974) where insensitive isolates had been introduced during flowering in 1973.

Although dichlofluanid sprays significantly reduced (P=0.01) the proportion of fruit infected (Table 1), there were some phytotoxic effects on the leaves.

Table 1

Effect of dichlofluanid on fruit infection by overwintering benomyl-insensitive Botrytis

Treatment		Fruit infection	
1973	1974	(%)	Angular transformation ∠
Benomyl	Dichlofluanid	2.9	8.46
Benomyl	Unsprayed	24.6	29.30
Unsprayed	Dichlofluanid	5.1	11.75
Unsprayed	Unsprayed	23.8	29.06
S.E.D.			+2.16

Statistical treatment as 2 x 2 factorial design

Effects

Spraying 1973	-1.52
Spraying 1974	-19.07**
Interaction	-1.76
S.E. (effect)	±1.53

∠ Data transformed $\sin^{-1}\sqrt{x}$

** Significant at P=0.01

Trial II

The effectiveness of benomyl and dichlofluanid in controlling benomyl-insensitive Botrytis was compared with chlorothalonil, DDAB, glyphosene, PP 073 and thiram. Benomyl and dichlofluanid were also evaluated as a tank mix (half strength of each fungicide) and as alternating sprays using all combinations in the three-spray programme.

Thirsm, dichlofluanid, chlorothalonil, and the tank mix all gave very good control; the latter two programmes completely suppressed fruit rot due to insensitive isolates. Alternating sprays of benomyl and dichlofluanid in the three-spray programme also effectively controlled fruit rot; however, all the Botrytis infecting rotted fruit was benomyl-insensitive (Table 2).

Table 2

Evaluation of spray programmes for the control of benomyl-insensitive Botrytis isolates (1974)

Treatment	Yield/ plot(g)	Fruit infection (%)	Fruit infection angular transformation≠	Causal isolate (%) sensitive	Causal isolate (%) insensitive
A - Thiram	695	4.07	0.193	50	50
E - Tank mix	657	5.46	0.210	100	0
B - Dichlofluanid	504	5.95	0.220	50	50
J - Ben/Ben/Dich	631	7.40	0.242	0	100
G - Chlorothalonil	810	8.23	0.280	100	0
H - Dich/Ben/Dich	537	8.99	0.280	0	100
C - DDAB	616	10.45	0.299	0	100
D - Ben/Dich/Ben	653	10.07	0.313	17	83
F - Dich/Dich/Ben	822	10.74	0.333	25	75
K - Glyphosene	811	20.14	0.415	0	100
L - PP 073	684	17.66	0.434	50	50
M - Benomyl	702	23.04	0.493	0	100
O - Unsprayed	793	36.13	0.647	33	67
S.E.D.	±97		±0.062		

≠ Data transformed $\sin^{-1}\sqrt{x}$

Trial III

In 1975, six spray programmes from trial II were tested again and compared with a commercial benomyl/thiram mixture, prothiocarb, experimental fungicide N-87, griseofulvin and vinclozolin.

Fruit infections on unsprayed plants were rather few (10%) probably because of dry weather. However, all treatments except benomyl alone and prothiocarb significantly reduced fruit infection (P=0.01)by B. cinerea (Table 3). Isolates from all infected fruit picked in this trial were benomyl-insensitive.

Table 3

Evaluation of spray programmes for the control of benomyl-insensitive Botrytis isolates (1975)

Treatment	Yield/ plot(g)	Fruit infection (%)	angular transformation*
B - Dichlofluanid	928	0.05	1.342
N - Vinclozolin	1115	0.09	1.707
A - Thiram	768	0.23	2.776
R - Griseofulvin	862	0.60	4.456
E - Tank mix	975	0.70	4.804
K - Glyphosene	1190	1.23	6.380
L - PP 073	1000	1.33	6.625
P - Benomyl/Thiram	706	1.82	7.756
S - N.87	832	2.46	9.018
Q - Prothiocarb	1053	4.70	12.520
M - Benomyl	808	9.11	17.570
O - Unsprayed	743	10.39	18.808
S.E.D.	±83		±1.772

*Data transformed $\sin^{-1}\sqrt{x}$

Post-harvest infection
Trial II

With the exception of the benomyl, PP 073, and glyphosene treatments and the benomyl/benomyl/dichlofluanid programme, more fruit sampled from the sprayed blocks remained free from all fungal infection than fruit from unsprayed blocks (Figure 1) and these differences were significant.

Post-harvest Botrytis infection was suppressed by all spray programmes except chlorothalonil and PP 073. Although Rhizopus infection on fruit from the chlorothalonil, dichlofluanid, and DDAB treatments was less than on unsprayed plants and infection was greater on fruit from all benomyl treatments, the differences were not significant.

Fig. 1

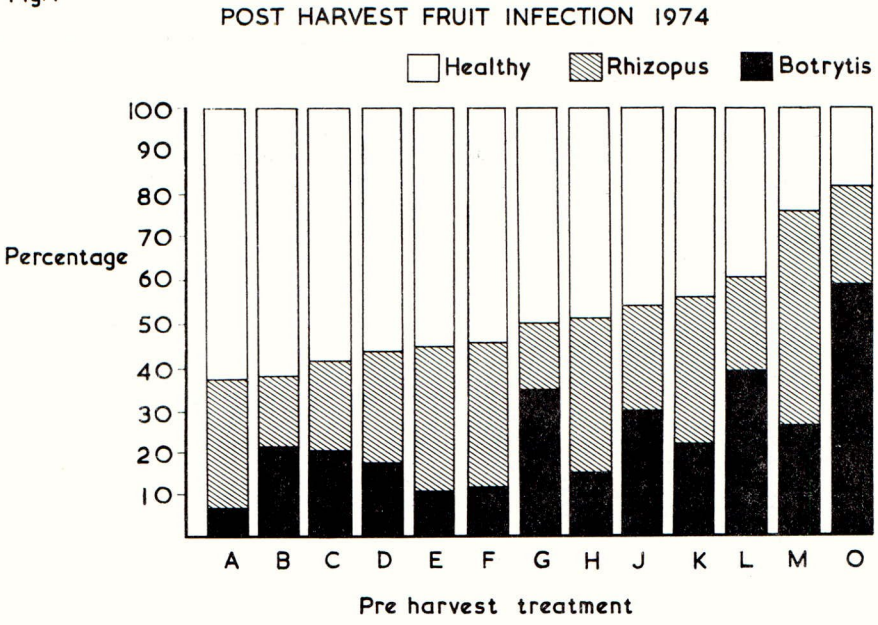
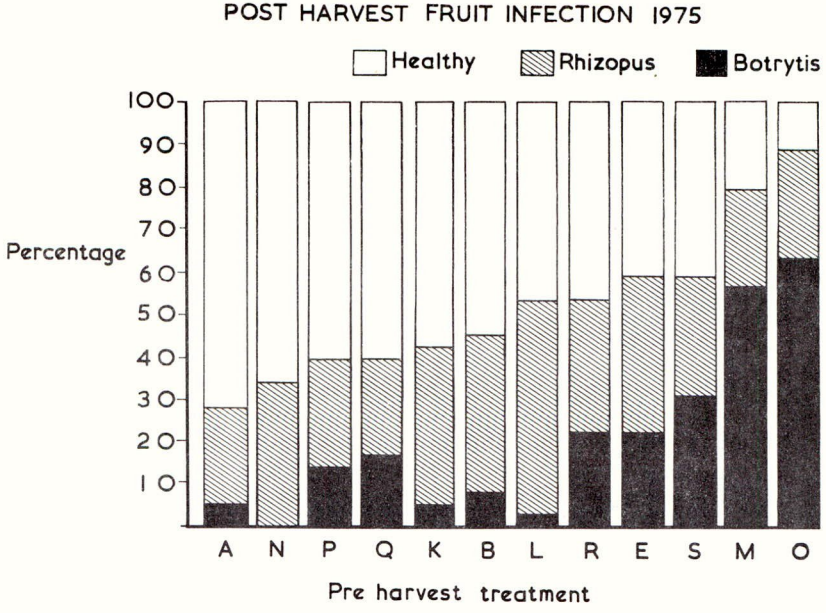

POST HARVEST FRUIT INFECTION 1974
Healthy
Rhizopus
Botrytis
Percentage
100
90
80
70
60
50
40
30
20
10
A B C D E F G H J K L M O
Pre harvest treatment

Fig. 2


POST HARVEST FRUIT INFECTION 1975
Healthy
Rhizopus
Botrytis
Percentage
100
90
80
70
60
50
40
30
20
10
A N P Q K B L R E S M O
Pre harvest treatment

Trial III

Significantly fewer healthy fruit were obtained from the benomyl, benomyl/dichlofluanid tank mix, N-87 treatments and unsprayed plants ($P = 0.05$) than all other treatments (Figure 2). All programmes, except benomyl alone, significantly decreased post-harvest Botrytis rot, and none occurred on fruit from vinclozolin treated plants. As with trial II there were no significant differences in post-harvest rotting caused by Mucor spp. or Rhizopus spp. Most soft rot infections were due to Rhizopus stolonifer, Mucor mucedo predominated on fruit from the last two pickings.

DISCUSSION

These results show that benomyl-insensitive strains of Botrytis can survive the winter on leaf debris and re-infect next summer. Although dichlofluanid suppressed sporulation of Botrytis for three months, it did not eliminate the insensitive strain. Treating leaf debris reduced the effect of overwintered inoculum.

Better control of fruit rot was obtained by using benomyl and dichlofluanid as a combined spray than by using the two fungicides alternately. Chlorothalonil and the benomyl/dichlofluanid tank mix completely eliminated the benomyl-insensitive strain in 1974; but not in 1975.

Since thiram gave good control of fruit rot in 1974, Benlate-T, a commercial mixture of benomyl and thiram, was included in the 1975 programme. This information gave good control but was not as effective as thiram alone or as the benomyl/dichlofluanid tank mix.

In both 1974 and 1975, less than 20 per cent of fruit from untreated plants remained healthy following incubation. The highest proportion of healthy fruit was obtained from thiram treated plants. Although pre-harvest treatment with vinclozolin completely eliminated post-harvest Botrytis rot, none of the spray treatments had any significant effect on post-harvest soft rot. Contrary to the findings of Dennis & Mountford (1975) Botrytis cinerea and Rhizopus stolonifer were the predominant spoilage organisms, and Mucor mucedo was isolated only from the two last pickings.

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References

BOLLEN, G.J. and SCHOLTEN, G. (1971). Acquired resistance to benomyl and some other systemic fungicides in a strain of Botrytis cinerea in cyclamen. Netherland Journal of Plant Pathology. 77, 83-90.

DENNIS, C. and MOUNTFORD, J. (1975). The fungal flora of soft fruits in relation to storage and spoilage. Annals of Applied Biology. 79, 141-147.

JARVIS, W.R. and HARGREAVES, A.J. (1973). Tolerance to benomyl in Botrytis cinerea and Penicillium corymbiferum. Plant Pathology. 22, 139-141.

JORDAN, V.W.L. and RICHMOND, D.V. (1974). The effects of benomyl on sensitive and tolerant isolates of Botrytis cinerea infecting strawberries. Plant Pathology. 23, 81-83.

MILLER, M.W. and FLETCHER, J.T. (1974). Benomyl tolerance in Botrytis cinerea isolates from glasshouse crops. Transactions of the British Mycological Society. 62, 99-103.

PERKINS, W.A. (1957). 2nd Semi-Annual Report, Aerosol Laboratory, Stanford University. C.M.L.186.

NOTES

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RESISTANCE TO ORGANOPHOSPHORUS INSECTICIDES OF PEACH-POTATO APHID (MYZUS PERSICAE) FROM SUGAR BEET IN 1975

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Summary Resistance to dimethoate and demeton-S-methyl was detected by laboratory tests in many of the 62 samples of peach-potato aphids collected from overwintering sites and from sugar beet during 1975, confirming findings of the smaller survey in 1974. One sample had a large proportion of very resistant aphids, but resistance was usually small and was detected by topical application of insecticides. The proportion of aphids with high carboxylesterase activity characteristic of resistance in most populations was measured.

INTRODUCTION

In 1973 there were complaints about the poor control of the peach-potato aphid (Myzus persicae) on sugar beet by organophosphorus insecticides. The following year, we examined the susceptibility to dimethoate of several samples originating from untreated fields and fields treated with one or more applications of insecticide (Needham and Devonshire, 1975). This survey, using topically applied discriminating doses of dimethoate, showed that resistant aphids were present in some of these populations. Clones established from some of the resistant populations were tested by topical application and systemic bioassays (Needham and Devonshire, 1976) which showed resistance factors of approximately 5-10-fold to demeton-S-methyl and dimethoate.

The present paper describes the findings of a similar but more extensive survey conducted in 1975, again in collaboration with Broom's Barn Experimental Station and the British Sugar Corporation agriculturists. We received samples of overwintering aphids, aphids from sugar beet before insecticide had been applied, and aphids which had survived insecticide treatment.

METHOD AND MATERIALS

Aphids from field samples were cultured on chinese cabbage to obtain sufficient numbers for bioassays. Dimethoate and demeton-S-methyl were applied topically (Needham and Devonshire, 1973), and populations were classified as resistant when more than 10% survived the dose of insecticide (25 ng dimethoate/aphid, or 10 ng demeton-S-methyl/aphid) which always killed 100% of susceptible populations. The proportions surviving these discriminating doses were determined from regression lines calculated from the response to a range of doses (400, 100, 25, 6.3 ng dimethoate per aphid, or 40, 10, 2.5 ng demeton-S-methyl per aphid), each applied to

a group of 30 aphids.

In addition, the total carboxylesterase activity was measured in each of 15 individuals from most samples (Devonshire, 1975). Not more than 15 individuals from each population could be examined because of the large number of samples received, but care was taken to avoid using the progeny of single aphids.

RESULTS AND DISCUSSION

The tables summarize the results of bioassays and esterase determinations.

Table 1

Resistance to dimethoate and demeton-S-methyl of *M. persicae* overwintering asexually

Sample	Source	Resistance status		
		dimethoate*	demeton-S-methyl*	% high esterase**
1	Northants., Rape	-	-	50
2	Notts., Mangold clamp	R	-	60
3	Cambs., Mangold clamp	S	-	70
4	Cambs., Mangold clamp	S	-	90
5	Cambs., Rape	R	R	85
6	Lincs., Mangold clamp	R	R	100
7	Beds., Beet seed crop (sprayed 1974)	R	R	100

* Assessed by topical application of discriminating doses. S = susceptible, R = more than 10% of population surviving a dose of 25 ng/aphid dimethoate or 10 ng/aphid demeton-S-methyl.

** Proportion of the population with total esterase activity typical of a resistant strain of aphids (MS1 clone G) isolated from sugar beet in 1974 (Needham and Devonshire, 1975).

There was a large proportion of resistant aphids in the seven samples of overwintering populations tested (Table 1). However, this may not represent the overall situation throughout all of the sugar beet growing areas because the number of samples was small. All the samples from overwintering sites contained a large proportion of individuals with total esterase activity characteristic of resistant aphids, and resistance was confirmed by bioassay in four of the samples. The first sample did not survive long enough for bioassay tests to be done. These results indicate that resistance is maintained in aphids overwintering asexually.

Twenty three samples were received from sugar beet before spraying and seventeen were reared successfully and tested (Table 2). Of these, 7 contained more than 25% of aphids with total esterase activity characteristic of resistance, and resistance was confirmed in four of these by bioassay together with another sample in which the esterase was not examined.

Table 2

Resistance to dimethoate and demeton-S-methyl of M. persicae from unsprayed sugar beet

Sample	Source	Resistance status		
		dimethoate*	demeton-S-methyl*	% high esterase**
2	Hunts.	R	R	85
3	Cambs.	R	S	80
4	Beds.	-	-	40
6	Norfolk	R	R	50
7	Lincs.	S	S	-
8	Norfolk	S	S	-
10	Salop.	S	S	35
11	Yorks.	-	-	65
12	Cambs.	-	-	0
13	Lincs.	S	S	20
14	Norfolk	S	-	20
15	Lincs.	R	-	0
19	Yorks.	R	S	0
20	Worcs.	R	R	-
21	Yorks.	S	S	-
22	Norfolk	R	-	65
23	Norfolk	S	S	-

*, ** see footnote for Table 1

Table 3 gives the results of tests on populations of aphids from sugar beet sprayed with the organophosphorus insecticides shown. Thirteen of the samples were from fields which had been sprayed between 3 and 5 times, but we did not detect resistance in some of these, and presume that resistance was not responsible for failure to control in these cases. In 23 of the 34 samples tested more than 25% of the population had high esterase activity, but resistance was detected by bioassay in only 13 of these.

Resistance in most samples was low and the largest doses of insecticide used in the bioassays killed all the aphids in every sample except number 1 in Table 3, in which a large proportion survived. Approximately half the aphids in this sample had esterase activity characteristic of very resistant aphids from glasshouses, and a clone with high esterase activity established from this population was approximately 30-40-fold resistant to demeton-S-methyl and dimethoate (Devonshire, 1975).

There are some apparent inconsistencies between the determinations of resistance by bioassay and esterase activity (Table 1, samples 3 and 4; Table 2, sample 10; Table 3, samples 17, 21, 27, 31, 36, 37 and 46). These suggest that the two methods might not be well correlated, but we believe the discrepancies result from the insensitivity of the bioassay technique to the small differences in the susceptibility of these aphids and the heterogeneity of the populations. A small proportion of aphids with enhanced esterase activity might not be revealed by bioassay because their weak resistance would give a range of response to the

Table 3

Resistance to dimethoate and demeton-S-methyl of M. persicae from sprayed sugar beet

Sample	Source	Spray treatment	Resistance status		
			dimethoate*	demeton-S-methyl*	% high esterase**
1	Beds.	Demeton-S-methyl	R	R	50
3	Bucks.	4 x Demeton-S-methyl	-	R	-
4	Essex	Phosphamidon	R	R	70
5	Notts.	2 x Phosphamidon	R	S	-
6	Cambs.	2 x Dimethoate, 2 x Demeton-S-methyl	R	S	-
7	Cambs.	2 x Demeton-S-methyl, 2 x Dimethoate	S	-	15
8	Lincs.	Phosphamidon	S	R	0
9	Essex	Demeton-S-methyl	R	R	30
10	Cambs.	4 x Demeton-S-methyl	R	S	35
11	Norfolk	Demeton-S-methyl	R	-	10
12	Lincs.	Demeton-S-methyl	S	-	0
13	Cambs.	2 x Demeton-S-methyl, 2 x Dimethoate	S	S	10
15	Suffolk	2 x Demeton-S-methyl	R	R	100
16	Suffolk	Demeton-S-methyl,Pirimicarb	R	R	100
17	Norfolk	3 x Demeton-S-methyl	S	S	70
18	Suffolk	2 x Demeton-S-methyl	S	S	35
19	Norfolk	Demeton-S-methyl	R	R	80
20	Norfolk	Phorate, Demeton-S-methyl	R	R	100
21	Norfolk	Demeton-S-methyl	R	R	0
22	Lincs.	3 x Demeton-S-methyl	R	R	35
24	Beds.	Dimethoate, 4 x Demeton-S-methyl	R	R	100
25	Norfolk	3 x Demeton-S-methyl	S	S	35
26	Norfolk	Demeton-S-methyl, Phosphamidon	S	-	0
27	Lincs.	4 x Demeton-S-methyl	S	S	100
28	Essex	4 x Dimethoate	S	S	15
29	Norfolk	2 x Demeton-S-methyl	S	S	15
31	Norfolk	2 x Demeton-S-methyl	S	S	65
32	Norfolk	2 x Demeton-S-methyl	R	R	100
35	Norfolk	Demeton-S-methyl, Phorate	R	-	35
36	Norfolk	Dimethoate, Demeton-S-methyl	S	S	100
37	Suffolk	Demeton-S-methyl	S	S	80
38	Norfolk	2 x Demeton-S-methyl	-	-	0
39	Suffolk	Demeton-S-methyl	R	R	-
40	Lincs.	2 x Demeton-S-methyl	-	-	0
41	Suffolk	4 x Demeton-S-methyl	-	-	65
42	Yorks.	2 x Demeton-S-methyl	-	-	25
46	Lincs.	Demeton-S-methyl	S	S	50
47	Norfolk	2 x Dimethoate	R	R	100

*, ** see footnote for Table 1

insecticides overlapping with that of the susceptible strains. This would explain the disagreement in some samples between the bioassay tests and the esterase determinations. In other confirmed cases of resistance of the peach-potato aphid to anticholinesterase insecticides, high esterase activity has always been associated with resistance (Needham and Sawicki, 1971; Beranek, 1974; Needham and Devonshire, 1975; Devonshire, 1975).

We therefore conclude that, although the results for some individual samples may be difficult to interpret, the overall situation is one of widespread resistance of peach-potato aphids associated with sugar beet to certain organophosphorus compounds and that these resistant aphids overwinter asexually without losing resistance.

Acknowledgements

We thank Mrs. S. Petzing and Mr. G. D. Moores for doing the laboratory tests, and all those who supplied samples of aphids; in particular, the agriculturalists of the British Sugar Corporation and our colleagues at Broom's Barn Experimental Station.

References

BERANEK, A.P. (1974) Esterase variation and organophosphate resistance in populations of Aphis fabae and Myzus persicae, Entomologia experimentalis et applicata, 17, 129-142

DEVONSHIRE, A.L. (1975) Studies of the carboxylesterases of Myzus persicae resistant and susceptible to organophosphorus insecticides, Proceedings 8th British Insecticide and Fungicide Conference Session 2A

NEEDHAM, P.H. and DEVONSHIRE, A.L. (1973) A technique for applying small drops of insecticide solution to Myzus persicae, Pesticide Science, 4, 107-111

NEEDHAM, P.H. and DEVONSHIRE, A.L. (1975) Resistance to organophosphorus insecticides in field populations of Myzus persicae from sugar beet, Pesticide Science, in the press

NEEDHAM, P.H. and DEVONSHIRE, A.L. (1976) Laboratory techniques for evaluating insecticides for aphid control and their application to studies on resistance, Crop Protection Agents and their Biological Evaluation ed. N.R. McFarlane, Academic Press, London

NEEDHAM, P.H. and SAWICKI, R.M. (1971) Diagnosis of resistance to organophosphorus insecticides in Myzus persicae, Nature, 230, 125-126

NOTES

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MONITORING FOR RESISTANCE TO ORGANOPHOSPHORUS INSECTICIDES IN MYZUS PERSICAE FROM SUGAR BEET

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Summary A survey in 1975 of the susceptibility of Myzus persicae has confirmed the presence of resistance to dimethoate and demeton-S-methyl on sugar beet first detected in 1974. Although the resistance levels found in these laboratory tests were low, a field trial showed that some dimethoate and demeton-S-methyl-resistant M. persicae can survive normal applications of demeton-S-methyl. The field resistant aphids are susceptible in laboratory bio-assays to acephate, carbofuran, "Croneton", methamidophos and pirimicarb. A simple and rapid technique has been developed for detecting resistance to dimethoate and demeton-S-methyl.

Resumé Des éssais en laboratoire et en plein air ont demontré que des éléments de Myzus persicae prélevés sur la betterave sucrière en 1974 et 1975 resistent au demeton-S-methyl et dimethoate. Ces élements sont restés susceptibles à l'acéphate, la carbofuran, le "Croneton", le methamidophos et le pirimiphos. Un essais simple et rapide decèle les pucerons resistants au demeton-S-methyl.

INTRODUCTION

In 1974, a survey of the response to insecticides of the peach-potato aphid (Myzus persicae) on sugar beet showed that a substantial proportion of the field populations tested contained individuals resistant to dimethoate. However this resistance was weak and it is uncertain how far it contributed to the poor control of aphids in 1974 particularly as the exceptional field conditions affected the performance of the insecticidal treatments.

In view of the serious implications of resistance to aphicides for the spread of virus yellows, in the autumn of 1974 we greatly expanded our investigations on resistance in aphids in co-operation with Broom's Barn Experimental Station and with support from the British Sugar Research and Education Committee (BSREC). The principal objectives of these investigations were to:-

1. evaluate new or alternative insecticides controlling resistant aphids;

2. determine if the low level of resistance found in laboratory tests affected aphid control in the field;

3. develop a simple bio-assay technique to be used by advisors in the field of the British Sugar Corporation (B.S.C.) for discriminating between susceptible and resistant aphids;

4. monitor resistance in M. persicae during the 1975 season by bio-assays and carboxyesterase determination.

Evaluation of insecticides for controlling demeton-S-methyl- and dimethoate-resistant aphids

The two strains of M. persicae used for the laboratory tests, MS1 (resistant) and US1L (susceptible) were bred from insects collected on sugar beet in East Anglia in the summer of 1974 (Needham and Devonshire, 1976). Strain US1L was bred from a single vivipara which had weak carboxylesterase (EC 3.1.1.1.) activity (Needham and Sawicki, 1971), MS1 was bred from a sample of aphids collected from sugar beet sprayed several times with aphicides. The US1L strain and MS1 represented the two extremes of the tolerance range to dimethoate and of esterase activity found in the populations tested in 1974 (Devonshire and Needham, 1975).

The aphids were reared on chinese cabbage in constant environment rooms under 16h daylength and were bioassayed either by the topical application of measured drops of solutions of insecticides (Needham and Devonshire, 1973), or by a systemic test in which they fed on seedlings of chinese cabbage treated with insecticides as described elsewhere (Needham and Devonshire, 1976).

The MS1 aphids were 5-6 times less susceptible to demeton-S-methyl, disulfoton, and dimethoate than the US1L aphids, but interstrain differences were small or absent when the aphids were bioassayed systemically with acephate, carbofuran, "Croneton", methamidophos or pirimicarb (Table 1). These tests showed that the levels of resistance to demeton-S-methyl and dimethoate of the field strain were considerably lower than those of some resistant strains of M. persicae from glasshouses (Needham and Sawicki, 1971) and that several insecticides, including organophosphorus compounds, should control field strains of M. persicae resistant to demeton-S-methyl and dimethoate.

Table 1

24 h results of systemic tests with several insecticides against susceptible (US1L) and resistant (MS1) aphids

Insecticide	Strain	LC_{50} ± S.E. w/v	Resistance factor
demeton-S-methyl	US1L	.000034 ± .000007	-
	MS1	.00013 ± .000031	8
dimethoate	US1L	.00058 ± .000057	-
	MS1	.0032 ± .00024	6
disulfoton	US1L	.0011 ± .00018	-
	MS1	.0052 ± .00090	5
acephate	US1L	.0071 ± .0012	-
	MS1	.0058 ± .0011	0.8
carbofuran	US1L	.011 ± .0022	-
	MS1	.023 ± -	2
"Croneton"	US1L	.00030 ± .000061	-
	MS1	.00051 ± .00010	1.7
methamidiphos	US1L	.0023 ± .00031	-
	MS1	.0034 ± .00062	1.5
pirimiphos	US1L	.00014 ± .000017	-
	MS1	.00018 ± .000029	1.3

Studies of the control of strains US1L and MS1 under field conditions

The resistant and susceptible aphids, reared on chinese cabbage in constant environment rooms were transferred onto sugar plants grown in pots in the glasshouse at the 4 leaf stage. The plants were left in the glasshouse to increase the infestation for two weeks and were then planted outside in 4 randomized blocks of 6 plots. The infested plants were enclosed in fine mesh muslin and each was surrounded by guard plants at the normal spacing. The number of aphids per infested plants were assessed in the evening 7 days after planting out, and plants were sprayed the following morning with Metasystox 55 at the standard rate (238g a.i./ha) twice or four times the standard rate using a knapsack sprayer. The volume of water used (1123 l/ha)greater than recommended ensured uniform covering but allowance was made for the greater dilution in determining the volume applied to each plant, and the muslin covers were lifted only during spraying and counting. On the 1st day after spraying 18% of the susceptible aphids survived the standard dose of demeton-S-methyl and 4% survived on plants treated with the largest dose (Table 2). In contrast 51-71% of the resistant aphids were alive on plants treated with the recommended or double dose and 15% survived the largest dose. Four days after treatment all the susceptible aphids were dead, except for a few on a single plant treated with a double dose of insecticide; 17% and 11% of the resistant aphids were alive on plants treated with the single and double dose but none survived treatment with the largest dose.

Table 2

Survival of susceptible and resistant aphids in the field on sugar beet sprayed with Metasystox 55

Day after treatment	Strain	% survivors after treatment in Metasystox 55		
		Standard rate	2 x Standard rate	4 x Standard rate
1	S	18	8	4
	R	51	71	16
4	S	0	1	0
	R	17	11	0

Survey of the susceptibility of M. persicae to dimethoate and demeton-S-methyl on sugar beet during 1974-1975

A survey was done to determine if resistant aphids were present in (a) overwintering populations in clamps and on rape and (b) among aphids invading the sugar beet before spraying. Tests were done to check for resistance when control in the field was inadequate.

The methods used and the results of this survey are presented in detail in an accompanying paper (Needham and Devonshire, 1975). Resistant individuals were present in overwintering populations and among aphids invading the sugar beet before spraying, and in most of the samples collected from fields where control with insecticidal sprays was inadequate.

Simple bioassay for discriminating between susceptible and resistant aphids

Topical application of measured drops of insecticides and systemic bioassays are generally used to determine resistance in aphids but require considerable skill, specialized equipment and are unsuitable for use outside the laboratory. We have therefore devised a simple bioassay to detect resistant aphids based on a dipping technique which will be evaluated in the field next year.

The aphids are placed in a short glass tube (25 mm high, 25 mm diameter) treated with Fluon® halfway up the internal wall and with the bottom end being covered with a fine-meshed nylon gauze secured by an elastic band. This cage is placed in a small narrow glass dish and 2 ml of 0.03% w/v a.i. of Metasystox 55 in water pipetted onto the aphids. After contact with the solution for 10 sec. the insects are dried by blotting the bottom end of the cage with filter paper. The top end of the tube is then covered with nylon gauze secured by an elastic band, and the tube is inverted and tapped sharply to tip the aphids onto the clean gauze. The gauze originally at the bottom end of the tube is replaced by a fresh gauze and kill is recorded every 15 min for 1 hour. Whenever possible 5 replicates of 10 insects are used. In preliminary tests, kill was assessed 16 hr after treatment, but this was decreased to 1 hr. when it was found that nearly all susceptible aphids died within 30 to 45 min of treatment, whilst many of the resistant aphids survive. Decreasing the time interval between treatment and check has the advantage that it decreases control mortality, simplifies post-treatment and emphasises the differences between susceptible and resistant aphids because resistant individuals continue to die as the interval after treatment increases. This technique is designed to detect the presence of resistant individuals in heterogeneous field populations. It cannot estimate the proportion of resistant individuals because dosage/response curves overlap so that resistant as well as all susceptible individuals die at any chosen dose level.

Conclusions

Several problems highlighted by the 1974 survey of the tolerance of field populations of M. persicae on sugar beet to dimethoate have now been investigated. Systemic bioassays showed that several insecticides are effective against both resistant and susceptible aphids and might be used where loss of control is directly attributable to the resistance of the aphids to demeton-S-methyl or dimethoate. Although the resistance detected by systemic tests in the laboratory was weak, the field experiments indicated that some resistant aphids can survive normal insecticidal treatment. A simple and rapid test has been devised to detect resistant aphids and this will be evaluated in the field in 1976. The latest (1975) survey has again shown that resistance to demeton-S-methyl and dimethoate is present in M. persicae on sugar beet (Devonshire and Needham, 1975). The presence of resistant individuals in overwintering populations demonstrated by this survey together with the studies of the heredity of resistance in M. persicae by R. Blackman of the Natural History Museum (1975, personal communication) indicates that resistant M. persicae are unlikely to disappear from one season to the next and that resistance must be regarded as an established problem which will remain with us. However nothing is yet known about the frequency and distribution of this resistance and in view of the particularly unusual weather in the last two years it is not possible to predict how the situation will evolve in the future.

Acknowledgements

We thank Mrs. S. Petzing and Mr. G. Moores for their help, the British Sugar Research and Education Committee for financial support, our colleagues of Broom's Barn Experimental Station and all the people who supplied us with the insecticides and aphids.

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DEGRADATION OF BUPIRIMATE FUNGICIDE ON APPLES AND IN WATER

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Summary Bupirimate, an apple powdery mildew fungicide, was shown to be volatile in a radioactive experiment. Such volatility would account for its vapour phase fungicidal activity.

There was little penetration of either ^{14}C-bupirimate or its radioactive conversion products into apple leaves and fruits. The main short-term degradation product was ethirimol which itself was further degraded.

At low concentration, bupirimate degraded rapidly in water exposed to sunlight. The main degradation product was ethirimol which is known to break down further, but more slowly, in water. A minor degradation product which was formed from bupirimate was identified as a bupirimate isomer.

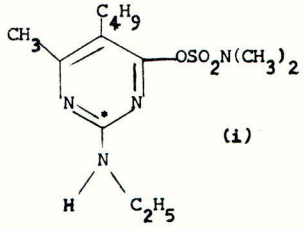
INTRODUCTION

Bupirimate (5-butyl-2-ethylamino-6-methylpyrimidin-4-yl dimethylsulphamate, (i), fig. 1) has proved to be very effective for controlling apple powdery mildew (Podosphaera leucotricha). (Finney J. R. 1975) It is a sulphamate ester of ethirimol ((ii), Fig 1) a systemic fungicide used for the control of powdery mildew of cereals. The metabolism of ethirimol in cereal plants has been reported previously (Cavell et al, 1971).

Bupirimate contrasts with ethirimol in respect of its vapour activity, and the composition of bupirimate vapour was therefore determined.

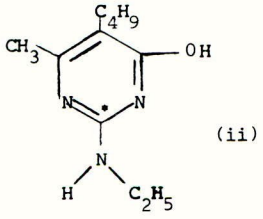
In this paper we also consider the breakdown of bupirimate after application to apple foliage and fruit and also in an aqueous environment.

Figure 1 Structures of bupirimate and its breakdown products



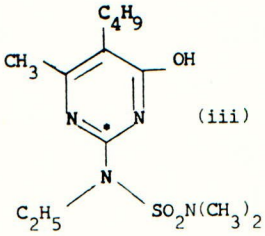
(i)

bupirimate

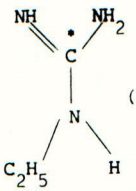


(ii)

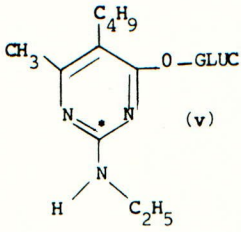
ethirimol



(iii)



(iv)



(v)

METHOD AND MATERIALS

The ^{14}C-bupirimate used in the study was labelled in the 2 position of the pyrimidine ring. (Shown by the asterisk in Fig 1). The specific activity varied for each experiment.

Thin layer chromatography was carried out on 0·25 mm silica F_{254} precoated chromatoplates (Merck A. G., Darmstadt, Germany).

Solvent systems used were:-

a1)	Chloroform : methanol	95 : 5	for less polar degradation products
a2)	" "	90 : 10	
b)	Acetone : water	95 : 5	
c)	Butanol : acetic acid : water	12 : 3 : 5	for more polar degradation products
d)	Butanol : ethanol : water	3 : 1 : 1	

Occasionally solvent system e was used

e) Chloroform : methanol : ethyl acetate 6 : 3 : 1

Analysis of bupirimate vapour

A thin film of ^{14}C-bupirimate (10uCi, 125 µg) was applied to the inside of a 2 litre flask by rotary evaporation of a methanolic solution of the radioactive fungicide.

Air was drawn through the flask (kept at 40°C) and then bubbled through methanol (20 ml) cooled to -70°C in a solid carbon dioxide/acetone bath.

After 24 hours the quantity of radioactivity in the methanol was measured and its composition determined by thin layer chromatography.

Application of ^{14}C-bupirimate to apple fruit and foliage

^{14}C-bupirimate was prepared as the normal commercial formulation containing 100 ppm bupirimate. The formulation (10 ml) was applied to the branch of an apple tree by an aerosol spray. When quantitative application was required, a microsyringe was to spot the material on to individual leaves and fruits. Analyses were performed at intervals after application. Leaves and apple fruit peel were washed with methanol and then homogenised in the same solvent.

The amount of radioactivity extracted by these techniques was determined and the extracts were chromatographed together with reference compounds.

Radioactivity which had penetrated into the apple flesh was similarly quantified following methanol extraction. Radioactivity which could not be extracted was determined by combustion analysis.

Degradation in water

Aqueous solutions containing ^{14}C-bupirimate (0·2 and 2 µg/ml) were placed in uncovered crystallisation dishes which were exposed to bright sunlight for 43 days. Aliquots were withdrawn at intervals and analysed quantitatively for ^{14}C-bupirimate and qualitatively for other reference compounds by two dimensional chromatography.

A solution (1 litre) containing bupirimate (15 µg/ml) was exposed to daylight for 80 hours. After removing the water in vacuo, bupirimate conversion products were separated by thin layer chromatography in solvent system a1). An isomer (iii, Fig 1) of bupirimate was isolated and purified by repeated chromatography in the same solvent system.

RESULTS AND DISCUSSION

Analysis of bupirimate vapour

A small but significant (<0·2%) volatilisation of ^{14}C-bupirimate occurred when a flask coated with a thin film of the fungicide was heated at 40°C for 24 hours. The volatile radioactivity was trapped in methanol and was shown to be mainly bupirimate by two way chromatography.

The fungicidal activity of the vapour is therefore due to bupirimate itself and not to its breakdown products.

Breakdown of bupirimate on apple fruit and leaves

Commercial conditions were simulated as closely as possible during the application of ^{14}C-bupirimate to apples in the field.

Little bupirimate or its breakdown products entered the leaf or fruit. (See autoradiogram Fig 2).

That only a small percentage of the radioactivity penetrated the leaf was confirmed by combustion of samples of apple flesh and measurement of any $^{14}CO_2$ evolved. The maximum amount of radioactivity found in the flesh of any apple up to 22 days after application was 9% of that applied.

Bupirimate was more stable on the surface of apple leaves and fruit than in aqueous solution (see later) and could still be detected after three weeks. Figure 3 shows that ethirimol is a major degradation product on apple leaves after three days. Two other major products have been identified as ethirimol glucoside and ethylguanidine. The rest of the mixture consists of a complex series of products most of which are likely to be breakdown products of ethirimol itself.

Many of the degradation products of ethirimol, formed in and on cereals, are known (Cavell et al 1971) and these are being evaluated to see if they are formed from bupirimate on apples.

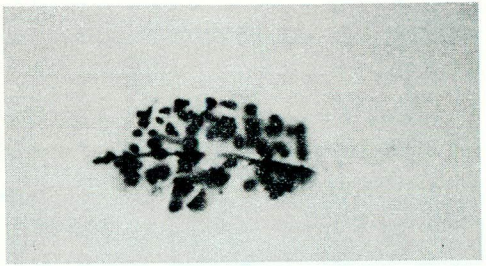
The presence of ethylguanidine demonstrates cleavage of the pyrimidine ring and the formation of the glucoside shows that bupirimate and/or ethirimol had penetrated the leaf.

The identification of breakdown products of a pesticide on plants is often difficult. The same products may be formed in aqueous solution where they are more readily identified.

Autoradiograms of apple fruit & foliage 22 days after treatment with ^{14}C—bupirimate

Cox

Golden Delicious



Cross sections of Golden delicious fruit

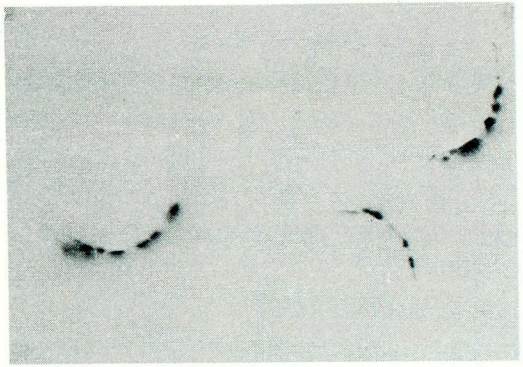
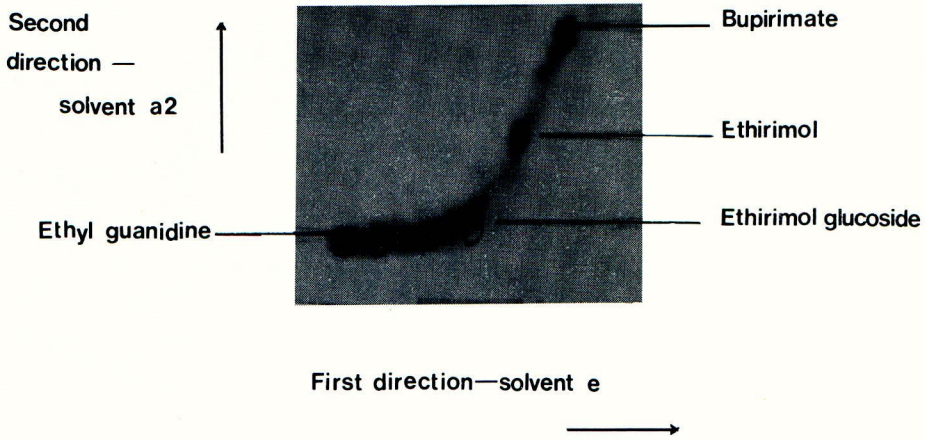
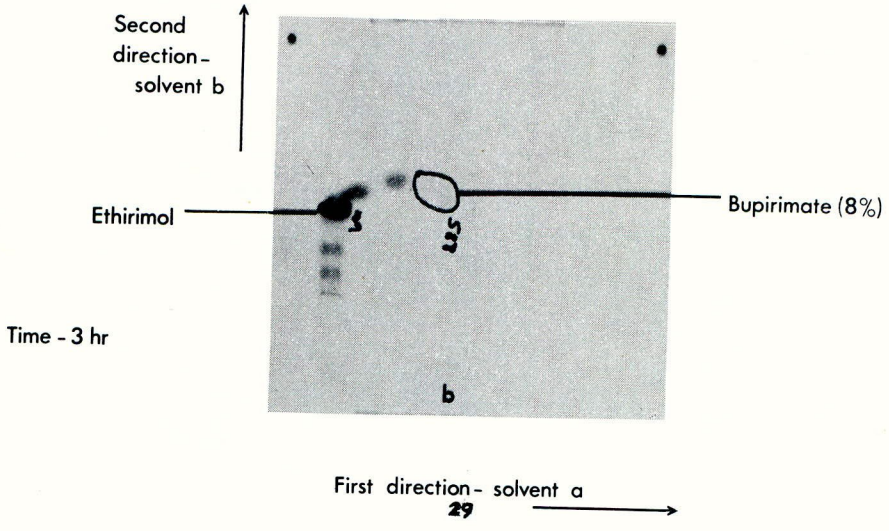


Fig. 2

Fig.3 Breakdown of ^{14}C-bupirimate on apple leaf (3 days)



Breakdown of bupirimate in aqueous solution exposed to sunlight — separation of the degradation products by T.L.C.
Fig.4



Bupirimate degradation in water

Experiments with tracer levels of bupirimate in water showed it to be highly unstable. Figure 4 shows that after three hours exposure of a solution of concentration of 0·2 µg/litre to natural daylight only 8% of the applied radioactivity remained as unchanged bupirimate. Ethirimol was the major product present and was still the main product after 6 days exposure. Little radioactivity was lost by volatilisation or adsorption since the average recovery of radioactivity was 94%.

The rate of degradation of bupirimate was slower in the second experiment probably due to the increased concentration (experiment 1 - 0·2 µg/ml, experiment 2 - 2 µg/ml) and the time of year (experiment 1 - May, experiment 2 - September). In spite of this only 12-14% of the radioactivity was accounted for as bupirimate after 3 days. Ethirimol was again the major degradation product.

Since bupirimate could be used in conjunction with a scab fungicide such as captan, the influence of captan present at an equal concentration of 2 µg/ml, was investigated. Captan was shown to have no effect on the rate of breakdown of bupirimate or the nature of the products produced.

The breakdown of ethirimol in aqueous solution has been studied previously and results in the ultimate production of ethylguanidine (Cavell et al 1974).

A secondary degradation pathway yielded three products which were derived directly from bupirimate and which chromatographed between bupirimate and ethirimol. The first of these, which chromatographed close to bupirimate, was the most stable and has been identified as the isomeric 5-butyl-2-(N-dimethylsulphamoyl-N-ethyl)-6-methyl-pyrimidin-4-ol (iii Fig 1) by nmr and mass spectrometry. Since there is no reaction between ethirimol and N-dimethyl sulphamic acid this compound may have been produced by intramolecular rearrangement. The product is non-fungicidal.

Acknowledgement

Mrs B. A. Cleverley, Miss M. S. May and Mr M. Skidmore assisted in the experimental work.

References

CAVELL, B. D., HEMINGWAY, R. J. and TEAL, G. (1971) Some Aspects of the Metabolism and Translocation of the Pyrimidine Fungicides (1971). Proc. 6th Br. Insectic. Fungic. Conf. 2, 431-437.

CAVELL, B. D. and TEAL, G. Unpublished results (1974).

FINNEY, J. R. PP588 (BUPIRIMATE): A New Fungicide for the Specific Control of Powdery Mildews on Apples and Other Crops (1975). 8th International Plant Protection Congress Reports and Information, Section III, Chemical Control Part 1, 235-236.

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TRIDEMORPH RESIDUES IN GRAIN FROM BARLEY PLANTS GROWN TO MATURITY

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Summary Samples of grain from commercial field crops of winter and spring barley sprayed with tridemorph at 0.6 lb/ac were assayed for tridemorph residues using a specific gas-liquid chromatographic method. Residues were detectable in all samples, but with a large range of variability. The highest levels (4.4 µg/g grain) found were in excess of the levels previously detected in grain samples from controlled test plots.

INTRODUCTION

Tridemorph (2,6-dimethyl-4-tridecylmorpholine) is the active ingredient of the systemic fungicide 'Calixin' which is widely used as a foliar spray against powdery mildew of barley (Erysiphe graminis f.sp. hordei). Previous work (Waring & Wolfe, 1975) had shown that residues of tridemorph could be found in grain from plants in field plots sprayed with the fungicide and then grown to maturity. It was considered necessary to test whether similar residues could be detected in material grown and sprayed under normal field conditions, and whether the varietal differences observed in the earlier tests were also apparent in commercially grown barley varieties.

METHOD AND MATERIALS

Ear samples were collected at random from commercial barley fields at dead ripe stage, shortly before harvest (Table 1). All fields had been sprayed with standard farm equipment, using recommended rates of application of the fungicide as part of the normal farm spray programme. Plots in the two fields of Maris Otter at Bayfordbury had been left unsprayed, sprayed in the autumn only, or sprayed in the spring only. The major area of both fields was sprayed both in autumn and spring.

The samples of grain were analysed for tridemorph residues using methanolic extraction followed by g.l.c. with an Alkali Flame Ionisation detector (Pye Unicam) to determine nitrogen-containing compounds (Waring & Wolfe, 1975). The limit of detection of tridemorph was 3 ng (equivalent to 0.03 ppm in a 1 g sample).

RESULTS

From Table 2 it can be seen that all samples had some tridemorph residues in the grain. The trace recorded in the control samples may have occurred as a result of spray drift since the unsprayed plot was bordered by large areas which received

two spray applications respectively in the autumn and spring. In the earlier work (Waring & Wolfe, 1975) no residues were found in control grain samples at the limit of detection (3 ng equivalent to 0.03 ppm in a 1 g sample).

The data show considerable variation in the level of residues, differing with both site and variety, and extending beyond the range observed in samples from trial plots in 1973, i.e. 0.3 to 4.4 µg/g grain in 1974, compared with 0.4 to 1.6 µg/g grain in 1973.

At Bayfordbury samples were taken from set points along a transect crossing the two fields of Maris Otter (A and B, Table 2). The data indicate considerable within-field variability (0.4 to 2.1 µg/g grain) although there was some evidence of a gradient along the transect in field A. Residues appeared to have persisted in the plot sprayed in the autumn only (0.5 µg/g grain) for a period of approximately nine months. The mean level of grain residues in the samples from the areas which received two sprays were considerably less than the levels detected in samples from crops of Maris Otter at Abbotsley and Elsworth, i.e. respectively, 1.1 against 2.6 µg/g grain. This difference may have been due in part to the spring sprays at the Cambridgeshire sites having been applied one month later than those at Bayfordbury.

Although there was considerable variation in the residues detected in the Choseley, Norfolk, samples (2.0 to 4.4 µg/g grain) all residue levels were in excess of the maximum obtained for this variety in the previous study (1.6 µg/g). The reason for this may have been due in part to the generally poor conditions for crop growth in this area in 1974. As a consequence, plant size was severely limited, thus reducing the volume of tissue available for distribution of the applied spray.

At the Balsham and St Albans sites, the crops were relatively close together and were well grown. The close similarity in residue levels of samples from within crops thus indicates that the differences between crops may have had a large varietal component.

DISCUSSION

It is apparent that tridemorph residues occur generally in barley grain at harvest: these may represent more than 10% of the material applied in a single spray (see Waring & Wolfe, 1975). The levels found were, however, highly variable and any varietal influence which may exist was largely obscured by environmental differences. It is likely that the range of variation in residues in crops in general will be in excess of that found in crops surveyed. Large numbers of samples would be required to confirm the varietal differences found in previous controlled tests. The technique for analysing residues, which is both sensitive and convenient, could be used to monitor grain samples for unacceptable levels of fungicide. It is also of value in determining tridemorph residues at various stages of bioassays, in order to find the critical levels in leaves for control of the pathogen. Differences between sensitive strains of the pathogen and those which exhibit insensitivity would be of interest, and some of this work is now being undertaken.

Reference

Waring, R.H. & Wolfe, M.S. (1975) Distribution of tridemorph and its metabolites in barley plants grown to maturity. Pesticide Science 6, 169-172.

TABLE 1 Conditions of field trials of barley plants sprayed with tridemorph and then grown to maturity

	SITE	NO. OF FIELDS	VARIETY	SPRAY DATE (0.6 lb/acre)	NO. OF SAMPLES
1.	Bayfordbury, Herts.	2	Maris Otter	1 Autumn 1973 2 8 April 1974 3 untreated	9
2.	Choseley, Norfolk	7	Proctor	16 May 1974	7
3.	Balsham, Cambs.	4	Berac	mid-May 1974	6
			Julia	"	"
			Mazurka	"	"
			Tern	"	"
4.	St. Albans, Herts.	3	Hassan	28 May 1974	4
			Maris mink	"	"
			Universe	"	"
5.	Elsworth, Cambs.	1	Maris Otter	early April 1974	8
6.	Abbotsley, Cambs.	1	Maris Otter	early April 1974	1
7.	Harpenden, Herts.	1	Sultan	May 1974 (ethirimol treated)	1

TABLE 2 Residue levels (µg/g grain) found in samples of grain from barley plants sprayed with tridemorph and then grown to maturity

Site Code (as Table 1)	Variety & Treatment		Residue levels (µg/g grain)	Mean
1	Maris Otter - no spray		0.1	0.1
	- autumn spray only		0.5	0.5
	- autumn and spring sprays	A	0.3, 0.8, 1.6, 1.8	1.1
		B	0.6, 0.4, 2.1, 1.8	1.2
5, 6	Maris Otter - spring spray		2.5, 2.6	2.6
2	Proctor - spring spray		3.5, 3.4, 2.2, 2.2 2.4, 2.4, 2.0, 4.4	2.8
	Berac - spring spray		1.3, 1.4	1.3
3	Julia - spring spray		1.1, 1.2	1.1
	Tern - spring spray		1.1	1.1
	Hassan - spring spray		0.9	0.9
4	Maris Mink - spring spray		1.8	1.8
	Universe - spring spray		0.8, 0.9	0.8
7	Sultan - spring spray (ethirimol treated)		1.2	1.2

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MBC RESIDUES AND PHYTOTOXICITY IN GHERKIN PLANTS BY SOIL AND FOLIAR SPRAY APPLICATIONS WITH MBC PRODUCING COMPOUNDS

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Summary Benomyl, thiophanate-methyl and MBC were applied to gherkin plants by soil and by foliar spray application in equivalent amounts calculated on the basis of the potentially available MBC.
Leaf development, as determined by weight, was considered with regard to product, dosage, and mode of application.
MBC residues were determined by UV-spectrophotometry and thiophanate-methyl residues by polarography for the spray applications only.
For all determinations, leaf samples from the 0-50 cm, the 50-100 cm and the 100-150 cm part of the plants were investigated separately. The highest MBC-residues resulting from soil application came from thiophanate-methyl. The same product was responsible for the lowest leaf development. A relation between dosage and leaf development could be demonstrated.

INTRODUCTION

The knowledge of side effects of systemic fungicides on plants is not new. Frahm (1973) mentioned some data in a review on side effects of benomyl. The aim of the present investigation was the comparison of three MBC producing compounds for their residue and their influence on leaf development. Therefore, these compounds were used in equivalent amounts calculated on basis of potentially available MBC. The following application modes were used : soil treatment by mixing or by drenching and foliar application by one spray or by repeated sprays.

MATERIALS AND METHODS

Benomyl, thiophanate-methyl and MBC were used as commercial wettable powders with respectively 50, 70 and 50 % a.i. at rates of 5, 4.2 and 3.3 g/100 l.

of soil as the lowest dosage for soil treatment. Tenfold and twentyfold rates were also applied. Foliar application on an analogous basis was done with 50 g of formulated benomyl per 100 l. of liquid at two weeks after planting, two and four, and two, four and six weeks after planting. Thiophanate-methyl was used at 42.1 g/100 l. and MBC at 32.7 g/100 l. The soil was a digested leaf soil of pH (H_2O) 6.45 and an organic matter content of 27.6 %. Gherkin seedlings were grown on the same soil in 12 cm diameter pots. Soil mixing with the products took place in the medium around the original potvolume after its transfer to a new pot of 18 cm diameter. Drenching application to soil was done in the original soil volume also after its transfer to the pots of 18 cm diameter. Soil treatments had three replicates, leaf treatment had four replicates and the number of check plants was six. The average relative air humidity was 85 % at an average temperature of 20°C. Residue analysis was done for MBC by the UV-spectrophotometric method (Nestres et al., 1971). Residue analysis for thiophanate-methyl after foliar application was done by polarography modified from Martens and Cus (1972). Harvest of leaves took place 8 weeks after transfer of the seedlings to the 18 cm diameter pots at a distance of 2 cm from the stem. Leaves were collected per stem section of 50 cm for all the replications per treatment. The number of the harvested and weighed leaves was noted to obtain the weight per leaf. Residue analysis was done on a maximum of 100 g from the bulk sample.

RESULTS

MBC and thiophanate-methyl residues.

The following tables present residues per mode of application of the systemic fungicides. The influence of dosage is clearly shown. The general decrease of MBC-residues in the leaves of higher stem sections is not so clear (Table 1) for the lowest dosage of thiophanate-methyl possibly due to a non-homogeneous mixing of the small quantity of product in the soil. The differences between MBC residues from benomyl, thiophanate-methyl and MBC itself are remarkable.
By drench treatment of the original soil volume (Table 2), the influence of dosage is not strongly manifested for the lowest leaves due to the penetration of roots into the untreated soil. Absolute residue values for the same stem section are lower than for the foregoing treatment for the same reason. Also lower total amounts of fungicides were available because of different soil volumes. A comparison of tables 1 and 2 proves once more what has been observed by Fuchs et al. (1972) namely the slower transformation rate of thiophanate-methyl which results in higher MBC residues (Table 1).

Table 1

MBC residues in gherkin leaves from 50 cm stem sections after mixing of soil around the original seedling pot volume with MBC producing compounds.

Product	Dosage (g/100 l. soil)	MBC residues (ppm) 0–50 cm	50 –100 cm	100–150 cm
Benomyl (50 % WP)	5.0	0.30	0.14	0.05
	50.0	2.51	0.99	0.31
	100.0	6.24	1.69	0.20
Thiophanate-M (70 % WP)	4.2	0.39	0.35	0.64
	42.1	5.57	1.07	0.20
	84.2	16.10	6.16	0.76
MBC (50 % WP)	3.3	0.47	0.26	0.08
	32.7	4.41	0.27	0.03
	65.5	4.82	2.09	0.12

Table 2

MBC residues in gherkin leaves from 50 cm stem sections after drenching of the original seedling soil volume with MBC producing compounds.

Product	Dosage (g/100 l. soil)	MBC residues (ppm) 0–50 cm	50–100 cm	100–150 cm
Benomyl (50 % WP)	5.0	1.87	0.10	0.08
	50.0	3.19	2.95	0.55
	100.0	4.07	3.24	0.37
Thiophanate-M (70 % WP)	4.2	1.35	0.12	0.04
	42.1	3.71	0.56	0.42
	84.2	3.28	1.15	0.77
MBC (50 % WP)	3.3	1.76	0.07	0.04
	32.7	1.58	0.24	0.07
	65.5	3.14	0.13	0.08

Late penetration of roots into the treated soil volume and retention of the product in (or on) the roots results in a constant supply of MBC.

Table 2 shows, for the leaves of stem sections from 0 to 50 and from 50 to 100 cm, higher residues resulting from benomyl applications than from thiophanate-methyl. This suggests that fungicidal protection is better from drench treatments of benomyl than from thiophanate-methyl drench because of more rapid accumulation in the leaves. The application of MBC as such, results in lower MBC residues in higher leaf positions because of the immediate translocation to lower leaves and leaf tips and margins.

Table 3

MBC and thiophanate-methyl (th-M) residues of gherkin leaves from 50 cm stem section after sprays with MBC producing compounds.

Product	time (weeks after plant.)	dosage/spray (g/100 l.)	Residues (ppm) 0-50 MBC	0-50 th-M	50-100 MBC	50-100 th-M	100-150 MBC	100-150 th-M
Benomyl (50% WP)	2	50.0	67.0		3.2		2.1	
	2 & 4	50.0	173.7		67.8		10.7	
	2, 4 & 6	50.0	178.4		187.9		85.2	
Thioph-M (70%WP)	2	42.1	7.7	6.4	1.1	2.0	0.2	traces*
	2 & 4	42.1	21.1	10.3	12.0	2.6	3.9	traces*
	2, 4 & 6	42.1	37.5	14.6	24.2	10.2	16.4	9.3
MBC (50% WP)	2	32.7	47.9		1.2		0.1	
	2 & 4	32.7	223.9		75.7		5.8	
	2, 4 & 6	32.7	431.0		201.0		79.4	

* traces thiophanate-M : < 0.5 ppm (classic polarography).

Because the spray treatments took place during plant development, higher leaves got less of the fungicides. Benomyl gives higher residues than thiophanate-methyl. MBC residues from benomyl are the sums of the MBC as the transformation product of benomyl produced during the analysis procedure, and the MBC already present on the leaves. By means of a NaOH reflux technique and radiolabeled materials, Baude et al. (1973) demonstrated benomyl possesses an excellent stability as a residue on treated plants. Thiophanate-methyl residues and MBC residues, added and calculated on equimolar base did not reach the total of MBC by benomyl or by MBC application. But no absolute comparison is possible because of

the different behaviour of the formulated products during application. Only relations between residues and inhibition of leaf development can be drawn from the latter results.

Influence of benomyl, thiophanate-methyl and MBC on leaf development.

Figures 1 and 2 present the average weight per leaf in grams for the different applications and leaf positions.

From figure 1 it seems that soil treatment with these systemic fungicides around the original pot volume causes a decrease of the leaf weight in all of the cases where the tenfold or the twentfold dosage was used. Treatments by soil drenching cause a stronger inhibition than the foregoing treatments although roots penetrate into the soil free of fungicides. For these applications, influence of dosage is not so pronounced just for that reason. Leaf weight from check plants raises with higher positions on the stem. Generally the inhibiting effect does not decrease with higher leaf positions when comparing proportionally the treated plants with the corresponding checks 50 cm sections. Thiophanate-methyl gives the strongest inhibition by mixing into the surrounding soil. Those leaves also contain the highest MBC residues. Benomyl and MBC have a less inhibitory effect and cause also lower MBC residues by the same application mode.

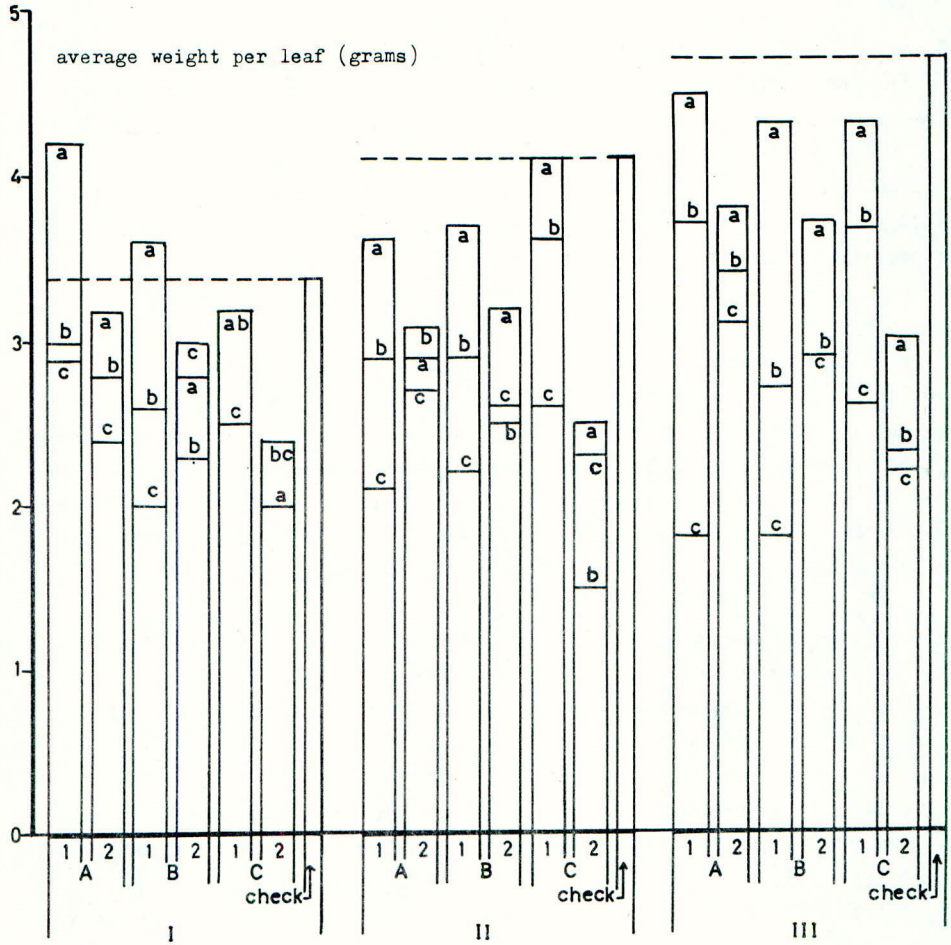
Original pot drenching with the MBC producing compounds gave most inhibition for the case of MBC although it does not cause the highest residues. Benomyl and thiophanate-methyl have also a reversed relation to the phytotoxic effect although higher dosagesare more phytotoxic.

Commercial rates of benomyl are 5-10 g w.p. with 50 % a.i. per 100 l. of soil. In these experiments the lowest rate caused inhibition in many cases.

Figure 2 shows the results after foliar spray applications. The leaves of the lowerest 50 cm undergo no harmful influence of spraying. The other 50 cm sections, however, are inhibited in their development. The influence of dosage, as a result of repeated application, is significant only for the highest two application rates.

The commercial rate for benomyl spray applications is 100 g of the 50 % product per 100 l. of water. The concentration in the experiments was only 50 g of the formulated benomyl per 100 l. of water.

Fig. 1. Weight of gherkin leaves after soil treatment with MBC producing compounds



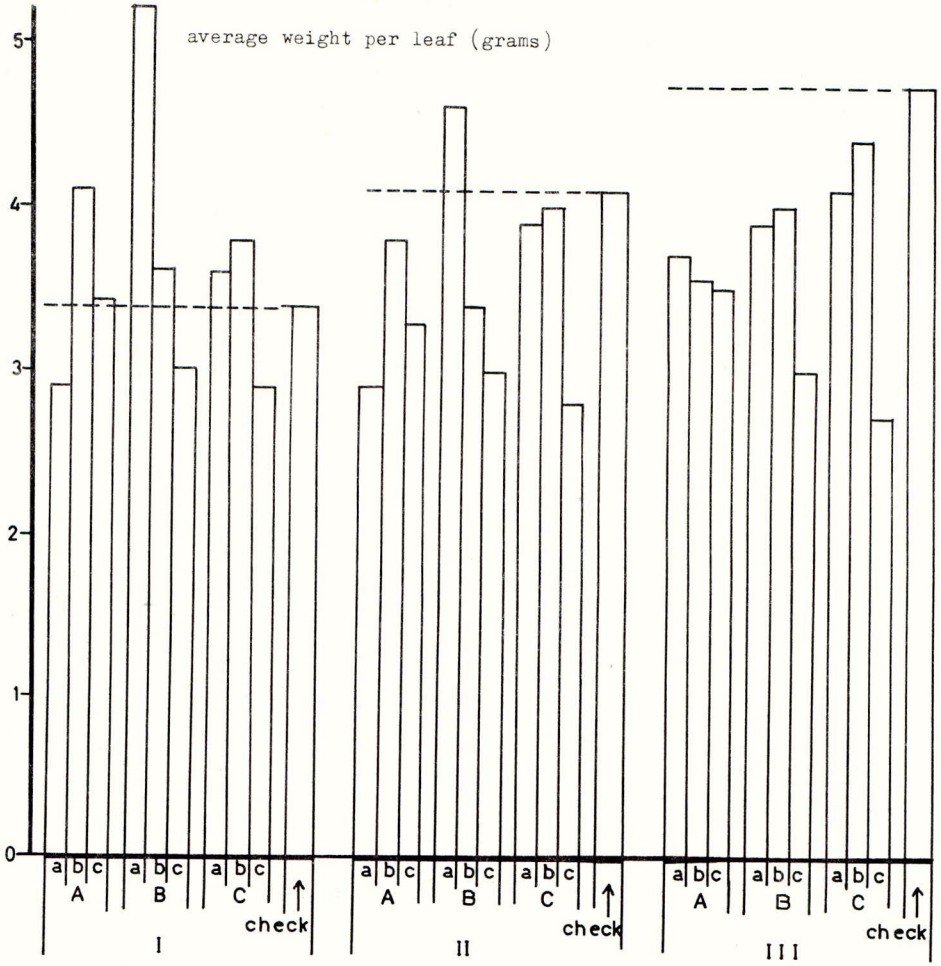
I leaves sampled between 0 and 50 cm
II leaves sampled between 50 and 100 cm
III leaves sampled between 100 and 150 cm

A benomyl (50 %)
B thiophanate-methyl (70 %)
C MBC (50 %)

1 systemic fungicides around the original soil volume
2 systemic fungicides in the original soil volume

a lowest dosage (benomyl: 5 g; thioph.-M: 4.2 g and MBC: 3.3 g/100 l.of soil)
b tenfold dosage
c twentyfold dosage

Fig. 2. Weight of gherkin leaves after foliar spray applications with MBC producing compounds



I leaves sampled between 0 and 50 cm
II leaves sampled between 50 and 100 cm
III leaves sampled between 100 and 150 cm

A benomyl (50 %) 50 g per 100 l.
B thiophanate-methyl (70 %) 42.1 g per 100 l.
C MBC (50 %) 32.7 g per 100 l.

a spray 2 weeks after planting
b spray 2 and 4 weeks after planting
c spray 2, 4 and 6 weeks after planting

CONCLUSION

Leaf development of gherkin plants was affected by treatment with MBC and the MBC generators benomyl and thiophanate-methyl, except for the lowest leaves (0-50 cm section) treated by the foliar spray. Dosage is of great importance in inhibition of leaf development and for residues. The method of soil treatment had a significant effect upon MBC residues produced by thiophanate-methyl. Mixing this fungicide with soil around the original seedling soil volume i.e. in the region of new root growth, gave much higher residues than from drench treatment of the original seedling soil.

In practice, choice of application mode and dosage may affect plant protection and plant production.

Acknowledgements

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References

BAUDE, F.J., GARDINER, J.A. and HAN, J.C.Y. (1973). Characterization of Residues on Plants Following Foliar Spray Applications of Benomyl. Journal of Agricultural and Food Chemistry, 21, 1084-1090.

FRAHN, J. (1973). Verhalten und Nebenwirkungen von Benomyl. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, 80, 431-446.

FUCHS, A., VAN DEN BERG, G.A. and DAVIDSE, L.C. (1972). Comparison of Benomyl and Thiophanates with Respect to Some Chemical and Systemic Fungitoxic Characteristics. Pesticide Biochemistry and Physiology, 2, 191-205.

MARTENS, P.H. et CUS, M. (1972). Détermination oscillopolarographique des résidus de thiophanate sur légumes divers. Mededelingen Fakulteit Landbouwwetenschappen, Gent, 37, 891-896.

MESTRES, R., TOURTE, J. et CAMPO, M. (1971). Dosage des résidus de bénomyl dans les fruits et les légumes. Travaux de la Société de Pharmacie de Montpellier, 31, Fasc. 1, 49-56.

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PROBLEMS IN THE ASSAY OF RESIDUES OF CARBENDAZIM AND ITS PRECURSORS

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Summary The properties of carbendazim make it difficult to extract from crops and soils and extracts contain substances which interfere with spectrometric and chromatographic assays. Commercially available fungicides which are carbendazim precursors may decompose during extraction, concentration and processing thus preventing unequivocal assay. High-speed liquid chromatography offers some promise but is not yet sufficiently selective and sensitive to overcome these problems.

Resume Les propriétés du carbendazim rendent son extraction du sol et du matérial végétal difficile car ces extraits contiennent des substances qui interfèrent le dosage spectrométrique et chromatographique. La décomposition des fongicides précurseurs du carbendazim durant l'extraction et la preparation empêchent le dosage certain. La chromatographie à haute pression offre des possibilités mais n'est pas encore suffisament sélective ou sensible.

INTRODUCTION

There is much current interest in the systemic fungicide carbendazim (methyl 2-benzimidazole carbamate) and the increasing variety of related commercially-available substances (Marsh 1972, Erwin 1973). These include benomyl, thiophanate methyl, "NF 48" (2-(3-methoxy-carbonyl-thioureido)-aniline), and "R 28291" (2-(3-methoxycarbonyl-thioureido)-O,O-diethylphosphoranilide) which are readily converted to carbendazim under mild conditions (Noguchi et al 1971, Fuchs et al 1972). Numerous assay methods have been published and are well reviewed elsewhere (Baker and Hoodless 1974). A variety of widely different procedures have been used including gas-liquid chromatography (GLC), thin-layer chromatography (TLC), high-speed liquid chromatography (HSLC), ultra-violet (UV) and fluor-spectrometry, polarography, the formation of coloured derivatives, and bioassay.

None of the methods is entirely satisfactory and in this paper we describe and discuss our investigations into various approaches to the assay of these substances.

EXPERIMENTAL

Assay Methods

U.V.Spectrometry. Carbendazim and its precursors have strong characteristic absorption in the range 260-300 nm which may be used for assaying pure solutions. For measuring residues in soils and plants, however, separation from other U.V.-absorbing materials in the extracts is essential.

Spectrofluorimetry. Although carbendazim exhibits fluorescence (λ excitation = 277 nm; λ emission = 394 nm in 0.1M aqueous HCl), the emission spectrum is broad and weak and is quenched by substances extracted from soils and plants.

Chromatography.

TLC. Carbendazim and related substances can be separated, usually on silica gel plates, using a variety of solvents such as:

1. methanol:acetic acid:chloroform = 10:1:89

2. ethyl acetate:methanol:0.88 ammonia = 100:25:1

3. ethyl acetate:chloroform = 1:9

4. methanol:chloroform = 1:19

Rf values from chromatograms on Merck Kieselgel 60 F254 plates (0.25 mm) are given in Table 1. Detection may be by fluorescence quenching or reaction with colour reagents. We used 0.5% w/v 2,6-dichloro-p-benzoquinone-4-chlorimine in cyclohexane for the thiophanates and "NF 48". Carbendazim gave a blue-green colour with Wood's reagent (0.2% bromophenol blue and 1% silver nitrate in 50% aqueous acetone) while 2-aminobenzimidazole (2-AB) and "NF 48" were found to give blue colours when sprayed with 0.5% N-1-naphthylethylenediamine dihydrochloride in 20% aqueous acetic acid followed by spraying with 6M aqueous HCl and heating to 110° for 10 to 15 minutes. No suitable spray reagent was found for unchanged benomyl.

Table 1

Thin-layer chromatography on silica gel

Rf values in different solvent systems

Compound	1	2	3	4
benomyl	0.81	0.82	0.59	0.77
carbendazim	0.60	0.66	0.03	0.33
thiophanate methyl	0.73	0.77	0.26	0.63
"NF 48"	0.69	0.77	0.23	0.59
thiophanate	0.79	0.81	0.54	0.76
2-AB	0.10	0.49	0	0

In practice, TLC is difficult to make quantitative but, by using marker spots and scraping off appropriate zones, it may be used as a clean-up procedure prior to assay by U.V. absorption.

GLC. Gas chromatography is not generally applicable to the assay of this group of compounds although a method for the assay of carbendazim, as its trifluoracetyl

derivative, has been reported (Rouchaud and Decallonne 1974).

HSLC. Carbendazim and its precursors were chromatographed by reverse-phase chromatography on Permaphase ODS or ETH-type columns using aqueous phospate buffer, pH7 (ionic strength 0.05)/methanol mixtures as solvents and by adsorption chromatography on silica gel columns using hexane/propan-2-ol mixtures. Using fixed and variable-wavelength U.V. detectors, as little as 1 ng of carbendazim may be measured in pure solution (Austin et al, in press).

Extraction Procedure

Extraction procedures for residues of carbendazim and its precursors have been reviewed in detail (Baker and Hoodless 1974, Fernandes and Cole 1974, Austin and Briggs, in press). Most use an organic solvent, frequently ethyl acetate or chloroform, but we showed that mixtures of 1 M aqueous ammonium chloride with acetone or alcohols extract soils more completely under all conditions examined and are also effective with plant materials. Extracts must be purified by partition between solvents. For plant materials, the fungicides are extracted with ethyl acetate from alkaline aqueous solutions and back-extracted into acid aqueous solutions. A final TLC chromatographic step may be required before U.V. spectrometric assay.

Octanol-water distribution coefficients

Octanol-water distribution coefficients (P values) were determined by the method of Fujita et al (1964). Before use, the solvents were adjusted to pH7 with aqueous sodium hydroxide solution.

RESULTS AND DISCUSSION

Carbendazim is relatively stable so that its solutions may be concentrated to improve sensitivity and treated to remove substances which interfere with the final assay without significant loss. We have investigated the potential of HSLC for residue determination using U.V.-absorption detectors. The method shows promise but is not yet at a suitable stage for routine residue measurements of carbendazim because, to obtain adequate sensitivity, it requires as much manipulation as direct U.V. spectrophotometry which is simpler and therefore preferable. HSLC can be used to separate and assay carbendazim and its precursors in "pure" solution, as is shown by the retention volumes (V_N) of some fungicides on a 1 m Permaphase ODS column with 25% methanol in buffer as solvent (Table 2).

Table 2

Chromatographic retention volumes (V_N ml) and octanol/water Partition coefficients (P values) for carbendazim and related fungicides

Compound	V_N *	P value	Soil Mobility Class +
"NF 48"	1.5	5.0	Mobile
thiophanate methyl	1.8	12.8	Mobile
thiophanate	3.4	54.2	Intermediate
benomyl	3.1	133	Low
carbendazim	5.3	29.8	Intermediate

* Flow-rate = 0.6 ml/min at 25°C.

\+ Classification according to Briggs (1973), using P values.

The value of HSLC is greatly limited by the conversion of precursors to carbendazim in the course of extraction and subsequent manipulation needed to obtain samples suitable for assay. Because of this difficulty, many residue methods do not attempt to measure the amounts of precursors but convert them to carbendazim. For example, Kirkland et al (1973) extracted benomyl-treated soils with acidic methanol and determined total (benomyl plus carbendazim) residues together as carbendazim by HSLC on a cation exchange resin. Co-occurring 2-AB was determined simultaneously. The problem of assaying benomyl and carbendazim residues simultaneously on plants was largely overcome by Baude et al (1973) using alkali-catalysed conversion during the course of extraction yielding characteristic stable derivatives, 2-(3-n-butylureido)-benzimidazole and 2-AB respectively. These were then separated by ion-exchange HSLC. Co-occurring 2-AB could not be measured. The usefulness of their procedure is limited by non-quantitative conversion of benomyl, by the possible presence of 2-AB as a natural breakdown product in the sample and by the difficulty of the chromatographic procedure. We know of no such methods for the thiophanate and other related fungicides.

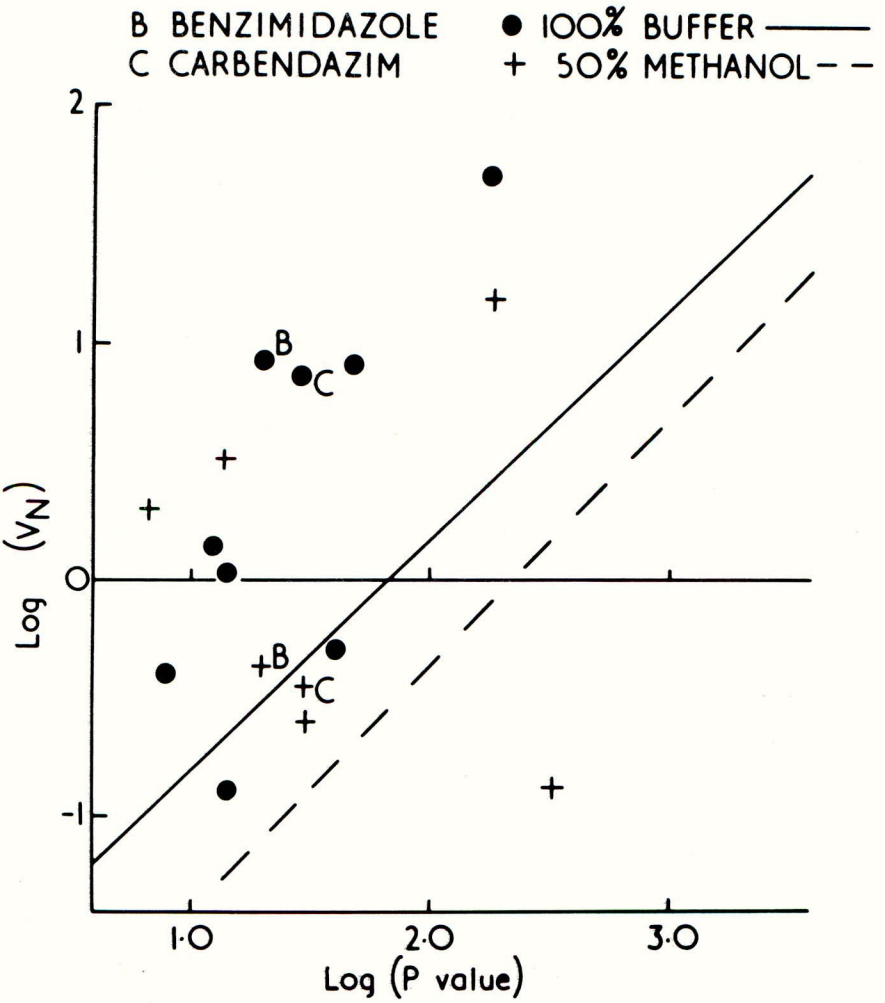
In our tests of leaching in soil using ^{14}C-labelled benomyl, carbendazim, thiophanate methyl, and "NF 48", none of the compounds moved downwards appreciably (Austin and Briggs, in press). This was quite unexpected in view of their soil mobility classification (Table 2), based on the established relationship between soil adsorption and P values (octanol/water partition coefficients) (Briggs, 1973). The movement of chemicals over soil organic matter may be regarded as similar to reverse-phase chromatography and we have found a simple relationship between P values and relative retention volumes (V_N) in reverse-phase systems for a wide range of compounds. This relationship is shown for 54 substances of various classes in Figure 1, expressed as lines of best fit which account for 98% of the variance. The figure includes our results for various benzimidazoles and thiophanates, which clearly behave in an unusual way, with little obvious relationship between polarity and retention volume. Explanations of anomalous soil mobility and chromatographic behaviour within this group of compounds are not yet available.

It is important to identify and measure both carbendazim and its precursors separately because their differing chemical and physical properties, such as solubilities and distribution coefficients, will result in varying uptake and distribution in soils, plants and pathogens which may well explain the differing biological effects observed (Bollen 1972, Fuchs et al 1974), even though their fungitoxicity may depend upon the formation of carbendazim. Apart from the implications for practical disease control, similar considerations also apply to the location and assay by fungal-growth inhibition of substances separated in TLC or other systems. Thus, the detection of benomyl or the thiophanates on TLC plates by bioassay may necessarily involve their conversion to carbendazim and the results may differ depending upon whether the change occurs on the plate or in the organism. Other difficulties may arise. For example, in our soil leaching tests, the major residue extracted in each case was authentic carbendazim. However, after one week, the extract of soil containing thiophanate-methyl residues contained a substance which co-chromatographed with carbendazim on silica-gel plates. It was distinguished from carbendazim by being extracted by ethyl acetate from aqueous acidic solutions as well as from alkaline solutions and by the colour reaction with 2,6-dichlorobenzoquinone-4-chlorimine, similar to the behaviour of thiophanate methyl and thiophanate. If the manipulation of the extract had been abbreviated by omission of the ethyl acetate extraction of the acidified aqueous extract, the compounds would not have been separated. Coincidence of biological activity from carbendazim with the colour reaction would not, in this case, demonstrate the presence of a new biologically-active substance.

So far we have discussed problems involved in measurement of carbendazim and its precursors, without considering the many problems of extraction from soils and crops. The efficacy of simple solvents such as chloroform and ethyl acetate for extracting carbendazim from soils is pH dependent, being greater with alkaline

Figure 1.

Relation between reverse-phase chromatography and octanol-water distribution coefficients. Comparison of benzimidazoles and thiophanates (• , +) with some phenyl ureas, oxime carbamates, and organic solvents (——,— —).



than acid or neutral soils with aged residues (Table 3), and it may be related to sorption of the ionic form of carbendazim by soil minerals or to formation of metal complexes (Austin and Briggs, in press). Kirkland et al (1973) used hot methanolic HCl which, though efficient, leads to severe problems through the formation of intractable metal hydroxide gels in subsequent processing. Our own method, which uses mixed solvents consisting of aqueous ammonium chloride (pH7) with equal volumes of acetone or methanol, avoids such difficulties and appears to be unaffected by the pH of the soil.

Table 3

Recovery of carbendazim from soil with acetone/1M NH_4Cl or chloroform

	Carbendazim recovered (per cent)	
Soil pH	Acetone/1M NH_4Cl	Chloroform
6.61	84.1	71.6
6.00	86.8	70.2
5.32	78.9	21.5
4.52	80.3	7.9

It is not yet possible to predict the rates of formation of carbendazim from its precursors in soils, crops or solvents, so that it is important both for better understanding of the underlying processes and for developing better methods of practical disease control to measure the amounts of carbendazim and its precursors. The extraction of the precursors of carbendazim presents great problems because partial or total degradation during the process is likely. A selective extraction method might be devised for some fungicides under particular circumstances to separate carbendazim from precursors used to treat crops or soils. However, with existing methods, conversion to carbendazim can occur during extraction and again during separation and analysis. The analytical problems are so complex that it may be necessary to accept a degree of uncertainty and, for routine determinations, measurement of total residues as carbendazim may have to suffice.

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References

AUSTIN, D.J. and BRIGGS, G.G. A new extraction method for benomyl residues in soil and its application in movement and leaching studies. Pesticide Science, in press.

AUSTIN, D.J., WILLIAMS, I.H., and LORD, K.A. High pressure liquid chromatography of benzimidazoles. Pesticide Science, in press.

BAKER, F.B. and HOODLESS, R.A. (1974). Analytical methods for the detection and determination of systemic fungicides. Pesticide Science, 5, 465-472.

BAUDE, F.J., GARDINER, J.A., and HAN, J.C.Y. (1973). Characterization of residues on plants following foliar spray applications of benomyl. Journal of Agricultural and Food Chemistry, 21, 1084-1090.

BOLLEN, G.J. (1972). A comparison of the in vitro antifungal spectra of thiophanates and benomyl. Netherlands Journal of Plant Pathology, 78, 55-64.

BRIGGS, G.G. (1973). A simple relationship between soil adsorption of organic chemicals and their octanol/water partition coefficients. Proceedings 7th British Insecticide and Fungicide Conference, 1, 83-86.

ERWIN, D.C. (1973). Systemic fungicides: disease control, translocation, and mode of action. Annual Review of Phytopathology, 11, 389-422.

FERNANDES, D.L. and COLE, J.S. (1974). Comparison of solvents and methods of extracting methylbenzimidazol-2-ylcarbamate (MBC) from tobacco. Pesticide Science, 5, 239-243.

FUCHS, A., FERNANDES, D.L., and DE VRIES, F.W. (1974). The function of an MBC-releasing deposit of benomyl and thiophanates in plant roots and soil. Netherlands Journal of Plant Pathology, 80, 7-18.

FUCHS, A., VAN DER BERG, G.A., and DAVIDSE, L.C. (1972). A comparison of benomyl and thiophanates with respect to some chemical and systemic fungitoxic characteristics. Pesticide Biochemistry and Physiology, 2, 191-205.

FUJITA, T., IWASA, J. and HANSCH, C. (1964). A new substituent constant, pi, derived from partition coefficients. Journal of the American Chemical Society, 86, 5175-5180.

KIRKLAND, J.J., HOLT, R.F. and PEASE, H.L. (1973). Determination of benomyl residues in soils and plant tissues by high-speed cation exchange liquid chromatography. Journal of Agricultural and Food Chemistry, 21, 368-371.

MARSH, R.W., editor (1972) 'Systemic Fungicides', (Longman, London).

NOGUCHI, T., OHKUMA, K., and KOSAKA, S. (1971). Chemistry and metabolism of thiophanate fungicides. In 'Pesticide Terminal Residues' pp.257-270, edited by TAHORI, A.S. (Butterworths, London).

ROUCHAUD, J.P. and DECALLONNE, J.R. (1974). A gas chromatographic method for the analysis of MBC in plants and soil. Journal of Agricultural and Food Chemistry, 22, 259-260.