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# MODE OF ACTION OF DIFLUBENZURON AS AN OVICIDE AND SOME FACTORS INFLUENCING ITS POTENCY

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Summary Electron microscopical observations of eggs of *Leptinotarsa decemlineata* treated with diflubenzuron through the female showed that the developing embryo formed an amorphous cuticular region instead of a normal lamellate cuticle. Treated embryos probably cannot use their muscles to leave the egg by virtue of a lack of rigidness in the cuticle. These symptoms are comparable with those studied in larvae and provide evidence that the ovicidal activity of diflubenzuron is caused also by its interference with chitin synthesis. Effects on fertilization, fecundity, and the role of males are discussed. In the case of direct contact of diflubenzuron with susceptible eggs, activity increases with smaller particle size and higher relative humidity of the air, and in some species is also affected by surfactants. Younger eggs are generally more sensitive than older eggs. Inhibition of egg hatch after treating female adults can be caused by oral uptake or by contact. After excretion of the compound, normal egg hatch is resumed. The rate of this reversibility depends on the dose received and, as reported in literature, can depend on the age of the adult. Ovicidal effects after treating female adults by contact are greatly dependent on the formulation of the compound.

## INTRODUCTION

Diflubenzuron is the common name for 1-(4-chlorophenyl)-3-(2,6-difluorbenzoyl)--urea. (Code names PH 60-40, TH 6040, Du 112.307, PDD 6040I, ENT-29054, OMS 1804. Trade name DIMILIN). Initially diflubenzuron was thought to be a stomach poison with larvicidal activity only (Mulder and Gijswijt, 1973), but has since proved to prevent egg hatch and to possess contact activity. In a previous paper we concluded that diflubenzuron was not a true chemosterilant but that the prevention of egg hatch could be attributed to an interference with chitin synthesis (Grosscurt, 1976). In the present paper, histological evidence is adduced in favour of that conclusion. Furthermore the effects of diflubenzuron on fertilization and fecundity and the role of males in the ovicidal effects are discussed.

During our experiments it became obvious that many factors influenced the ovicidal activity of diflubenzuron. In the second part of this paper these factors are evaluated and summarized with previously published data.

## MODE OF ACTION OF DIFLUBENZURON

### Histology

Ovicidal effects of diflubenzuron can be obtained either by topical application to the eggs or by treating female insects. In either case the symptoms are similar.

The larva in the egg develops fully but is unable to leave the egg, though it sometimes ruptures the egg wall. For Musca domestica photographs of this phenomenon were presented by Grosscurt (1976). In accordance with our knowledge concerning the mode of action of diflubenzuron and the related compound Du 19.111 (1-(2,6-dichlorobenzoyl) -3-(3,4-dichlorophenyl)-urea) in larvae (Mulder and Gijswijt, 1973; Post and Vincent, 1973; Post, de Jong, and Vincent, 1974) we assumed that the formation of cuticular chitin in the embryo was blocked by diflubenzuron. This was likely to prevent the larva using its muscles to leave the egg. To check this hypothesis, electron microscopic preparations were made of embryos of Leptinotarsa decemlineata, after the adults had been given 1000 ppm diflubenzuron in the food for 3 days. The tissue was fixed and stained by the method of Akster and Smit (1975), first with saturated uranyl acetate and then with a lead solution. Micrographs were made with a Philips EM 201C electron microscope by Miss E.H. Velzing and Drs. W.A. Smit, Zoological Laboratory, University of Amsterdam, The Netherlands.

The embryos had been fixed a few hours before hatching. Embryos in the eggs, both from treated and from untreated females could be seen moving inside the egg at that moment. In diflubenzuron-contaminated eggs we saw instead of the normal patterns of lamellate cuticle deposition the formation of an amorphous cuticular region. Globular structures were vaguely discernible. They were possibly similar to the globules which in treated larvae could be observed under a light microscope (Mulder and Gijswijt, 1973). The microvilli of the epidermal surface of larvae in diflubenzuron-treated eggs had a more irregular shape than microvilli in normal eggs.

All cells were not equally affected, suggesting an individual response of the epidermal cells to diflubenzuron. In untreated embryos, dark spots consisting of dense, granular material occurred in the tips of the microvilli. These findings are similar to those of Delachambre (1970) in a study of epicuticle formation in adult Tenebrio molitor.

In diflubenzuron-treated embryos the dark spots in the tips of the microvilli were less clear. For unknown reasons the contrast in the affected tissue was less than in normal tissue. The micrographs made it clear that the ovicidal effects of diflubenzuron were caused by interference with chitin synthesis in the developing embryo. After exposure to diflubenzuron, the larva probably could not use it muscles to leave the egg because now they were attached to a cuticle which lacked the normal structure and strength.

## Effects on fertilization

In Musca domestica we found no effect of diflubenzuron on fertilization after 7 days of feeding with 1000 ppm diflubenzuron in the food (Grosscurt, 1976).

## Effects on fecundity

We shall define fecundity as the number of eggs produced per female. Findings concerning the effects of diflubenzuron on fecundity are not unanimous, but a decrease in fecundity has been reported in some insect species including Epilachna varivestis (Holst, 1974), Eurydema oleraceum (Leuschner, 1974), and Dacus oleae (Fytizas, 1976).

With Epilachna varivestis, Holst found that the reduction in fecundity after three days' oral administration of diflubenzuron was 65% on treatment of 3-day-old adults and 37% on treatment of 11-day-old adults. In Dacus oleae a reduction of fecundity was found after dipping the last larval instar into a suspension of diflubenzuron in water (Fytizas, 1976). However with Tribolium castaneum (Carter, 1975), Anthonomus grandis (Moore and Taft, 1975), Culex tarsalis (Arias and Mulla, 1975), Stomoxys calcitrans and Musca domestica (Wright and Spates, 1976), Musca domestica (Grosscurt, 1976) and Pectinophora gossypiella (Flint and Smith, 1977) no effects of diflubenzuron on fecundity were observed. The maximum exposure to the compound was 7 days at 3.0 ppm for Tribolium castaneum. With Anthonomus grandis the weevils were dipped into a 0.1% acetone solution of diflubenzuron; with Pectinophora gossypiella a maximum of 200 ppm diflubenzuron was fed for 6 days, and with Musca domestica the compound was offered 7 days at a concentration of 1000 ppm in the food. Wright and Spates (1976) give no information about their methods with Stomoxys calcitrans and Musca domestica. With Culex tarsalis 4th-instar larvae were treated 48 hours with 0.4 ppm diflubenzuron.

### The role of males in the ovicidal phenomenon

Moore and Taft (1975) found a reduced hatch of eggs from untreated females of Anthonomus grandis that had been mated with males fed with diflubenzuron.

McGregor and Kramer (1976) concluded from feeding experiments that the ovicidal effects of diflubenzuron in Sitophilus granarius could be attributed primarily to an effect on the females and to a lesser extent to the males. In experiments with Stomoxys calcitrans, Wright and Spates (1976) found that the egg hatch was reduced by diflubenzuron after dipping males into aqueous suspensions or after topical application of an acetone solution.

In these experiments, the techniques used might have allowed direct uptake by the female insects as well as transfer from the male to the female by body contact in the cage or during copulation, as also suggested by Moore and Taft (1975). To avoid this possibility, we injected males of Musca domestica (Grosscurt, 1976) and males of Leptinotarsa decemlineata with 5 µg of diflubenzuron and allowed them to mate with untreated females. In these experiments we found no influence on fertilization and egg mortality after the males had been treated with diflubenzuron. We therefore concluded that in Musca domestica and in Leptinotarsa decemlineata diflubenzuron neither prevented the development of sperm nor caused the death of sperm after it had been produced.

## FACTORS INFLUENCING THE DIRECT CONTACT ACTIVITY OF DIFLUBENZURON ON EGGS

### The formulation of the compound

The formulation of diflubenzuron has two important aspects: the particle size and the presence of surfactants.

With larvae, the effect of the particle size of diflubenzuron was published first by Mulder and Gijswijt (1973). Ascher and Nemny (1974) studied the effect of particle size with eggs of Spodoptera littoralis. They found that the LC100 of dilutions of the liquid formulation was ten times lower than the LC100 of aqueous suspensions of the dispersible powder.

Depending on the insect species and the host plant, surfactants can play an important role to obtain good covering of the eggs. For example eggs of Leptinotarsa decemlineata on potato leaves can be sufficiently covered without a surfactant, but in the case of eggs of Pieris brassicae on cabbage leaves addition of a surfactant to the spray liquid is absolutely necessary.

### The age of the eggs

The ovicidal activity of diflubenzuron by direct contact with the eggs decreases with increasing age of the eggs. With Spodoptera littoralis, Ascher and Nemny (1974) found 0-1 day old and 1-2 day old eggs to be equally susceptible, whereas 2-3 day old eggs were much less susceptible.

Similar results were obtained in our laboratory with eggs of Pieris brassicae (Table 1).

Table 1

The effect of various ages of eggs of *Pieris brassicae* on the ovicidal effect of a 0.3 ppm flowable of diflubenzuron in water containing 250 ppm Citowett as a surfactant and sprayed till run-off.

| age in days | mean % nonhatching eggs | number of experiments |
|---|---|---|
| 0-1 | 97 | 2 |
| 1-2 | 95 | 4 |
| 2-3 | 82 | 3 |
| 3-4 | 54 | 4 |

Average temperature 25°C, relative humidity 100%. Data have been corrected for mortality in the control treatments (Abbott, 1925). The mean precentage of nonhatching eggs denotes the average value of the experiments in which 170-300 eggs per treatment were used.

Table 1 indicates that younger eggs are more susceptible to diflubenzuron than older eggs. With the parameter free trend-test of Page (1963) this appeared to be significant at $P = 0.06$ (two-sided).

Miura et al. (1976) reported that younger eggs of *Culex pipiens quinquefasciatus* were more sensitive than older ones. This was only true, however, of the percentage of abnormal hatch and not of the percentage of unhatched eggs.

With *Culex pipiens fatigans*, *Anopheles gambiae*, and *Anopheles quadrimaculatus*, Busvine et al. (1976) found 12-hour-old eggs to be somewhat less susceptible than 8-hour-old eggs.

Experiments with eggs of *Epilachna varivestis* (Holst, 1974), however, gave results which were contrary to those mentioned above: eggs of between 0 and 12 hours were less susceptible than older eggs.

## The relative humidity

The relative humidity greatly affects egg hatch after treatment with diflubenzuron (Table 2).

Table 2

The effect of the relative humidity on the mean percentage of nonhatching eggs after spraying eggs of *Leptinotarsa decemlineata* aged between 0 and 24 hours till run-off with suspensions of diflubenzuron made from the wettable powder.

| % relative humidity | mean % nonhatching eggs | | | | | |
|---|---|---|---|---|---|---|
| | concentrations in ppm | | | | | |
| | 300 | 100 | 30 | 10 | 3 | 0 |
| 30 | 70 | 56 | 59 | 31 | 40 | 21 |
| 50 | 85 | 59 | 44 | 33 | 39 | 32 |
| 80 | 96 | 78 | 66 | 47 | 53 | 24 |
| 100 | 99 | 97 | 100 | 97 | 76 | 21 |

Average temperature 25°C. The mean percentage of nonhatching eggs denotes the average value of 3 experiments in which 35-84 eggs per treatment were used.

Table 2 indicates that the mortality caused by diflubenzuron increases with increasing relative humidity. By means of the parameter free trend-test of Page (1973) this increase was found to be significant at P = 0.001 (two-sided).

## FACTORS INFLUENCING THE OVICIDAL ACTIVITY OF DIFLUBENZURON APPLIED TO ADULT FEMALE INSECTS

### Oral uptake of the compound

#### The formulation

Just as the formulation, including particle size, has an important bearing on the ovicidal activity of diflubenzuron by contact activity, so it is likely to be of importance, too, after oral uptake by adults. However, no information is available yet about this topic.

#### The concentration

*Eurydema oleraceum*, the ovicidal activity increased from one egg batch to the next when the food was sprayed with 250 ppm. In the fifth egg batch eclosion was fully blocked. With the 1000 ppm treatment, egg eclosion was blocked almost completely in the second egg batch (Leuschner, 1974).

When diflubenzuron treatment is discontinued, egg eclosion in newly laid egg batches can increase again. In *Musca domestica* the rate of this increase depends on the concentration of diflubenzuron in the previous treatment (Grosscurt, 1976).

#### The age of the adult

The effects on egg hatch after feeding of diflubenzuron to adults aged 3 or 11 days was studied with *Epilachna varivestis* (Holst, 1974). The adults were fed on a diet containing diflubenzuron for 3 days. After this treatment the ovicidal effects of diflubenzuron decreased much more quickly in the older adults than they did in the younger ones.

### Treatment of the adults by contact with the compound

#### The formulation

When adults of *Musca domestica* were treated topically with an acetone solution of diflubenzuron, recrystallization of the compound on the integument was observed after evaporation of the acetone.

After application of 1 µg diflubenzuron in acetone to adult females the effect on egg hatch lasted for four days after treatment (Grosscurt, 1976). Though egg hatch returned to normal after this period, diflubenzuron was still present as crystals on the outside of the integument. Apparently diflubenzuron can penetrate the integument of *Musca domestica* as an acetone solution, but the diflubenzuron crystals formed after evaporation of the acetone are incapable of penetrating. In that case the rate of evaporation of acetone, is of great importance to the effect of the solution of diflubenzuron.

After topical application of diflubenzuron, ovicidal effects were obtained with *Musca domestica* (Grosscurt, 1976), *Hylemya brassicae* (Van de Veire and Delcour, 1976), and *Haematobia irritans* (Wright and Harris, 1976).

Adults can also be treated with diflubenzuron by contact with a treated surface. Uptake of the compound in this case might be by penetration through the cuticle and, in some cases orally.

The ovicidal activity during the first four days of egg laying was assessed after putting houseflies in cages treated with an aqueous suspension of diflubenzuron (formulated as a w.p.). After one month, more flies were introduced and the ovicidal activity was assessed in the same way.

At a dose of 1.0 g a.i./$m^2$ the initial activity and the activity after one month were both 100%. At a dose of 0.1 g a.i./$m^2$ the initial activity was 88% and after one month it was 57%. This experiment was performed in duplicate for each concentration, always with a cage having an inner surface of 0.5 $m^2$ and containing 400 flies of both sexes. The temperature was 28°C and the relative humidity 50%. Wright and Harris (1976) released Stomoxys calcitrans and Haematobia irritans into holding cages attached to the winter coat of a Hereford steer which had been sprayed with a 1% aqueous suspension of diflubenzuron (formulated as a w.p.). After 4 days of continuous exposure, starting just after spraying, egg hatch of S. calcitrans was inhibited only 83%. With H. irritans however, complete prevention of egg hatch was obtained for 5 weeks, and some effects on hatchability (data not shown) were apparent for another 6 weeks. Flint and Smith (1977) exposed adults of Pectinophora gossypiella to a surface treated with diflubenzuron (0.02 g/$cm^2$). Egg hatch in several batches had decreased to 0% after 6 days exposure. The residue of a suspension has a particle size equal to that of the original formulation, but the residue of a solution always has an unknown particle size on recrystallization of the compound after evaporation of the solvent. This factor might account for the difference in residual activity of an acetone solution applied to the adults of Musca domestica and the activity by contact with a spray suspension made from the w.p.

## References


ABBOTT, W.B. (1925 A method for computing the effectiveness of an insecticide, Journal of Economic Entomology, 18, 265-267.

AKSTER, H.A. and SMIT, W.A. (1975) Bridges between microtubules and desmosomes in the terminal filament-cuticle connection and in the muscle-cuticle connection (tendon cell) of the Colorado beetle (Leptinotarsa decemlineata Say), Netherlands Journal of Zoology, 25, 122-124.

ARIAS, J.R. and MULLA, M.S. (1975) Postemergence effects of two insect growth regulators on the mosquito Culex tarsalis (Diptera; Culicidae), Journal of Medical Entomology, 12, 317-322.

ASCHER, K.R.S. and NEMNY, N.E. (1974) The ovicidal effect of PH 60-40 (1-(4-chlorophenyl)-3-(2,6-difluorbenzoyl)-urea) in Spodoptera littoralis Boisd., Phytoparasitica, 2, 131-133.

BUSVINE, J.R., RONGSRIYAM, Y., and BRUNO, D. (1976) Effects of some insect development inhibitors on mosquito larvae, Pesticide Science, 7, 153-160.

CARTER, S.W. (1975) Laboratory evaluation of three novel insecticides inhibiting cuticle formation against some susceptible and resistant stored products beetles, Journal of Stored Products Research, 11, 187-193.

DELACHAMBRE, J. (1970) Etudes sur l'épicuticule des insectes. I. Le développement de l'epicuticule chez l'adulte de Tenebrio molitor L., Zeitschrift für Zellforschung und mikroskopische Anatomie, 108, 380-396.

FLINT, H.M. and SMITH, R.L. (1977) Laboratory evaluation of TH 6040 against the pink bollworm, Journal of Economic Entomology, 70, 51-53.

FYTIZAS, E. (1976) L'action du TH 6040 sur la métamorphose de Dacus oleae Gmel. (Diptera, Trypetidae), Zeitschrift für Angewandte Entomologie, 81, 440-444.

GROSSCURT, A.C. (1976) Ovicidal effects of diflubenzuron on the housefly (Musca domestica), Mededelingen van de Faculteit voor Landbouwwetenschappen, Gent, 41, 949-963.

HOLST, H. (1975) Die fertilitätsbeeinflussende Wirkung des neuen Insektizids PDD 60-40 bei Epilachna varivestis Muls. (Col. Coccinellidae) und Leptinotarsa decemlineata Say. (Col. Chrysomelidae), Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, 82, 1-7.

LEUSCHNER, K. (1974) Wirkung des neuen Insektizids PDD 6040 auf Larven und Imagines der Kohlwanze Eurydema oleraceum L. (Heteroptera, Pentatomidae), Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, 81, 8-12.

McGREGOR, H.E. and KRAMER, K.J. (1976) Activity of Dimilin (TH 6040) against Coleoptera in stored wheat and corn, Journal of Economic Entomology, 69, 479-480.

MIURA, I., SCHAEFER, C.H., TAKAHASHI, R.M. and MULLIGAN, F.S. (1976) Effects of the insect growth inhibitor, Dimilin®, on hatching of mosquito eggs, Journal of Economic Entomology, 69, 655-658.

MOORE, R.F. and TAFT, H.M. (1975) Boll weevils: Chemosterilization of both sexes with busulfan plus Thompson-Hayward TH-6040, Journal of Economic Entomology, 68, 96-98.

MULDER, R. and GIJSWIJT, M.J. (1973) The laboratory evaluation of two promising new insecticides which interfere with cuticle deposition, Pesticide Science, 4, 737-745.

PAGE, E.B. (1963) Ordered hypothesis for multiple treatments: A significance test for linear ranks, Journal of the American Statistical Association, 58, 216-230.

POST, L.C. and VINCENT, W.R. (1973) A new insecticide inhibits chitin synthesis, Naturwissenschaften, 60, 431-432.

POST, L.C., DE JONG, B.J. and VINCENT, W.R. (1974) 1-(2,6-disubstituted benzoyl)-3-phenylurea insecticides: Inhibitors of chitin synthesis, Pesticide Biochemistry and Physiology, 4, 473-483.

VEIRE, M. VAN DE, and DELCOUR, E. (1976) Effect of diflubenzuron on the off-spring after treatment of the adult cabbage root fly Hylemya brassicae, Mededelingen van de Faculteit voor Landbouwwetenschappen, Gent, 41, 965-974.

WRIGHT, J.E. and SPATES, G.E. (1976) Reproductive inhibition activity of the insect growth regulator TH 6040 against the stable fly and the house fly: effects on hatchability, Journal of Economic Entomology, 69, 365-368.

NOTES

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# THE EFFECT OF VARIOUS INTERVALS BETWEEN TREATMENTS ON THE SYNERGISM OF A PYRETHROID INSECTICIDE, (1R, TRANS) - TETRAMETHRIN, IN HOUSEFLIES

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Summary Susceptible houseflies were treated with the insecticide (1R,trans)-tetramethrin either before or after the synergists n-propyl 2-propynyl phenylphosphonate (NIA 16388) or piperonyl butoxide (pb). NIA 16388 at 1 µg per fly was most effective when the insecticide was applied approximately 3h after the synergist, but with 0.09 µg NIA 16388 or 1 µg pb per fly, simultaneous treatment was most effective. The effect of pretreating the flies with 1 µg NIA 16388 gradually declined as the interval before applying the insecticide was increased from 3h to 48h, and with 1 µg pb to 30h. When the flies were dosed first with the insecticide, its activity could still be synergised by either additive 16h after application suggesting that substantial amounts of insecticide persist in the insect during this period.

## INTRODUCTION

Pyrethroid insecticides can be synergised by various chemicals with widely differing chemical structures (Casida, 1970), especially methylenedioxyphenyl compounds such as piperonyl butoxide (pb) and sesamex. Early work with pb and natural pyrethrins indicated that knock-down activity is not affected by applying the synergist after the toxicant (Lindquist et al., 1947, Chamberlain 1950) but Blum and Kearns (1956) showed that kill could be synergised by applying pb up to 8h after natural pyrethrins.

Among chemicals other than methylenedioxyphenyl compounds which have been shown to have strong synergistic activity, n-propyl 2-propynyl phenylphosphonate (NIA 16388) (Niagara Chemical Div., 1967) is particularly effective as a synergist for tetramethrin (Miyamoto and Suzuki, 1973).

The present work with (1R, trans)-tetramethrin, pb and NIA 16388 is part of a wider study to investigate principles governing the relative effectiveness of synergists on pyrethroids of different structure.

## MATERIALS

A susceptible strain of houseflies originally obtained from the Wellcome Foundation, Berkhamsted, Herts., and bred without exposure to insecticide was used for all tests. The chemicals (1R,trans)-tetramethrin and piperonyl butoxide (pb) were also kindly given by the Wellcome Foundation, and n-propyl 2-propynyl phenylphosphonate (NIA 16388) was given by FMC Corporation, Middleport, U.S.A.

## METHODS

3-4 day-old adult female flies bred at 28°C±1 were lightly anaesthetised with diethyl ether and treated topically with 0.5 µl drops of chemical in acetone (1.0 µl per fly for the simultaneous treatment). For each test a single dose of synergist was applied at the appropriate time before or after the insecticide. Four or five doses of (1R,trans)-tetramethrin within the kill range for the chosen interval were applied using 15 flies per replicate and two replicates per dose. Each test was done twice. The flies were dosed at room temperature; after the first treatment they were kept at 20°C±1, and fed from a wick of cotton wool soaked in 2% sucrose solution. Mortality was assessed 24h after the second treatment, which was after kill end-point. The mortality data were subjected to probit analysis (Finney, 1971) to obtain LD50 values using a maximum likelihood computer programme (Ross, 1975). From these figures, the weighted mean LD50 of the two experiments for each time interval and insecticide/synergist combination was calculated. 57 such duplicate tests at 19 different time intervals and 3 different synergist treatments were done. Also, for each synergist treatment a test with (1R,trans)-tetramethrin only was included: additional flies were dosed with (1R,trans)-tetramethrin coincidentally with those pretreated with synergist to establish variations in LD50s throughout the test period due to diurnal rhythms, pot effects etc.

Relative potencies at each time interval were calculated from the ratio of the weighted mean LD50 values of (1R,trans)-tetramethrin with and without the synergist. The response to NIA 16388 and pb was measured separately.

## RESULTS

Figure 1 shows the results of the bioassays. Each point represents a weighted mean LD50 whose standard error was less than 10%. The LD50 at 48h between synergist and insecticide treatments was not significantly different from that of the standard in each test. Fluctuation in LD50 to flies treated only with (1R,trans)-tetramethrin during the duration of twotests was assessed but the maximum variation was less than two-fold and not significant at P=0.05 (t=2.6 with 4 degrees of freedom).

At 1µg NIA 16388 per fly the effect of (1R,trans)-tetramethrin was greatest when it was applied 2-4h after the synergist. However with approximately one tenth of this dose of NIA 16388, the maximum effect occurred when the interval between treatments was 0-1h. With 1.0µg pb per fly, maximum effect also occurred with simultaneous treatment but there was little change in response up to 4h between treatments. LD50s of the compounds alone are shown in Table 1.

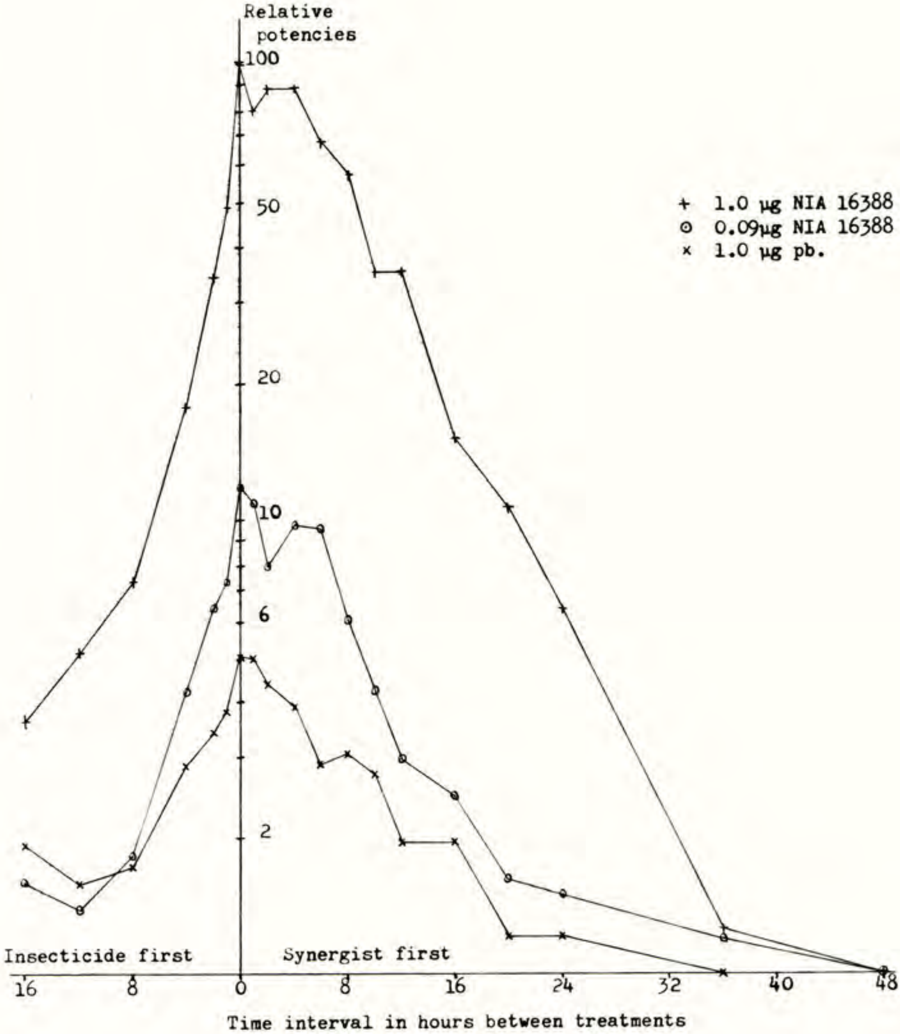
Table 1

LD50 (µg per female fly) of chemicals tested against susceptible houseflies

| Compound | LD50 ± S.E. | Number of Assays |
|---|---|---|
| (1R trans)-tetramethrin | 0.126 ± 0.010 | 4 |
| NIA 16388 | 7.3 ± 0.60 | 2 |
| Piperonyl Butoxide | 12.0 ± 1.0 | 2 |

## Figure 1

Graph of the relative potencies of synergised to unsynergised [1R,trans]-tetramethrin at LD50 against time interval between treatments for three synergist applications



## DISCUSSION

Although these results are clear cut, they conflict with some aspects of previous work. Synergists are frequently applied simultaneously with an insecticide at constant ratio, typically between 1:1 and 20:1. Pretreatment with a constant amount of synergist has also been used to inhibit any protective mechanism to a constant extent so that variations in response are caused only by the insecticide. However, the quantity of additive used by different workers varies, for example NIA 16388 was applied both at 5.0µg per fly and at a constant ratio of 5:1 with tetramethrin by Miyamoto and Suzuki (1973) while Jao and Casida (1974) used 0.6µg per fly of an analogue (2-methylpropyl 2-propynyl phenylphosphonate) of NIA 16388. The larger doses of synergist were inappropriate to the present study because they killed the flies and a maximum of 1.0µg per fly was chosen as this is well below the LD50 for the more toxic synergist (Table 1).

The delay in the time for the larger dose of NIA 16388 to reach its maximum effect in the presence of the insecticide is probably due to the time taken for each chemical to reach an optimum concentration at its site of action. Farnham (1973) measured the rate of penetration of $^{3}$H-pyrethrin I and showed that 70% of a topically-applied dose of 0.1µg per fly could not be recovered by external washings 4h after treatment, and Bridges (1957) reported that pb can slow the rate of penetration of $^{14}$C-allethrin. However, the effects on rate of penetration by NIA 16388 on (1R,trans)-tetramethrin have not been studied.

After the first four hours, the effectiveness of both synergists declined (Figure 1) but the time taken for complete loss of effectiveness in susceptible flies was up to 48h. For the larger dose of NIA 16388 effectiveness persisted between 36 and 48h but declined faster for the 0.09µg dose. The decay in effectiveness of 1.0µg pb per fly was more rapid than that of the same dose of NIA 16388. The causes for this loss of activity were not investigated but are likely to involve metabolism and excretion of the synergist.

The results on pretreating with (1R,trans)-tetramethrin show that its activity, revealed by treating subsequently with synergist, persisted more than 16h. Jao and Casida (1974) reported that measurable quantities of unmetabolised bioresmethrin and cismethrin can persist up to 96h after injection into milkweed bugs and that each has a half-life of approximately 48h. However, responses of milkweed bugs and houseflies to insecticides and synergists applied topically including (1R,trans)-tetramethrin and NIA 16388 were quite different, indicating the dangers of generalising from results obtained with one insect.

The results of Miyamoto and Suzuki (1973) cannot be compared readily with those presented here since they injected tetramethrin (0.5µg per fly) then topically applied NIA 16388 (5µg per fly). Each of these treatments alone would be lethal to the strain used here. However the SK strain they tested showed a four-fold tolerance to tetramethrin compared with the Lab-em-7-em strain, another susceptible strain, so fundamental differences between their strain and the one used here are possible.

The right half of the curves shown in Figure 1 is an indication of the amount of synergist remaining at each treatment interval while the left half indicates the amount of insecticide. The rate of loss of activity of the synergist should be independent of the insecticide used to detect its presence, and likewise, the rate of loss of activity of the insecticide, detected by applying the synergist should be independent of the nature of the synergist. This latter part is supported by the results for the synergists used here, since when the insecticide was applied first each revealed similar proportions of the maximum synergism after 16h period.

The results of this work indicate that the degree of synergism of (1R,trans)-tetramethrin depends on the additive and the time between treatments. Similar studies with other insecticides, synergists and strains of fly are needed to verify the theories of characteristic decay proposed, as well as investigations on the penetration of insecticide and synergist. The techniques described show how bioassays can indicate the rate of loss of activity of either insecticide or synergist, which can be of value in establishing the importance of various protective systems in the insect.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## References


BLUM, M.S. and KEARNS, C.W. (1956) The synergistic activity of piperonyl butoxide applied at different intervals to pyrethrum-treated houseflies, Journal of Economic Entomology, 49, 496-497.

CASIDA, J.E. (1970) Mixed-function oxidase involvement in the biochemistry of insecticide synergists, Journal of Agricultural and Food Chemistry, 18, 753-772.

CHAMBERLAIN, R.W. (1950) An investigation on the action of piperonyl butoxide with pyrethrum, American Journal of Hygiene, 52, 153-183.

FARNHAM, A.W. (1973) Genetics of resistance of pyrethroid-selected houseflies, Musca domestica L., Pesticide Science, 4, 513-520.

FINNEY, D.J. (1971) Probit Analysis, 3rd edition, Cambridge University Press, Cambridge.

JAO, L.T. and CASIDA, J.E. (1974) Esterase inhibitors as synergists for (+)-trans-chrysanthemate insecticide chemicals, Pesticide Biochemistry and Physiology, 4, 456-464.

LINDQUIST, A.W., MADDEN, A.H. and WILSON, H.G. (1947) Pre-treating houseflies with synergists before applying pyrethrum sprays, Journal of Economic Entomology, 40, 426-427.

MIYAMOTO, J. and SUZUKI, T. (1973) Metabolism of tetramethrin in houseflies in vivo, Pesticide Biochemistry and Physiology, 3, 30-41.

NIAGARA CHEMICAL DIVISION (1967) Technical Data Sheet on NIA 16388, FMC Corporation, Middleport, New York, U.S.A.

ROSS, G.J.S. (1975) Simple non-linear modelling for the general user, Bulletin of the International Statistical Institute, 46, 585-593.

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# MOLECULAR MODIFICATION OF CARBAMATES IN RELATION TO THEIR SELECTIVE TOXICITY[(1)]

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Summary Comparative activity of aldicarb, methomyl and N-desmethyl methomyl to honey bee and cotton leafworm were investigated. Except for aldicarb, these compounds are extremely toxic to both insects, with undesirable selectivity. Detailed kinetic studies show that this selectivity may be due in part to anticholinesterase specificity. Selective toxicity of these oxime carbamates to these insects parallels their ability to carbamylate their cholinesterases. N-substitution of carbaryl and carbofuran with formyl, acetyl, and phenylsulfenyl groups is accompanied by a marked decrease in anticholinesterase activity against both insects. This decreased activity is mainly due to decreased carbamylating ability rather than complex formation. All derivatized compounds are less toxic than their parents by varying degrees particularly to honey bee.

## INTRODUCTION

Studies of the selective toxicity of insecticides between harmful and beneficial insects are not as comprehensive as those between insects and mammals. Carbamates are known to have a typically erratic pattern of selective toxicity between insects. Honey bee and other hymenopterous insects are extremely susceptible to a wide variety of carbamates (Georghiou & Atkins, 1964 and Abdel-Raof, 1974), whereas some lepidopterous larvae such as the southern armyworm (Weiden, 1971) and the Egyptian cotton leafworm (Abdel-Aal, 1975) are extremely tolerant to many carbamates.

This study is aimed at understanding the relationship between comparative toxicity and anticholinesterase activity of some oxime and aryl carbamates to honey bee (*Apis mellifera*) and cotton leafworm (*Spodoptera littoralis*).

## METHODS AND MATERIALS

Insecticides: Compounds used in this study were: carbaryl (1-naphthyl methylcarbamate); carbofuran (2,3-dihydro-2,2-dimethyl-benzofuran-7-yl methylcarbamate); aldicarb[2-methyl-2-(methylthio)propionaldehyde O-(methylcarbamoyl) oxime]; methomyl (S-methyl-N-[(methylcarbamoyl)oxy] thioacetimidate), and N-desmethyl methomyl (S-methyl-N-[(carbamoyl)oxy]thioacetimidate). All these compounds were obtained from their commercial sources and purified by recrystallisation to their reported melting points.

Acylation of carbaryl and carbofuran was carried out according to methods described by Fraser et al (1965), and Fahmy (1968). N-phenylsulfenyl derivatives

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of carbaryl and carbofuran were synthesized by the procedure described by Black et al. (1973).

Bioassay: Carbamates were dissolved in acetone and applied to the notum of bee workers anaesthetised with $CO_2$ or to cotton leafworm (third instar larvae) reared as previously described (Eldefrawi et al 1964). Mortality of both insect species was assessed 24 hours after treatment. Synergised toxicity was determined with a simultaneous constant dose of piperonyl butoxide (P. B.) of 16 µg per insect. The average weights of treated insects were 14.5 mg per larva and 100 mg per bee.

Cholinesterase preparations: Adult bee workere were frozen at -20°C and heads removed and homogenized in 0.1 M sodium phosphate buffer, pH 7.0 (25 heads/ml). The slurry was centrifuged at 6,400 r.p.m. for 15 minutes and the supernatant was used directly or stored at -20°C. Cotton leafworm cholinesterase was prepared from heads of the 6th instar larvae by the same technique (40 heads/ml).

Measurement of cholinesterase activity: The colorimetric method of Ellman et al (1961) was used for determining enzyme activity. Final concentrations of acetylthiocholine and 5,5'-dithiobis (2-nitrobenzoate) (DTNB) were $2\times10^{-3}$ M and $2\times10^{-4}$ M respectively. The final dilutions of enzyme preparations made for measurement of activity were 0.25 head/ml and 0.4 head/ml for honey bee and cotton leafworm respectively. The reaction was monitored in pH 7.6 phosphate buffer 0.1 M, at 30 ± 0.5°C, and activity was measured spectrophotometrically at 412 nm.

Inhibition constants: Affinity ($K_a$), and carbamylation ($k_2$) and bimolecular ($k_i$) rate constants were calculated according to the procedure of Main & Iverson (1966). $I_{50}$ values were measured within one hour after preincubating enzyme and inhibitor for one and five minutes for bee and leafworm cholinesterase respectively.

## RESULTS AND DISCUSSION

Toxicity and anticholinesterase activity of oxime carbamates: Table 1 shows the kinetic constants for the inhibition of cholinesterases from honey bee (H.B.) and cotton leafworm (L.W.) by three oxime carbamates, and their toxicity to these insects. It is clear that these carbamates are potent insecticides for honey bee and except for aldicarb are also effective against the cotton leafworm. While aldicarb is extremely poisonous to bee and is not synergised by piperonyl butoxide (P.B.), it is virtually non-toxic to the leafworm even in the presence of the synergist. This undesirable selective toxicity of aldicarb between honey bee and leafworm is rather interesting. It seems to occur partly because aldicarb is approximately 8 times more effective against honey bee cholinesterase than against that of cotton leafworm. Although aldicarb has 4 times more affinity ($K_a$) to cotton leafworm cholinesterase than to that from honey bee, its carbamylates ($k_2$) the latter 32.5 times faster than the former. If cholinesterase inhibition contributes significantly to this selectivity, these results would indicate that carbamylation of the enzyme is more important in toxicity than complex formation. It could be argued that selective toxicity of these oxime carbamates between honey bee and cotton leafworm parallels the ability to carbamylate their enzymes. This is substantiated by comparing the relative synergised $LD_{50}$ (L.W./H.B.), >2500, 5, and 2.6 for aldicarb, methomyl, and N-desmethyl methomyl respectively with their respective relative $k_2$ values (H.B./L.W.), 32.5, 9 and 1.7. Also the high toxicity of aldicarb to mice and rats ($LD_{50}$ 0.3 - 1.0 mg/kg) is associated with a very high rate of carbamylation of mammalian cholinesterase; $k_2$ equals 135 $min^{-1}$ for bovine erythrocyte acetylcholinesterase (Hastings et al 1970).

Table 1

Anticholinesterase activity and toxicity of some oxime carbamates to honey bee and cotton leafworm

| Compound | Insect | $K_a \times 10^5$ (M) | $k_2$ ($min^{-1}$) | $k_i \times 10^{-3}$ ($M^{-1}min^{-1}$) | $LD_{50}$ (µg/g) Alone | + P.B. |
|---|---|---|---|---|---|---|
| Aldicarb | H.B. | 237 | 3.25 | 1.4 | 0.50 | 0.40 |
| | L.W. | 60 | 0.10 | 0.17 | >1000.00 | >1000.00 |
| Methomyl | H.B. | 7.75 | 10.00 | 125 | 0.62 | 0.19 |
| | L.W. | 1.84 | 1.10 | 60.6 | 3.38 | 0.97 |
| N-desmethyl methomyl | H.B. | 2.24 | 6.67 | 300 | 2.20 | 1.05 |
| | L.W. | 3.40 | 4.00 | 112 | 24.84 | 2.76 |

However, it seems likely that the virtual immunity of the leafworm to aldicarb is greatly affected by some factor other than cholinesterase sensitivity or oxidative metabolism. It is also evident that methomyl and its N-desmethyl derivative have greater affinities and carbamylation rates to both insect cholinesterases than does aldicarb. This is reflected in the high toxicity of these two compounds to both insects.

N-derivatisation of arylcarbamates: N-derivatization of carbamates is usually associated with a marked decrease in anticholinesterase potency (Fraser *et al* 1965 and Black *et al* 1973), and this was also found in the present studies (Table 2).

Table 2

Anticholinesterase activity and toxicity of some N-substituted arylcarbamates to honey bee and cotton leafworm

| Compound | Insect | $I_{50}$(M) $\times 10^7$ | $LD_{50}$ (µg/g) Alone | + P.B. |
|---|---|---|---|---|
| Carbaryl | H.B. | 1.7 | 1.9 | 0.37 |
| | L.W. | 30 | >1000 | 518 |
| Carbaryl N-formyl | H.B. | 1100 | >480 | 92 |
| | L.W. | >3000 | >1000 | >1000 |
| Carbaryl N-acetyl | H.B. | >3000 | 8.0 | 3.4 |
| | L.W. | 1700 | >1000 | >1000 |
| Carbaryl N-phenyl-Sulfenyl | H.B. | 20 | 3.5 | 2.9 |
| | L.W. | >3000 | >1000 | 759 |
| Carbofuran | H.B. | 1.2 | 0.094 | 0.075 |
| | L.W. | 370 | >1000 | >1000 |
| Carbofuran N-acetyl | H.B. | >3000 | 240 | 105 |
| | L.W. | >3000 | >1000 | >1000 |
| Carbofuran N-phenyl-Sulfenyl | H.B. | 120 | 35 | 20 |
| | L.W. | >3000 | >1000 | >1000 |

It was hoped that honey bee would be unable to release the potent anticholinesterase parent carbamate from its N-substituted derivatives as effectively as leafworm larvae because honey bees are known to metabolise carbamates more slowly.

Table 2 shows the kinetic data for such derivatives of carbaryl and carbofuran. All acylated and phenylsulfenylated derivatives of carbaryl and carbofuran were less toxic than the parent carbamates by varying degrees. Also, except for the phenylsulfenyl derivative of carbaryl, all derivatives were poor inhibitors for both insect cholinesterases. The toxicity data indicate that deacylation in the bee is rather slow and dependent on the nature of the carbamate and the acyl moiety. For example, N-deacetylation of N-acetyl carbaryl appears to be much faster than that of N-acetyl carbofuran as evidenced by their relative toxicity. However, N-formyl carbaryl is 60 times less toxic to bee than its N-acetyl analogue, which indicates that N-formyl is cleaved in this insect much more slowly. These findings are rather interesting since the opposite is known to occur in the cotton leafworm for the same derivatives (Fahmy and Fukuto, 1970). Unfortunately the toxicity of the parent carbaryl to leafworm is relatively low compared to its toxicity to honey bee, a fact that limits the usefulness of this finding. However derivatization of some of the oxime carbamates which are active against cotton leafworm would seem worthwhile.

In order to understand the reason for the low anticholinesterase activity of the N-phenylsulfenyl derivatives of carbaryl and carbofuran, affinity and carbamylation rate constants were examined for honey bee cholinesterase (Table 3). Derivatization of carbaryl did not affect affinity while carbamylation was decreased by a factor of 2.3. The marked decrease in anticholinesterase potency ($k_i$) of carbofuran (76-fold) resulting from derivatization is mainly due to reduced carbamylation rate constant (13.3-fold) with a somewhat smaller decrease in affinity (6.0-fold).

Table 3

Effect of N-phenylsulfenyl substitution of carbaryl and carbofuran on affinity and carbamylation of honey bee cholinesterase

| Compound | $K_a \times 10^7$ (M) | $k_2$ ($min^{-1}$) | $k_i \times 10^{-5}$ ($M^{-1}\ min^{-1}$) |
|---|---|---|---|
| Carbaryl | 4.7 | 2.55 | 55.6 |
| Carbaryl N-phenyl-sulfenyl | 5.0 | 1.1 | 22.2 |
| Carbofuran | 15 | 12 | 80 |
| Carbofuran N-phenyl-sulfenyl | 90 | 0.9 | 1.05 |

Therefore, it may be concluded that the decreased anticholinesterase activity of the N-substituted derivatives is associated primarily with their lower ability to carbamylate the enzyme. This indicates the importance of the N-proton in the carbamylation process and confirms previous observations that N-methyl carbamates are in general better carbamylating agents than N,N-diacyl derivatives (O'Brien et al 1966).

## References

ABDEL-AAL, Y.A.I. (1975) Relation between chemical structure and biological activity of certain insecticides, Ph.D. Thesis, Assuit University, 1975.

ABDEL-RAOF, T.K. (1974) Effect of some pesticides on soil microorganisms and their kinetic parameters in the inhibition reaction of insect cholinesterases, Ph.D. Thesis, Assiut University, 1974.

BLACK, A.L., CHIU, Y.C., FAHMY, M.A.H. & FUKUTO, T.R. (1973) Selective toxicity of N-sulfenylated derivatives of insecticidal methylcarbamate esters, Journal of Agriculture and Food Chemistry, 21, 747-51.

ELDEFRAWI, M.E., TOPPOZADA, A., MANSOUR, N. & ZEID, M. (1964) Susceptibility of different larval instars of Prodenia to insecticides, Journal of Economic Entomology, 57, 391-3.

ELLMAN, G.L., COURTNEY, K.D., ANDRES,Jr., V. & FEATHERSTONE, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity, Biochemical Pharmacology, 7, 88-95.

FAHMY, M.A.H. (1968) A new method for the preparation of N-formylcarbamates, Egyptian Patent 8749, (Sept. 8, 1968).

FAHMY, M.A.H. & FUKUTO, T.R. (1970) Insecticidal activity of N-acylcarbamates on susceptible and carbaryl-resistant strains of the Egyptian cotton leafworm, Journal of Economic Entomology, 63, 1783-86.

FRASER, J., CLINCH, P.J. & REAY, O.C. (1965) N-acylation of N-methylcarbamate insecticides and its effect on biological activity, Journal of Science, Food and Agriculture, 16, 615-8.

GEORGHIOU,G.P. & ATKINS, E.L. (1964) Temperature coefficient of toxicity of certain N-methylcarbamates against honey bees and the effect of synergist piperonyl butoxide, Journal of Apiculture Research, 3, 31-5.

HASTINGS, F.L., MAIN, A.R. & IVERSON, F. (1970) Carbamylation and affinity constants of some carbamate inhibitors of acetylcholinesterase and their relation to analogous substrate constants, Journal of Agriculture and Food Chemistry, 18, 497-502.

MAIN, A.R. & IVERSON, F. (1966) Measurements of the affinity and phosphorylation constants governing irreversible inhibition of cholinesterase by di-isopropyl phosphorofluoridate, Biochemical Journal, 100, 525-31.

O'BRIEN, R.D., HILTON, B.D. & GILMOUR, L. (1966) The reaction of carbamates with cholinesterase, Molecular Pharmacology, 2, 593-605.

WEIDEN, M.H.J. (1971) Toxicity of carbamates to insects, Bulletin of the World Health Organisation, 44, 203-213.

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# THE CONTROL OF MITES IN STORED OILSEED RAPE

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Summary Increased production of oilseed rape has resulted in extended storage periods and a greater risk from mite attack. Mites are unlikely to breed on seed dried to 8% moisture content or below, but the surface of dry bulks may absorb atmospheric moisture.

Chemical control in the UK is restricted to HCH admixture, but resistance in *Acarus siro* and *Glycyphagus destructor* has reduced the value of this acaricide. Several compounds tested in the laboratory showed promise as alternative acaricides, and the application of 2% pirimiphos-methyl dust successfully controlled a heavy surface infestation in farm-stored seed. Residue analysis showed an average level of 8.1 ppm in the treated seed.

In a laboratory experiment, most of a dose of 4 ppm pirimiphos-methyl remained on the seed 9 months after treatment. However, during a simulated commercial oil extraction and refinement, the level of pirimiphos-methyl decreased from an average of 14.7 ppm in the crude oil to 1.5 ppm in the final deodorised oil.

## INTRODUCTION

Oilseed rape, *Brassica napus*, is grown chiefly for the oil which constitutes about 40% of the seed. Some oil is used in industry, but the bulk goes into the manufacture of edible oils and fats. The residue remaining after the oil has been extracted is converted into rapeseed meal for animal feed.

The acreage grown has expanded considerably in England and Wales from 5130 ha in 1971 to an estimated 47,800 ha in 1976, and the increase is expected to continue. This expansion has led to a considerable change in the pattern of storage. Previously most of the crop was processed shortly after harvest and little storage on the farm was necessary. However, the current harvest is too large for immediate processing and substantial quantities of seed are now stored on farms for up to 7 months.

During storage, oilseed rape can become infested by several species of mites. A study carried out in 1974-75 of 14 English farms storing oilseed rape revealed some mites on most farms and serious infestations on 3 of them (ANON, 1977). The principal species were *Acarus siro* L and *Glycyphagus destructor* (Schrank). Mites in oilseed rape have also been reported from Canada and France. MILLS (1976) in a survey of Canadian stores found that about 25% contained infested seed during 1974-75. In France, FLEURAT LESSARD and ANGLADE (1973) reported that serious mite infestations commonly occur in seed stored at moisture contents of 8% or above.

The presence of mites is undesirable for several reasons. When a heavy infestation is present, the contents of many of the seeds are completely eaten away, leaving only the hollow testa. In addition, mites are more noticeable on the black seeds than on cereals, making rejection by buyers more probable.

Little information exists on the prevention of mite infestations during storage. APPELQVIST and OHLSON (1972) suggest that if the seed is dried to a moisture content of 7% it can be stored safely for long periods. The same authors however, point out that trading agreements are frequently based on moisture contents of 9 - 10%. PIXTON and WARBURTON (1977) found that at 15°C, a moisture content of between 7.7 and 8.4%, depending on the variety of the seed, was at equilibrium with 70% r.h. This relative humidity is close to the minimum level required by A. siro and G. destructor to complete their development under otherwise optimal laboratory conditions (CUNNINGTON, 1976). In England and Wales oilseed rape is often harvested at high moisture contents. Some excess water is removed by artificial drying, but commercial pressures tend to encourage farmers to store the seed at 8 - 9% moisture content, thereby placing it at risk from mite attack. Even when the crop is put into store sufficiently dry, the surface layer of seed frequently absorbs moisture from the atmosphere during storage and becomes prone to mite attack.

Chemical control of mites in stored oilseed rape in the UK is restricted to admixture of HCH (ANON, 1973), the only suitable acaricide cleared by the UK Pesticides Safety Precautions Scheme for safe admixture to oilseeds. Unfortunately the widespread resistance to HCH in A. siro and G. destructor in Britain has considerably reduced the value of this acaricide (WILKIN, 1975a). At present, the only treatment that can be advised is to re-dry or turn the bulk (WILKIN, 1975b). Work has therefore been started to assess the effectiveness of alternative acaricides for use on stored oilseed rape and to determine their commercial suitability.

Although it may be necessary to apply pesticides to the seed to control infestation, it is important to monitor the residue levels to prevent toxic hazards. It is particularly important to ascertain the residue level in the final product after it has been processed for human or animal consumption. The ideal pesticide would protect the product from infestation and would break down during storage and processing.

The present studies involved laboratory trials to assess the value of potential acaricides. One promising compound, pirimiphos-methyl was subsequently tested under field conditions. In addition the breakdown of pirimiphos-methyl applied to oilseed rape was investigated, both during storage and during a simulated commercial oil extraction.

## LABORATORY TRIALS OF CANDIDATE ACARICIDES

Materials and methods Seven compounds possessing acaricidal activity (see Table 1) were applied to English oilseed rape of 8 - 9% moisture content, and tested against laboratory strains of A. siro and G. destructor. Four of the acaricides were known to be effective against HCH-resistant mites. The compounds were applied to the seed at a dosage rate considered suitable for application to cereal grains, and at double that dose, using the method described by WILKIN (1973). Dust or emulsifiable concentrate formulations were used, and approximately 50 g lots of treated seed were placed in 100 g wide-necked jars. About 0.1 g of vigorous mite culture was added, the jars were sealed with filter paper tops and then stored at 17.5°C and 75% r.h. Controls were set up in the same way but using untreated seed.

After 7 and 14 days, 2 replicates of each treatment and of the control were examined. The mites were extracted from the seed by sieving, and mortality was assessed as one of 5 gradings: (0 - 4), which approximated to $<$10% kill (0); $\simeq$ 25% kill (1); $\simeq$ 50% kill (2); $>$ 75% kill (3); and 100% kill (4).

Results The 7 and 14 day results are given in Table 1, with the compounds arranged in approximate order of effectiveness. After 7 days, no compound was completely effective against both species, but after 14 days, both doses of etrimfos had killed all the mites. The higher doses of pirimiphos-methyl and bioresmethrin/pb were also totally effective against both species after 14 days. All these acaricides are effective against HCH resistant mites (ANON, 1977). Chlorpyrifos-methyl, pyrethrins/ pb, fenitrothion and iodofenphos did not kill all the mites. Control mortality never exceeded category 0.

## FIELD TRIAL

Materials and methods Since pirimiphos-methyl was already commercially available in the UK and was cleared for safe use by the UK Pesticides Safety Precautions Scheme as an insecticide for admixture to cereals, it was selected for a field trial. In November 1976 a serious mite infestation developed in the surface of 200 tonnes of oilseed rape stored on a Berkshire farm. The seed was stored between bulkheads on the floor of a soundly constructed barn. According to the farmer, the bulk had been dried to below 8% moisture content immediately after the harvest.

Two adjacent areas, each approximately 4.5 m x 6.5 m, were marked on the surface of the bulk and used for the experiment. Both areas were heavily infested with *A. siro*, and small numbers of *G. destructor* were also present. A sampling spear was used to collect 200 g samples from the surface and a depth of 1 m at 4 positions in each area. Mites were removed from the samples by sieving, and counted. The moisture content of the sieved seed was recorded using an electrical resistance meter. A thermistor probe was used to record temperatures at each position. Samples were collected at 14 and 7 days before treatment and then at 8, 16, 24 and 31 days after treatment.

Two per cent pirimiphos-methyl dust was applied to the surface of one area at the rate of 86 $g/m^2$. The dust was then mixed into the surface by means of shovels aiming at a treatment of 8 ppm in the top 0.3 m of seed. Chemical analysis gave an average level of 8.1 ppm pirimiphos-methyl. The other area was left untreated as a control.

Results Eight days after treatment, the mite population in the surface of the treated area was reduced from a mean of 23280/kg to 303/kg; a reduction of about 99% (see Table 2). By the end of the experiment virtually 100% kill was achieved, whereas a very heavy surface infestation remained in the control. At 1 m a similar reduction in numbers occurred in the treated area. Throughout the experiment a difference in mite population from one side of the store to the other and in both areas was observed. The higher populations appeared to be correlated with a higher moisture content. During the experiment the mean surface moisture content in both areas rose from about 8% to about 9.5%. The moisture content at 1 m remained constant at about 7.3%. The mean temperature of the bulk remained around 8°C.

## BREAKDOWN OF PIRIMIPHOS-METHYL ON STORED OILSEED RAPE

Materials and methods Oilseed rape of 8% moisture content was treated with 2% pirimiphos-methyl dust to give a dose of 4 ppm and stored in sealed glass jars at a temperature of 17.5°C. Samples were taken at intervals over a nine-month period and the pirimiphos-methyl residues determined in both the whole seed and the extracted oil, obtained by crushing.

In addition rapeseed oil was extracted and refined on a laboratory scale, simulating as far as possible, the conditions used in the commercial process. Samples of oilseed rape treated with 8 ppm pirimiphos-methyl, using both dust and e.c. formulations, were ground to a meal and the oil extracted with n-hexane using the Soxhlet technique. The free fatty acid content of the crude oil was determined by the method described in the British Pharmacopoeia (1973). The acids were neutralised by the gradual addition of a 20% excess of 2N sodium hydroxide with stirring at 70 - 80°C for 30 minutes. The mixture was allowed to stand until the soapstock had separated out and the oil was then washed 3 times with hot distilled water before drying under vacuum. Two to three per cent of bleaching earth was added to the neutralised oil and the mixture was agitated under reduced pressure for 30 minutes before filtering. Finally the refined and bleached oil was heated to 170 - 190°C and freshly generated steam was injected into the heating chamber for 2 hours. The impurities were removed by steam distillation under reduced pressure. Samples were collected at each stage of the process and subjected to residue analysis.

Residue Analysis: Samples were analysed by gas-liquid chromatography on a 1 m glass column, packed with 5% OV - 17 + 0.02% Epikote 1001 on Diatomite CLQ mounted in a Perkin-Elmer F33 gas chromatograph equipped with a phosphorus-sensitive thermionic detector. Oily samples were cleaned-up before injection by n-hexane-acetonitrile counter-current partition (BULLOCK, 1976).

Results The breakdown of pirimiphos-methyl on oilseed rape under laboratory storage conditions is illustrated in Fig. 1. Nine months after the initial treatment, most of the applied dose was still present in the seed and most of this was concentrated in the oil.

The breakdown of pirimiphos-methyl in rapeseed oil during the simulated commercial extraction and refinement process in shown in Table 3. The levels of pirimiphos-methyl decreased with each processing step. The greatest losses occurred during the bleaching and steam distillation processes where on average 67% and 69% were lost respectively. However, a measurable amount was still present in the final oil (8.5% of amount in the crude oil). No residues of pirimiphos-methyl were found in any of the spent meal samples.

## DISCUSSION

Oilseed rape can become infested with mites during farm storage and physical control methods are not always adequate or convenient. The extended storage period due to expanded production has increased the likelihood of infestation. There is thus a need for acaricides which can be safely admixed with the stored seed and which will give satisfactory control of mites, including strains resistant to HCH.

Etrimfos and pirimiphos-methyl showed considerable promise in laboratory trials and both chemicals are effective against H-CH resistant strains of A. siro and G. destructor (ANON, 1977). The selectivity of action of pesticides in tests using wheat (WILKIN, 1973, ANON, 1977) was also demonstrated with chemicals applied to oilseed rape. Chlorpyrifos-methyl, iodofenphos and fenitrothion controlled G. destructor but not A. siro. Most chemicals tested were less effective when applied to oilseed rape than when applied to wheat, and a higher dose was required to give complete kill. This could be due, in part, to the much greater surface area provided by the smaller seeds, which would tend to reduce the effective dose. The rapid uptake of pesticides by the oil in the seed may also have reduced their availability to the mites. Etrimfos was, however, very effective even at a dose lower than that tested on wheat. This compound is not yet commercially available, but it clearly merits further evaluation.

The field trial demonstrated that effective control of a serious mite infestation can be obtained using pirimiphos-methyl. Almost complete control was achieved 3 days after treatment. The very small and diminishing numbers of survivors which were found at subsequent samplings were probably due to a few mites having escaped direct contact with the acaricide within hollowed out seeds.

Although the main infestation was confined to the surface layers of the bulk, small numbers of mites were also found at 1 m. These mites were most probably strays from the surface layers rather than a breeding population and this would account for the apparent control obtained at 1 m by the surface treatment.

Several workers have studied the effects of simulated commercial processing techniques on the level of organochlorine pesticide residues in vegetable oils and two reviews of the subject have been published (MEEMKEN, 1975, WOLFF, 1974). Most workers reported that pesticide levels decreased with each stage of the refining process and no organochlorine residues could be detected in the processed oil (GOODING, 1966, MOUNTS et al., 1969, SMITH et al., 1968). In a study of the distribution of aldrin and dieldrin in soybean oil (CHAUDRY et al., 1976) a residue of aldrin was noted in one sample of the deodorised oil. Alkali-refining and bleaching had little or no effect on HCH and DDT residues in rapeseed oil (SAHA et al., 1970). However, deodorisation of the oil by heating with steam at 230-260°C and 6 torr for 4 hours removed 95 - 99% of the residues. No comparable studies with organophosphorus insecticides appear to have been published.

Pirimiphos-methyl is very stable when applied to oilseed rape and extremely good persistence and long-term protection should be obtained from a single treatment. However, the compound is degraded during commercial processing of the seed and the residue levels found in the refined oil were very low. No residues could be detected in the spent meal. KROGER (1968) determined the experimental conditions necessary for the reduction and elimination of heptachlor oxide and dieldrin from butter oil. He found that mild deodorisation treatments were not effective. More severe deodorisation reduced the insecticide levels appreciably. Steam distillation at 180-195°C and 0.01-0.5 torr for five hours was necessary to remove all traces of the insecticides. This is in broad agreement with the findings of the present work in which a different class of compound was used.

The good results obtained in the field trial demonstrate the value of treatment with pirimiphos-methyl under practical conditions. This compound is readily available and widely used but at present it is not cleared for safe use, by the UK Pesticide Safety Precautions Scheme, as an insecticide for admixture with oilseeds.


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## References


ANON (1977) Pest Infestation Control Laboratory Report 1974-76, H.M.S.O., London. In press.

ANON (1973) Oilseed Rape. Billingham Press Ltd, for United Oilseeds Ltd, Billingham.

APPELQVIST, L.-Å. and OHLSON, R. (1972) Rapeseed. Elsevier Pub. Co, Amsterdam.

BRITISH PHARMACOPOEIA (1973), H.M.S.O., London, A 84.

BULLOCK, D.W.J. (1976) Pirimiphos-methyl, Analytical methods for pesticides and plant growth regulators, 8, 185-205.

CHAUDRY, M.M. et al. (1976) Distribution of aldrin and dieldrin in soybeans, oil and by-products during processing, Journal of the American Oil Chemists' Society, 53, 695-697.

CUNNINGTON, A.M. (1976) The effect of physical conditions on the development and increase of some important storage mites, Annals of Applied Biology, 82, 175-178.

GOODING, C.M.B. (1966) Fate of chlorinated organic pesticide residues in the production of edible vegetable oils, Chemistry and Industry, 344.

FLEURAT LESSARD, F. and ANGLADE, P. (1973) Influence de la teneur en eau des grains stockes sur le developpement des populations des acariens, Compte Rendus du Symposium International 'Preservation of Wet Harvested Grains', edited by J.-L. Multon and A. Guilbot published in Annales de Technologie Agricole numero hors serie, 1973, 391-400 and 22, 531-540.

KROGER, M. (1968) Effect of various physical treatments on certain organochlorine hydrocarbon insecticides found in milk fat, Journal of Dairy Science, 51, 196-198.

MEEMKEN, H.-A. (1975) Rückstände von Pesticiden in fetthaltigen Lebensmitteln unter besonderer Berücksichtigung der tierischen und pflanzlichen Fette und Öle, Fette, Seifen, Anstrichmittel, 77, 290-296.

MILLS, J.T. (1976) Spoilage of rapeseed in elevator and farm storage in Western Canada, Canadian Plant Diseases Survey, 56, 95-103.

MOUNTS, T.L. et al. (1969) Radiochemical experiments on minor constituents in soybean oil, Journal of the American Oil Chemists' Society, 46, 482-484.

PIXTON, S.W. and WARBURTON, S. (1977) The moisture content/equilibrium relative humidity relationship and oil composition of rapeseed, Journal of Stored Products Research, 13, 77-81.

SAHA, J.G. et al. (1970) Effect of commercial processing techniques on lindane- and DDT- $^{14}C$ residues in rapeseed oil, Journal of Agricultural and Food Chemistry, 18, 43-44.

SMITH, K.J. et al. (1968) Removal of chlorinated pesticides from crude vegetable oils by simulated commercial processing procedures, Journal of the American Oil Chemists' Society, 45, 866-869.

WILKIN, D.R. and HOPE, J.A. (1973) Evaluation of pesticides against stored product mites, Journal of Stored Products Research, 8, 323-327.

WILKIN, D.R. (1975) The effects of mechanical handling and the admixture of acaricides on mites in farm-stored barley, Journal of Stored Products Research, 11, 87-95.

WILKIN, D.R. (1975) The control of stored product mites by contact acaricides, Proceedings of the 8th British Insecticide and Fungicide Conference, 1, 355-364.

WOLFF, J.P. (1974) Evolution des pesticides au cours des operations industrielles d'obtention et de raffinage des corps gras, Revue française des corps gras, 21, 161-164.

Table 1 The effectiveness of compounds applied to oilseed rape against Acarus siro and Glycyphagus destructor

| Compound (formulation) | Dose (ppm) | Mortality grading* A. siro 7 days | A. siro 14 days | G. destructor 7 days | G. destructor 14 days |
|---|---|---|---|---|---|
| Etrimfos (e.c.) | 4 | 3 | 4 | 3 | 4 |
| | 8 | 3 | 4 | 3 | 4 |
| Pirimiphos-methyl (dust) | 4 | 3 | 4 | 2 | 3 |
| | 8 | 3 | 4 | 3 | 4 |
| Bioresmethrin/pb‡ (e.c.) | 2 + 10 | 3 | 4 | 2 | 3 |
| | 4 + 20 | 3 | 4 | 3 | 4 |
| Chlorpyrifos-methyl (e.c.) | 4 | 3 | 3 | 4 | 4 |
| | 8 | 3 | 3 | 4 | 4 |
| Iodofenphos (dust) | 10 | 0 | 0 | 3 | 4 |
| | 20 | 1 | 1 | 3 | 4 |
| Fenitrothion (dust) | 4 | 0 | 0 | 3 | 3 |
| | 8 | 1 | 1 | 3 | 4 |
| Pyrethrins+/pb‡ (dust) | 1.25 + 10 | 1 | 2 | 0 | 0 |
| | 2.5 + 20 | 2 | 3 | 1 | 3 |

* <10% (0); ≏ 25% (1); ≏ 50% (2); > 75% (3); 100% (4)

+ pesticide lacking an ISO name ‡ piperonyl butoxide

Table 2 The effect of pirimiphos-methyl dust admixture on an A. siro infestation in farm-stored oilseed rape

Mean no. of A. siro/kg in the 4 samples (Range)

| | | Pirimiphos-methyl Surface | Pirimiphos-methyl 1 m | Control Surface | Control 1 m |
|---|---|---|---|---|---|
| Days before treatment | 14 | 9510 (280-20480) | 610 (0-1160) | 6640 (160-23360) | 95 (0-280) |
| | 7 | 23280 (960-58880) | 1860 (120-3840) | 17720 (240-62080) | 295 (0-740) |
| Days after treatment | 8 | 303 (50-440) | 10 (0-20) | 13843 (90-27200) | 2768 (0-8000) |
| | 16 | 133 (5-440) | 3 (0-5) | 21621 (5-62700) | 673 (0-1680) |
| | 24 | 11 (0-35) | 0 | 12404 (15-30080) | 1155 (0-2720) |
| | 31 | 10 (0-30) | 0 | 62621 (5-168960) | 135 (0-370) |

Table 3 The concentration of pirimiphos-methyl in oilseed rape and during processing of rapeseed oil (ppm)

| Formulation used | Whole seed | Crude oil | Neutralised oil | Bleached oil | Oil after steam distillation |
|---|---|---|---|---|---|
| e.c. | 7.7 | 17.7 | 17.0 | 6.9 | 2.3 |
| dust | 6.1 | 11.7 | 9.7 | 2.4 | 0.7 |

All results are the mean of at least two determinations

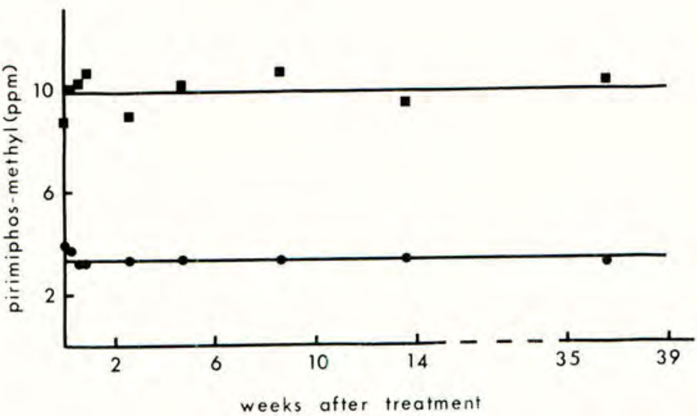


Figure 1 The concentration of pirimiphos-methyl in

■ rapeseed oil and

● oilseed rape

Proceedings 1977 British Crop Protection Conference - Pests and Diseases

# THE EFFECTIVENESS OF THREE CONTACT INSECTICIDES AGAINST A SUSCEPTIBLE AND A MALATHION-RESISTANT STRAIN OF THE SAW-TOOTHED GRAIN BEETLE (*Oryzaephilus surinamensis*)

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Summary The efficacy of fenitrothion, pirimiphos-methyl and malathion for the control of a susceptible strain, and a malathion-resistant strain of *Oryzaephilus surinamensis* was assessed. Exposures were on filter papers treated with emulsifiable concentrate formulations of the insecticides. Some exposures were in arenas provided with refuges containing food. The presence of a refuge significantly reduced the mortality of both strains in arenas treated with each of the three insecticides.

Pirimiphos-methyl was most effective against the resistant strain, and fenitrothion against the susceptible strain.

Sommaire L'efficacité du fénitrothion, du méthyl-pirimiphos et du malathion pour le contrôle d'une espèce susceptible, ainsi qu'une espèce de l'*Oryzaephilus surinamensis* présentant une résistance au malathion fut évaluée. Les expositions étaient sur papiers filtres traités avec une des formulations de concentrats émulsifiables des insecticides. Certaines expositions étaient dans des zones où des refuges contenaient de la nourriture. La présence d'un refuge eut comme effet de réduire d'une façon importante le taux de mortalité dans le cas des deux espèces, dans ces zones traitées avec chacun des trois insecticides.

Le méthyl-pirimiphos s'avéra très effectif contre l'espèce resistante, et il en fut de même dans le cas du fénitrothion pour l'espèce susceptible.

## INTRODUCTION

The saw-toothed grain beetle (*Oryzaephilus surinamensis*) is a major pest of farm-stored grain in Britain (GREEN, 1972). Methods of preventing its establishment include storage of grain at low temperature and moisture content (below 18°C and 14% m.c.), cleaning and pre-harvest treatment of the grain store with a residual insecticide, and admixture of the grain with insecticide. For the last ten years, malathion has been the most commonly used insecticide for such fabric and grain treatments in this country (PAPWORTH *et al.*, 1975), and it has been exceptionally effective against indigenous strains. However, the FAO survey carried out in 1972-73 (CHAMP and DYTE, 1976) revealed the existence of malathion-resistant strains of *O. surinamensis* in overseas countries (Figure 1) which may constitute a serious threat should they become established in British grain stores.

Figure 1

The occurrence of malathion resistance in Oryzaephilus surinamensis to 1973 (after CHAMP & DYTE 1976)

Susceptible ◆ Resistant ⊙

For many years staff of the U.K. Ministry of Agriculture, Fisheries and Food have been monitoring the incidence of infestations of O. surinamensis in imported foodstuffs, and since 1973 the number of resistant strains thus intercepted has been determined (Table 1). The aim has been to prevent or at least delay the introduction of resistant strains by fumigation of infested commodities before release from the port area, while alternative methods of control are investigated (DYTE et al., 1975; GREEN, 1975).

Table 1

The number of inland and imported strains of Oryzaephilus surinamensis tested for malathion resistance since 1973

| | INLAND STRAINS | | | IMPORTED STRAINS | | |
|---|---|---|---|---|---|---|
| | Tested | Resistant | % | Tested | Resistant | % |
| 1974 | 187 | 1 | 0.5 | 49 | 9 | 19 |
| 1975 | 163 | 5 | 3 | 71 | 12 | 17 |
| 1976 | 190 | 2 | 1 | 41 | 18 | 44 |
| 1977* | (87) | (1) | (1) | (15) | (8) | (53) |

* First 6 months

PINNIGER (1974; 1975) working with susceptible strains of Sitophilus granarius (the Grain weevil) and O. surinamensis demonstrated that behaviour is an important factor in the control of residual populations of stored-product pests. He subsequently showed that the behaviour of resistant and susceptible strains of Tribolium castaneum (the Rust-red flour beetle) could differ, and that refuge seeking behaviour was a component to be considered in the evaluation of insecticides against this species. The present paper summarises the results of a similar investigation of the susceptibility of a susceptible and a malathion-resistant strain of O. surinamensis to malathion, fenitrothion and pirimiphos-methyl.

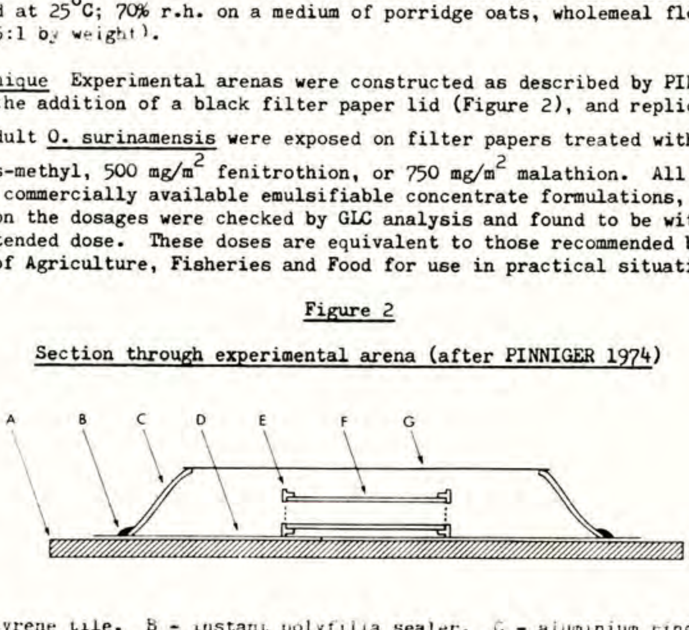
## METHODS AND MATERIALS

Insects The test insects (1-3 weeks old) were taken from cultures of a susceptible strain of O. surinamensis originating from an English farm, and a malathion-resistant strain (S484) collected from a rice store in India in 1972. The cultures were maintained at 25°C; 70% r.h. on a medium of porridge oats, wholemeal flour, and yeast (5:5:1 by weight).

Test technique Experimental arenas were constructed as described by PINNIGER (1974) but with the addition of a black filter paper lid (Figure 2), and replicates of 50 unsexed adult O. surinamensis were exposed on filter papers treated with 500 mg/m$^2$ pirimiphos-methyl, 500 mg/m$^2$ fenitrothion, or 750 mg/m$^2$ malathion. All insecticides used were commercially available emulsifiable concentrate formulations, and after application the dosages were checked by GLC analysis and found to be within ± 10% of the intended dose. These doses are equivalent to those recommended by the U.K. Ministry of Agriculture, Fisheries and Food for use in practical situations.

Figure 2

Section through experimental arena (after PINNIGER 1974)




A - polystyrene tile. B - instant polyfilla sealer. C - aluminium ring. D - filter paper (base). E - end stop. F - aluminium refuge tube. G - filter paper (top).

Sixteen replicates were set up for each test, eight being provided with a food refuge containing 8 g of kibbled English wheat, and eight being without a refuge. Insects to be tested in the presence of a refuge were left to settle in untreated arenas (transfer arenas) with the food refuge for 24 hrs before transfer of refuge and all insects to the treated surface. Insects in replicates without a refuge were placed directly on the treated surface with 0.5 g of kibbled wheat. All tests were carried out in dark cabinets at 25°C; 70% r.h.

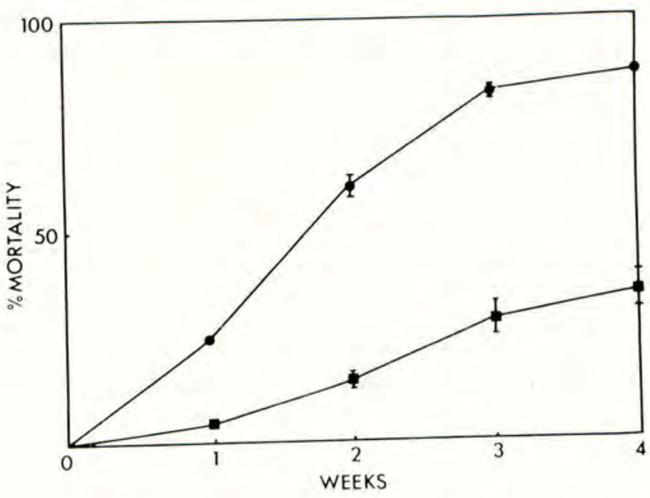
Assessment The numbers of live and dead insects in the arenas were counted daily and the dead beetles removed. At 7, 14, 21 and 28 days the refuges were removed from the arenas, carefully emptied and the number of live and dead beetles therein recorded. The live beetles were then returned to the untreated transfer arena with the original food refuge and allowed to settle for 24 hrs, before they and the refuge were replaced in the treated arena.

## RESULTS

As is often the case in experiments in which this species is handled, control mortalities were rather high. However in untreated arenas without a refuge mortality was less than 1% for the susceptible strain within day 1 and for the resistant strain 15% at day 15. Observations were continued over longer periods in the arenas containing refuges and in these, control mortalities were rather high in the last two weeks of test (Figure 3). An average mortality of 87% for the susceptible strain and 35% for the resistant strain was recorded after 28 days.

Figure 3

The control mortality of a malathion-resistant and a susceptible strain of Oryzaephilus surinamensis in untreated arenas with refuges

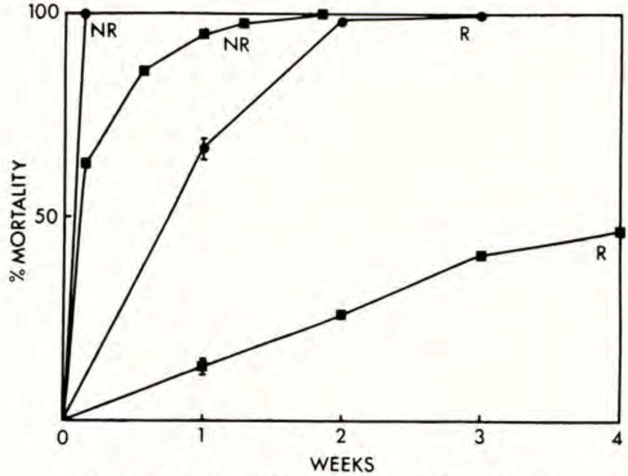



Susceptible ● Resistant ■

The presence of a refuge in arenas treated with malathion resulted in an increase in the exposure time for 100% mortality from under 1 day to 14 - 21 days for the susceptible strain (Figure 4). The resistant strain took 15 days to reach 100% mortality in a malathion-treated arena without a refuge, whereas even after 28 days only 47% mortality was achieved in arenas provided with refuges. In untreated control arenas many insects of both strains were inside the refuges, with an average over the first seven days of assessment of 89% for the resistant strain and 94% for the susceptible strain.

Figure 4

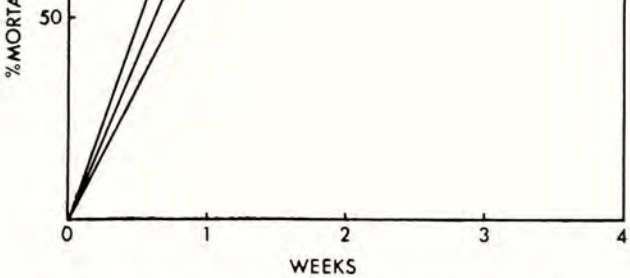
The effect of a refuge on the mortality of resistant and susceptible *Oryzaephilus surinamensis* exposed to 750 mg/m$^2$ malathion




NR - without a refuge. R - with a refuge
Susceptible ● Resistant ■

Figure 5

The mortality of susceptible *Oryzaephilus surinamensis* in arenas treated with fenitrothion, malathion or pirimiphos-methyl and provided with a refuge



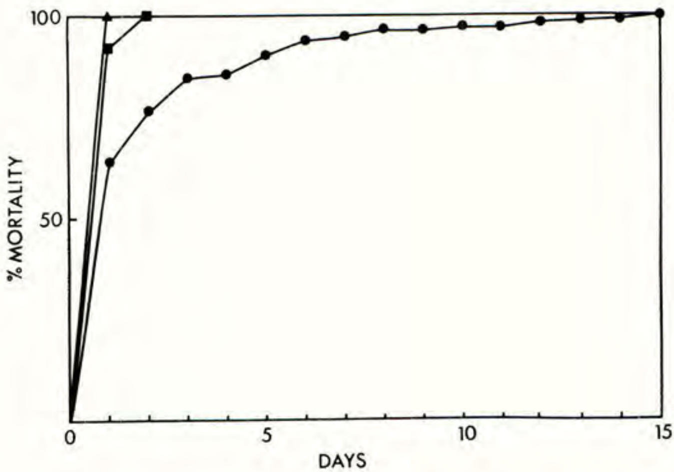

**Fenitrothion ■ Pirimiphos-methyl ▲ Malathion ●**

All the insecticides killed the susceptible strain within a few hours in arenas without refuges. In arenas with refuges fenitrothion was the most effective insecticide against the susceptible strain (Figure 5). Mortality was slower on malathion and pirimiphos-methyl, though both achieved 99% mortality by 14 days.

In arenas without refuges, both pirimiphos-methyl and fenitrothion were more effective than malathion against the resistant strain, achieving 100% kill in 1 and 2 days respectively compared to 15 days for malathion (Figure 6). In a refuge situation the order of potency against the resistant strain was the same, but none of the three insecticides gave 100% kill even after 4 weeks (Figure 7).

Figure 6

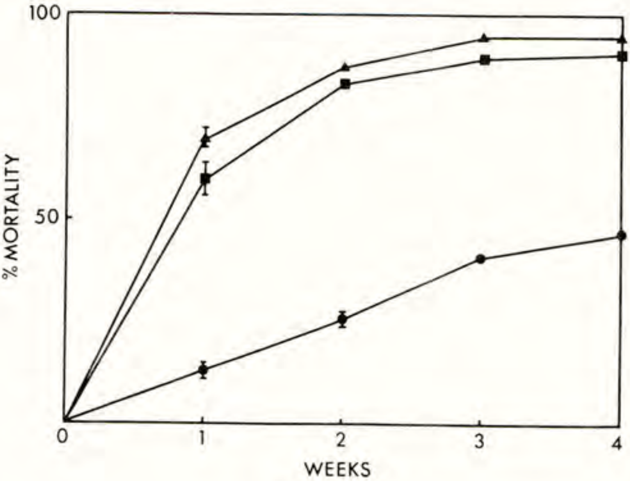
The mortality of resistant Oryzaephilus surinamensis in arenas treated with pirimiphos-methyl, fenitrothion or malathion without a refuge




Pirimiphos-methyl ▲ Fenitrothion ■ Malathion ●

Figure 7

The mortality of resistant Oryzaephilus surinamensis on pirimiphos-methyl, fenitrothion or malathion in arenas with a refuge




Pirimiphos-methyl ▲ Fenitrothion ■ Malathion ●

DISCUSSION

As expected, the availability of a refuge significantly reduced the mortality of both strains in arenas treated with each of the three insecticides. It appears that the behaviour of this species reinforces the effect of resistance in the overseas strain by further increasing survival in an insecticidal environment.

Although results from only one resistant strain and one susceptible strain of O. surinamensis are presented here, laboratory tests indicate similar behaviour patterns in one other malathion-resistant and one other susceptible strain. Cross resistance studies of 7 other malathion resistant strains (DYTE et el. 1975) indicate resistance to 6 other organophosphate insecticides, and CHAMP and DYTE (1976) record that two malathion-resistant strains were resistant to a total of 28 insecticides tested including organochlorines, carbamates, and pyrethroids. The response of this resistant strain to malathion (Figure 6) showed that a small percentage of beetles exhibit a high level of tolerance to the insecticide, indicating a heterogenous population which may well respond to selection for increased physiological resistance.

The present studies show that when refuges are available, pirimiphos-methyl (the insecticide now recommended for control of resistant strains) is less effective against the resistant strain (Figure 7) than is malathion against the susceptible strain (Figure 6). If similar resistant strains were introduced into this country, eradication of infestations may be difficult, if not impossible, using currently available insecticides and techniques. Furthermore the differing control mortalities in the two strains indicate physiological differences which may increase the survival of the resistant strain compared to the susceptible strain under practical conditions. Since insects are unlikely to stay in the cracks and crevices of a grain store permanently due to lack of food or the attraction of a fresh harvest, the speed of action of an insecticide should be considered as an important factor along with persistence and toxicity in assessing an insecticide's potential. A quick acting persistant insecticide should be able to prevent insects which leave a refuge and cross the insecticidally treated surface of the store from reaching newly stored goods.

Although behaviour attributed to the presence of a refuge is a complicating factor in control of this species, avoidance of the insecticidally treated surface may be yet another factor to be considered in assessing insecticidal efficacy. When testing the resistant strain in malathion treated arenas without a refuge, it was noted that the small number of beetles surviving after 8 days were immobile on top of the few grains of food provided. If dislodged they immediately attempted to climb back on to the grains, suggesting a tendency to avoid the treated surface.

Behaviour and physiological resistance in a population depends upon selection of individuals in a population. One may select for physiological resistance by exposing a population to a sub-lethal dose of insecticide and breeding the survivors. Similarly one may select for an increased tendency to avoid contact with an insecticide. Although these two components may develop separately, it is reasonable to expect strains exhibiting physiological resistance to be more readily selected for avoidance of insecticidally treated surfaces than susceptible strains. A resistant insect attempting to avoid a toxic surface is more likely to survive the effort than a susceptible beetle, thus increasing the frequency of resistant individuals displaying such a behaviour pattern.

A strain of O. surinamensis which exhibited a high level of physiological resistance and a marked tendency to avoid a treated surface would present serious control problems in this country.

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## References

CHAMP, B.R., and DYTE, C.E. (1976) Report of the FAO global survey of pesticide susceptibility of stored grain pests. FAO, Rome.

DYTE, C.E., GREEN, A.A., and PINNIGER, D.B. (1975) Some consequences of the development of insecticide resistance in stored-product beetles. Proceedings of 1st International Working Conference on Stored-Product Entomology, Savannah 1974 261-271.

DYTE, C.E., TYLER, P.S., BLACKMAN, D.G., FORSTER, R., COGAN, P.M., BINNS, T.J. and PECKOVER, J. (1976) Cross resistance studies. Pest Infestation Control Laboratory Report 1971-73, 74.

GREEN, A.A. (1972) The protection of cereals stored on farms. Proceedings of 3rd

British Pest Control Conference. St. Helier 1971, 5th Sess., Paper No. 13, 76-82.

GREEN, A.A. (1975) Resistance as a problem in the control of insects and mites infesting stored products in Britain. Proceedings of 4th British Pest Control Conference. St. Helier 1975, 7th Sess., Paper No. 14, 6 pp.

PAPWORTH, D.S., MILWARD, A.F., TAYLOR, J.K. and MAYHEW, A.W. (1975) Second survey of pesticides used for protection of stored grain and the control of harmful mammals on British farms. International Pest Control, 1975, 17 (2), 12, 14-16.

PINNIGER, D.B. (1974) A laboratory simulation of residual populations of stored product pests and an assessment of their susceptibility to a contact insecticide. Journal of Stored Product Research 10 217-223.

PINNIGER, D.B. (1975) The behaviour of insects in the presence of insecticides: the effect of fenitrothion and malathion on resistant and susceptible strains of Tribolium castaneum (Herbst). Proceedings of 1st International Working Conference on Stored-Product Entomology, Savannah 1974 301-308.

NOTES

Proceedings 1977 British Crop Protection Conference - Pests and Diseases

# RECENT ADVANCES IN CONTROL OF POST-HARVEST DETERIORATION USING THIABENDAZOLE*, WITH SPECIAL REFERENCE TO TROPICAL AND SUB-TROPICAL CROPS

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Summary Thiabendazole is used extensively for the control of post-harvest diseases of banana, citrus, apples and potatoes. Recent investigations indicate that the compound is also effective against post-harvest diseases of other tropical fruits (e.g. pineapple, avocado, mango, papaya) and various root crops (e.g. yam, sweet potato). Thiabendazole is particularly effective against certain species of *Aspergillus*, *Botrytis*, *Colletotrichum*, *Fusarium* and *Penicillium*, especially when they are wound-pathogens. There are some instances of fungal resistance to thiabendazole, notably *Penicillium* spp. on citrus. However, this problem can be eliminated or avoided by maintaining a high standard of packinghouse sanitation and by alternating thiabendazole with non-benzimidazole fungicides.

## INTRODUCTION

Thiabendazole (2-4'-thiazolyl)-benzimidazole), widely used as an anthelmintic since 1961, has a relatively broad antifungal spectrum (Weinke *et al.*, 1969). Early investigations describing the efficacy of thiabendazole against fungi causing decay of citrus and banana were reviewed by Eckert (1969). It soon became evident that control by thiabendazole was considerably better than with conventional protectant fungicides. Approval for the use of thiabendazole as a post-harvest fungicide was first obtained in the United States and elsewhere in 1968. Shortly afterwards it was introduced for commercial use in many countries for the control of post-harvest decay of citrus and banana fruits. Subsequent studies have shown that thiabendazole is a very versatile fungicide, being both curative and protectant against many fungus diseases on a wide range of plants. The present paper reviews the potential of thiabendazole as a general post-harvest fungicide.

## FRUITS

### a) Bananas

In most commercial banana operations, fruit is exported in the form of individual "hands" or clusters of "fingers" which are packed in boxes at packing stations in the plantations. The fresh wound where the "crown" of the hand is severed from the stalk is susceptible to invasion by several fungi resulting in crown rot. During early stages of boxing plant development (1960-63), crown rot

* Thiabendazole is marketed by Merck Sharp & Dohme under the registered trademarks of TECTO and MERTECT.

was a major cause of reduction of fruit quality in transit (Meredith, 1971). Fungicides available at that time gave only partial control of crown rot. However, with the introduction of thiabendazole, excellent control was easily achieved (Stover, 1972). The fungicide is applied by dipping fruit in an aqueous suspension, or by means of a recirculating spray, or by brushing or sponging the freshly severed crown tissue. It is used at a rate of 200-400 ppm a.i. thiabendazole. In the United States a tolerance of 3 ppm for the whole fruit has been approved, of which no more than 0.4 ppm shall be in the pulp after the peel is removed.

Starting in 1971, there have been several reports about the development of resistance to benomyl and related fungicides by fungi which were initially found to be sensitive to these materials (Dekker, 1976; Ogawa et al., 1976). The spread of some of these resistant strains has led to difficulties in disease control. One of the most important crown rot pathogens is Colletotrichum musae. In St. Lucia, West Indies, Griffee (1973) obtained many isolates of C. musae from bananas which had received pre-harvest benomyl sprays at 3 weekly intervals from the time of bunch emergence to that of harvest (approximately 3 months). One isolate was resistant to benomyl, thiabendazole and methyl thiophanate, in vitro, at concentrations up to 8000 ppm. In contrast, isolates of C. musae from fruit not treated with benomyl were completely inhibited by 1 ppm or less of each fungicide. Griffee (1973) suggested that the resistant C. musae strain was a benomyl-selected or benomyl-induced mutant. It should be noted that the conditions under which Griffee obtained a resistant strain of C. musae in St. Lucia were somewhat artificial, because it is not normal plantation practice to spray bunches of fruit with benomyl or any other benzimidazole fungicide at such frequent intervals. However, the fact that resistance can occur is significant since benomyl has been used for several years to control banana leaf spot disease (Sigatoka) caused by Mycosphaerella musicola in many major banana-growing countries.

So far there are no published reports indicating that failure to control crown rot can be attributed to thiabendazole-resistant strains of C. musae or other crown rot pathogens. One possible means of preventing this is to change the Sigatoka spray programme by alternating cycles of benzimidazole fungicides with non-benzimidazole fungicides and, in fact, this is being done in some Central American banana plantations. The primary objective is to prevent the development of benzimidazole-resistant strains of M. musicola, but at the same time it should do the same against crown rot pathogens.

b) Citrus

During the past decade, thiabendazole has been granted registration and used extensively in all major citrus-growing countries to control green mould (Penicillium digitatum), blue mould (P. italicum), and stem-end rots (Diplodia natalensis and Phomopsis citri) on harvested fruit (McCornack et al., 1976). Stem-end rot is the major post-harvest problem on citrus grown in warm humid areas, whereas green and blue moulds are responsible for major losses of fruit produced in arid sub-tropical areas (Eckert & Kolbezin, 1971). Thiabendazole not only controls primary decay but it also retards the growth and sporulation of P. digitatum on the surface of decaying fruits, so that sound fruit in the same container is protected against spoilage.

Thiabendazole is easy to apply to citrus and is compatible with all major citrus wax formulations. It also reduces chilling injury (pitting) on grapefruits during long-term storage (Schiffmann-Nadel et al., 1973; Wardowski et al., 1975).

A recent problem in the United States, Japan, and Israel is the occurence of benzimidazole-resistant strains of P. digitatum and P. italicum (Gutter, 1973; Smoot & Brown, 1974; Wild & Rippon, 1975; Kuramoto, 1976). It is believed that some of these strains have developed because of poor packinghouse sanitation. Rotten and mouldy fruit previously treated with thiabendazole or other benzimidazole fungicides and held in or near the packinghouse can provide a good source of resistant spores. Brown et al. (1976) recommend that all cull fruit should be removed daily and the area surrounding the packinghouse should be maintained in a clean condition. Successive treatments with thiabendazole may have to be avoided. Alternation with non-benzimidazole compounds could aid in preventing a build-up of resistant spores. Possible compounds are sodium o-phenylphenate, diphenyl, or 2-aminobutane. It may also be helpful to wash floors, walls, equipment and pallet boxes with suitable sterilant chemicals in order to eliminate resistant Penicillium spores. Formaldehyde has proved successful for this purpose in Florida, although use of this compound requires certain precautions and adequate airing after treatment.

Another possible cause of development of benzimidazole resistance in Penicillium spp. is the continued and extensive use of these fungicides for pre-harvest spraying of citrus groves. It is known that this can lead to a build-up of resistant strains from benzimidazole-selected or benzimidazole-induced mutants.

If resistance problems in citrus increase then it will be necessary to alter existing recommendations for fungicide use. Kuramoto & Yamada (1976) suggest that fungicides having a mode of action different from benzimidazoles are needed. However, the high efficacy of thiabendazole against citrus decay fungi and its great safety to humans and animals, makes it one of the best choices for this crop. It may be possible to overcome the resistance problem by rotating or alternating fungicides with different modes of action, for example thiabendazole and 2-aminobutane. For pre-harvest spraying, alternation of fungicides or use of tank-mix combinations may prevent the build-up of thiabendazole-resistant Penicillium spp. in the field.

c) Pineapple

The most important post-harvest decay of pineapples is "black-heart" caused by Ceratocystis paradoxa. This fungus is widely distributed in the tropics and attacks sugar-cane, coconuts, and bananas. It enters the pineapple fruit through the cut stem and, at tropical temperatures, rapidly invades the entire fruit. Other fungi which invade wounds in harvested fruit are Cladosporium spp., Penicillium spp. and Trichoderma spp., causing "surface mould".

Frossard (1968) found that direct application of a thiabendazole suspension (1600 ppm a.i.) to the cut stalk, within 30 min of inoculation with C. paradoxa, prevented development of "black heart". In later studies Frossard (1970) found that dipping fruit in 1500-3000 ppm a.i. thiabendazole, immediately after harvest, also gave good control of C. paradoxa. However, the efficacy of thiabendazole decreased rapidly as the time between harvest and treatment increased; after 4 h the treatment was ineffective at 1500 ppm.

In South Africa, a post-harvest dip in 1500-2000 ppm a.i. thiabendazole gave almost complete control of "surface mould" (B.M. Myhill, 1972, personal communication).

These preliminary observations are promising, but the full potential of thiabendazole as a post-harvest fungicide for pineapple has yet to be determined. More information about minimum effective dose rate, method of application, timing of treatment, need for spreader-stickers, etc., is needed.

d) Papaya and mangos

In Brazil, papaya is grown as a cash crop mainly for the local market. Yield losses due to post-harvest fungal decay are estimated to exceed 50% of total yield (Bolkan et al., 1976). The most important post-harvest pathogens are Colletotrichum gloeosporioides, Ascochyta caricae and Gloeosporium spp. Before the introduction of thiabendazole, the recommended method for control of post-harvest fruit rots was by submerging fruits in hot water (47.8-48.9°C) for 20 min. However, Bolkan et al. (1976) found that a post-harvest dip for 3 min in an aqueous suspension of thiabendazole (500 ppm a.i.), plus a spreader-sticker, reduced the incidence of all fruit rots by 80%. Further work is in progress to determine the minimum effective dose rate of thiabendazole, and to establish residue tolerances.

In South Africa, a post-harvest dip treatment for 3 min in 1000 ppm a.i. thiabendazole suspension at 52°C gave 33-90% control of papaya anthracnose (B. M. Myhill, 1973, personal communication). Further trials are in progress to determine the optimum temperature of the thiabendazole suspension, and the optimum dipping time.

Anthracnose (C. gloeosporioides) and stem-end rot (Diplodia natalensis) are two of the most common and important post-harvest disorders of mangos in most countries where they are grown. Various post-harvest hot water treatments have been tested to control these diseases, but a completely safe, convenient and effective one has not been developed. Spalding & Reeder (1972) found that a post-harvest dip for 2 min in an aqueous suspension of thiabendazole (1000 ppm a.i.) at room temperature failed to control these diseases; immersion for 5 min in thiabendazole at 54°C controlled anthracnose but not stem-end rot, for 4 weeks. Hot water treatment alone was only effective for 3 weeks.

In India, Pathak et al. (1971) obtained complete control of Diplodia rot when fruits were treated with 500 ppm a.i. thiabendazole before wound-inoculation. Treatment 24-48 h after inoculation was not effective. In Mexico, a 2 min post-harvest dip in 100, 200 or 400 ppm a.i. thiabendazole failed to control mango anthracnose (C. gloeosporioides). However, good control was obtained by an 8 min dip in water at 54oC (J. T. Vazquez, 1971, personal communication).

Results obtained so far using thiabendazole for control of post-harvest diseases of mangos are not outstanding. More needs to be known about the infection biology of the causal fungi in order to improve the timing of fungicide application. Some infections are probably initiated at an early stage of fruit development, and remain latent in a sub-cuticular position until maturity is approached (Simmonds, 1963). One or more timely pre-harvest applications of fungicide might help. This was demonstrated by McMillan (1973) who obtained good control of anthracnose by spraying with benomyl (300 ppm a.i.) at weekly intervals before fruit set, and at monthly intervals thereafter until harvest. The relative importance of wound versus non-wound infections has to be determined in each area. If wound infection predominates, then a single application of thiabendazole shortly before harvest and another one immediately after harvest may be effective.

e) Avocado

Anthracnose (Colletotrichum gloeosporioides) is one of the most important diseases of avocados wherever the crop is grown. In Peru, post-harvest losses of up to 70% have been reported (R. Talavera, 1976, personal communication). Stem-end rot caused by Diplodia natalensis is also important, especially in Israel (Zauberman et al., 1975). In Israel, starting in 1972, pre-harvest thiabendazole sprays were made 4 or 5 times during the development of the fruit to control anthracnose. A post-harvest dip treatment in 1500-2000 ppm a.i. thiabendazole was also made to control stem-end rot (Zauberman et al., 1975). Good control of both diseases was obtained for 3 years. However, the incidence of post-harvest rots then began to increase, not due to Diplodia or Colletotrichum, but due to Alternaria spp. One way to overcome this problem is to use combinations of benzimidazole and non-benzimidazole fungicides in the field, or to alternate applications, in order to broaden the spectrum of activity to include Alternaria spp., and to reduce the risk of development of benzimidazole-resistant strains of Colletotrichum and Diplodia.

In Peru, good control of avocado anthracnose was obtained by dipping fruits in aqueous suspension containing 1200 ppm a.i. thiabendazole for 1.5 min, followed by drying (R. Talavera, 1976, personal communication).

In South Africa, both stem-end rot and anthracnose have been controlled during transport to the U.K. by dipping in a water-wax emulsion containing 3000 ppm a.i. thiabendazole (B. M. Myhill, 1977, personal communication). A major advantage of thiabendazole is its effect in reducing chilling injury inside refrigerated ships. Although other benzimidazole fungicides give good disease control, they do not reduce susceptibility to chilling.

## ROOT CROPS

### a) Potatoes

Many recent investigations have shown that the incidence of Fusarium dry rot in stored potatoes can be reduced considerably by treating with thiabendazole soon after harvest (Boyd, 1975; Henriksen, 1975; Leach, 1975; Meijers, 1975; Meredith, 1975). Good control can be obtained with as little as 6-7 g a.i. thiabendazole/t of potatoes. Gangrene (Phoma exigua var. foveata and P. exigua var. exigua) is also controlled in stored potatoes by treating with thiabendazole shortly after harvest. However, it is necessary to increase the rate of application to about 42 g a.i./t (Logan & Copeland, 1975). This treatment also provides excellent control of skin spot (Oospora pustulans) and silver scurf (Helminthosporium solani) in stored potatoes (Jouan et al., 1974; Copeland & Logan, 1975; Anon., 1976; Jellis & Taylor, 1977). The incidence of these two diseases in progeny tubers is also markedly reduced as a result of post-harvest thiabendazole treatment, and in the case of silver scurf, the low incidence of disease on progeny tubers at the time of lifting is well maintained during subsequent storage.

Commercial treatment of harvested potatoes with thiabendazole is currently being carried out in North America, Mexico, Brazil and several European countries. The most effective and economical method of treatment is by means of ULV mist-spraying as soon as possible after lifting.

### b) Yams and sweet potatoes

The edible yams (Dioscorea spp.) of the tropics, of which there are 50 or more species, are an important food crop in many tropical and sub-tropical countries (Coursey, 1967). Soft rot caused by fungi is one of the most important causes of losses during storage of yams (Coursey, 1961). The fungi enter through wounds and natural openings on the surface of the tubers. Fungi most frequently associated with soft rot are Botryodiplodia theobromae, Penicillium spp., Aspergillus spp. and Fusarium spp. (Thompson et al., 1977).

In Nigeria, Ogundana (1972) wound-inoculated tubers with spores and/or mycelium of B. theobromae, F. moniliforme, P. sclerotigenum or A. niger. After 3 h incubation inoculated tubers were dipped for 30 min in aqueous suspensions of thiabendazole at 250, 500 or 1000 ppm a.i. After 16 weeks storage in a barn 100% of inoculated, untreated tubers were rotted whereas all of those treated with 1000 ppm a.i. thiabendazole remained healthy. At 500 ppm there was complete control of F. moniliforme and P. sclerotigenum and 96% and 94% control of B. theobromae and A. niger, respectively. At 250 ppm there was 40-50% control of all fungi. On the basis of these results, a recommendation was made for routine thiabendazole treatment of yams before storage.

In Jamaica, Thompson et al. (1977) found that soft rot of yams in cool storage (12.5°C) is much more severe than at ambient temperatures. Almost complete control of rot was obtained by dipping tubers soon after harvest in an aqueous suspension of thiabendazole (1000 ppm a.i.), followed by air drying. Control of rot resulted in marked and significant reductions of fresh weight losses. Fourteen days after treatment with either 750 or 1500 ppm a.i. thiabendazole, the residues in the edible portion of the tubers were 0.09 and 0.15 ppm, respectively, on a fresh weight basis. These low residues are well within the limits approved for other crops.

In Trinidad, sweet potatoes (Ipomoea spp.) also subject to dry rot (Fusarium spp.) during storage. R. H. Phelps (1975, personal communication) reported approximately 60% reduction in dry rot when tubers were dipped for 3 min in 100 ppm a.i. thiabendazole before being placed in storage.

## OTHERS

### a) Eggplant

One of the major causes of loss of eggplant shipped from the French Antilles to Europe is anthracnose caused by Colletotrichum gloeosporioides (J. Fournet, 1973, personal communication). Experiments in Martinique showed that a post-harvest dip for 2 min in an aqueous suspension of thiabendazole (200-500 ppm a.i.) at 50°C was superior to dipping in water at 50°C (F. Daly, 1973, personal communication). Thiabendazole treatment was effective for up to 48 h after harvest, which would allow treatment to be carried out at a central packinghouse close to the wharf, rather than immediately after harvest in the field. Although the installation of a thermostatically controlled fungicide tank is rather expensive, it is considered that the outlay would be recovered within 1 year as a result of increased marketability of exported eggplants.

### b) Reconstituted tobacco

A special formulation of thiabendazole has been developed to retard or inhibit mould growth in reconstituted tobacco leaf. In vitro evaluations using organisms isolated from mouldy tobacco showed that thiabendazole was effective at 2.5 ppm a.i. (Moshy et al., 1968). In vivo studies showed that good control of Penicillium spp., Aspergillus spp. and Scopulariopsis spp. was obtained with 600 ppm a.i. thiabendazole. In practice, thiabendazole is added to the tobacco water stream which is used to re-moisten the leaf (before shredding in the case of cigarettes, or wrapping in the case of cigars). The inhibition of mould growth allows long-term storage of tobacco at relatively high moisture content, that is, at a level required by the manufacturer of smoking articles. Pyrolysis experiments showed that thiabendazole tends to sublime unchanged at a cigar periphery temperature of 700°C.

## DISCUSSION

Thiabendazole is a unique fungicide in regard to safety and versatility. Its safety to animal and human health is well established (Campbell & Cuckler, 1969; Eaton et al., 1969), and it is non-phytotoxic to most crop plants. It is available as dusts, wettable powders, flowable formulations, an ULV formulation, and thermal dusting tablets for fumigation purposes.

Although thiabendazole is used to control several pre-harvest diseases (e.g. Sigatoka of bananas, Septoria spp. on cereals) it has, so far, been used most extensively for control of post-harvest disorders. It is particularly active against certain species of Aspergillus, Botrytis, Colletotrichum, Fusarium, Gloeosporium and Penicillium, expecially when they invade the host as post-harvest wound-pathogens.

The problem of fungal resistance to thiabendazole and other benzimidazole compounds is not insurmountable. These compounds will continue to have great value for plant protection, but they will have to be managed more discriminately in future in order to reduce selection pressure favouring resistant strains of pathogens (Dekker, 1976).

## References


ANON. (1976) Skin spot and silver scurf of potatoes. Ministry of Agriculture, Fisheries and Food, Agricultural Development and Advisory Service, Advisory Leaflet 279, 4p.

BOLKAN, H.A., CUPERTINO, F.P., DIANESE, J.C. & TAKATSU, A. (1976) Fungi associated with pre- and post-harvest fruit rots of papaya and their control in Central Brazil. Plant Disease Reporter, 60, 605-609.

BOYD, A.E.W. (1975) Fungicides for potato tubers. Proceedings 8th British Insecticide and Fungicide Conference, 1035-1044.

BROWN, G.E., McCORNACK, A.A. & SMOOT, J.J. (1976) Means of avoiding resistance: removal of resistant spores. Citrus Packinghouse Newsletter No. 78.

BUGBEE, W.M. (1975) Penicillium claviforme and Penicillium variabile: pathogens of stored sugar beets. Phytopathology, 65, 926-927.

CAMPBELL, W.C. & CUCKLER, A.C. (1969) Thiabendazole in the treatment and control of parasitic infections in man. Texas Reports on Biology and Medicine, 27, 665-692.

COURSEY, D.G. (1961) The magnitude and origins of storage losses in Nigerian yams. Journal of the Science of Food and Agriculture, 12, 574-580.

DEKKER, J. (1976) Acquired resistance to fungicides. Annual Review of Phytopathology, 14, 405-528.

EATON, L.G., SIEGMUND, O.H., RANKIN, A.D. & BRAMEL, R.G. (1969) The veterinary uses of thiabendazole. Texas Reports on Biology and Medicine, 27, 693-708.

ECKERT, J.W. (1969) Chemical treatments for control of postharvest diseases. World Review of Pest Control, 8, 116-137.

ECKERT, J.W. & KOLBEZEN, M.J. (1971) Chemical treatments for the control of postharvest diseases of citrus fruits. Proceedings 6th British Insecticide and Fungicide Conference, 683-693.

FROSSARD, P. (1968) Essais de désinfection des pédoncules d'ananas contre le Thielaviopsis paradoxa. Fruits de'outre Mer, 23, 207-215.

FROSSARD, P. (1970) Désinfection des ananas contre Thielaviopsis paradoxa. Fruits d'outre Mer, 25, 785-791.

GRIFFEE, P.J. (1973) Resistance to benomyl and related fungicides in Colletotrichum musae. Transactions of the British Mycological Society, 60, 433-439.

GUTTER, Y. (1973) Benzimidazole-resistant strains of citrus fruit pathogens. Research Summaries 1971-1973, Division of Fruit and Vegetable Storage, The Volcani Center, Bet Dagan, Israel.

HENRIKSEN, J.B. (1975) Prevention of gangrene and Fusarium dry rot by physical means and with thiabendazole. Proceedings 8th British Insecticide and Fungicide Conference, 603-608.

JELLIS, G.J. & TAYLOR, G.S. (1977) Control of silver scurf (Helminthosporium solani) disease of potato with benomyl and thiabendazole. Annals of Applied Biology, 86, 59-67.

JOUAN, B., LE MAIRE, J.M., PERENNEC, P. & SAILLY, M. (1974) Le gale argentee de la pomme de terre. L'Institut Technique de la Pomme de Terre, Paris, Brochure No. 32, 87-94.

KURAMOTO, T. (1976) Resistance to benomyl and thiophanate-methyl in strains of Penicillium digitatum and P. italicum in Japan. Plant Disease Reporter, 60, 168-172.

KURAMOTO, T. & YAMADA, S. (1976) DF-125, a new experimental fungicide for the control of Satsuma mandarin postharvest decays. Plant Disease Reporter, 60, 809-812.

LEACH, S.S. (1975) Control of postharvest Fusarium tuber dry rot of white potatoes. United States Department of Agriculture, Publication ARS-NE-55, 19pp.

LOGAN, C. & COPELAND, R.B. (1975) Potato gangrene - its control during storage by mist application of thiabendazole. Proceedings 8th British Insecticide and Fungicide Conference, 589-595.

McCORNACK, A.A., WARDOWSKI, W.F. & BROWN, G.E. (1976) Postharvest decay control: recommendations for Florida citrus fruit. Florida Agricultural Research and Education Center, Circular 359-A.

McMILLAN, R.T. (1973) Control of anthracnose and powdery mildew of mango with systemic and non-systemic fungicides. Tropical Agriculture, Trinidad, 50, 245-248.

MEIJERS, C.P. (1975) Experiences with thiabendazole in control of potato storage diseases in Holland. Proceedings 8th British Insecticide and Fungicide Conference, 597-602.

MEREDITH, D.S. (1971) Transport and storage diseases of bananas: biology and control. Tropical Agriculture, Trinidad, 48, 35-50.

MEREDITH, D.S. (1975) Control of fungal diseases of seed potatoes with thiabendazole. Proceedings 8th British Insecticide and Fungicide Conference, 581-587.

MOSHY, R.J., FIORE, J.V., HALTER, H.M. & GREENWALD, B.W. (1968) 2(4-thiazolyl)-benzimidazole: an antimycotic agent for reconstituted tobacco. 22nd Tobacco Chemists Research Conference, Richmond, Virginia, U.S.A.

OGAWA, J.M., MANJI, B.T. & CHASTAGNER, G.A. (1976) Field problems due to chemical tolerance of plant pathogens. Proceedings American Phytopathological Society, 3, 47-53.

OGUNDANA, S.K. (1972) The control of soft rot of yams in Nigeria. International Biodeterioration Bulletin, 8, 75-78.

PATHAK, U.N., GOYAL, J.P. & SHARMA, H.C. (1971) Screening trials for control of Diplodia and Rhizopus rots of mango fruits. Plant Disease Reporter, 55, 752.

SCHIFFMANN-NADEL, M., CHALUTZ, E., WAKS, J., LATTAR, F.S. & DAGAN, M. (1973) Reduction of pitting and rots of grapefruit by thiabendazole and benomyl during long-term storage. Research Summaries 1971-1973, Division of Fruit and Vegetable

Storage, The Volcani Center, Bet Dagan, Israel.

SIMMONDS, J.H. (1963) Studies in the latent phase of Colletotrichum species causing ripe rots of tropical fruits. Queensland Journal of Agricultural Science, 20, 373-424.

SMOOT, J.J. & BROWN, G.E. (1974) Occurrence of benzimidazole resistant strains of Penicillium digitatum in Florida citrus packinghouses. Plant Disease Reporter, 58, 933-934.

SPALDING, D.H. & REEDER, W.F. (1972) Postharvest disorders of mangos as affected by fungicides and heat treatments. Plant Disease Reporter, 56, 751-753.

STOVER, R.H. (1972) Banana, plantain and abaca diseases. Commonwealth Mycological Institute, Kew, England.

THOMPSON, A.K., BEEN, B.O. & PERKINS, C. (1977) Fungicidal treatments of stored yams. Tropical Agriculture, Trinidad, 54, 179-183.

WARDOWSKI, W.F., ALBRIGO, L.G., GRIERSON, W., BARMORE, C.R. & WHEATON, T.A. (1975) Chilling injury and decay of grapefruit as affected by thiabendazole, benomyl, and $CO_2$. Hortscience, 10, 381-383.

WEINKE, K.E., LAUBER, J.J., GREENWALD, B.W. & PREISER, F.A. (1969) Thiabendazole, a new systemic fungicide. Proceedings 5th British Insecticide and Fungicide Conference 340-343.

WILD, B.L. & RIPPON, L.E. (1975) Response of Penicillium digitatum strains to benomyl, thiabendazole, and sodium o-phenylphenate. Phytopathology, 65, 1176-1177.

ZAUBERMAN, G., SCHIFFMANN-NADEL, M., FUCHS, Y. & YANKO, U. (1975) La lutte contre les pourritures de l'avocat et son effet sur le changement de la flore des champignons pathogenes des fruits. Fruits d'outre Mer, 30, 503-504.

NOTES

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# THE EFFECT OF IPRODIONE ON THE FUNGAL DETERIORATION OF STORED WHITE CABBAGE

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Summary The external fungal rots caused by *Botrytis cinerea* and *Alternaria brassicicola* are an important type of deterioration of white cabbage during cold storage. In four commercial storage trials of four to six months' duration, the fungicide iprodione, applied either as a dust or as ultra-low volume or high volume sprays, successfully controlled *Botrytis* rots, and was usually more effective than the other fungicide used, thiabendazole dust. *Alternaria* rots were consistently controlled by iprodione dust. During preparation of white cabbage for market from three cold storage trials, trimming losses were reduced by the iprodione treatments and marketable yields increased by 9-19%. In the fourth trial with barn-stored cabbages, these results were not achieved. Rots caused by *Phytophthora porri* and bacteria (*Pseudomonas marginalis*) were not controlled.

Résumé Les pourritures externes produites par les champignons *Botrytis cinerea* et *Alternaria brassicicola* sont importantes dans la détérioration des choux blancs lorsqu'ils sont stockés en chambre froide. Dans les quatre essais de stockage, qui ont duré quatre à six mois conformément aux conditions commerciales, le fongicide iprodione -appliqué sous forme de poudre ou pulverisé en ULV ou à haut volume- a donné de bons résultats sur les pourritures du *Botrytis* et s'est montré souvent plus efficace que le thiabendazole en poudre. Iprodione en poudre a révélé une efficacité constante sur les pourritures d'*Alternaria*. Pendant la préparation du chou blanc pour la vente aprés stockage, les pertes en feuilles contaminées ont été réduites dans les lots traités avec iprodione et de ce fait les quantités négotiables ont été augmentées de 9% a 19%. Cependant, cette augmentation n'a pas eu lieu dans le quatrième essai où les choux ont été engrangés. Iprodione ne s'est montré efficace ni sur *Phytophthora porri*, ni sur la bactérie *Pseudomonas marginalis*.

## INTRODUCTION

The production of white storage (Dutch white) cabbage in the U.K. has increased considerably over the last ten years, and since 1972, has exceeded 100,000 tonnes annually. Storage cabbage is sold in nets on the open market or as pre-packaged heads and segments in supermarkets. It is also used for the manufacture of processed foods such as cole-slaw. Storage is needed to improve continuity of supply to these outlets, and to extend the availability of white cabbage into the summer months, particularly for processors. Storage can be in refrigerated chambers using bulk bins or a stacking system; in barns with forced ventilation using the cold ambient air of the winter months; or in clamps. Only refrigeration allows long-term storage in excess of three to four months without serious wastage. Losses of 5 - 20% often occur from bacterial and fungal spoilage, either of the whole head or from trimming, even with ideal conditions, and total losses of up to 40% can readily occur in commercial practice. Spoilage from the spreading soft rot caused by grey mould (Botrytis cinerea) is one of the most important factors limiting successful storage (Brown et al., 1975; Bobbin and Geeson, 1976, 1977). Deterioration from Alternaria brassicicola can also be important. Rots caused by these fungi frequently occur, but rarely result in total loss of the head. This is in contrast to infections by Phytophthora porri and bacteria (predominantly Pseudomonas marginalis) which often cause complete loss and which vary in incidence between seasons. Symptomatology of these and other cabbage storage diseases and disorders such as pepper spotting, are given elsewhere (Geeson and Robinson, 1975; Geeson, 1976; Walkey and Webb, 1977).

Previous work with fungicides on stored white cabbage has been carried out. For example, Brown et al., (1975) showed that thiabendazole and dicloran dusts controlled B.cinerea and increased marketable yields, and Bobbin and Geeson (1976 ; 1977) used benzimidazole fungicides as drenches to control B.cinerea; A.brassicicola and P.porri were not controlled by these fungicides. A new fungicide, iprodione, has recently been developed by May & Baker Ltd., in the U.K. It is highly active against B.cinerea and also A.brassicicola (Burgaud et al., 1975), and it was considered to be a suitable candidate for testing on stored white cabbage. Several methods of application were considered, and the results from using iprodione applied as a dust, ultra-low volume and high volume sprays in barn and refrigerated storage, are described. Results from using iprodione drench on stored white cabbage will be published elsewhere (Geeson, 1977).

## METHODS AND MATERIALS

Cabbages for the four trials were grown in the areas around Boston and Spalding in Lincolnshire, using standard cultivation procedures. They were harvested during November before the first frosts, and stored by the grower at three sites in the areas in which they were grown. Commercial harvesting, handling and storage were carried out, and rather than attempt to extend the storage life of the cabbage, the trials were completed at the same time as the grower marketed his produce. Further details are given in Table 1. Any loose leaves left on field-trimmed cabbages were usually removed at the storage site. Excessively bruised or rotten heads were omitted from the trials. The weight of the cabbages used was typically in the range 1.0 - 2.0 kg, destined for distribution to supermarkets after storage.

The fungicides used were: iprodione as a 50% w.p. formulation ('Rovral'*) and a 5% dust in talc; thiabendazole as a 30% dust in talc (Merck, Sharpe and Dohme Ltd.). Details are presented in Table 2. Thiabendazole, shown to be active against B.cinerea on stored white cabbage (Brown et al., 1975), was included as a reference standard, although it has not been approved under the Agricultural Chemicals Approval Scheme for use on storage cabbage. Owing to the size of the trials, control treatments were limited to untreated cabbages. Cabbages were treated whilst being trimmed and loaded into bulk bins, or stacked onto the face for barn-stored cabbage, by alternating a layer of cabbages with a fungicide application.

* 'Rovral' is a registered trademark of May & Baker Ltd.

TABLE 1

Site and storage details of trials

| Trial | Season | Cultivar | Storage period (wk) | Storage regime | Storage unit | Amount stored (t) |
|---|---|---|---|---|---|---|
| 1+ | 1975-76 | Langendijk type: unknown selection | 26 | $1-2^{o}$ 90-95% r.h. refrigerated store | ½t bulk bin | 0.75 |
| 2 | 1976-77 | Late Winter Special: Elsom's (Spalding) Ltd. | 18 | (as for Trial 1) | | 3.0 |
| 3 | 1976-77 | Late Green Winter: Elsom's (Spalding) Ltd. | 19 | Barn storage*: ambient temp: heads stored 1.5m high. | part of* stacked face | 5.0 |
| 4+ | 1976-77 | Langendijk type: unknown selection | 20 | (as for Trial 1) | | 1.0 |

\+ Trials 1 and 4 were carried out at the same site.

* Temperatures fluctuated between 2°C and 10°C; the storage unit was a section 2.1m x 2.0 x 0.75m deep of the stacked cabbages.

TABLE 2

Fungicidal treatments and dose rates

| Treatment and application | Applied at site | Dilution rate (a.i.) | Mean dose rate (g a.i./t) | Method of application |
|---|---|---|---|---|
| Thiabendazole dust | 1,2,3,4 | 30% | 110 | Fiddle brush hand duster (K6, Kyoritsu Noki Co. Ltd) |
| Iprodione dust | 1,2,3,4 | 5% | 30 | |
| Iprodione ultra-low volume spray | 2 | 0.1% | 0.8 | Spinning disc ultra-low volume applicator (ULVA, Micron Sprayers Ltd) |
| | 3,4 | 10% | 24 | |
| Iprodione high volume spray | 2,3,4 | 0.1% | 2.5 | Knapsack sprayer (Polypak, Allman Patents Ltd) |

The experimental design was a randomized block, and sub-samples of 50-100 cabbages per replicate were taken at the end of the storage period for a detailed assessment of each cabbage. The assessment techniques were those used previously (Kear and Symons, 1973; Anon., 1974a; Brown et al., 1975). A preliminary investigation of the residues of iprodione was made at the end of the storage period on cabbage heads trimmed for market.

## RESULTS

For the three trials in refrigerated stores, the most important and widespread spoilage organism in the untreated control was B.cinerea (Table 3), and a significant reduction in its incidence was achieved by all the fungicides used, though it was not eradicated. In Trials 1 and 4 the iprodione fungicides gave significantly better control than thiabendazole dust. There was also a high incidence of A.brassicicola in the three trials. Partial control was recorded for the iprodione fungicides, and iprodione dust gave consistently good control of A.brassicicola. Thiabendazole did not reduce the incidence of A.brassicicola, which is known to be insensitive to this fungicide, though apparently some effect occurred in Trial 2. For the barn-stored cabbage (Trial 3), the spoilage was caused predominantly by bacterial soft rots (usually Pseudomonas marginalis) and P.porri. In this trial, the Botrytis and Alternaria lesions tended to be masked by rots caused by these organisms. However, control of B.cinerea was still achieved by all fungicides, and of A.brassicicola by iprodione ULV and dusts only. Alternaria rot was superficially less extensive than Botrytis rotting but it often penetrated deeply into the cabbage, which consequently needed more trimming. Every head infected by B.cinerea and A.brassicicola could be trimmed to a marketable condition.

### TABLE 3

Incidence of B.cinerea and A.brassicicola (mean % infected heads)

| | B.cinerea | | | | A.brassicicola | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 1 | Trial 2 | Trial 3 | Trial 4 |
| Untreated (control) | 88a* | 74a | 49a | 84a | 83a | 72a | 61a | 56ab |
| Thiabendazole dust | 14b | 8c | 10c | 12bc | 79a | 37bc | 52ab | 71a |
| Iprodione dust | 10b | 8c | 2c | 4c | 31b | 15d | 20c | 6c |
| Iprodione ULV | - | 39b+ | 24b | 6c | - | 43b+ | 35bc | 47b |
| Iprodione HV | - | 38b | 4c | 22b | - | 23cd | 56a | 58ab |

* Means within each column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

\+ Iprodione ULV was applied at 1/100th of the intended rate at this site.

During market preparation, the infected leaves were removed and weighed. For the three cold-storage trials, the fungicidally-treated cabbages had significantly less trimming losses, and significantly higher marketable yields than the untreated controls (Table 4), despite the incidence of bacterial soft rotting and P.porri (Table 5). The fungicides did not have a significant effect on trimming losses or marketable yield for the barn-stored cabbages (Trial 3) where most of the losses were from these two spoilage organisms. The iprodione treatments gave significantly lower trimming losses than thiabendazole in Trials 1 and 4, and significantly higher marketable yields than thiabendazole in Trial 4. The increased marketable yield by using iprodione treatments, apart from Trial 3, ranged from 9.1 - 18.6%.

TABLE 4

Trimming losses and marketable yield (mean % by weight) of cabbage

| Treatment | Trimming losses from rots Trial 1 | Trial 2 | Trial 3** | Trial 4 | Marketable yield Trial 1 | Trial 2 | Trial 3** | Trial 4 |
|---|---|---|---|---|---|---|---|---|
| Untreated (control) | 25.2a* | 25.9a | 25.3a | 29.4a | 67.0a | 64.1a | 63.2a | 60.1a |
| Thiabendazole dust | 15.3b | 11.3cd | 21.6a | 21.4b | 74.2b | 77.4b | 66.1a | 69.1b |
| Iprodione dust | 9.9c | 8.5d | 23.7a | 7.0d | 76.1b | 78.4b | 63.6a | 78.7c |
| Iprodione ULV | - | 19.4b+ | 19.2a | 11.1cd | - | 75.2b+ | 69.9a | 77.3c |
| Iprodione HV | - | 15.5bc | 21.9a | 14.3c | - | 75.0b | 64.9a | 75.2c |

* Means within each column followed by the same letter are not significantly different at the 1% level using Duncan's multiple range test.

**Bacterial soft rots and Phytophthora porri were predominant at this site.
+ Iprodione ULV was applied at 1/100th of the intended rate at this site.

Total loss of cabbage heads from P.porri was high in the wet 1976 harvest period (Table 5) but it was completely absent in the drier 1975 conditions.

TABLE 5

Occurrence of P.porri (mean % number of total)

| Treatment | Heads infected Trial 2 | Trial 3 | Trial 4 | Heads lost Trial 2* | Trial 3* | Trial 4 |
|---|---|---|---|---|---|---|
| Untreated (control) | 42 | 8 | 6 | 20 | 6 | 4 |
| Thiabendazole dust | 24 | 25 | 12 | 14 | 16 | 8 |
| Iprodione dust | 16 | 10 | 4 | 8 | 6 | 4 |
| Iprodione ULV | 31 | 21 | 0 | 12 | 12 | 0 |
| Iprodione HV | 35 | 12 | 15 | 12 | 8 | 2 |

* Loss of heads predominantly from infection by P.porri, but severe secondary infection by pseudomonads also present.

The incidence of bacterial soft rots in the three trials in refrigerated stores was variable, with a mean incidence of 22.9% (range 0 - 56.7%). In the barn-stored cabbages (Trial 3), with the wet harvest conditions of the 1976 autumn and high storage temperatures, the incidence was high (90.0 - 98.0% for all treatments). Complete loss of heads from bacterial soft-rotting sometimes occurred. In Trials 1 and 4 the mean loss was 2.6%, though no losses occurred for Trial 2. The mean losses were high again for Trial 3 at 5.7%. None of the fungicidal treatments affected losses from Pseudomonas marginalis, and there were no significant differences between treatments for all trials.

Other losses that occurred in the trials were caused by internal necrotic lesions (pepper-spotting, viruses and vascular streaking); and by evaporation and respiration during storage. Evaporative losses were acceptably low, compared with, for example, data from Shipway (1977), and were similar for the three trials of cabbages stored under refrigeration (Trials 1, 2 and 4), ranging from 1.3 to 1.7% $30d^{-1}$. The losses for the barn-stored cabbages (Trial 3) were higher at 2.2% $30d^{-1}$ because of the higher storage temperatures. There were no significant differences between the treatments of each trial. Trimming losses from internal necrotic lesions after removal of diseased leaves were slight. The mean incidence of pepper spotting on outer leaves was high, however, ranging from 65.4% - 98.1% in all trials. There were no significant differences in the incidence of pepper spotting between treatments. Complete loss of heads was rare from pepper spotting (1.9% in Trial 4), and from vascular streaking in Trials 3 and 4 (2.1% and 1.9% respectively). In previous reports (Kear and Symons, 1973; Anon., 1974b; Bobbin and Geeson, 1977) it was suggested that benzimidazole dips and drenches increased pepper-spotting symptoms or encouraged their development at the start of the storage period. There was no evidence from these trials that the high volume iprodione spray had any effect on pepper spotting. Viral lesions, probably caused by the turnip mosaic virus (Walkey and Webb, 1977), were also observed during trimming cabbages for market. The incidence was variable (19.6 - 88.1% for all trials).

## DISCUSSION

Clearly it has been shown that by using iprodione a significant reduction in rotting from B.cinerea and A.brassicicola, and an increased marketable yield of stored white cabbage, is obtained after four to six months' refrigerated storage. Other advantages are:-

1. the possibility of mid-term trimming to remove fungal rots may be unnecessary, thus saving a labour intensive practice and the likelihood of re-infection;

2. the stored product can be more quickly trimmed after storage and is more pleasant to handle;

3. the trimmed cabbage has usually lost less leaves and therefore has a better colour, size and shape (Anon., 1974a).

On the basis of one trial only, these advantages were not found for barn-stored cabbages. During the mild winter of 1976-77, forced ventilation with ambient air did not reduce the temperature sufficiently to maintain optimum storage conditions. It was hoped that the use of a fungicide would allow this system to be used without fungal deterioration becoming too severe, but the wet harvesting conditions and relatively high storage temperatures predisposed the stored cabbages to infection from bacterial soft rots and P.porri. Conversely, there is no suggestion from these trials that losses from bacterial soft-rotting were higher for the iprodione high volume spray treatment, which is the "wettest" of the fungicide applications. Further large-scale work is in progress to evaluate the performance of iprodione using various application methods in barns and refrigerated stores.

There have been no reports of iprodione-insensitive strains of fungi. Residue levels from two trials on cabbages treated with 5% iprodione dust, stored for six months and subsequently prepared for market, did not exceed 0.85 ppm. Additional analyses are in progress and iprodione has been granted a limited clearance under the Pesticides Safety Precautions Scheme.

Shipway (1977) has shown that cabbages can be successfully stored without fungicides by careful handling and harvesting, and good storage practice. However, it is not always possible to adopt these ideal operations commercially, particularly if piece-work is involved or where wet conditions prevail during harvest. The application of a fungicide such as iprodione to stored white cabbage should allow growers to carry out a more flexible programme of harvesting and storage.

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## References

ANON. (1974a) E.E.C. Standards for cabbage (round-headed), Ministry of Agriculture, Fisheries and Food.

ANON. (1974b) Dutch white cabbage : Control of Botrytis in clamp store and cold store, Report of the Stockbridge House Experimental Horticulture Station for 1973, 20-22.

BOBBIN, P. and GEESON, J.D. (1976) Effects of benomyl drench on storage cabbage, Agricultural Development and Advisory Service, Experiments and Development in the Eastern Region 1975, 284-286.

BOBBIN, P. and GEESON, J.D. (1977) Effects of benzimidazole fungicide drenches on cabbage storage disorders, Agricultural Development and Advisory Service, Experiments and Development in the Eastern Region 1976, 151-154.

BROWN, A.C., KEAR, R.W. and SYMONS, J.P. (1975) Fungicidal control of Botrytis on cold-stored white cabbage, Proceedings 8th British Insecticide and Fungicide Conference, 1, 339-346.

BURGAUD, L., CHEVREL, J., GUILLOT, M., MARECHAL, G., THIOLLIERE, J. and COLE, R.J. (1975) The hydantoin 26,019 RP, a new polyvalent fungicide, Proceedings 8th British Insecticide and Fungicide Conference, 2, 645-652.

GEESON, J.D. (1976) Storage rot of white cabbage caused by Phytophthora porri, Plant Pathology, 25, 115-116.

GEESON, J.D. (1977) Personal communication.

GEESON, J.D. and ROBINSON, J.E. (1975) Damage will mean trouble in store, Commercial Grower, No. 4147, 1245-1246 (June 27th, 1975).

KEAR, R.W. and SYMONS, J.P. (1973) Post-harvest deterioration of stored Dutch white cabbage, Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent, 38, 1549-1560.

SHIPWAY, M.R. (1977) Notes on white cabbage storage 1977, Storage Cabbage Open Day, 12th May, 1977, Kirton Experimental Horticultural Station.

WALKEY, D.G.A. and WEBB, M.J.W. (1977) Virus infection of Dutch white cabbage, Report of the National Vegetable Research Station for 1976, 109.

NOTES

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# EVALUATION OF CHEMICALS FOR CONTROL OF PHYTOPHTHORA FRUIT ROT IN STORED APPLES

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Summary Fungicides were evaluated as pre-harvest or post-harvest treatments to control secondary spread of Phytophthora fruit rot (*P. syringae*) from inoculated fruits during refrigerated storage. Pre-harvest sprays of captan or mancozeb with zineb showed slight control when applied 2-3 or 8 days before harvest. Captan and etridiazole and to a lesser extent captafol and chlorothalonil showed some activity in reducing secondary spread of the disease when used as a post-harvest dip treatment, but no chemical was fully effective. Application of a water based synthetic resin emulsion, Vinamul, to stabilise soil below fruit trees and reduce splash infection from soil showed some promise in reducing the disease.

Résumé Les fongicides ont été evalués comme traitement d'avant-récolte ou d'après-récolte pour reduire la contagion secondaire du dégât par *Phytophthora syringae* sur le fruit en refrigeration. L'arrosage avant-récolte avec captan ou mancozeb avec zineb a indiqué un peu d'activité quand appliqué 2-3 ou 8 jours avant la récolte. Captan et etridiazole et un peu moins dans le cas de captafol et chlorothalonil ont exhibé quelque activité quand appliqués après-récolte. L'application d'une résine synthetique, Vinamul, pour stabilizer la terre sous les arbres et empêcher l'infection a indiqué quelques progrès.

## INTRODUCTION

Since 1973 Phytophthora fruit rot caused by *P. syringae* has become prevalent in commercial apple crops in all the main apple growing areas of the U.K. (Anon., 1975, 1976) and the disease is now regarded as the main cause of fruit wastage during storage. The occurrence of the disease is closely associated with rain during the harvest period September-October (Upstone and Gunn, in press) and two distinct phases of the disease can be recognised; initial infection in the orchard prior to or at harvest as a result of soil contamination or rain splash from soil, and secondary spread to adjacent fruit during refrigerated storage. Losses of 10-12% have sometimes occurred without secondary spread but fruit stored for more than three months, particularly cv. Cox's Orange Pippin, has frequently shown losses of over 30%. Post-harvest fungicide treatment using benzimidazole or related fungicide (Wiggell and Derbyshire, 1973) is now standard commercial practice for control of *Gloeosporium* spp. and *Monilia fructigena* on dessert apples but this treatment has no effect on the development of Phytophthora fruit rot (Upstone, in press). Edney (1976) reported that a number of fungicides, of which captan was the most effective, was able to reduce rotting if allowed to dry on fruit before treatment with a slurry of soil to initiate the disease. This paper describes attempts to control the secondary spread of Phytophthora fruit rot using orchard sprays and post-harvest treatments and in addition preliminary trials using a synthetic resin emulsion as a soil stabiliser to reduce the initial infection.

## METHODS AND MATERIALS

Apple fruits cv. Cox's Orange Pippin were inoculated by inserting a small piece of mycelium of P. syringae beneath the skin and covering with adhesive tape. After a few days at room temperature, inoculated fruits were spaced among freshly harvested healthy fruits in wooden storage containers which were then stored at 3.3°C until mid March under controlled atmosphere conditions of 2% oxygen and less than 1% carbon dioxide as normal commercial practice. On removal from store, the numbers of fruit affected by Phytophthora and other storage rots were assessed. Owing to the large amount of fruit involved it was not possible to replicate the treatments.

### Pre-harvest fungicide treatments

Fruiting apple trees cv. Cox's Orange Pippin were treated with high volume sprays of captan (2.4 g/l.) or mancozeb with zineb (as Dicamate, 2.0 g/l.) applied 2-3 or 8 days before harvest on 1-2 October 1975. Twenty inoculated fruits were spaced amongst approximately 3,000 treated fruits in one wooden bulk storage bin per treatment and the bins subsequently drenched in 0.05% benomyl as normal commercial practice and placed in store until 19 March 1976.

### Post-harvest fungicide treatments

Eight inoculated fruits were spaced among approximately 160 healthy fruit cv. Cox's Orange Pippin in a wooden container which was immersed for one minute in 90 l. of a suspension of fungicide in water. Two containers were used for each treatment, the immersion being made on 30 September 1975. On removal, all containers were placed in store until 16 March 1976. The following fungicides were used (for rates of use see Table 2):

captafol (Sanspor, 50% aq. soln)
captan (PP Captan, 50% w.p.)
captan/benomyl product (DPX 115 B, Du Pont (U.K.) Ltd, w.p.)
chlorothalonil (Daconil Flowable, 54% liquid)
dichlorophen (Panacide, 40% soln)
etridiazole (Aaterra, 35% w.p.)

### Soil stabiliser treatment

Water based polyvinyl acetate emulsions Vinamul 8170 and Vinamul 3270 (Vinyl Products Ltd, Carshalton, Surrey) were applied to soil under fruiting apple trees cv. Cox's Orange Pippin in an attempt to reduce soil splash on to fruit and the initial infection of Phytophthora syringae. In each trial 0.05-0.10 ha of orchard soil was treated. In 1975, Vinamul 8170 was applied on three farms in Kent at HV using a herbicide applicator mounted on a tractor. The product was applied at 168 and 336 l/ha in approximately 1,000 l. water/ha 6 weeks before harvest. Fruit was picked into bulk bins, drenched with benomyl as normal commercial practice and stored until 12 April (trial 1), 26 February (trial 2) or 3 February 1976 (trial 3).

In 1976, Vinamul 3270 (a successor product) was applied in a similar manner to the previous season (trial 4), using hand-lances for application (trial 5), and also at 336 and 600 l/ha in approximately 3,000 l. water/ha applied by tractor-mounted herbicide applicator (trial 6). The spray was applied 4 weeks before harvest. Fruit was picked, drenched in benomyl and stored until 14 March (trial 4), 17 January (trial 5) or 21 March 1977 (trial 6).

## RESULTS

### Pre-harvest fungicide treatments

The numbers (and percentage) of fruit affected by Phytophthora fruit rot (omitting the 20 inoculated fruits) and by other rots (mainly brown rot caused by Monilia fructigena) on removal from store are shown in Table 1. Both spray treatments gave a slight reduction in the spread of Phytophthora compared with untreated.

Table 1

Numbers (and %) of fruit affected by rots on removal from store (out of approximately 3,000)

| Treatment | Days before harvest | Phytophthora | | Other rots | |
|---|---|---|---|---|---|
| | | Number affected | % affected | Number affected | % affected |
| Captan | 8 | 167 | 5.6 | 58 | 1.9 |
| | 2-3 | 113 | 3.8 | 59 | 2.0 |
| Mancozeb with zineb | 8 | 122 | 4.0 | 67 | 2.2 |
| | 2-3 | 138 | 4.6 | 44 | 1.5 |
| Unsprayed (mean of 2) | | 181 | 6.2 | 87 | 2.5 |

### Post-harvest fungicide treatments

The numbers (and mean percentages) of fruit affected by Phytophthora fruit rot (omitting the 8 inoculated fruits) and by other rots on removal from store are shown in Table 2. Statistical analysis of the results is not possible since only two replicates were made. None of the treatments appeared to affect the development of Phytophthora in the inoculated fruits but captan and etridiazole, and to a lesser extent captafol and chorothalonil, gave some reduction in the secondary spread of the disease. Dichlorophen was ineffective and may have increased the disease.

Table 2

Numbers (and %) of fruit affected by rots on removal from store (mean of 2 replicates)

| Treatment | % a.i. | Phytophthora Number affected | Phytophthora % affected | Other rots Number affected | Other rots % affected |
|---|---|---|---|---|---|
| Captafol | 0.1 | 10.0 | 6.5 | 2.5 | 1.6 |
| | 0.2 | 8.5 | 5.0 | 1.5 | 0.9 |
| Captan | 0.1 | 7.0 | 4.6 | 2.0 | 1.3 |
| Captan/benomyl | 0.05 | 14.0 | 9.1 | 2.0 | 1.3 |
| | 0.1 | 14.5 | 8.9 | 3.0 | 1.8 |
| Chlorothalonil | 0.14 | 9.5 | 5.8 | 4.5 | 2.7 |
| Dichlorophen | 0.1 | 21.5 | 13.1 | 3.0 | 1.8 |
| Etridiazole | 0.035 | 8.0 | 4.8 | 3.5 | 2.1 |
| | 0.07 | 6.0 | 3.7 | 4.5 | 2.8 |
| Water (control) | - | 17.5 | 11.3 | 4.5 | 2.8 |

Soil stabiliser treatment

The results of the trials in 1975-76 using Vinamul 8170 are shown in Table 3, and trials in 1976-77 using Vinamul 3270 in Table 4. With the exception of trial 6, the levels of Phytophthora fruit rot were very low but in all trials fruit picked from trees in areas treated with one of the soil stabilisers showed less infection compared with untreated control fruit. There were no consistent effects on the incidence of other fruit rots.

Table 3

Trials with Vinamul 8170. % fruit rot on removal from store (approximately 3,000 fruit per treatment)

| Treatment | % Phytophthora fruit rot Trial 1 | Trial 2 | Trial 3 |
|---|---|---|---|
| Vinamul 168 l/ha | 0.9 | 1.1 | 1.5 |
| Vinamul 336 l/ha | 0.7 | - | 1.9 |
| Untreated | 1.0 | 1.7 | 2.8 |

Table 4

Trials with Vinamul 3270. % fruit rot on removal from store (approximately 3,000 fruit per treatment)

| Treatment | % Phytophthora fruit rot | | |
|---|---|---|---|
| | Trial 4 | Trial 5 | Trial 6 |
| Vinamul 168 l/ha | 0.6 | 0.5 | - |
| Vinamul 336 l/ha | 0.6 | 0.9 | 8.1 |
| Vinamul 600 l/ha | - | - | 6.9 |
| Untreated | 1.8 | 1.0 | 10.8 |

## DISCUSSION

The trials using fungicides either as a pre-harvest spray or post-harvest dip treatment indicate that although some of the chemicals showed activity against secondary spread of Phytophthora fruit rot, it is doubtful whether any showed sufficient control to warrant commercial development. None of the fungicides used as post-harvest treatment has been cleared for this use under the Ministry of Agriculture's Pesticides Safety Precautions Scheme, and there is an urgent need for an effective chemical safe to use in this manner.

The trials using the soil stabiliser product Vinamul demonstrate the unpredictable nature of the disease since these were all carried out on farms with a previous history of the disease and wet weather conditions occurred in both seasons. In only one trial (6) were high levels of disease recorded but in all trials there was some reduction in Phytophthora infection and further work with these materials is indicated.


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## References


ANON. (1975) Disease Intelligence. Fruit and Hops. Agricultural Development and Advisory Service (Science Service) Annual Report for 1974, 148.

ANON. (1976) Advisory Work and Disease Intelligence. Fruit and Hops. Agricultural Development and Advisory Service (Science Service) Annual Report for 1975, 214.

EDNEY, K.L. (1976) Storage rots. Report East Malling Research Station for 1975, 116.

UPSTONE, M.E. (in press) Phytophthora fruit rot (P. syringae) of apples.

UPSTONE, M.E. and GUNN, E. (in press) Relationship between rain and the occurrence of Phytophthora fruit rot of apples in Kent, 1973-75.

WIGGELL, D. and DERBYSHIRE, D.M. (1973) Control of fruit and vegetable storage diseases by post-harvest treatment with fungicides, 1970-72. Agricultural Development and Advisory Service (Science Arm) Annual Report for 1972, 153-159.

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# THE SELECTIVE EFFECT OF FUNGICIDES ON POST-HARVEST SPOILAGE FUNGI OF STRAWBERRIES

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Summary The action of pre-harvest fungicides currently available for use on strawberries and on other soft fruit has been studied. Whereas, dichlofluanid, thiram, chlorothalonil and dichlofluanid + thiram reduced the count of the grey mould fungus, Botrytis cinerea, on freshly harvested fruit in the first week of the picking season, the incidence of grey mould developing on the stored fruit was decreased throughout the season. Dichlofluanid was the most efficient single fungicide in reducing this disease with dichlofluanid and dichlofluanid + thiram giving the best control and chlorothalonil or thiram alone the least control. However, a greater development of the Phycomycetes Mucor mucedo and Rhizopus sexualis occurred in all cases where there was a reduced incidence of grey mould.

Further tests of a range of fungicides on detached strawberry fruits showed that iprodione and vinclozolin as well as dichlofluanid were highly effective against grey mould but that none had marked activity against Mucor and Rhizopus species.

Résumé Effet sélèctif des fungicides sur les champignons nocifs aux fraises dès récolte

Nous avons etudié l'action des fungicides qu'on trouve actuellement dans le commerce pour le traitement avant la récolte des fraises et des autres fruits charnus. Etant donné que, pendant la première semaine de la saison de cueillette, le dichlofluanide, le thiram, le chlorothalonil et le dichlofluanide + thiram réduisent le nombre de moisissures grises, Botrytis cinerea, sur les fruits fraichement récoltés, l'incidence de la moisissure grise sur les fruits entreposés est en baisse tout au cours de la saison. Si on n'a recours qu'a un seul fungicide, le dichlofluanide est le plus efficace; on obtient les meilleurs résultats en utilisant le dichlofluanide et le dichlofluanide + thiram, tandis que le chlorothalonil ou thiram utilises seuls sont les moins efficaces. Néanmoins, il y an un developpement plus poussé des Phycomycètes Mucor mucedo et Rhizopus sexualis dans tous les cas où l'incidence de la moisissure grise est réduite.

D'autres essais de toute une gamme de fungicides sur les fraises après cuillette ont montré que le iprodione et le vinclozolin sont aussi efficaces que le dichlofluanide pour réprimer la moisissure grise, mais que nul fungicide n'agit efficacement sur les espèces Mucor et Rhizopus.

## INTRODUCTION

At present pre-harvest fungicides are used on strawberries for the control of fruit rots caused by the grey mould fungus, Botrytis cinerea. Previous work has shown a selective action of benomyl and dichlofluanid on soft fruit spoilage fungi, in that both fungicides although reducing the incidence of grey mould allowed greater development of Phycomycetes compared to unsprayed fruit (Dennis, 1975). After testing further fungicides in culture for activity against all of the spoilage fungi Cohen & Dennis (1975) reported dichlorophen and thiram to be most active against the Phycomycetes. In preliminary field trials, dichlorophen caused taint of the fresh fruit (Dennis, unpublished) and therefore further trials were not carried out.

The present paper reports field trials with thiram, dichlofluanid, thiram + dichlofluanid and chlorothalonil, comparing their effectiveness in reducing post-harvest rots of strawberries caused by grey mould and the Phycomycetes. More recent tests of other fungicides on detached strawberry fruits are also reported.

## METHODS AND MATERIALS

### Effect of fungicides on post-harvest rots

The strawberry plots, cv. Cambridge Favourite, were situated at Burlingham, Norfolk. The plots (0.006 ha) were sprayed three times during the flowering period. Dichlofluanid was used at the rate of 4.5 kg/2250 l/ha, chlorothalonil at 3.4 kg/2250 l/ha in 1975 and 3.4 l/2250 l/ha in 1976, thiram at 11 kg/2250 l/ha for the first spray and at 5.6 kg/2250 l/ha for the remaining two sprays. A treatment of dichlofluanid + thiram was also used, each fungicide being applied at the above rates. The fungicides were applied to all plots on the same day using a hand operated ASL Killaspray commencing at early flower and then at 10 - 14 day intervals, the last spray being applied 14 days before the first fruit was harvested. Additional plots were left unsprayed.

Fruit was picked in the 1975 and 1976 seasons into 454g fibre punnets and transported to the laboratory within 2 h of harvesting. At each harvest, four 100 g samples of whole strawberries (with calyx) were taken at random from at least 9 kg of fruit from each of the unsprayed and fungicide sprayed plots. Isolation and identification of the moulds were carried out as described by Dennis & Mountford (1975).

Fruit from each harvest was stored for 14 days at 10°C and 21 days at 5°C and the incidence of the spoilage fungi determined as described by Dennis (1975).

### Activity of fungicides against spoilage fungi on detached fruits

Detached ripe strawberries with calyx, cv. Cambridge Favourite, were sprayed to run-off with solutions of each fungicide (5-100 ppm a.i.) in 0.01% Tween 80, control berries being sprayed with 0.01% Tween 80. After allowing to dry, the berries (10 per treatment) were sprayed to run-off with spore suspensions ($10^4$ spores/ml in 0.01% Tween 80) of single strains of B. cinerea, Mucor mucedo, Rhizopus sexualis and R. stolonifer. After the berries had dried they were incubated at 15°C, 95-97% r.h. for up to 4 days after which time the development of rots was recorded. All the control berries were infected at this time. Duplicate experiments were carried out with each fungicide. The fungicides tested were dichlofluanid, chlorothalonil, vinclozolin, iprodione, LS73-783, A5430, triadimefon, potassium hydroxyquinoline sulphate and pimaricin.

## RESULTS

### Effect of fungicides on the fungi associated with freshly harvested fruit

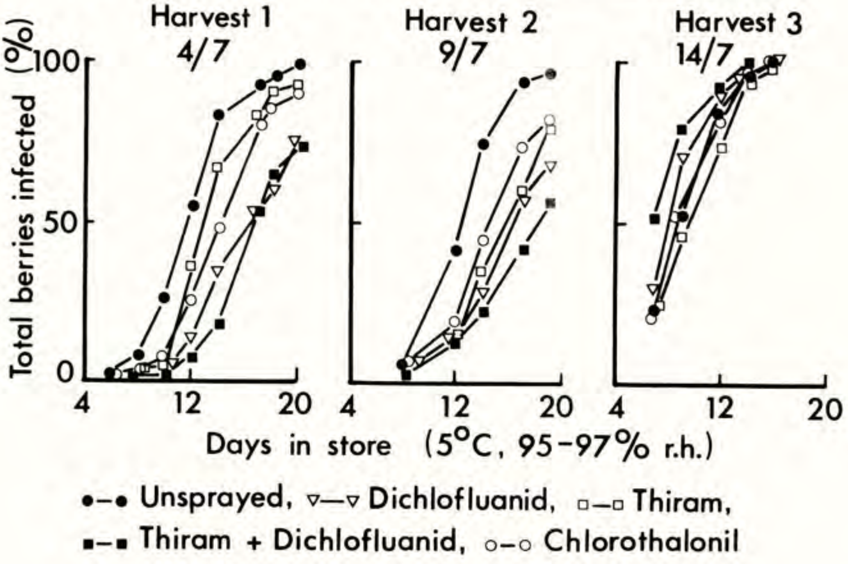
The type and incidence of the predominant fungi associated with freshly harvested fruit was similar to those reported by Dennis (1975). The incidence of these fungi was not affected by any of the fungicides apart from Aureobasidium pullulans and B. cinerea. All fungicides reduced the incidence of A. pullulans on fruit at all harvests and of B. cinerea at the first harvest only, in both seasons. The counts of the other fungi were not consistently affected by any of the fungicide treatments.

### Effect of fungicides on fungal spoilage of stored strawberries

The rate of fungal spoilage of strawberries stored singly at 5°C (100 berries per treatment) in the 1975 season is shown in Figure 1. Similar rates of spoilage were observed on berries stored in punnets. There was a marked increase in the rate of spoilage as the season progressed together with a change in the relative importance of the spoilage fungi (Figure 2). In the first two harvests the predominant spoilage organism of fruit from all treatments was grey mould, whereas the Phycomycete fungi increased on all fruit at the third harvest and predominated on fruit from the dichlofluanid and dichlofluanid + thiram treatments. At 10°C, the Phycomycetes predominated on fruit of these treatments at both the second and third harvests and also on the thiram sprayed fruit at the third harvest.

Berries from dichlofluanid and dichlofluanid + thiram sprayed plots consistently showed the lowest incidence of grey mould whereas thiram alone and chlorothalonil gave least control of this disease at all harvests. However, wherever the incidence of grey mould was reduced, a greater development of the Phycomycetes occurred. A similar pattern of development of spoilage fungi occurred on fruit in the 1976 season.

Fig.1 Spoilage of stored strawberries - 1975 season



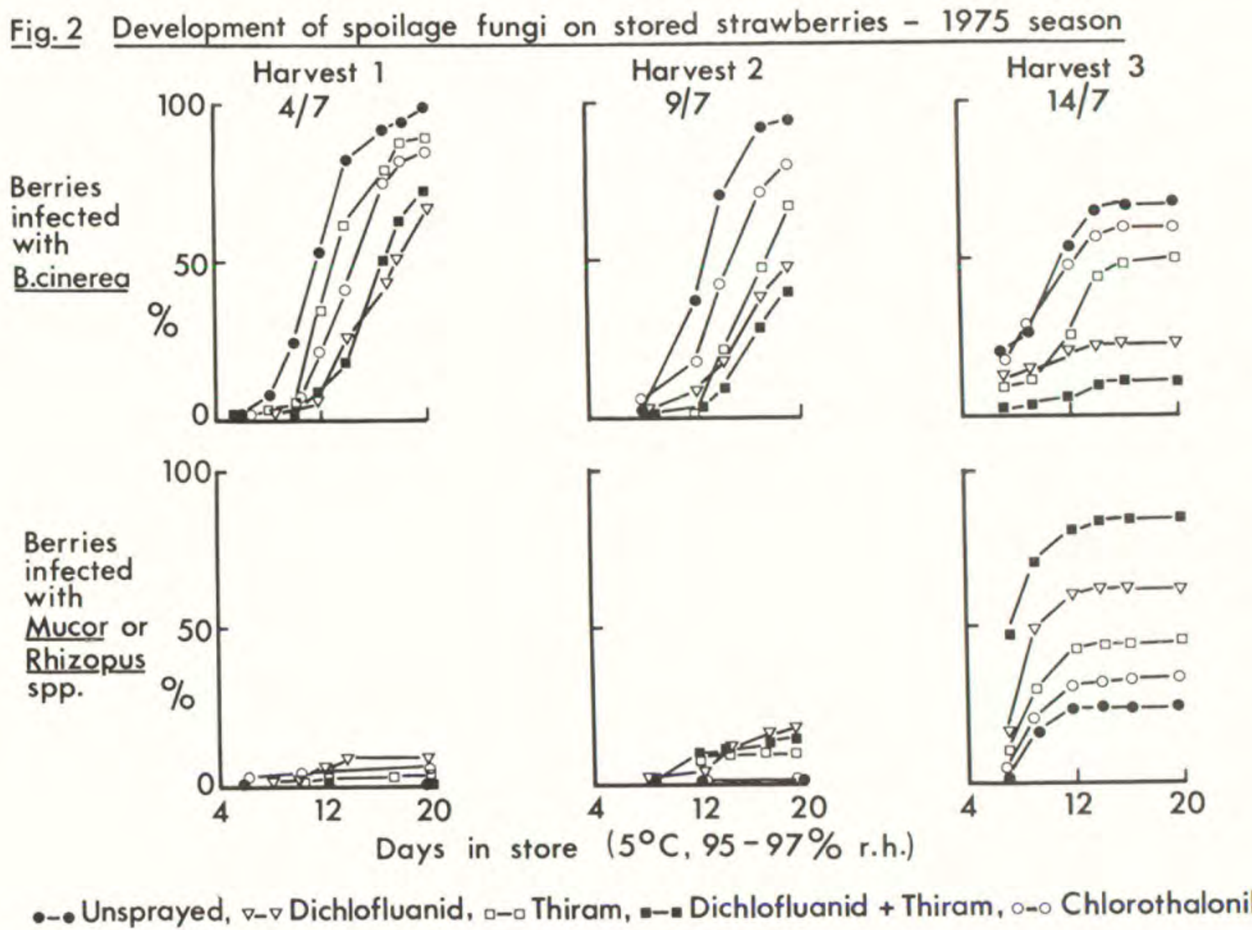


Fig. 2 Development of spoilage fungi on stored strawberries - 1975 season
Harvest 1
4/7
Harvest 2
9/7
Harvest 3
14/7
Berries infected with B.cinerea
Berries infected with Mucor or Rhizopus spp.
%
100
50
0
4
12
20
Days in store (5°C, 95 - 97% r.h.)
●-● Unsprayed, ▽-▽ Dichlofluanid, □-□ Thiram, ■-■ Dichlofluanid + Thiram, ○-○ Chlorothalonil

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## Table 1

**Incidence of M. mucedo and R. sexualis on stored strawberries in the 1975 and 1976 seasons**

% berries infected with M.mucedo

| | 1975 | | | | | | 1976 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Harvest 1 | | Harvest 2 | | Harvest 3 | | Harvest 1 | | Harvest 2 | |
| | 10° | 5° | 10° | 5° | 10° | 5° | 10° | 5° | 10° | 5° |
| Unsprayed | 1 | 0 | 30 | 2 | 33 | 25 | 0 | 0 | 0 | 0 |
| Chlorothalonil | 8 | 3 | 12 | 6 | 60 | 30 | 0 | 0 | 0 | 2 |
| Thiram | 11 | 1 | 19 | 32 | 56 | 61 | 0 | 0 | 1 | 4 |
| Dichlofluanid | 7 | 22 | 62 | 22 | 87 | 87 | 10 | 0 | 4 | 6 |
| Dichlofluanid + Thiram | 22 | 15 | 48 | 55 | 83 | 89 | 5 | 5 | 3 | 3 |

% berries infected with R. sexualis

| | 1975 | | | | | | 1976 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Harvest 1 | | Harvest 2 | | Harvest 3 | | Harvest 1 | | Harvest 3 | |
| | 10° | 5° | 10° | 5° | 10° | 5° | 10° | 5° | 10° | 5° |
| Unsprayed | 16 | 1 | 24 | 4 | 27 | 3 | 20 | 0 | 22 | 2 |
| Chlorothalonil | 42 | 4 | 20 | 1 | 30 | 10 | 23 | 0 | 48 | 4 |
| Thiram | 21 | 2 | 34 | 0 | 17 | 0 | 7 | 1 | 34 | 2 |
| Dichlofluanid | 30 | 2 | 16 | 2 | 4 | 1 | 51 | 0 | 67 | 4 |
| Dichlofluanid + Thiram | 25 | 0 | 13 | 0 | 9 | 0 | 43 | 3 | 43 | 5 |

Mucor mucedo and R. sexualis were the only Phycomycetes isolated from the leading edge of fruit rots in both seasons. Table 1 shows the relative frequency of these fungi on berries stored at 10°C and 5°C. At 10°C R. sexualis predominated on the fruit from all treatments at the first harvest in 1975 and both harvests in 1976, while M. mucedo predominated on fruit from most treatments stored at both 10°C and 5°C in the second and third harvests in 1975 but was recorded infrequently in 1976. The higher incidence of Phycomycetes developing on fruit stored at 10°C, as opposed to that stored at 5°C is due to the increased development of R. sexualis at the higher temperature, whereas the incidence of M. mucedo was often similar at both temperatures.

## Activity of fungicides on spoilage fungi on detached strawberries

Dichlofluanid, iprodione and vinclozolin were highly effective against grey mould whereas none of the fungicides tested showed marked activity against Mucor and Rhizopus (Table 2). Rhizopus sexualis showed greater sensitivity to chlorothalonil and triadimefon than the other Phycomycetes and Rhizopus spp. were more sensitive than M. mucedo to iprodione. Pimaricin although being highly active against grey mould and also having activity against Mucor and Rhizopus is unstable when exposed to light and rapidly loses its activity (Raab, 1972).

Table 2

Effect of fungicides on infection of detached strawberry fruits by spoilage fungi

| | % infection after 4 days incubation | | | | | | |
|---|---|---|---|---|---|---|---|
| | B. cinerea | | | | M. mucedo | R. sexualis | R.stolonifer |
| | concentration of active ingredient (ppm) | | | | | | |
| | 5 | 10 | 50 | 100 | 100 | 100 | 100 |
| Dichlofluanid | 20 | 30 | 0 | 0 | 60 | 70 | 50 |
| Chlorothalonil | 90 | 100 | 70 | 60 | 90 | 30 | 80 |
| Iprodione | 20 | 10 | 0 | 0 | 100 | 50 | 30 |
| Vinclozolin | 10 | 20 | 10 | 0 | 100 | 100 | 100 |
| Pimaricin | 10 | 0 | 0 | 0 | 40 | 20 | 20 |
| LS 74 - 783 | NT | NT | NT | 90 | 100 | 100 | 100 |
| A 5430 | NT | NT | NT | 50 | 90 | 100 | 90 |
| Triadimefon | 90 | 100 | 60 | 70 | 100 | 10 | 90 |
| Potassium hydroxquinoline sulphate | 80 | 90 | 70 | 40 | 90 | 60 | 90 |

N.T. - not tested

## DISCUSSION

Although the count of B. cinerea on the fruit was decreased by the fungicides only in the first week of the picking season the incidence of grey mould developing on stored fruit was reduced throughout the season. Dichlofluanid was the most efficient fungicide in reducing this disease both alone or mixed with thiram while thiram alone and chlorothalonil gave the least control. The relatively poor control of grey mould by chlorothalonil both in the field trial and on detached fruits is in agreement with the results of Jordan & Richmond (1975) but contrary to those of Freeman & Pepin (1977) where post-harvest grey mould was reduced to a similar level as that achieved by iprodione (RP 26019).

In the present experiments the last field spray of all fungicides was applied 14 days before the first harvest. Although it is permitted to spray chlorothalonil up to 3 days before the first pick, it appears that this fungicide would still be less effective than dichlofluanid as was indicated from the early harvests where the fruit would be derived from early flowers which did receive all applications of the fungicide.

The relative frequency of the Phycomycetes at 10°C and 5°C is due to the very slow growth of R. sexualis at 5°C compared to M. mucedo and B. cinerea (Dennis & Cohen, 1976). It appears that the recent warmer, drier seasons have favoured the development of Rhizopus spp. relative to M. mucedo since there was a higher incidence of R. sexualis in this study compared to that reported by Dennis (1975), and also a higher incidence of this fungus relative to M. mucedo in 1976 compared to 1975. Freeman & Pepin (1977) also report this seasonal influence on the incidence of Rhizopus in the U.S.A.

Records suggest that the Phycomycetes have attained greater significance in stored produce in recent years. Apart from causing fruit rots of soft fruit, Mucor sp. has recently caused severe post-harvest losses of peaches in the U.S.A. (Smith, personal communication) while Rhizopus species, especially R. stolonifer cause spoilage of a range of fruits and vegetables (Eckert 1975). Both Mucor and Rhizopus have also recently been reported to produce pectolytic enzymes which have caused disintegration of commercial samples of strawberries preserved in sulphite liquor (Dennis, 1976, 1977) and the breakdown of canned apricots (Harper et al. 1973; Sommer et al, 1974).

Thus the increased awareness of the importance of Mucor and Rhizopus species in spoilage, both pre- and post-harvest emphasizes the need for a chemical effective against these fungi. Of the fungicides tested in the present work, dichlofluanid, iprodione and vinclozolin gave effective control of grey mould, but none of the fungicides had the same order of activity against Mucor or Rhizopus.

## Acknowledgements

The authors thank Mr. P. Withers for use of the strawberry plots and the chemical firms for supplying samples of fungicides used in the present work.

## References

COHEN, E. & DENNIS, C. (1975) Effect of fungicides on the mycelial growth of soft fruit spoilage fungi, Annals of applied Biology, 80, 237-242.

DENNIS, C. (1975) Effect of pre-harvest fungicides on the spoilage of soft fruit after harvest, Annals of Applied Biology, 81, 227-234.

DENNIS, C. (1976) Fungi in preserved soft fruit, Grower, 85, 1123-25.

DENNIS, C. (1977) The involvement of fungi in the breakdown of sulphited strawberries, Annals of Applied Biology (In Press).

DENNIS, C. & COHEN, E. (1976) The effect of temperature on strains of soft fruit spoilage fungi, Annals of Applied Biology, 82, 51-56.

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

ECKERT, J.W. (1975) Post-harvest biology and handling of fruits and vegetables. Eds. N.F. HAARD & D.K. SALUNKE AVI, New York, 81-117.

FREEMAN, J.A. & PEPIN, H.S. (1977) Control of post-harvest fruit rot of strawberries by field sprays, Canadian Journal of Plant Science, 57, 75-80.

HARPER, K.A., BEATTIE, B.B., & BEST, D.J. (1973) Texture changes in canned apricots II. Study of infection with Rhizopus stolonifer under commercial conditions. Journal of Science Food and Agriculture, 24, 527-531.

JORDAN, V.W.L. & RICHMOND, D.V. (1975) Perennation and control of benomyl-insensitive Botrytis affecting strawberries. Proceedings 8th British Insecticide and Fungicide Conference Volume 1, pp 5-13.

SOMMER, N.F., BUCHANAN, J.R. & FORTLAGE, R.J. (1974) Fungus and enzyme activity in fresh apricots as related to softening of canned fruits, California Agriculture, 28, 8.

RAAB, W.P. (1972) Natamycin (Pimaricin) Its properties and possibilities in Medicine. Georg Thieme Publishers Stuttgart.

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# RELATIONSHIPS BETWEEN NUMBERS OF ADULT SPODOPTERA LITTORALIS (BOISD.) CAUGHT IN PHEROMONE TRAPS AND SUBSEQUENT LARVAL POPULATIONS

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Summary Adult and larval populations of Spodoptera littoralis were sampled at two areas in Crete. Adults were sampled by pheromone traps and larvae by quadrats. The adult samples were positively correlated with populations of early instar larvae in adjacent areas two weeks later. Correlations with older instar larvae were less good.

## INTRODUCTION

The Egyptian Cotton Leafworm, *Spodoptera littoralis* (Boisd.), is a serious and widespread pest of cotton and other crops in the Middle East and North Africa.

It has been shown that traps baited with the synthetic female sex attractant of *S. littoralis* *cis*-9, *trans*-11, tetradecadien-1-yl acetate (Nesbitt *et al*., 1973)is an effective method of trapping adult male moths (Campion *et al*., 1974).

Pheromone traps may therefore be used as a means of monitoring increases in insect populations in the field and so to time more precisely when to spray insecticides.

A correlation between light trap catches of adults of *S.littoralis* and changes in larval populations in Cyprus was reported in Anon.(1974). Experiments were therefore carried out in certain areas of Crete during 1976 to determine whether a similar relationship could be shown between catches of moths in pheromone traps and subsequent increases of larvae.

## METHODS AND MATERIALS

All the experiments were carried outin lucerne fields situated in two relatively isolated areas of north west Crete. None of the fields sampled was bigger than 5000 $m^2$ so the areas being sampled could be clearly defined.

Each field was sampled for larvae at least once each week. Square metal quadrats, 50cm x 50cm,were used to define the area sampled and six quadrats were taken from each field, approximately equidistant from each other across a diagonal of the field. Within each quadrat, the larger larvae were first picked off the plants and counted, then the smaller larvae were counted and finally the ground below the plants was thoroughly searched with particular attention being paid to the leaf litter and cracks in the ground. The larvae were recorded as small (first to third instar) or large (fourth to sixth instar). All the larvae were returned to the quadrat after counting. The number of larval samples taken in each area ranged from 30 to 180 each week.

The adult pheromone traps used were those described by Campion et al. (1974). All the traps were baited with 2 µg of the synthetic sex attractant dispensed in polythene vials. All the attractant vials were replaced every four weeks. Daily records of moth catches were made throughout the experimental period of 13 weeks.

At the Episcopi site, one trap was maintained and no field sampled was more than 800m from the trap. At the Maleme site, seven traps were maintained and all the fields sampled were within 200m of any one trap.

## RESULTS

The weekly numbers of larvae recorded each week together with the mean totals of moths caught in the pheromone traps at the Episcopi site are shown in Table 1. Possible relationships between the numbers of moths caught in traps and numbers of larvae in the fields were investigated for the Episcopi site by plotting moth numbers against larval numbers at intervals of 0, 1 and 2 weeks. The best relationship was observed using a time interval between moth catches and larval counts of two weeks. A significant regression ($p<001$) was obtained by plotting moth numbers against numbers of small (first to third instar) larvae using a two week interval (Figure 1). A regression for moth numbers against larger larvae was also obtained although of less significance ($p<01$)(Figure 2).

Similar treatment of the data for the Maleme site (Table 2) is shown in Figure 3. A significant regression is again obtained by plotting moth numbers against small larvae. The plots for large larvae show much more scatter. Owing to the lack of irrigation towards the end of the experimental period in the Maleme site, larval sampling was terminated in Week 11.

## DISCUSSION

The correlation between moth numbers and subsequent numbers of first-third instar larvae was highly significant at both sites. The results from the two areas were, however, not comparable and this may account for the different slopes of the regression lines. At the Episcopi site, comparisons were made of catches in one trap with larval samples from several nearby fields at varying distances from the trap. At the Maleme site, the traps were several km apart with the fields sampled relatively close to any one trap. Sufficient larval sampling data was available to make direct comparisons with moth populations in any one field.

The relationships between moth catches and the subsequent numbers of fourth-sixth instar larvae were not so highly correlated at either site. This may be the result of varying predation, insecticide application or the relative inefficiency of the sampling technique for the larger larvae. The larger larvae spend much of the day buried in the surface trash and are consequently more difficult to find than the smaller larvae which remain within the plant complex. Sampling procedures based on small larvae may therefore be more efficient.

## CONCLUSIONS

The relationship shown between the numbers of moths caught in pheromone traps and the subsequent larval populations may provide the basis for an efficient monitoring system.

The time interval of two weeks before significant relationships emerge between moth catches and small larvae would provide farmers with ample warning of incipient larval infestations.

These results were obtained on relatively low insect populations and it remains to be seen whether similar relationships can be demonstrated at higher infestation levels and in larger agricultural areas (see Kehat et al, 1975).


## Acknowledgements

This work formed part of a joint collaborative project between the Centre for Overseas Pest Research and the Greek Ministry of Agriculture. We therefore thank Mr N.Psillakiss, Director of the Agricultural Research Station, Chania, Crete, Greece for his help and for providing us with work facilities, and his staff for assisting us in many other ways.


## References


ANON. (1974) COPR Interim Report, Cyprus Armyworm Project 1974.

CAMPION, D.G., BETTANY, B.W., NESBITT, B.F., BEEVOR, P.S., LESTER, R. & PAPPI, R.G. (1974) Field studies of the sex pheromone of the cotton leafworm Spodoptera littoralis (Boisd.) in Cyprus. Bulletin of Entomological Research, 64, 89 - 96.

NESBITT, B.F., BEEVOR, P.S., COLE, R.A., LESTER, R. & PAPPI, R.G. (1973) Sex pheromones of two Noctuid moths. Nature - New Biology, 244, 208 - 209.

KEHAT, M., GENIZI, A. & GREENBERG, S. (1975). The use of traps baited with live females or synthetic pheromone as a tool for improving control programs of the cotton leafworm Spodoptera littoralis (Boisd.) in cotton fields in Israel. Phytoparasitica 3 (1), 3 - 18.

Table 1

The number of larvae recorded in successive weeks in lucerne fields at the Episcopi site together with the weekly totals of moths caught in the pheromone trap. The larvae are grouped as small (1st to 3rd instar) or large (4th to 6th instar).

| Week | Dates | Number of samples | Number of small larvae/ $m^2$ | Number of large larvae/ $m^2$ | Numbers of moths in pheromone trap |
|---|---|---|---|---|---|
| 1 | 1/8 - 7/8 | 30 | 0 | 0 | 44 |
| 2 | 8/8 - 14/8 | 42 | 0 | 0 | 67 |
| 3 | 15/8 - 21/8 | 48 | 0 | 0 | 143 |
| 4 | 22/8 - 28/8 | 60 | 0 | 0.2 | 67 |
| 5 | 29/8 - 4/9 | 108 | 0.8 | 2.3 | 20 |
| 6 | 5/9 - 11/9 | 96 | 0.2 | 1.0 | 22 |
| 7 | 12/9 - 18/9 | 114 | 0 | 2.4 | 103 |
| 8 | 19/9 - 25/9 | 36 | 0 | 0.7 | 496 |
| 9 | 26/9 - 2/10 | 108 | 1 | 1.2 | 1003 |
| 10 | 3/10 - 9/10 | 78 | 4.9 | 1.3 | 527 |
| 11 | 10/10 - 16/10 | 126 | 9.4 | 4.9 | 599 |
| 12 | 17/10 - 23/10 | 90 | 4.9 | 2.4 | 117 |
| 13 | 24/10 - 30/10 | 162 | 1.9 | 1.8 | 110 |

Table 2

The number of *Spodoptera* larvae recorded in successive weeks in lucerne fields at the Maleme site together with the weekly mean totals of moths caught in seven pheromone traps. Larvae grouped as small (1st to 3rd instar) and large (4th to 6th instar).

| Week | Dates | No. of samples | Number of small larvae/ $m^2$ | Number of large larvae/ $m^2$ | Mean No. moths in pheromone traps |
|---|---|---|---|---|---|
| 1 | 1/8 - 7/8 | 30 | 0 | 0 | 7 |
| 2 | 8/8 - 14/8 | 24 | 0 | 0 | 7 |
| 3 | 15/8 - 21/8 | 42 | 0 | 0 | 42 |
| 4 | 22/8 - 28/8 | 60 | 0 | 0 | 154 |
| 5 | 29/8 - 4/9 | 72 | 3.2 | 4.3 | 97 |
| 6 | 5/9 - 11/9 | 72 | 6.8 | 5.7 | 92 |
| 7 | 12/9 - 18/9 | 18 | 1.0 | 0.4 | 38 |
| 8 | 19/9 - 25/9 | N.S. | - | - | 129 |
| 9 | 26/9 - 2/10 | 30 | 0.3 | 2.2 | 256 |
| 10 | 3/10 - 9/10 | 18 | 4.6 | 0* | 449 |
| 11 | 10/10 - 16/10 | 30 | 0 | 0* | 789 |

* Owing to lack of irrigation, most of the lucerne fields had dried up by this time and further larvae sampling was discontinued.

Figure 3. Relationship between weekly catches of moths (mean of 7 traps) at the Maleme site with numbers of early instar larvae (1st - 3rd) and later instars (4th - 6th) sampled from surrounding fields two weeks later.



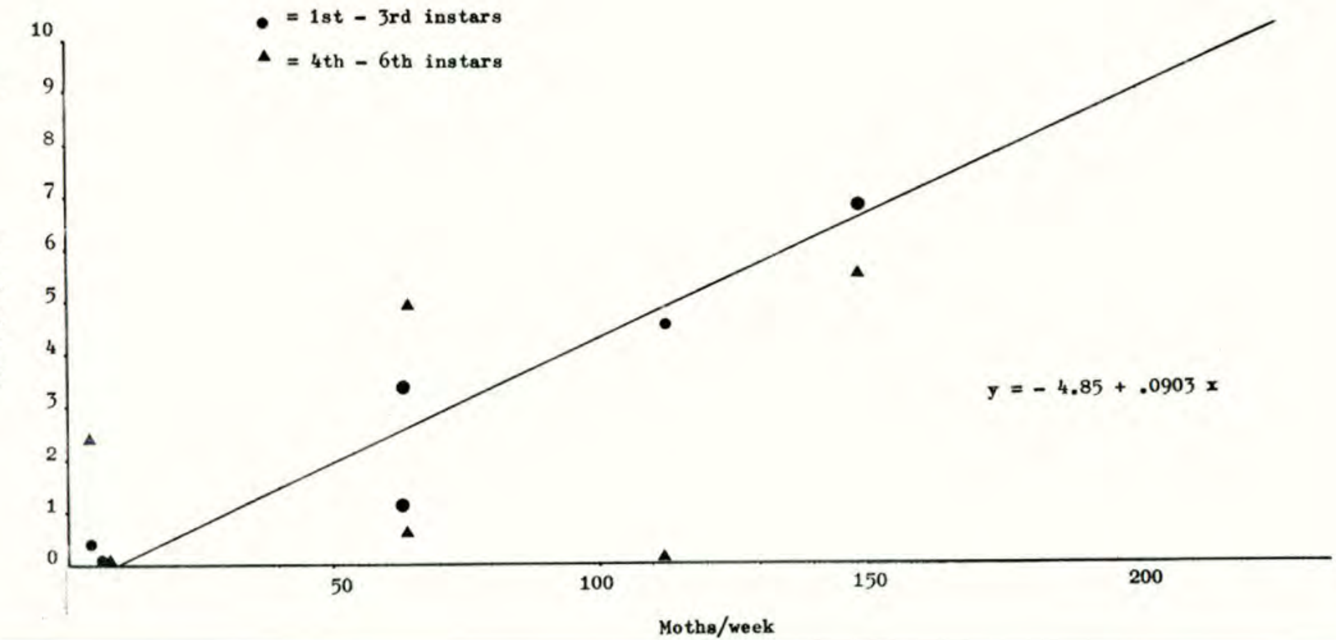


**Figure 2. Relationship between weekly catches of moths in a pheromone trap at the Episcopi site with numbers of large larvae (4th - 6th instar) sampled from surrounding fields two weeks later.**

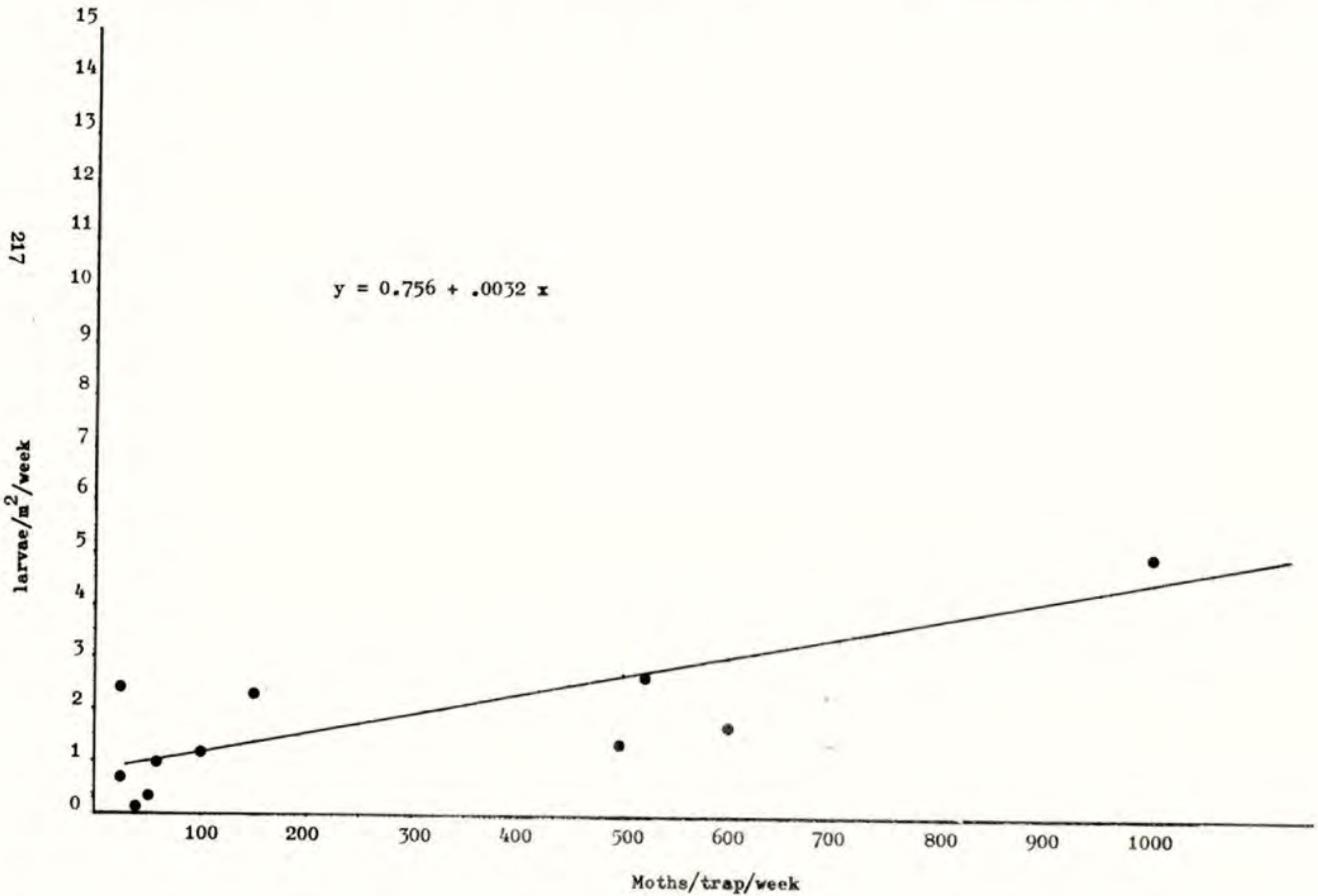
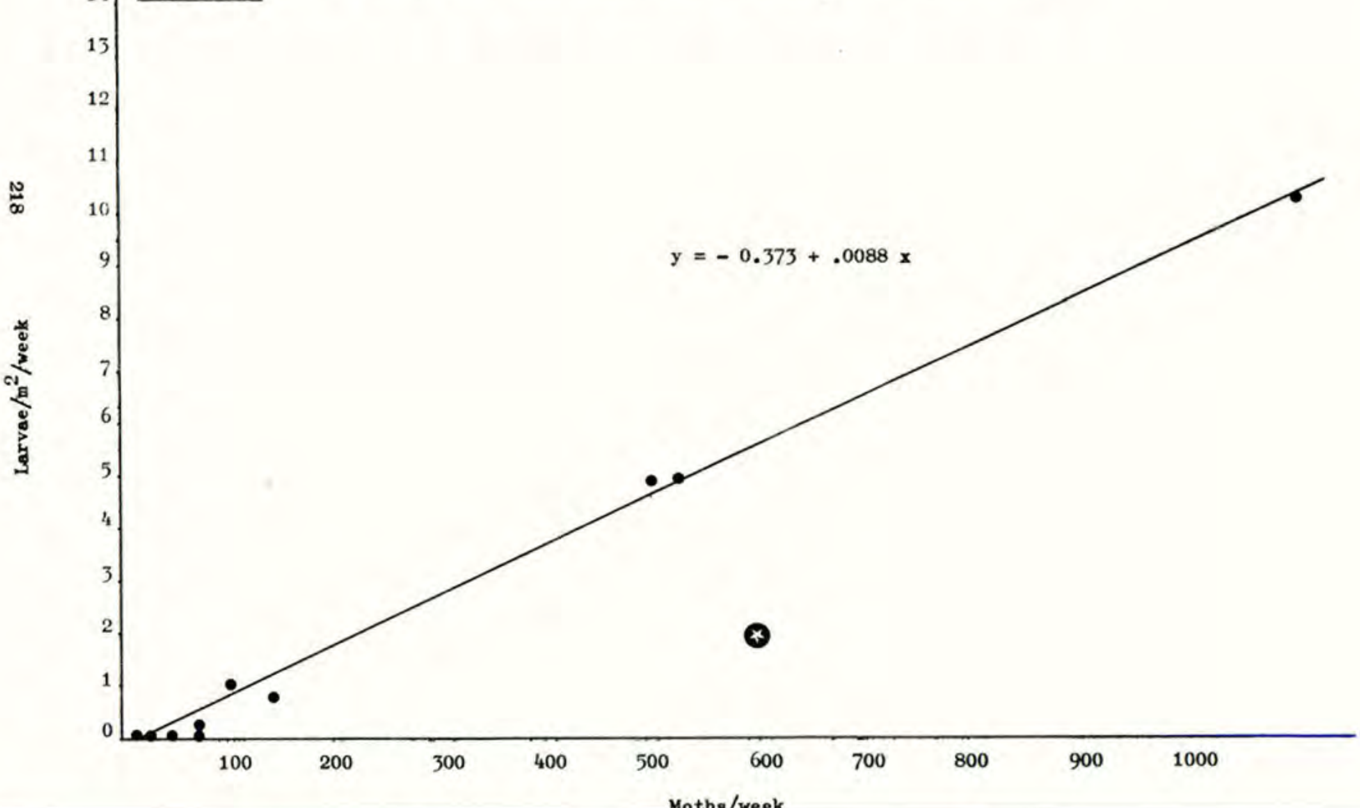


Figure 1. Relationship between weekly numbers of moths in one phenomone trap at the Episcopi stie with numbers of early instar larvae (1st - 3rd) sampled from surrounding fields two weeks later. The point indicated by ✪ was a larval count in the last week of October and larval numbers may have been falling due to low temperature.



# MONITORING INSECT PESTS OF CRUCIFEROUS CROPS

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Summary Peak activity of the adults of the three annual generations of cabbage root flies can be predicted using day degrees ($D^{o}$) accumulated from 1 January. From 1970-1976 the peak of females of the first generation occurred in May after a mean accumulation of 227 $D^{o}$ above 6°C. When air temperatures exceeded 21°C in early June, the second generation entered aestivation. Without aestivation, the second and third generations peaked after means of 601 and 1115 $D^{o}$ respectively. With aestivation, 802 and 1428 $D^{o}$ were required. Insecticide to kill the adult cabbage root fly should be applied to crops requiring long term protection if more than 30 females are caught/ANCS-trap during the week of the predicted peak. The ANCS-trap can also assess populations of six other cruciferous pests with minimum interference to many beneficial insects.

Résumé On peut prédire le maximum de l'activité des adultes de trois générations annuelles des mouches du chou en employant les degrés de jour ($D^{o}$) accumulés depuis le 1ier janvier. De 1970 à 1976 le sommet d'activité des femelles de la première génération a en lieu au mois de mai après une accumulation moyenne de 227 $D^{o}$ au dessus de 6°C. Au moment où les températures de l'air ont dépassé 21°C au début de juin, la deuxième génération a commencé l'estivation. Sans estivation les deuxième et troisième générations ont atteint leurs sommets après des moyennes respectivement de 601 et 1115 $D^{o}$. Avec l'estivation, on avait besoin de 802 et de 1428 $D^{o}$. L'insecticide pour tuer la mouche du chou adulte devrait être appliqué aux récoltes ayant besoin de protection à long terme si on attrape plus de trente femelles/piège allylisothiocyanate pendant la semaine du sommet prédit de l'activité. La piège allylisothiocyanate peut également évaluer les populations de six autres insectes nuisibles aux crucifères avec le minimum d'intervention envers bien d'insectes avantageux.

## INTRODUCTION

Interest in trapping to monitor populations of insects has been stimulated both by the isolation of specific attractants and by improvement in trap design. A trap was developed at Wellesbourne for monitoring changes in cabbage root fly (*Delia brassicae*) populations, a task previously carried out laboriously by extracting fly eggs from soil samples. Although it is relatively easy to monitor fly populations using a trap, it is still time consuming. Consequently, if the peaks of activity of the pest could be predicted, it may then only be necessary to trap for a few days to determine whether the size of the insect population merits the application of insecticide.

In southern England there are normally three generations of the cabbage root fly each year. Phenological relationships of fly emergence with accumulated day degrees ($D^o$) and the flowering of Anthriscus sylvestris appear to provide reliable methods for the prediction of the first flies in the field (Coaker & Wright, 1963). Recently Eckenrode & Chapman (1972) showed that successive fly peaks (generations) were separated by a mean of 1176 (662) $D^o$ above $43^oF$ ($6^oC$) in Wisconsin whereas, in contrast, Nair & McEwen (1975) concluded that the variation in thermal units in Ontario was too large to be useful in predicting fly peaks. If it is possible to predict fly peaks in the British Isles from $D^o$, then such forecasts would allow more effective application of insecticide sprays against cabbage root fly adults.

In the British Isles, short-season cruciferous crops are at present protected effectively from the cabbage root fly by soil applied insecticides. For longer-season crops (eg. turnips and swedes) where the pest damages the plant part used for human consumption, the soil-applied insecticides available are not sufficiently persistent to be satisfactory. Consequently, supplementary sprays against the adult flies are now being applied prophylactically to keep such crops 'damage free'. If the peaks of activity of the second and third generations of flies in the British Isles could be predicted, then according to Wyman et al. (1977) a single spray can be as, or more,effective than the soil treatments applied at planting.

This paper describes the accuracy with which the peaks of cabbage root fly activity can be predicted using trapping data from Wellesbourne, and also the efficiency of the traps for monitoring populations of other insect pests of cruciferous crops.

## MATERIALS AND METHODS

Calculation of thermal units. Two methods were used for defining the accumulative temperatures, namely 1) the method proposed by the Meteorological Office in Britain (Agro. Met.) (Anon. 1946), and 2) the method used in Canada for converting temperatures to 'Ontario units'. The Agro. Met. method is a refinement of the simplest method, which is to accumulate the mean differences when the base temperatures ($6^oC$ here) is subtracted from the maximum and minimum air temperatures. It now includes a contribution from negative values and is therefore comparable to the method used by Eckenrode & Chapman (1972). Ontario units are also obtained from maximum and minimum air temperatures but a separate contribution for each is calculated, and the mean is then taken (see Bunting, 1976). Coaker & Wright (1963) concluded that in the Wellesbourne locality, cabbage root fly emergence is best associated with accumulated $D^o$ commencing from 1 February rather than from 1 January or 1 March. Comparisons were made, therefore, to see if this was also true for the peaks of adult activity.

Sampling eggs. The plots used in 1970 and 1971 were well separated across the 160 ha area of the Research Station at Wellesbourne. Cauliflowers (cv. Finney's 110) were planted at 0.6 x 0.6 m spacing on all plots except one, Plot 3, which was planted with cabbage (cv. Avon Coronet) on 17 June 1971. Eggs were sampled each Monday, Wednesday and Friday by collecting a 1 - 2 cm deep layer of soil from within a 5 cm radius of 40 plants/plot (Finch et al., 1975). The eggs were extracted from the soil by flotation and counted.

Sampling flies. Traps consisted of 15 cm diameter plastic dishes sprayed fluorescent 'saturn' yellow and filled 4 cm deep with a solution of 20 ml Teepol/l. Allyisothiocyanate (ANCS), an attractant for the cabbage root fly was allowed to evaporate from vials supported above the middles of the traps (Finch & Skinner, 1974). Three traps, spaced 5 m apart, were placed along two opposite sides of each plot. The numbers of flies captured were recorded throughout the season from four plots in each of years 1970 - 1972 and 1976. During 1973 - 1975 flies were trapped from a further eight plots but only during the first generation.

Sampling other insects. In 1976, 50 traps were evenly-spaced 20 m apart around the perimeter of a wheat field which had been cropped with Brussels sprouts in 1975. ANCS was ommitted from every fifth trap. The traps were maintained throughout April and May to monitor the rate of emergence of cabbage root flies from over-wintering pupae. Counts were also made during 6 - 12 May for other pest insects caught in the traps including cabbage seed weevils (*Ceutorhynchus assimilis*), cabbage stem weevils (*Ceutorhynchus quadridens*), turnip flea beetles (*Phyllotreta cruciferae*), small striped flea beetles (*Phyllotreta undulata*), the small cabbage white butterfly (*Pieris rapae*) and blossom beetles (*Meligethes aeneus*). During May, two ANCS-traps and two water-traps were also alternated 20 m apart, in a line, in each of 10 fields of winter-sown oil-seed rape. To determine the effect of trapping on beneficial insects, further batches of four traps were placed through a crop each of cabbage, cauliflower and swede. The numbers of pest insects, hover flies (Syrphids), bumble bees (*Bombus* spp.), seven-spot ladybirds (*Coccinella septempunctata*) and eleven-spot ladybirds (*Coccinella unidecempunctata*) caught during July were recorded.

In 1976 large numbers of cabbage seed weevils were trapped during a 3-day period in May, but few were trapped either before or afterward. To determine whether this was monitoring a definite pronounced annual dispersal flight, and whether it was predictable, the numbers of this weevil caught were counted during May and early June 1977 at 32 small ($3\ m^2$) isolated radish plots, each containing one ANCS-trap.

## RESULTS

### Cabbage root fly

The $D^o$ accumulated by the time of peak activity of the first generation of cabbage root flies at the 24 different sites in the Wellesbourne area during 1970 - 1976 were calculated (Table 1). The 95% fiducial limits of the estimate of $D^o$ to peak flight activity did not decrease when the accumulation was calculated from 1 February rather than 1 January and so, for completeness, future computations were taken from 1 January. Since a daily average of about 11 Ontario units or 6 $D^o$ can be expected at the time of the first generation, the 95% fiducial limits can be expressed as not more than $\pm 3$ days on both scales. There was little difference in the accuracy of prediction of peak fly activity between the two methods of computation. Most published work on the cabbage root fly has been based on Agro. Met. -type units rather than 'Ontario units' and so only Agro. Met. $D^o$ (base $6^oC$) will be used here. The 95% fiducial limits for the mean number of days to the peak of the first generation were greater than those for $D^o$. These differences became progressively larger for the second and third generation results, indicating that peaks of fly activity were predicted more accurately from $D^o$ than as time.

Table 1

The accumulated thermal units for peak activity of the first generation of cabbage root flies (1970 - 1976) at Wellesbourne.

| | A. Thermal units accumulated from 1 January | | | |
|---|---|---|---|---|
| | Ontario units | | Agro. Met. $D^o$ | |
| Flies | Male | Female | Male | Female |
| Range | 187-459 | 280-459 | 155-285 | 202-285 |
| Mean $\pm$95% fiducial limits | 319 $\pm$31 | 345 $\pm$28 | 214 $\pm$15 | 227 $\pm$14 |
| | B. Thermal units accumulated from 1 February | | | |
| Range | 160-450 | 233-450 | 129-268 | 176-268 |
| Mean $\pm$95% fiducial limits | 303 $\pm$33 | 329 $\pm$31 | 195 $\pm$15 | 208 $\pm$15 |

Fig. 1. The numbers of cabbage root flies trapped at one plot in 1970 (O) with aestivation and at the same plot in 1971 (●) without aestivation. The values are mean D° (base 6°C) accumulated between successive generations.

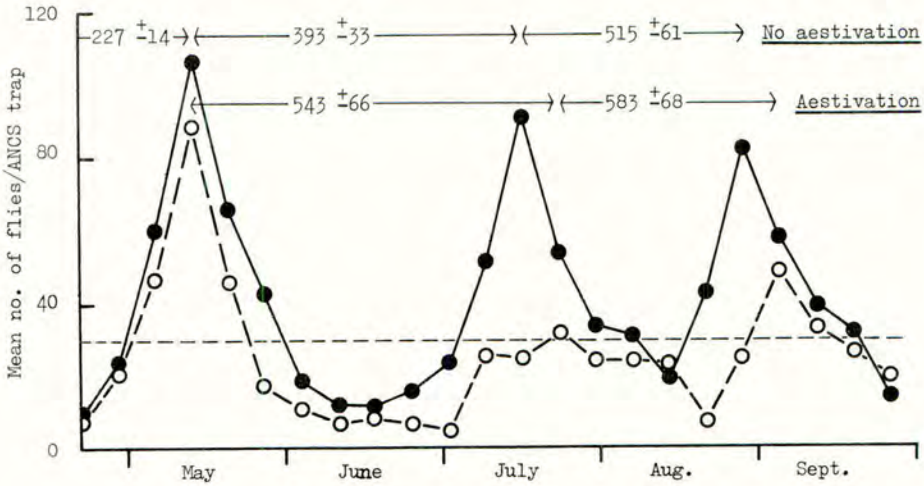
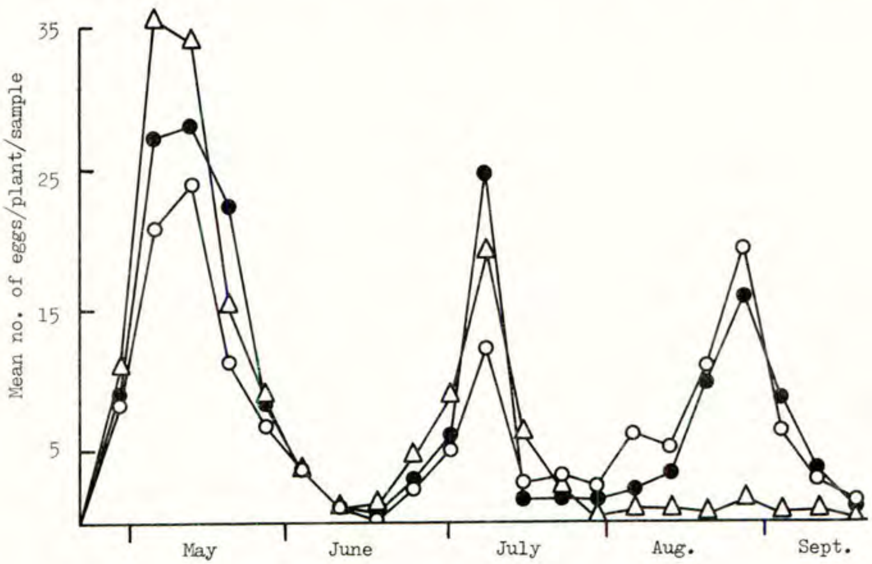



Fig. 2. Seasonal changes in the mean numbers of cabbage root fly eggs collected/plant/week from plots 1 (O), 2 (●), and 3(△) during 1971.



Unlike the first generation where the peak of males preceded that of the females (Table 1), the male and female peaks during both the second and third generations were more or less coincident. Only the results for the females are therefore shown (Table 2). Pooling the data for all 4 years revealed large variability with, for example, the prediction for the third generation being 1280 $\pm$90D$^o$, or $\pm$12 days. Aestivation of some of the second generation of pupae was probably responsible for much of this. The numbers of flies trapped at one plot in 1970 and 1971 (Fig. 1) showed that, when aestivation occurred in the first generation of pupae, as in 1970, the second generation of flies was protracted, its peak was difficult to define and the peak of the third generation of flies was delayed. Analysing the data according to whether or not the second generation had aestivated improved the accuracy of prediction, reducing the 95% fiducial limits to $\pm$3-5 days (Table 2). However, an accurate prediction may be of little practical use without also a direct form of population assessment, since few eggs were laid on Plot 3 in 1971 where cauliflowers were replaced by cabbage (Fig. 2). On plots 1 and 2 cauliflowers were replaced by cauliflowers and the expected peaks of eggs occurred.

Table 2

Accumulated temperatures (D$^o$) and peak activity of the second and third generations of cabbage root flies (1970 - 1972 and 1976)

| | All 4 years | | 1971 & 1972 (NO AESTIVATION) | | 1970 & 1976 (AESTIVATION) | |
|---|---|---|---|---|---|---|
| Generation of flies | 2nd | 3rd | 2nd | 3rd | 2nd | 3rd |
| Range | 535-902 | 1039-1496 | 535-633 | 1039-1158 | 688-902 | 1297-1496 |
| Units/day | 10.1 | 7.4 | 9.2 | 7.3 | 11.0 | 7.5 |
| Mean $\pm$95% fiducial limits | 733 $\pm$58 | 1280 $\pm$90 | 601 $\pm$30 | 1115 $\pm$37 | 804 $\pm$54 | 1428 $\pm$30 |
| 95% fiducial limits (days) | $\pm$5.7 | $\pm$12.2 | $\pm$3.3 | $\pm$5.1 | $\pm$4.9 | $\pm$4.0 |

Unlike the data of Eckenrode & Chapman (1972) from Wisconsin, the D$^o$ accumulated between successive generations at Wellesbourne was not constant (Fig. 1). The D$^o$ accumulated between generations were also lower than the constant value of 662 (base 6$^o$C) recorded at Wisconsin.

Other insects

1976. The mean numbers of insects caught daily are shown in Table 3. Few insects were caught in the traps in the oil-seed rape crops and these data are not included. Prior to 8 May, and from 11 May onwards, the numbers of cabbage seed weevils caught around the 1975 Brussels sprouts field never exceeded 10 weevils/ANCS trap. During 8-10 May, however, each of the ANCS traps caught 36 times as many cabbage seed weevils and nine times as many cabbage stem weevils as the comparable water-traps (Table 3). More flea beetles and blossom beetles were also caught in the ANCS-traps.

During July, similar numbers of bumble bees, ladybirds and hover flies were caught in the cabbage, cauliflower and swede crops and so the data were pooled (Table 3). Similar numbers of ladybirds were caught in both types of trap whereas only half as many bumble bees and hover flies were caught when ANCS was present.

1977. The numbers of cruciferous pest insects caught at the 32 radish plots during May and early June are shown in Table 4. There was a peak of blossom beetles during the week ending 6 May and a peak for all four species during the week ending 3 June. As in 1976, the peak of seed weevils was brief, occurring on 28-29 May.

Table 3

Mean number of insects caught/trap/day during 1976 using ANCS traps and water traps

| Trapping period | | No. of insects caught/trap/day ANCS trap | Water trap | ANCS/water trap ratio |
|---|---|---|---|---|
| 8-10 May | Cabbage seed weevils | 183 (40) | 21 (10) | 9 |
| | Cabbage stem weevils | 36 | 1 | 36 |
| | Blossom beetles | 60 | 26 | 2 |
| | Turnip flea beetles | 9 | 1 | 9 |
| | Small striped flea beetles | 50 | 4 | 12 |
| July | Bumble bees | 2 (12) | 5 (12) | 0.4 |
| | Seven-spot ladybirds | 19 | 18 | 1 |
| | Eleven-spot ladybirds | 33 | 41 | 1 |
| | Blossom beetles | 6617 | 1413 | 5 |
| | Cabbage white butterflies | 3 | 1 | 3 |
| | Hover flies | 7 | 12 | 0.6 |

Numbers of traps are shown in parentheses.

Table 4

Mean number of insects caught/ANCS trap/week during 1977 at 32 radish plots

| Week ending (Date) | May 6 | 13 | 20 | 27 | June 3 | 10 |
|---|---|---|---|---|---|---|
| Cabbage seed weevils | 3 | 5 | 6 | 8 | 129 | 3 |
| Cabbage stem weevils | 11 | 5 | 5 | 2 | 27 | 4 |
| Blossom beetles | 280 | 97 | 74 | 40 | 293 | 52 |
| Flea beetles | 22 | 21 | 10 | 121 | 492 | 36 |

DISCUSSION

Cabbage root fly. Several authors have developed their own methods of calculating D° and hence direct comparisons of results are not always possible. Consequently, to make more valid comparisons, many of the values quoted here have been calculated from different starting dates or base temperatures to those preferred by the original authors. Using a base temperature of 6°C, the number of D° required from 1 January for emergence of cabbage root flies of the first generation ranged from 101-116 (Nair & McEwen, 1975), 134-194 (Coaker & Wright, 1963) and 166-208 (Eckenrode & Chapman, 1972). Although these values are not within the same range, the individual ranges are relatively small. It would therefore appear that, for any given locality, emergence of the first generation of flies could be predicted with reasonable accuracy using an appropriate thermal unit. The same is not true, however, for the peak of adult activity of the first generation. In Ontario it ranged from 162 to 322 thermal units over a 4-year period and could not be satisfactorily predicted (Nair & McEwen, 1975). At both Wisconsin (Eckenrode & Chapman, 1972) and Wellesbourne, however, the peak can be predicted and is 172 and 227 D° respectively, from the base temperature of 6°C. Furthermore, according to Eckenrode & Chapman (1972), if the time of the first generation peak is known, the number of D° between successive generations is constant and in their case is 662. At Wellesbourne, however, when aestivation did not occur there were many fewer D° between the first and second generation peaks than between the second and third (Fig. 1). A similar situation occurred annually in Ontario (Nair & McEwen, 1972). In addition, fewer D° were recorded between generations at Wellesbourne than at Wisconson (Fig. 1), indicating that the Wisconsin data were obtained under conditions that would induce aestivation at Wellesbourne. In contrast, the Ontario data were collected under non-aestivating conditions. The Wellesbourne data thus appear to be intermediate between the two extremes. Furthermore, unlike Wisconsin, the prediction of the generation peaks at Wellesbourne are more accurate when calculated from 1 January than from the peak of the preceding generation, probably because there is considerable movement between cabbage root fly populations (Finch & Skinner, 1975). Consequently, an early peak of the first generation does not necessarily mean that the second and third generation peaks will also be early. It is also apparent that under cooler conditions air thermal units alone are not satisfactory for predicting peaks of adult activity. Contributions from other factors such as soil moisture, which is known to have considerable effects on the developmental period of this insect (Coaker & Finch, 1971; Nair & McEwen, 1975) will probably have to be taken into account before more accurate predictions can be obtained. The accuracy of the present results, however, is adequate to indicate clearly when traps should be deployed in the field to monitor cabbage root fly activity, even though an accurate threshold temperature has not been obtained. Little plant damage appears to result unless more than 30 female flies are caught/ ANCS trap/week.

Other insects. Attempts to predict the peak period of immigration of cabbage seed weevils into flowering crops of oil-seed rape failed because few were caught in the ANCS traps sited in these crops. The results indicated that using ANCS traps at the site of an overwintering population or at a new crop other than oil-seed rape appears to be a suitable method for monitoring when this weevil is migrating into susceptible oil-seed rape crops and hence to indicate when sprays should be applied to be most effective against this pest.

A trap developed for field use should not catch large numbers of beneficial insects, especially parasites and predators of the pests concerned. During these experiments, no parasites or predators of the cabbage root fly were caught in the traps. In addition, only half as many hover flies and bumble bees were caught in ANCS-traps as in water-traps, apparently because these insects were repelled by the ANCS in the traps. Some beneficial insects, for example the seven-spot lady-bird, seemed unable to change their approach to avoid a trap and so were caught in similar numbers in both ANCS and water-traps.

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## References

ANON., (1946). Tables for the evaluation of daily values of accumulated temperature above 42°F from daily values of maximum and minimum temperatures. Meteorological Office Leaflet, No. 10, 5pp.

BUNTING, E.S. (1976) Accumulated temperature and maize development in England Journal of Agricultural Science, Cambridge, 87, 577-583.

COAKER, T.H. and FINCH, S. (1971) The cabbage root fly Erioischia brassicae (Bouché). Report of the National Vegetable Research Station for 1970, 23-42.

COAKER, T.H. and WRIGHT, D.W. (1963) The influence of temperature on the emergence of the cabbage root fly Erioischia brassicae (Bouché) from overwintering pupae. Annals of Applied Biology, 52, 337-343.

ECKENRODE, C.J. and CHAPMAN, R.K. (1972) Seasonal adult cabbage maggot populations in the field in relation to thermal unit accumulations. Annals of the Entomological Society of America, 65, 151-156.

FINCH, S. and SKINNER, G. (1974) Some factors affecting the efficiency of water-traps for capturing cabbage root flies. Annals of Applied Biology, 77, 213-226.

FINCH, S. and SKINNER, G. (1975) Dispersal of the cabbage root fly. Annals of Applied Biology, 81, 1-19.

FINCH, S. SKINNER, G. and FREEMAN, G.H. (1975) The distribution and analysis of cabbage root fly egg populations. Annals of Applied Biology, 79, 1-18.

NAIR, K.S. and McEWEN, F.L. (1975) Ecology of the cabbage maggot, Hylemya brassicae (Diptera : Anthomiidae) in rutabaga in southwestern Ontario, with some observations on other root maggots. Canadian Entomologist, 107, 343-354.

WYMAN, J.A. LIBBY, J.L. and CHAPMAN, R.K. (1977) Cabbage maggot management aided by predictions of adult emergence. Journal of Economic Entomology, 70, 327-331.

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# MONITORING AND FORECASTING INSECT PESTS OF CEREALS

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Summary Data on the numbers (/$m^2$) of stem-boring Diptera and cereal aphids found in cereals in the Game Conservancy West Sussex study area between 1972 and 1977 were considered. Oscinella frit was the dominant species in 1976, Opomyza florum in 1972, 1973 and 1975, O.germinationis in 1974 and Chlorops pumilionis in 1977. Most stem-borers were found in 1976 and fewest in 1977. The distribution of the species in the different cereal crops was also described.

Significant negative relationships were found between the numbers (/$m^2$) of cereal aphids in winter wheat crops in the third week of June and deviations from the long term mean March temperature ($r= -0.93$, $P < 0.01$) and between the peak numbers (/$m^2$) in the different years and deviations from the long term mean April temperature ($r= -0.86$, $P < 0.05$). There was also a highly significant relationship ($r= 0.98$, $P < 0.001$) between the number of 'air frost days' in April and the peak numbers (/$m^2$) of aphids.

## INTRODUCTION

By contrast with most other terrestrial habitats farmland has changed considerably over the past one hundred years and still continues to do so. These changes have included the polarisation of cereal and livestock production on a farm and regional scale, less rigid adherence to rotations, the decline in the practice of establishing grass leys by undersowing spring sown cereals and the increasing use of pesticides (Potts, 1977). More recently direct drilling techniques have been introduced and insecticides used on a large scale to control outbreaks of cereal aphids.

Whilst most of these changes have been quantified the consequences of them on the farmland ecosystem are largely unknown because there was no quantitative information available about the ground zone and field layer faunas of cereals and grasses until the mid 1960's when the effects of weed removal on insects were investigated (Southwood & Cross, 1969). Consequently we can only speculate about the effects of most of these changes on the fauna. We do not know whether any species have disappeared from the farm ecosystem or whether the changes have disrupted or destroyed natural control systems and consequently exacerbated pest problems. For example, we do not know whether populations of frit flies (Oscinella frit) have changed on farmland as a result of the decline in the national oat crop. Similarly, although there is some evidence (Baranyovits, 1973), we do not know with certainty whether the cereal aphid problem has increased in Britain and in other European countries in recent years (and if it has we do not know why) or if current agricultural practices will result in more or less cereal aphids.

There is therefore a clear need from an agricultural point of view to monitor the insect fauna of cereals and the effects of changes in agricultural practices on the fauna and to introduce forecasting schemes for the more important pest species, such as cereal aphids. The Rothamsted aphid monitoring service, which was established in 1968, has provided much useful information about changes in the distribution and abundance of many aphid species throughout Britain but, as Taylor (1974) pointed out, the disadvantage of aerial sampling is that the relationship between the parent population on the ground and the ephemeral aerial fraction is not sufficiently understood. For instance, Dean (1974a) could find no relationship between the abundance of cereal aphids in aerial traps and the severity of crop infestations in different years.

Over the period 1969-1977 insect populations present in cereal fields in the Game Conservancy West Sussex study area have been monitored and the effects of some agricultural practices on the more important species investigated. In the present account data on the densities of some insect pest species over the period 1972-1977 are considered and the possibilities of forecasting cereal aphid populations are also explored.

## MATERIALS AND METHODS

The West Sussex study area consists of a 62 $km^2$ area of the South Downs and has already been described (Potts & Vickerman, 1974). The area is made up of seventeen farms and about three hundred fields. Each year, since 1969, all the cereal fields in the study area have been sampled with a Dietrick vacuum insect net in the third week of June. Insects present in the samples are identified and counted. Such samples are used to provide annual indices of the abundance of the different insect species in oats, wheat and barley crops. In addition, since 1972 vacuum net samples have also been taken at regular intervals from cereal and grass fields on two contrasting farms on the study area to obtain more detailed information on the density of insects. The cereal aphid problem is most acute in crops of winter wheat and only the regular samples taken from these crops are considered in the section on cereal aphids. Weather records were obtained from the Worthing meteorological station, which is situated 8 km to the south of the study area.

## RESULTS AND DISCUSSION

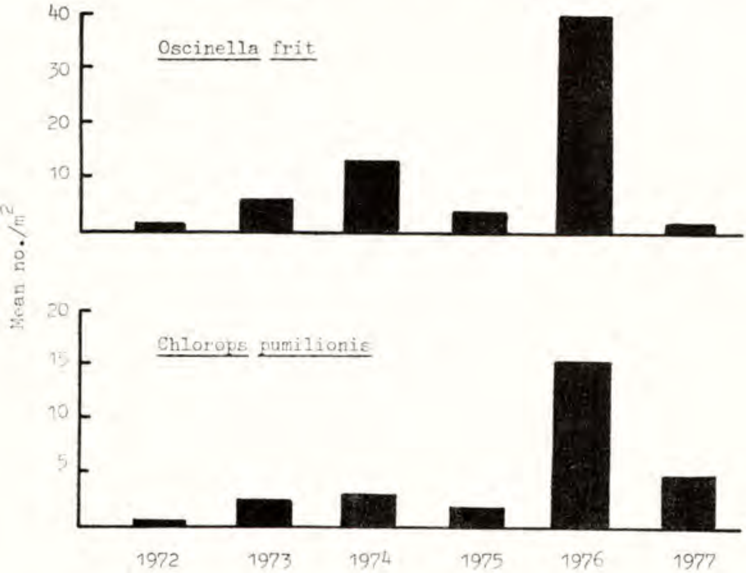
### Stem-boring Diptera

Changes in the numbers (/$m^2$) of the adults of the more common stem-boring Diptera, Oscinella frit, Chlorops pumilionis, Geomyza tripunctata, Opomyza florum and O.geminationis, found in cereal crops over the period 1972-1977 are shown in Figs. 1 & 2. Although O.frit is perhaps the best known of all these species as a pest of cereals, it was the dominant species only in 1976, O.florum was the dominant species in 1972, 1973 and 1975, O.germinationis in 1974 and C.pumilionis in 1977.

Over the period the fewest individuals were found in 1977 and the most in 1976, when stem-boring Diptera constituted about 80% of the dipterous fauna. Both O.frit and C. pumilionis were particularly abundant in 1976 and the average numbers of the two species in cereals were 40/$m^2$ and 15/$m^2$ respectively (Fig. 1). About 10% of the vacuum net samples contained more than 100 O.frit/$m^2$ and 7% more than 50 C.pumilionis/$m^2$.

Most O.frit adults were usually found in spring wheat and undersown spring barley crops. Over the period 1972-1977 other Oscinella species were surprisingly scarce in samples taken from cereals. Even on grassland O.frit was by far the dominant species; specimens of O.vastator and O.nitidissima were found occasionally whilst O.nigerrima and O.hortensis were rare. No specimens of O.albiseta or O.pusilla were found in samples taken from either cereals or grasses throughout the period.

Fig. 1. Mean numbers ($/m^2$) O.frit and C.pumilionis found in cereal crops in late June, 1972-1977



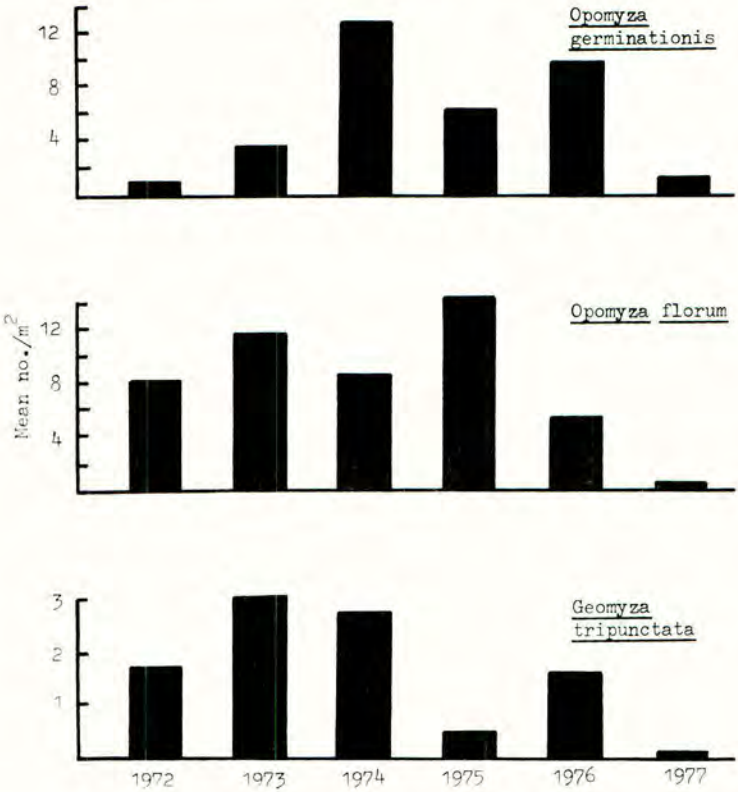

Of the Opomyza species O.germinationis was most numerous in 1974 and O.florum in 1975 (Fig. 2). In all years O.florum was rarely found in crops other than winter wheat. By contrast, although there was a tendency to find more O.germinationis in barley crops, this was not true in all years.

Numbers of Geomyza tripunctata were generally low ($< 3/m^2$) in all years (Fig.2) and this species was most common in spring and winter wheat crops. Other species such as Cetema elongata and the wheat bulb fly (Leptohylemyia coarctata) were generally scarce in the study area.

## Cereal aphids

Following the outbreaks of cereal aphids in 1968 various aspects of their biology in the field in Britain have been investigated by Jones (1972), Dean (1973, 1974 a & b), Sparrow (1974) and Potts & Vickerman (1974). The possibility of predicting cereal aphid populations was considered by Sparrow (1974) and Dean (1974 a) and has attracted much attention following the outbreaks in 1975, 76 and 77. Infestations of strawberry crops by the shallot aphid (Myzus ascalonicus) (Hurst, 1969) and the incidence of yellowing viruses of sugar beet (Hurst, 1965; Watson et al, 1975), of which Myzus persicae is a vector, are both correlated with mild weather between January and April and similar correlations have been sought with cereal aphids. Sparrow (1974) considered that a combination of above average temperatures in March and June was required to produce large populations of cereal aphids in spring cereals over the period 1969-1972 in Scotland. On the other hand Dean (1973, 1974 a) could find no relationship between the annual abundance of alatae and the number of days on which there was frost between January and March or between their abundance and the temperature during April. Dean noted that there

Fig. 2. Mean numbers (/$m^2$) O.florum, O.germinationis and G.tripunctata found in cereal crops in late June, 1972-1977



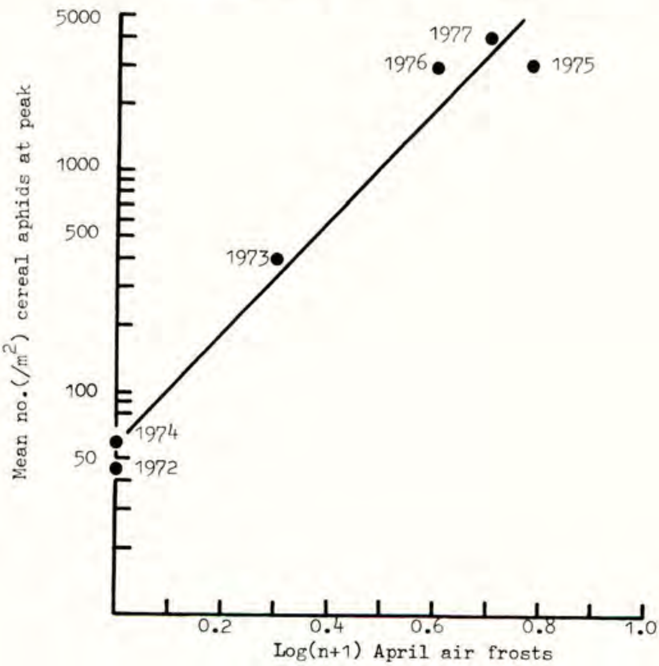

often seemed to be more aphids in years with most days of frost.

A preliminary attempt was therefore made to correlate cereal aphid populations (mainly Sitobion avenae) found in winter wheat crops in the West Sussex study area over the period 1972-1977 with a variety of meteorological parameters. Initially regression analyses were used to investigate the relationships between warm weather in winter and spring and the size of the populations in June and July; it seemed a reasonable hypothesis that warm winter weather would favour the survival and reproduction of overwintering virginoparae on cereals and grasses whilst warm spring weather would favour a rapid build up in these populations and consequently high numbers in June and July.

Warm winter and early spring weather appeared to increase the size of the overwintering population and in particular the numbers of the grass aphid (Metopolophium festucae) present in the spring. However, no positive relationships could be found between either the mean temperatures or deviations from the long term (1931-1960) mean temperatures in the months January - May and the numbers of cereal aphids present in wheat crops in June and July. Rather surprisingly a highly significant negative relationship ($r = -0.93$, $P < 0.01$) was found between the deviations from the long term mean March temperature and the numbers ($/m^2$) of cereal aphids in winter wheat crops in the study area in the third week of June. High aphid numbers in June were therefore associated with colder than average March weather.

The relationship between the logarithm of the total number of 'frost days' in the months January - May and the size of the aphid population was subsequently investigated. Although no relationship was apparent between the numbers of cereal aphids and the number of frost days, either alone or in combination, in January, February and March, the relationship between the number of 'air frost days' in April and the peak numbers ($/m^2$) of aphids in winter wheat crops in the different years was very highly significant ($r = 0.98$, $P < 0.001$) (Fig. 3). Similarly there was also a significant relationship ($r = -0.86$, $P < 0.05$) between the deviations from the long term April mean temperature and the peak number of aphids.

Fig. 3. Relationship between the number of 'air frost days' in April and the peak numbers ($/m^2$) of cereal aphids in winter wheat fields, 1972-1977




No consistent relationship could be found between temperatures in May, June and July, either alone or in combination, and cereal aphid numbers.

A possible explanation for the observed correlations is that warm winter and spring weather results in higher than average overwintering and early spring populations of cereal aphids and also the early appearance of parasites and predators. With an abundance of prey, parasite and predator populations would increase rapidly and large populations would be present in cereal fields when alatae of S.avenae arrive in May. A combination of parasitism and predation would then either significantly reduce the aphid population or check it sufficiently so that by the time population levels began to increase, the crop would be no longer suitable for reproduction. By contrast, in years with cold winters and springs early spring populations of cereal aphids would be very low and predator and parasite emergence would be delayed, or if it were on time prey would be scarce. The late invasion of cereal crops by S.avenae alatae at the end of May and in early June would be more synchronous, the population being almost entirely derived from overwintering eggs, and the population would be able to increase rapidly in the absence of predators and parasites.

Observed events in the West Sussex study area over the period 1972-1977 support the above hypothesis. The pattern of events over the six year period will be published in detail elsewhere but the situation in the two extreme years, 1974 and 1977, when aphid populations in June and July were exceptionally low and high respectively, will be briefly considered.

There were only nine 'air frost days' in January, February, March and April 1974 and the April mean temperature (10°C) was high. Populations of overwintering aphids were relatively high and in early April there were large outbreaks of M.festucae on grasses and winter cereals and of M.ascalonicus on weeds in the two types of crops. For example, in one of the grass study fields there were 6,000 M.festucae/$m^2$ in the third week of April and 25,000/$m^2$ on 30 May. The first cereal aphid parasites (Aphidius sp.) were found in the third week of April and the first parasitised aphid on 1 May. A new generation of parasites began to emerge on 16 May and by 30 May there were c.200 Aphidius/$m^2$ on the grassland. The first coccinellid (Coccinella 7-punctata) adults were found in April, the first larvae on 17 May and the first adults of the new generation on 4 June. Polyphagous predators were also abundant in late April/early May. Populations of M.festucae collapsed in early June and after this time predators and parasites were abundant in both winter and spring cereal crops. Populations of S.avenae remained very low throughout June and July in winter wheat crops.

By contrast, in 1977 there were twenty 'air frost days' and the April mean temperature (7.6°C) was much lower than average. After the middle of February no aphids were found until the last week of May, when S.avenae alates invaded the crops. The first Aphidius sp. were not found until 17 June and their density remained low ($< 5/m^2$) throughout the month. Parasitised aphids were not found until the first week of July and only one generation was completed in cereals, compared with three or four in 1974. Whilst coccinellid adults were common in cereal fields in March and April, there was no prey to support them and by the time aphids invaded the crops their numbers had dwindled. Numbers of S.avenae increased rapidly on winter wheat crops throughout June, at temperatures lower than those in 1974, and reached a peak in the second week of July.

As indicated previously the absence of quantitative information on cereal aphid densities prior to the 1970's makes it difficult to establish how well the hypothesis would account for events in previous years. However, whilst there is no quantitative information, subjective assessments of the severity of aphid outbreaks in different years may be obtained from the M.A.F.F. Monthly Summaries (e.g. see Dean, 1974 a) and this was done for S.avenae on wheat over the period 1948-1968. Interpretation of the records on infestations of grassland by M.festucae in April and May was made difficult in the early years by taxonomic uncertainty. As there was a correlation between the severity of M.festucae and M.ascalonicus attacks in those years when comparisons were possible, the severity of the infestation of strawberry crops in different years (see Hurst, 1969) by the latter species was chosen as an indicator of the likely level of abundance of grass and weed feeding aphids present on farmland in April.

Over the period 1948-1968 75% of the 'heavy' to 'severe' outbreaks of S.avenae occurred in years with slight infestations of M.ascalonicus and 25% in years with moderate M.ascalonicus infestations. No heavy or severe infestations of cereal aphids occurred in years with severe M.ascalonicus infestations (Table 1). A similar analysis was made of the data obtained from the West Sussex study area over the period 1969-1977. The four severe cereal aphid outbreaks all occurred in years when M.ascalonicus populations on weeds and M.festucae populations on grasses in the spring were low.

The data presented here indicate the possibility of establishing a tentative forecasting scheme for cereal aphids in southern England. Obviously it would be optimistic to expect the same sort of precision normally associated with black bean aphid (A.fabae) or peach-potato aphid (M.persicae) forecasts on sugar beet until many more years data have been collected. However it is hoped that the data will provide a basis for such a scheme and also stimulate long-term studies on the ecology and fluctuations in the abundance of pests such as cereal aphids.

Table 1

Relationship between severity of attacks by M.ascalonicus on strawberry crops and by S.avenae on winter wheat crops over the period 1948-1968 (based on M.A.F.F. Monthly Summaries)

| | | M.ascalonicus infestations | | |
|---|---|---|---|---|
| | | Light | Moderate | Severe |
| No. years | | 9 | 6 | 6 |
| S.avenae infestations | Light | 3 | 4 | 6 |
| | Heavy/severe | 6 | 2 | 0 |

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## References

BARANYOVITS, F. (1973) The increasing problem of aphids in agriculture and horticulture, Outlook on Agriculture, 7, 102-108.

DEAN, G.J. (1973) Aphid colonization of spring cereals, Annals of Applied Biology, 75, 183-193.

DEAN, G.J. (1974 a) The overwintering and abundance of cereal aphids, Annals of Applied Biology, 76, 1-7.

DEAN, G.J. (1974 b) The four dimensions of cereal aphids, Annals of Applied Biology, 77, 74-78.

HURST, G.W. (1965) Forecasting the severity of sugar beet yellows, Plant Pathology, 14, 47-53.

HURST, G.W. (1969) Shallot aphid in south-east England, Plant Pathology, 18, 62-66.

JONES, M.G. (1972) Cereal aphids, their parasites and predators caught in cages over oat and winter wheat crops, Annals of Applied Biology, 72, 13-25.

POTTS, G.R. (1977) Some effects of increasing the monoculture of cereals, in Origins of Pest, Parasite, Disease and Weed Problems, eds. J.M. Cherrett & G.R. Sagar, Blackwell Scientific Publications, Oxford, 183-202.

POTTS, G.R. & VICKERMAN, G.P. (1974) Studies on the cereal ecosystem, Advances in Ecological Research, 8, 107-197.

SOUTHWOOD, T.R.E. & CROSS, D.J. (1969) The ecology of the partridge. III. Breeding success and the abundance of insects in natural habitats, Journal of Animal Ecology, 38, 497-509.

SPARROW, L.A.D. (1974) Observations on aphid populations on spring-sown cereals and their epidemiology in south-east Scotland, Annals of Applied Biology, 77, 79-84.

TAYLOR, L.R. (1974) Monitoring change in the distribution and abundance of insects, Report of Rothamsted Experimental Station for 1973, 2, 202-239.

WATSON, M.A., HEATHCOTE, G.D., LAUCKNER, F.B. & SOWRAY, P.A. (1975) The use of weather data and counts of aphids in the field to predict the incidence of yellowing viruses of sugar-beet crops in England in relation to the use of insecticides, Annals of Applied Biology, 81, 181-198.

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# THE USE OF FORECASTING AS A METHOD OF RATIONALISING PESTICIDE APPLICATIONS

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Summary Economic and sociological aspects of the use of pest and disease forecasts are considered. The difficulty of persuading farmers to act in the more rational manner made possible by the forecast is discussed, with the usage of fungicide to control potato blight (*Phytophthora infestans*) as an example. In the calculation of the economic value of a forecast, the risk-aversion of the forecasters is noted, and also the importance of the pest control method used for comparison. The ADAS should be given more financial resources to enable them to promote more vigorously the uptake of the forecasts which they provide, and also a clearer political commitment to the rationalisation of pesticide usage.

## INTRODUCTION

It is appropriate to introduce a paper under this heading with a prediction: that the forecasting of pest and disease attacks and the monitoring of pest and pathogen populations will play an expanding role in future pest control systems (Wheatley, 1974; Mathys, 1970). Forecasts of pest or disease incidence are two-dimensional, covering both space (Wildbolz, 1970) and time (Way and Cammell, 1973), as shown in Table 1.

Table 1

Types of Forecast

| | | Spatial Dimension | |
|---|---|---|---|
| | | Large Scale | Small Scale |
| Temporal | Long-term | Regional Forecast | Local Forecast |
| Dimension | Short-term | Monitoring | Scouting |

The temporal dimension varies according to the time scale within which the farmer is expected to act, long-term forecasts allowing time for him to anticipate his needs for pest control measures whereas short-term forecasts demand almost immediate action. Large-scale forecasts, particularly if they are long-term, are most useful to pesticide distributors, but they have to be supplemented by more local information to be useful to individual farmers.

To be effective, a forecasting or warning scheme demands a high degree of interdisciplinary co-operation, not only among those concerned with insect pest and disease control, and meteorologists (Mathys, 1970), but also including economists and social psychologists (Norton, 1976), since the farmer's willingness to behave in the desired manner will depend on his attitudes to risk and to the required pest control tactic, and also on his economic situation. This paper will deal with the problems of encouraging farmers to use a forecasting or warning scheme as a basis for their actions, and of evaluating forecasting schemes in economic terms.

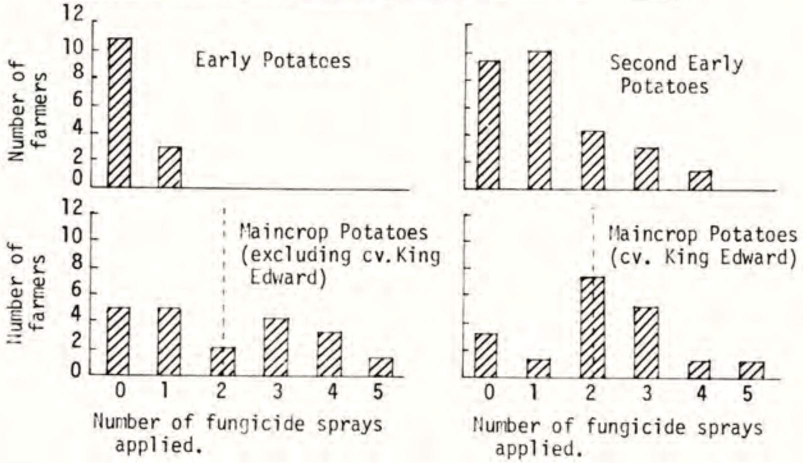
## ECONOMIC AND SOCIOLOGICAL ASPECTS OF FORECASTING

Forecasting or warning schemes aim to provide the grower with a more rational basis for his actions, either to increase the efficiency of pest control or to maintain pest control efficiency with a lower level of pesticide usage. A key consideration in this exercise is that of the economic threshold - the level of pest or disease infestation at which a pesticide treatment will provide an economic benefit to the grower. It is common, with monitoring schemes, to issue a warning to farmers when pests reach a level which is capable of causing economic damage (Cammell and Way, 1973; Lewis et al., 1975). However, in some cases, for example with forecasts of wheat bulb fly (Leptohylemyia coarctata) infestation levels (Bardner, 1974), a consideration of economic thresholds is of limited use in determining the need for pesticide usage. There are other situations where information on the likelihood of economically damaging infestations is available but is not incorporated into a monitoring scheme. Thus, potato blight infection period warnings are issued purely on the basis of meteorological and cultural data, (Croxall and Smith, 1976), although the information is available which relates the level of blight infestation to economic loss in the crop (Grainger, 1967; Large, 1958; James, 1974).

One of the most persistent difficulties with forecasting or monitoring schemes is that of persuading farmers to behave in a more rational manner, as indicated by the warning. It has been shown (Tait, 1977 a) that farmers in a compact area of Bedfordshire were very variable in their usage of fungicide to control blight on potato crops. Figure 1 summarises the fungicide usage of the farmers surveyed, in relation to the expected fungicide usage on the basis of the Beaumont period warnings issued. Three Beaumont periods were recorded at Cardington in Bedfordshire during 1973, between June 19th and 22nd, June 26th and 28th, and July 27th and 29th, and farmers should have applied two blight sprays, one in the second half of June as the haulms closed over the rows, and one in late July, there being no justification for applying fungicide to early or second early potatoes (D. Humphreys Jones, pers. commun.). On maincrop potatoes, excluding cv. King Edward, there were fewer farmers applying two blight sprays than in any other category except five sprays. On the other hand, for cv. King Edward potatoes, which are more blight susceptible than other maincrop varieties, farmers applying two blight sprays were in the most common category.

Figure 1

Fungicide Usage on Potato Crops in Bedfordshire in 1973




----- Level of fungicide usage based on Beaumont Period warnings

It is usually assumed that farmers are risk-averse in their approach to pest control (Norton, 1976; Cammell and Way, 1977) and this was apparently the case for many of the farmers studied - all those who applied pesticide to early or second early potatoes, 40% of those growing maincrop potatoes, excluding cv. King Edward, and 39% of those growing cv. King Edward. However, in the case of early and second early potatoes, this kind of effect could also be due to the adoption of standard operating procedures, all potato crops being treated in the same way to cut down on the time and effort of decision-making (Tait, 1977b). Many of the farmers appeared to be risk-prone, 50% of those growing maincrop varieties other than cv. King Edward, but only 22% of those growing the more blight-susceptible cv. King Edward.

The variable reactions of farmers may be due in part to the ambiguous attitude of the ADAS itself to the blight warnings. This is illustrated by an exercise which was carried out to encourage the routine spraying of potatoes and reduce losses caused by blight (Gough, 1977). As a result of a promotional campaign, the acreage of potatoes sprayed was approximately doubled and the exercise was considered a great success. However, at the same time as one group of ADAS advisers was promoting routine spraying of the potato crop, presumably another group was continuing to issue Beaumont period warnings as usual.

It may be desirable to regard a forecast in the same way as a presticide manufacturer would regard a new product. This would mean giving detailed attention to the marketing strategy at an early stage in the development process, with increasing promotional effort as the forecast or warning system is perfected and ready for routine use by farmers, as opposed to the current "take it or leave it" approach. It may also mean that the attention given to a forecast by farmers is subject to fluctuations similar to those which occur in the life cycle of a manufactured product. Typically, there is an introductory phase of slow growth in use of the product, followed by an expansion phase of more rapid growth, leading on to maturity and saturation with no further growth possible. The next stage in a product life cycle is the decline phase where buyers abandon a product and find other ways of fulfilling their needs, and it is likely that a forecasting system which has been in existence for a considerable time will begin to suffer the same kind of decline as farmers revert to the routine use or non-use of pesticides. This could be the case currently with blight infection period warnings and the occurrence of this period of decline could perhaps be arrested by a return to more vigorous promotional tactics.

The assessment of the economic value of a forecast or warning system has been attempted on several occasions. One of the most widely-used techniques for studying decision-making and the value of information under conditions of uncertainty is Bayesian decision analysis. Using this technique, for example, it has been calculated that the value of a forecast for brown rot on peaches in California was \$5 to \$25 per acre for individual farmers (Carlson, 1971). Also, for frost forecasts provided to fruit growers in Oregon, the average seasonal value per day was \$5.39 (Baquet et al., 1976). While these results are interesting academically, the method is not applicable to the routine evaluation of forecasting schemes, principally because of the time and expense inherent in the calculation of utility functions for individual farmers. It is more generally relevant to important investment decisions where considerable sums of money are involved, rather than repetitive small-scale decisions such as occur in pest control at the farm level.

Another possible approach is the comparison of the gross margin of the forecasting scheme with an alternative pest control strategy. Cammell and Way (1977) have compared the cost of the forecasting scheme for black bean aphid (Aphis fabae), with that for routine preventive treatment, which was the most acceptable alternative to the grower. Using this method, the derived value of the forecasting scheme is dependent mainly on the choice of alternative strategy for comparison. It would be interesting to know the value of the forecast, compared to the most rational alternative - eradicant insecticide treatment, applied when aphid colonies are observed on the crop. The other important factor in the calculation of the gross margin of a forecasting scheme is the level at which the economic threshold is set. Cammell and Way, and most other forecasters, admit that their forecasts are conservative (i.e. the economic threshold is set at a lower level of pest infestation than should rationally be the case) because it is assumed that farmers are not prepared to tolerate crop losses, even when these are less than the cost of insecticide treatment. In the case of the black bean aphid forecast, with an economic threshold of 5% of stems infested, the maximum crop loss experienced by farmers was 0.7% of the total yield, which was equivalent to only 10% of the cost of treatment in most years, and so a large number of farmers still treated their crops needlessly. This difference may represent the risk aversion of the forecasters, the risk being that farmers will cease to pay attention to the forecast if crop losses become too obvious to them. If the economic threshold level were allowed to increase, the probability of a forecast indicating no significant aphid attack would also increase, and it is necessary to know the relationship between these two factors in order to assess fully the potential economic value of a forecast.

## DISCUSSION

It is interesting to speculate on what will be the end result in pest control terms if farmers apply their assumed risk aversion to a forecast which is also risk averse. The result may be less rational than if no forecast were issued. If more information were available on farmers' attitudes to risk in pest control, the economic threshold could be modified or weighted to take account of the farmers' likely reactions to the forecast, giving an "action" threshold. This is one of the reasons for advocating the inclusion of an economist or social psychologist at an early stage in the development of a forecasting system.

The effect on the overall pest control situation of various levels of compliance with a forecast should also be considered. Where the pest is confined to a single crop, as with the pea moth (Cydia nigricana) (Lewis et al., 1975) more rational and effective pesticide usage could reduce pest levels to the extent that control measures are not necessary every year. Thus, a high level of observance of the forecasts could produce considerable public benefits as well as the private benefits to the individual grower. This possibility does not exist with a more ubiquitous pest such as the black bean aphid.

If the number of forecasts issued to farmers is to increase in the future, it will be necessary to know the likely effect of this on farmers' behaviour - increased compliance, or contempt bred by familiarity. Increased compliance is more likely to occur where a sustained effort is made to educate farmers to do their own scouting where this is feasible, and to encourage the acceptance of crop losses up to the economic threshold.

The resources currently allocated to the ADAS are probably not sufficient to permit them to put the necessary effort into promoting the uptake of more rational pest control systems, even those which are currently available. In a small-scale study of factors affecting the usage of pesticides by fruit and vegetable farmers (Tait, 1977b), those who relied primarily on the ADAS for advice were not enabled to use less pesticide. Also, ADAS have not been given any clear guidelines on their approach to the more rational use of pesticides. A clear definition of aims and priorities, inevitably politically-inspired, is necessary before the current somewhat chaotic situation can begin to be remedied.

## References


BARDNER, R. (1974) Leptohylemyia coarctata (Full.) - Integrated control and the use of economic thresholds, EPPO Bull., 4(3), 329-334.

BAQUET, A.E. et al. (1976) The value of frost forecasting: a Bayesian appraisal, Am. J. Agr. Econ., 58(3), 511-520.

CAMMELL, M.E. and WAY, M.J. (1977) Economics of forecasting for chemical control of the black bean aphid, Aphis fabae, on the field bean, Vicia faba, Ann. Appl. Biol., 85, 333-343.

CARLSON, G.A. (1971) The microeconomics of crop losses. In Economic Research on Pesticides for Policy Decision Making, USDA, Economic Research Service, Washington, D.C., 89-101.

CROXALL, H.E. and SMITH, L.P. "The epidemiology of potato blight in the East Midlands, 1923-74, Ann. Appl. Biol., 82, 451-466.

GOUGH, H.C. (1977) Advice and development in applied biology, Ann. Appl. Biol., 85, 1-12.

GRAINGER, J. (1967) Economic aspects of crop losses caused by diseases, Proc. FAO Symp. on Crop Losses, 55-98.

JAMES, W.C. (1974) The assessment of economic losses due to late blight of potato, EPPO Bull., 4(3), 335-338.

LARGE, E.C. (1958) Losses caused by potato blight in England and Wales, Pl. Path., 7, 39-48.

LEWIS, T., WALL, C., MACAULAY, E.D.M. and GREENWAY, A.R. (1975). The behavioural basis of a pheromone monitoring system for pea moth, Cydia nigricana, Ann. Appl. Biol., 80, 257-274.

MATHYS, G. (1970) Co-ordination of plant protection means, EPPO Public. Ser. A, No. 57, 9-11.

NORTON, G.A. (1976) Analysis of decision making in crop protection, Agroecosystems, 3, 27-44.

TAIT, E.J. (1977a) A method of comparing pesticide usage patterns between farmers, Ann. Appl. Biol. 86(2), 229-240.

TAIT, E.J. (1977b) Factors affecting the usage of insecticides and fungicides on fruit and vegetable crops in Great Britain; I, Crop-specific factors. J. Environ. Managemt., in press.

WAY, M.J. and CAMMELL, M.E. (1973) The problem of pest and disease forecasting - possibilities and limitations as exemplified by work on the bean aphid, Aphis fabae, Proc. 7th Br. Insectic. Fungic. Conf., 933-954.

WHEATLEY, G.A. (1974) The role of biology in the control of pests and diseases of vegetable crops. In Biology in pest and disease control, eds. D. Price Jones and M.E. Solomon, Blackwell Sci. Pub., Oxford, 277-293.

WILDBOLZ, Th. (1970) Large scale or local forecasts and warnings: important factors, EPPO Public Ser. A, No. 57, 85-87.

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# PEST ATTACK FORECASTS: ARE THEY OF DUBIOUS VALUE?

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Summary The annual incidence of damage by some sugar beet pests, as recorded by the British Sugar Corporation field staff since 1947, fluctuates considerably. Results are given for Apodemus sylvaticus, Atomaria linearis, Aphis fabae and Pegomya betae. Their forecasting and/or predictive monitoring has been or is being studied but the value of forecasting appears dubious, especially for P.betae and A.fabae. Forecasts can lead to growers' complacency, or consternation and misuse of pesticides, and are invariably too late for pesticide manufacturers, but their study does enable the research worker to better understand the pest's epidemiology. Predictive monitoring is more valuable but should not take precedence over work on grower's methods of determining economic thresholds, or on pesticide materials, methods and time of application, and, especially, on alternative methods of control.

Résumé Depuis 1947, selon les comptes rendus du personnel agricole de la British Sugar Corporation, les niveaux annuels des dégâts occasionés par des parasites betteravières ont variés considérablement. Les résultats sont donnés à propos d'Apodemus sylvaticus, Atomaria linearis, Aphis fabae et Pegomya betae. La prévoyance et/ou l'avertissement-prédit ont été recherchés mais leur valeur paraît douteux, surtout vis à vis de P.betae et A.fabae. La prévoyance peut rendre les planteurs complaisants ou effrayés, et peut aussi mener au mauvais emploi des pesticides, et elle est toujours trop tard pour les fabricants. Mais les études peuvent aider la connaissance de l'épidemiology des parasites. L'avertissement-prédit a plus de valeur mais ne doit pas devenir plus important que la recherche fait au sujet de la détermination des seuils economiques, aux pesticides eux-mêmes, aux moyens et aux moments des traitements, et surtout aux autres méthodes de l'elimination des parasites.

## INTRODUCTION

The 205,000 ha of sugar beet in England suffer damage from many vertebrate and invertebrate pests (Jones and Dunning, 1972), all of which are similarly damaging in Europe (Dunning, 1972, a). Since 1947 the British Sugar Corporation (B.S.C.) field staff have made monthly records of pest damage to the crop (Dunning, 1975). The national incidence of damage by each pest fluctuates greatly, reflecting mainly the pest's abundance, but perhaps also in part the differing susceptibility of the crop to damage in different seasons.

Data for four major pests, with contrasting epidemiology and damage, are presented and discussed in relation to possible forecasting of damage. The value of forecasts to the adviser, and especially to the pesticide manufacturer and the grower, is questioned.

## METHODS

From 1947 to 1956 the Agriculturist at each of the seventeen sugar factories in England reported for each month, April - September inclusive, the areas of sugar beet that failed, or were "severely", "moderately" or "slightly" affected, due to pest damage, despite any control measures taken. From 1957 the quality of recording was improved by obtaining monthly reports (April - July only) from each fieldman (150 in 1957 but decreasing gradually to about 90 in the last few years) and by specifying more precisely the differing degrees of damage.

Each fieldman today is responsible for 800 - 2000 ha; he knows the crop problems of his area well. His best estimate each month of the different categories of pest damage is a subjective but practical record. The monthly data are converted into an annual damage index for each pest by summing, for each of the four months, the number of hectares failed plus one tenth of the number of hectares severely damaged plus one hundredth of the number of hectares moderately damaged plus one thousandth of the number of hectares slightly damaged (see Dunning, 1975, for fuller details and explanation).

Of the pests considered here, damage by the wood mouse (Apodemus sylvaticus) (Figure 1) has been recorded only since 1971; previous to this minor damage had occasionally been suspected but the area affected was not recorded. The annual damage indices for pygmy beetle (Atomaria linearis), black bean aphid (Aphis fabae) and beet leaf miner (Pegomya betae) (Figures 2 - 4) are each averaged over the decade 1947 to 1956 when the recording system was less accurate; this average gives an indication of the pest's status at that time, i.e. largely before the use of pesticides.

## RESULTS AND DISCUSSION

Apodemus sylvaticus Taking of seed by wood mice was first recorded in 1971 (Dunning, 1972, b) and was particularly severe in 1974 (Figure 1). It occurred earlier on a small scale but was dismissed as of no significance when seeds were sown closer. Wood mice occur in many arable fields; they are most numerous in autumn but decline thereafter until successful breeding recommences the following summer (Green, 1975, 1976, 1977). Sugar beet seed is not taken after germination; the earlier the seed is sown, the longer it is likely to take to germinate and the longer it is "at risk".

Despite wood mice being present in a field, seed taking is not inevitable but seems to be triggered by the mice first finding uncovered seeds. Damage is controlled by poisoning, using about three bait points/ha; prophylactic treatment is not recommended, but curative treatment must be applied immediately damage occurs because the effect of the poison is not immediate.

Warning in March that wood mice are numerous and that control is necessary, especially if the beet is to be sown early, is very desirable. Practicable methods of population-assessment in the different sugar beet areas in February are needed and are being tested; a warning system, operated by the B.S.C., is envisaged.

Atomaria linearis The considerable fluctuations in pygmy beetle damage (Figure 2) led to a study, with the Government Meteorologists, of the relationship between the damage index and weather conditions. It was assumed that April - May weather suitable for dispersal from old to new beet fields was necessary; no correlation was found, but damage was most severe in years when the previous autumn was dry (G.W. Hurst, in litt.). Further work on the data is in progress, including consideration of sticky and suction-trap catches (Thornhill and Dunning, 1977). The results obtained by the meteorologist suggest that the entomologist must reassess the epidemiology of pygmy beetle attacks. Forecasting damage, on the basis of the previous summer weather, may be possible; the dubious value of such a forecast is discussed below.

Aphis fabae Alternation, in successive years, of more and less damage by black bean aphid on sugar beet was noted in 1941 - 1946 (Jones and Dunning, 1972) and is apparent in the more recent damage indices (1957 - 1965, Figure 3). Data are not available for 1966 - 1967 because no records were made in July; since then the alternation has been in two year periods. Forecasting damage to field beans is being done (Way et al, 1977); the reliability of these forecasts, and their possible value for sugar beet, are discussed below.

Pegomya betae Beet leaf miner seems no longer to be an important pest of sugar beet in England, the last major damage occurring in 1963 and 1964 (Figure 4: the damage index for 1966 - 1967 is imprecise because no record was made of second generation attacks in July). No attempt to forecast damage has been made in England but it has been done in W. Germany, and is based on overwintering populations of pupae collected either from wash water at sugar factories or from soil samples. Four categories of expected injury are specified, ranging from "of no economic importance" to "pest likely to be prevalent, causing widespread losses" (Winner and Schäufele, 1973).

## GENERAL DISCUSSION

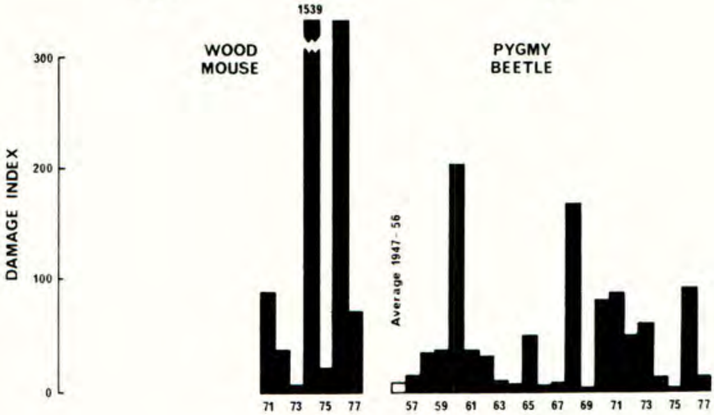
Pest problems on sugar beet, as on other crops, fluctuate annually. The desire to forecast damage seems innate in the applied zoologists' current thinking, but is forecasting worthwhile, especially to the recipient? The answer should be 'yes' but many qualifications must be made.

Where prophylactic measures must be applied well in advance of pest damage, the need to be able to forecast that damage appears obvious; "avoiding action" can surely then be taken in those seasons when necessary. However, despite our increasing ability to forecast pygmy beetle damage to sugar beet, would long-term forecasts to the B.S.C. be worthwhile? Gamma HCH applied to the seedbed, or aldicarb applied in the seed furrow at sowing, give the best, though not complete, control. But the former can have adverse side effects (e.g. increase of subsequent virus yellows incidence) and excess use of the latter must be discouraged because of the risk of hastening resistance to carbamates in aphids. Damage by pygmy beetle has not been sufficiently great to justify advising use of these chemicals. Giving the B.S.C. forecasts of pygmy beetle damage is not envisaged.

It is difficult to justify the forecasting, by research workers, of pest attacks where the grower can readily assess the need for treatment, economic thresholds having been established, and where curative treatment is effective. Beet leaf miner is such a pest, and yet forecasting is attempted in Germany in February for attacks that occur in late May. The accuracy of such general and early forecasts applied to individual fields must be in doubt, especially as the level of attack and severity of damage is much influenced by plant population, and stage and rate of growth. Control of beet leaf miner is only worthwhile with severe and early attacks and, if aphids are beginning to infest the crop, there is a risk of adverse side effects from using the pesticide normally recommended, trichlorphon (Dunning and Winder, 1965).

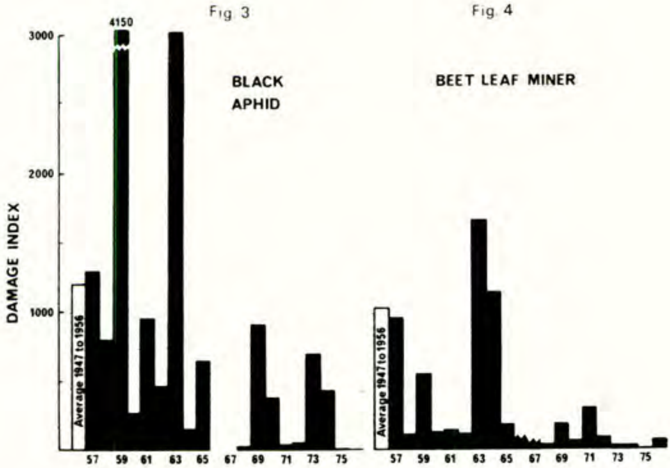
Similarly dubious is the value of forecasting, in March, black bean aphid infestation of field beans, in June, on the basis of winter egg counts on spindle (Euonymus spp.). Area forecasts, with all the opportunities for error, must be much inferior to crop inspection and the grower's decision to treat on the basis of readily determined economic thresholds, i.e. when 5 - 10% of bean plants are seen to be infested on the S.W. headland; the grower can also take into account stage of

Fig 1 Fig 2




Mean damage index, 1947 - 56, for pygmy beetle and annual index, 1957 - 77, for wood mouse and pygmy beetle.

Fig 3 Fig 4




Mean damage index, 1947 - 56, and annual damage index, 1957 - 76, for black bean aphid and beet leaf miner.

crop growth, likely water stress, etc. Or does the black bean aphid forecast aim merely to advise the adviser? Forecasts of black bean aphid attacks were given for sugar beet in the early 1940s when control hinged on the efficient deployment of a very limited number of nicotine fumigation machines. Such constraints no longer apply and, for sugar beet, the grower is advised by the sugar factory of the need for pesticide application when aphid populations, determined by daily counts in 10 - 50 random beet fields examined in the factory area, reach critical levels. "Spray warning postcards" almost invariably advise the grower to inspect his own crop before deciding whether or not to spray. Surely the farmer should be encouraged to do this, rather than rely solely on a general area forecast?

There is also the risk that work devoted to forecasting is at the expense of more pressing problems. Are we sure that black bean aphid is not developing resistance to pesticides? Should not more work be done on choice of pesticides, methods of application and time of application? These are certainly our current thoughts at Broom's Barn in relation to the sugar beet aphid spray warning system, aimed mainly at control of virus yellows. The ability to forecast incidence of yellows is improving, but the forecast is not available until early May (Cochrane and Heathcote, pers. comm.). This is far too late to advise seed-furrow application of systemic aphicides. The forecast appears only to help interpret better the threat from the developing aphid infestation on the crop, as determined by B.S.C. fieldmen.

Although further work on forecasting might help improve the efficiency and economical use of pesticides, it seems preferable to concentrate our longer term research on alternative control measures.

## References

DUNNING, R.A. (1972, a) A survey of sugar beet pest and disease incidence and damage, and pesticide usage in Europe. Journal of the International Institute for Sugar Beet Research, 6, 19-34.

DUNNING, R.A. (1972, b) Seedling pests and diseases. Rothamsted Report for 1971, 271.

DUNNING, R.A. (1975) Arthropod pest damage to sugar beet in England and Wales, 1947-74. Rothamsted Report for 1974, Part 2, 171-185.

DUNNING, R.A. and WINDER, G.H. (1965) The effect of insecticide applications to the sugar beet crop early in the season on aphid and yellows incidence. Plant Pathology, 14, 30-36.

GREEN, R.E. (1975, 1976, 1977) Wood mouse studies. Rothamsted Reports for 1974, 1975, 1976, pp. 51, 56, 54, respectively.

JONES, F.G.W. and DUNNING, R.A. (1972) Sugar beet pests. Ministry of Agriculture, Fisheries and Food. Bulletin 162. 3rd Ed. London, H.M.S.O., 113 pp.

THORNHILL, W.A. and DUNNING, R.A. (1977) Arthropod pests of seedlings. Rothamsted Report for 1976, Part 1, 52.

WAY, M.J., CAMMELL, M.E., ALFORD, D.V., GOULD, H.J., GRAHAM, C.W., LANE, A., LIGHT, W.I.St.G., RAYNER, J.M., HEATHCOTE, G.D., FLETCHER, K.E. and SEAL, K. (1977) Use of forecasting in chemical control of black bean aphid, *Aphis fabae* Scop., on spring-sown field beans, *Vicia faba* L. Plant Pathology, 26, 1-7.

WINNER, C. and SCHÄUFELE, W. (1973) *Pegomya betae* in Crop Loss Assessment Methods, F.A.O., Rome.

Notes

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# THE POTATO APHID SPRAY WARNING SCHEME IN SCOTLAND, 1975 - 1977

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Summary A "threshing method", used to detect the presence of aphids on young potato plants, showed that many Scottish potato crops were colonised by aphids shortly after plant emergence in 1975 and 1976 but that aphids arrived on crops later in 1977. Spray warnings were sent to local growers when the populations of Myzus persicae or Macrosiphum euphorbiae exceeded low threshold levels. The relationship between the date when the first potato aphids were caught in 12.2 m suction traps and the date of detection in adjacent potato crops was very variable. In general, suction traps caught few potato aphids during the early migration when emerging potato crops were colonised.

Résumé Plusieurs plantations de pomme de terre en Ecosse furent envahies quelque temps après la levée par des pucerons. On a utilizé la methode suivante pour detecter la présence de pucerons sur de jeunes plantes - à savoir secouer vigoureusement les fanes. Quand le nombre de Myzus persicae ou Macrosiphum euphorbiae a dépassé la valeur minimale les planteurs des environs furent avisés que le moment était venu de pulveriser les plantations. Le rapport entre la date de la presence des premiers pucerons dans les pièges à succion du type 12.2 m Rothamsted Insect Survey et la date ou ils ont été decouverts dans les plantations locales était très variable. En general, peu de pucerons sont captés par les pièges a succion durant le debut des migrations à un moment ou les jeunes plantations sont déja envahies de pucerons.

## INTRODUCTION

The low rate of spread of potato leaf roll virus (PLRV) and potato virus Y was one of the main reasons why a seed potato industry developed in Scotland. Until recently there had been no serious epidemic of aphid-borne virus in Scotland since 1945 (Todd, 1961), and there was no need for the routine use of aphicides because cultural methods were effective in preventing virus spread. The combined effects of roguing to remove virus-infected plants and eliminating crops above virus tolerance levels from seed production, by means of the Department of Agriculture and Fisheries for Scotland (D.A.F.S.) Seed Potato Certification Scheme, led to a progressive decline in the amount of PLRV in seed crops during the 1960s. By 1970 PLRV had been reduced to negligible levels (Howell, 1973).

In 1973 the level of PLRV in seed potato crops started to increase considerably and by 1976 PLRV was present in over 50% of the crops entered for certification (Howell, 1977). The increase in PLRV was clearly associated with the milder winters since 1971 which allowed more aphids to survive and infest potato crops earlier than in previous years. Early colonisation decreases the effectiveness of roguing because virus may spread before the plants growing from infected seed develop obvious symptoms. In these circumstances early aphid control is essential if virus spread is to be prevented. Unfortunately, when the recent epidemic of PLRV started there was little contemporary information about aphid infestations on seed potato crops in Scotland. The 12.2 m high Rothamsted Insect Survey (R.I.S.) Suction Traps, operated in Scotland since 1965, provided historical evidence of earlier migrations of potato aphids (Taylor & French, 1975), but it was not known how this information related to crop infestations or virus spread.

Work was started in 1975 to monitor the arrival of aphids on unprotected potato crops, to follow the early stages of crop colonisation and to advise seed potato growers of the need for aphid control. The numbers and date of arrival of aphids on potato crops were compared with the catches of aphids in 12.2 m suction traps in Scotland operated by the R.I.S. and D.A.F.S.

## METHODS

Crop sampling Potato crops within 100 km of 12.2 m suction traps were sampled regularly for aphids. We sampled 24 crops in 1975 and 1977 and 28 crops in 1976 (Fig. 1). At most sites maincrop potatoes (most commonly Maris Piper or Pentland Crown) were sampled. The main objective was to discover when aphids first arrived on the crops. The "threshing method" (Hille Ris Lambers, 1972) was used to facilitate rapid sampling while potato plants were small. In this method aphids were collected from random samples of 100 hills (complete potato plants) by tapping the foliage for a few seconds over two, white painted, rimmed boards held closely against the emerging haulms. When plants became too large to sample in this way aphids were counted on 50 or 100 haulms, or the "100 leaf" method (Broadbent, 1948) was used. These aphid counts were converted to "numbers per 100 hills". In 1975 and 1976 crops were sampled at weekly intervals, and when 5 or more adult Myzus persicae or 10 or more adult Macrosiphum euphorbiae per 100 potato hills were found a spray warning was issued. In view of the high incidence of PLRV in 1976 the threshold for issuing a spray warning in 1977 was decreased to 2 adult M. persicae or 10 adult M. euphorbiae per 100 potato hills. Few Aulacorthum solani were found and they were not taken into consideration when issuing spray warnings.

Aphid trapping Fig. 1 shows the location of the six 12.2 m suction traps in Scotland. The trap which started at Musselburgh on 14 June 1976, was operated at Pathhead (Dalkeith) in 1977. The 1976 catches from this trap are not considered here as migration started before the trap was operated.

Spray warnings Growers were advised to spray with an approved aphicide when aphid numbers in their area exceeded the threshold levels. In the North College area warnings were given in the local press and on radio and TV. In the East and West College areas cards were posted to individual growers, merchants and spray contractors. Warnings were also issued in the local press and on radio in the East College area. Starting in 1977 aphid spray warnings were publicised nationally in the press and on radio and TV.

Fig. 1. The distribution of potato crops sampled in 1975-77 (●) and 12.2 m suction traps (★). Musselburgh trap (☆) moved in 1977.


RESULTS AND DISCUSSION

Aphid arrival in potato crops Using similar sampling methods on crops from Easter Ross in the north to Dumfriesshire in the south west we were able to compare the dates on which potato crops throughout Scotland were colonised by aphids. In 1975 aphids were found on potato crops shortly after plant emergence as far north as Angus and Perthshire. Only in Aberdeenshire were crops mature before they became infested. In 1976 aphids arrived even earlier (Table 1) and were found on potato crops throughout Scotland at or soon after emergence. In Aberdeenshire crops were colonised six weeks earlier than in 1975. In 1977, after a more severe winter, aphid arrival was considerably later than in 1975 and 1976, and the pattern of colonisation was much more like that described by Shaw (1955) and Fisken (1959), with crops in the more traditional seed growing areas remaining free of aphids until mid to late July. To some extent we may have detected aphids earlier than previous workers in Scotland because we used the threshing method, but few early aphids were found by threshing in 1977. The method has been used in The Netherlands for 40 years to assess aphid arrival on seed potato crops (Hille Ris Lambers, 1972) and we found it ideal for advisory work. Staff could be trained quickly and one person could sample 100 potato hills in about 1 hour. Winged aphids were easily detected, but the method was not suitable for very small or large plants.

Within all areas the dates on which the first aphid was found varied widely in different fields, but the dates when threshold levels were reached were more consistent within an area. M. persicae usually reached threshold levels before M. euphorbiae, but in 1977 when the threshold for M. persicae was lowered, there was little difference in threshold dates and in parts of the West College area the first spray warnings were issued when threshold counts of M. euphorbiae were reached although the numbers of M. persicae were still low.

Table 1
Dates of spray thresholds and first records of M. persicae in suction traps

| Area[1] | 1975 | | 1976 | | 1977 | |
|---|---|---|---|---|---|---|
| | Crop[a] | S.T.[b] | Crop[a] | S.T.[b] | Crop[a] | S.T.[b] |
| Moray coast/Black Isle/Easter Ross | July 23 | July 25 | June 12 | May 21 | July 25 | April 25 |
| Aberdeenshire | July 31 | | June 15 | | * | |
| Kincardineshire | | | June 11 | | July 25 | |
| Angus/E. Perths. | June 25 | | June 6 | | July 7 | |
| Fife/S. Angus/ Carse of Gowrie | June 7 | June 23 | June 1 | May 31 | June 15 | June 30 |
| Lothians | June 9 | June 6 | May 21 | June 9 | July 8 | July 8 |
| Borders | June 9 | | June 1 | | July 15 | † |
| W. Perthshire/ Stirlingshire | June 6 | | June 8 | May 25 | July 21 | † |
| Lanarkshire | June 23 | | June 8 | | July 14 | |
| Ayrshire/ Renfrewshire | June 6 | June 7 | June 3 | July 2 | June 15 | July 31 |
| Dumfriesshire/ Kirkcudbrights. | June 9 | | June 4 | | July 15 | |

[1] Areas arranged in order moving south and west down the table

[a] First spray threshold date determined by aphid counts on potato crops

[b] Date of first M. persicae in local suction trap

* Threshold not reached by 4 August 1977

† No M. persicae up to 31 July 1977

Comparison of crop sampling and suction trap catches In 1976, when potato aphids were most abundant, they were caught in suction traps before thresholds were reached on local crops at all sites except Auchincruive. In 1975, however, winged M. persicae were found on crops in Fife and South Angus almost three weeks before the first specimen was caught in the suction trap at SHRI, and the spray threshold was reached two weeks earlier (Table 1). Similarly in 1977, when potato aphids were much scarcer, some crops were colonised before suction traps registered their arrival.

The main difficulty in issuing spray warnings based on the dates when potato aphids were first caught in suction traps was the unpredictable variation between crop colonisation and trap catch from site to site and year to year. At SHRI in 1976 there was a very close agreement between the spray threshold date (1 June) and the first M. persicae caught in the suction trap (31 May), but no more specimens were caught while the population of winged M. persicae on the crop (King Edward) rose in the next two weeks to 26 per 100 hills. At the same time the numbers of wingless aphids were increasing, and by early July the total population of M. persicae reached an estimated peak of over 10,000 per 100 hills. It was not until 2 July when winged M. persicae started to migrate from potato that the next specimens were caught in the suction trap.

Table 2 shows the total numbers of three aphids (M. persicae, Myzus certus which is morphologically very similar, and M. euphorbiae) caught during the primary (Spring migration). In 1976 potato crops throughout Scotland were colonised during the primary migration, broadly defined here as 14 May - 21 July (31 July in 1977), when aphids were dispersing from their overwintering hosts, but the traps caught relatively few aphids at the time when threshold populations appeared on potato crops and most were caught towards the end of the primary migration. Similar results were noted from most trap sites in the other two years. The exception to this pattern occurred at Elgin in 1975 where the trap caught many M. persicae and M. euphorbiae at the time when local crops were colonised but this was not until late July when the secondary migration of aphids further south had started.

M. certus was relatively common in the Scottish suction traps during the period when potato crops were colonised by M. persicae (Table 2). M. certus does not commonly infest potato and is not thought to transmit PLRV but until recently it was often mis-identified from trap catches as M. persicae, and also proved a problem for advisers in The Netherlands (Hille Ris Lambers, 1972).

12.2 m suction traps sample widely-dispersing airborne insects rather than local populations (Taylor, 1974). Some of the fields we sampled were nearly 100 km from the nearest trap (Fig. 1), yet there was often a fairly close agreement between the first potato aphids caught in traps and their detection in potato crops. The important question to consider is whether the effort put into crop sampling was necessary for advisory purposes, or whether the suction trap information would have been adequate. Heathcote et al (1969) showed that local R.I.S. suction traps were more effective in detecting the arrival of winged M. persicae than the routine inspection of sugar beet crops carried out by the British Sugar Corporation fieldmen. In The Netherlands, however, a similar trap failed to catch M. persicae during the primary migration even though large numbers were threshed from local potato crops (Taylor, 1974). Our results suggest that suction traps are potentially unreliable, particularly for M. persicae, because they catch very few aphids during the primary migration when, in early aphid years such as 1975 and 1976, many potato crops are colonised (Table 2). If the failure of suction traps to catch primary migrants merely reflects the small numbers of aphids flying at that time of year detection could be improved by increasing the volume of air sampled by the traps. However, any behavioural differences between migrants at different times of the year (eg. low level flights of early migrants) would probably go undetected by 12.2 m suction traps (Taylor, 1977).

Table 2

Numbers of aphids in 12.2 m suction traps during the primary migration period (a) and at the spraying threshold (b, c)

| Suction trap site | 1975 a | 1975 b | 1975 c | 1976 a | 1976 b | 1976 c | 1977 a | 1977 b | 1977 c |
|---|---|---|---|---|---|---|---|---|---|
| M. persicae | | | | | | | | | |
| Elgin | 0 | 0 | 26 | 12 | 0 | 2 | 0 | 0 | 0 |
| Dundee | 16 | 0 | 2 | 40 | 1 | 0 | 2 | 0 | 0 |
| East Craigs | 47 | 1 | 2 | 50 | 2 | 5 | 3 | (0) | (3) |
| Auchincruive | 10 | 0 | 1 | 9 | 0 | 0 | 1 | 0 | 0 |
| Stirling | - | - | - | 25 | 1 | 9 | 0 | 0 | 0 |
| M. certus | | | | | | | | | |
| Elgin | 1 | 0 | 5 | 5 | 0 | 3 | 0 | 0 | 0 |
| Dundee | 6 | 0 | 4 | 9 | 0 | 3 | 0 | 0 | 0 |
| East Craigs | 14 | 0 | 4 | 30 | 2 | 10 | 2 | (1) | (1) |
| Auchincruive | 5 | 0 | 1 | 7 | 0 | 4 | 1 | 0 | 0 |
| Stirling | - | - | - | 32 | 4 | 10 | 0 | 0 | 0 |
| M. euphorbiae | | | | | | | | | |
| Elgin | 3 | 2 | 84 | 53 | 4 | 34 | 1 | 0 | 1 |
| Dundee | 44 | 3 | 15 | 54 | 2 | 5 | 15 | 1 | 0 |
| East Craigs | 73 | 5 | 16 | 85 | 1 | 7 | 11 | (5) | (6) |
| Auchincruive | 15 | 0 | 2 | 22 | 0 | 2 | 6 | 0 | 0 |
| Stirling | - | - | - | 41 | 1 | 16 | 2 | 0 | 0 |

a Primary migration period = 14 May - 21 July in 1975, 1976; 14 May - 31 July 1977

b Number of aphids caught in 14 days preceding spray threshold

c Number of aphids caught in 21 days following spray threshold

\- No data; ( ) 1977 data incomplete at date of publication

Trap catches are of limited value if they detect early aphid flights long before potato crops emerge. In 1977, for example, the first M. persicae was caught in the Elgin trap on 25 April several weeks before crop emergence and almost three months before the first M. persicae was found on potatoes at Elgin. Admittedly, it is difficult to assess how many crops were infested at the time spray threshold levels of aphids were detected on sampled fields. Thresholds might indicate little more than the enthusiasm of local entomologists in finding and sampling early crops, but they do at least demonstrate the existence of aphid populations on potato crops. Although the threshold levels used were arbitrary they seemed to give a reasonable picture of alate deposition and population increase in 1975 and 1976.

We need more information about the amounts of virus spread during the early stages of aphid increase on potato before we can fully establish the risk posed by these small numbers of aphids. In England early spread of PLRV is important (Burt et al, 1964), and Cadman and Chambers (1960) suggested that the spread of PLRV in eastern Scotland was greatest in the areas where aphids colonised potato crops early in the season. Nevertheless, whether early migrating aphids or subsequent generations spread PLRV prompt treatment is desirable. Before 1975 aphids were not recognised as a limiting factor in seed potato production and very few growers used aphicides (Potato Marketing Board, 1975). The original purpose of the present advisory campaign was to promote their use, and this has been largely achieved. There has been a considerable increase in the amount of insecticides used on potatoes since 1973. In 1975, 41% of the main crop acreage in Scotland was treated with insecticides (Potato Marketing Board, 1976) and in 1976 a survey of pesticide usage on potato in Scotland showed that 80% of the grower dealers who were interviewed sprayed aphicides, and 15% used granular aphicides at planting (G. G. Tucker, personal communication). In many areas of the south, east and west of Scotland it was difficult to find untreated fields to sample for aphids in 1977. Efficient chemical control of aphids on seed potato crops will continue to be essential while levels of PLRV remain high. The use of granular aphicides at planting may become more widespread but such materials are unlikely to give full protection throughout the growing season and information on aphid colonisation of potato crops will still be necessary. Even in recent years there were some seed-growing areas, particularly in the north east of Scotland where potato aphids remained scarce and the value of insecticidal control was questionable. The potato aphid spray warning scheme provides a method for avoiding the unnecessary use of insecticides and thus promotes their rational use. With the sampling methods and administrative procedures firmly established the scheme has already been enlarged to provide additional information (eg. on roguing and haulm destruction) and could well be modified in the future to give advice on the incidence of insecticide-resistant aphids in Scottish seed potato crops and the use of selected pesticides for their control.

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## References


BROADBENT, L. (1948) Methods of recording aphid populations for use in research on potato virus diseases, Annals of Applied Biology, 35, 551-566.

BURT, P.E., HEATHCOTE, G.D. & BROADBENT, L. (1964) The use of insecticides to find when leaf roll and Y viruses spread within potato crops, Annals of Applied Biology, 54, 13-22.

CADMAN, C.H. & CHAMBERS, J. (1960) Factors affecting the spread of aphid-borne viruses in potato in Eastern Scotland. III. Effects of planting date, roguing and age of crop on the spread of potato leaf roll and Y viruses, Annals of Applied Biology, 48, 729-738.

FISKEN, A.G. (1959) Factors affecting the spread of aphid-borne viruses in potato in Eastern Scotland. II. Infestation of the potato crop by potato aphids, particularly *Myzus persicae* (Sulzer), Annals of Applied Biology, 47, 274-286.

HEATHCOTE, G.D., PALMER, J.M.P. & TAYLOR, L.R. (1969) Sampling for aphids by traps and by crop inspection, Annals of Applied Biology, 63, 155-166.

HILLE RIS LAMBERS, D. (1972) Viruses of potatoes and seed potato production PUDOC, Wageningen, 36-56.

HOWELL, P.J. (1973) The relationship between winter temperatures and the extent of potato leaf-roll virus in seed potatoes in Scotland, Potato Research, 16, 30-42.

HOWELL, P.J. (1977) Recent trends in the incidence of aphid-borne viruses in Scotland. Proceedings of the Symposium on Problems of Pest and Disease Control in Northern Britain, Dundee 1977, 26-28.

POTATO MARKETING BOARD (1975) Report on the Survey of main crop potato production 1973. P.M.B. Oxford.

POTATO MARKETING BOARD (1976) Report on the survey of main crop potato production 1975/6. P.M.B. Oxford.

SHAW, M.W. (1955) Preliminary studies on potato aphids in north and north-east Scotland, Annals of Applied Biology, 43, 37-50.

TAYLOR, L.R. (1974) Monitoring change in the distribution and abundance of insects, Report of Rothamsted Experimental Station for 1973, Part 2, 202-239.

TAYLOR, L.R. (1977) Migration and the spatial dynamics of an aphid, *Myzus persicae*, Journal of Animal Ecology, 46, 411-423.

TAYLOR, L.R. & FRENCH, R.A. (1975) The Rothamsted Insect Survey, Rothamsted Experimental Station Report for 1974, 104-106.

TODD, J.M. (1961) The incidence and control of aphid-borne potato virus diseases in Scotland, European Potato Journal, 4, 316-329.

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# CHANGES IN COMPOSITION IN POPULATIONS OF THE PEACH POTATO APHID, MYZUS PERSICAE, OVERWINTERING IN SCOTLAND IN 1976-7

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Summary Measurements of the genetic composition of successive samples of *Myzus persicae* (Sulz.) collected from two populations (on white mustard and on swedes) were carried out last winter, during the critical period November/December/January, to assess the differential effect of frost on the different clones in the populations. The short spell of frost at the beginning of December appeared to have little selective effect, whereas the much longer period of frost in January appeared to select strongly for one clone on the mustard and for another clone on the swedes. The correctness of this interpretation is supported by the predominance of these clones in samples from nettle populations collected shortly after this date. Because the common OP resistant clone may be a mutant of one of these frost survivors, this result raises the possibility that frost may select for and not against OP resistance on certain overwintering hosts.

## INTRODUCTION

A single *Myzus persicae* specimen contains enough protein and enzymic activity to make it possible to carry out electrophoretic assays for genetic variation at a number of protein and enzyme loci. Three of these assayable loci are polymorphic, that is, their protein or enzyme product can have a different electrophoretic mobility or activity in different *M. persicae* specimens. It has therefore been possible to classify these aphids on the basis of their electrophoretic phenotype (electromorph) at each of these loci. These electromorphs are inherited unchanged during parthenogenesis, so that all the members of a particular clone have the same combination of electromorphs, and each combination must be characteristic of specimens belonging to a subset of the total set of clones. Thus groups of clones can be typed biochemically, providing a useful tool for investigating changes in the proportions of different clones in a population (Baker, 1977a & 1977b).

The polymorphic loci are:

(a) An esterase sensitive to eserine. This is found in three electromorphs which appear to be the electrophoretically slow homozygote (ss), the fast homozygote (ff), and the heterozygote (sf) of a dimeric enzyme. An electrophoretically very slow allele has also been detected in (putative) heterozygotes (Baker, 1977b).

(b) A second esterase, insensitive to eserine, present (+) in some aphids and (almost completely) absent (-) in others. Some specimens have substantially higher activity (+++) (Baker, 1977a), and a few have the enzyme in an electrophoretically slower form (+)s. This or another slower form has very high activity indeed in some glasshouse specimens. Presence of activity at this locus is associated with resistance to organophosphorus (OP) insecticides and there is evidence that this enzyme is directly responsible for OP detoxification (Beranek & Oppenoorth, 1977). The very high activity observed in some glasshouse specimens is associated with great resistance to OPs and with cross resistance to carbamates (Devonshire, Foster & Sawicki, 1977).

(c) A protein. This is found in two electromorphs which appear to be the heterozygote (SF) and an electrophoretically fast homozygote (FF) of a monomeric protein (Baker, 1977b).

Investigation of electromorph combinations in the same aphid, by double staining each gel after electrophoresis, shows widespread associations: electromorphs are almost invariably present in a M. persicae specimen in a small number of favoured combinations while other combinations are rarely or never found. (ff), for example, has never been found with (+) or with (FF); (sf) is only occasionally found with (+); and (ss)(-) has only been found with (SF) in two aphids. Because a mixture of combinations would be expected in a mixture of different clones, the simplest explanation for the favoured combinations is that these are markers not for a group of clones but for single clones. Otherwise it is necessary to presuppose some biochemical interlocus interaction which affects fitness favourably or unfavourably depending on the electromorph combination. The electromorph associations are therefore evidence that these populations of M. persicae are oligogeneous, that is, composed of large numbers of copies of a few favoured genetic forms (Baker, 1977b). Similar populations have been reported in several other species (see Brown, Nevo & Zohary, 1977).

Different clones or groups of clones distinguished by a particular electromorph combination, have different biological properties. For instance, (ff)(-)(SF) aphids on some crops, appear to have a higher rate of multiplication than others in young populations in which predators and parasites are not common, because they consistently come to predominate in these populations at this stage in the life cycle (Baker, 1977b). This report describes the results of sequential electrophoretic monitoring of some populations of M. persicae, before, during, and after the relatively severe winter of 1976-7, to assess the effect of frost on the differential survival of electrophoretically distinguishable clones and groups of clones.

## METHODS AND MATERIALS

Samples of M. persicae were collected from two populations on Nov. 15/16th, Dec. 7/8th and Jan. 26/27th. These were on a crop of white mustard at Haddington, East Lothian, and on a swede crop at Bridge of Earn, near Perth. The weather remained mild until the first week of December when there was a short spell of severe frost. This killed off very large numbers of aphids and on Dec. 7/8th it was possible to collect samples of frost killed aphids as well as samples of survivors. A period of prolonged cold began towards the end of December and lasted through most of January. The weather after this became gradually warmer.

No M. persicae could be found on subsequent visits to either site because the mustard had been killed in the frost and the swedes had nearly all lost their leaves, but specimens could be found on nettles (Urtica urens) over the next few months, and samples were collected from these populations at sites at Dirleton, East Lothian; Musselburgh, East Lothian; and St Monance, Fife.

Methods of sampling (1 aphid/plant and plants 4 m. or more apart) and analysis were as described previously (Baker, 1977b).

## RESULTS

The numbers of aphids with each combination of electromorphs in each sample are shown in Tables 1 (white mustard crop), 2 (swede crop) and 3 (nettles). The Dec. 7/8th samples were survivors from a short period of frost and the Jan. 26/27th samples were survivors from frost over a much longer period. Because the protein assay was less sensitive than the esterase test, not all the aphids which could be scored for esterase could be unambiguously scored at the variable protein locus. The total numbers of each esterase combination have therefore been recorded in the Tables and in brackets after each of these numbers are the numbers having that esterase combination which could be unambiguously identified as SF or FF (in that order) at the variable protein locus. An * by a number indicates that one of the aphids with that electromorph combination was winged, ** that two of these aphids were winged.

Table 1

Numbers of each electromorph combination detected in samples of Myzus persicae collected from white mustard at Haddington

| | OP susceptible | | | OP resistant | |
|---|---|---|---|---|---|
| Sampling date | (ss)(-) | (sf)(-) | (ff)(-) | (ss)(+) | Total |
| Nov. 16 | 13(0,13) | 15(4,10) | 15**(13,0) | 1(0,1) | 44 |
| Dec. 7 | 17(0,16) | 18(7,10) | 16(13,0) | 1(0,1) | 52 |
| Dec. 7, frost killed | 9(0,9) | 12(1,7) | 11(8,0) | 1(0,1) | 33 |
| Jan. 26 | 20(0,20) | 4(0,4) | 6(5,0) | 1(1,0) | 31 |

In brackets are the numbers of each esterase combination which could be identified as (SF) or (FF) at the variable protein locus in that order.

** includes two winged specimens.

Table 2

Numbers of each electromorph combination detected in samples of Myzus persicae collected from swedes at Bridge of Earn

| | OP susceptible | | | OP resistant | |
|---|---|---|---|---|---|
| Sampling date | (ss)(-) | (sf)(-) | (ff)(-) | (ss)(+) | Total |
| Nov. 15 | 7(0,2) | 10(2,5) | 23**(8,0) | 2*(0,1) | 42 |
| Dec. 8 | 6(0,3) | 17(7,9**) | 10(6,0) | 1(0,1) | 34 |
| Dec. 8 frost killed | 5(0,4) | 9(0,4) | 11(4,0) | - | 25 |
| Jan. 27 | 1(0,1) | 2(0,2) | 5(1,0) | - | 8 |

* includes one winged aphid

** includes two winged aphids

Table 3

Numbers of each electromorph combination detected in samples of Myzus persicae collected from nettles (Urtica urens)

| Site and Sampling Date | OP susceptible | | | OP resistant | | Total |
|---|---|---|---|---|---|---|
| | (ss)(-) | (sf)(-) | (ff)(-) | (ss)(+) | (sf)(+) | |
| Dirleton site 1: | | | | | | |
| Feb. 21 | 1(0,1) | 2(0,2) | 4(3,0) | - | - | 7 |
| Mar. 15 | 2(0,2) | - | 3(2,0) | - | - | 5 |
| Dirleton site 2: | | | | | | |
| Mar. 16 | 13(0,5) | 1(0,0) | 10(5,0) | 5(0,4) | 2(0,0) | 31 |
| Apr. 4 | - | 1(0,1) | 6(2,0) | 1(0,0) | - | 8 |
| Apr. 25 | 3(0,2) | 1(0,0) | 6(2,0) | 3(0,2) | - | 13 |
| Musselburgh: | | | | | | |
| Jan. 26 | 3(0,1) | - | 1(1,0) | - | 1(0,1) | 5 |
| Feb. 21 | 4(0,4) | - | - | 1(0,1) | - | 5 |
| Mar. 15 | - | - | - | 1(0,1) | - | 1 |
| St. Monance: | | | | | | |
| Feb. 22 | - | - | 2(1,0) | 1(0,1) | - | 3 |

## DISCUSSION

All 83 (ss)(-) specimens which could be scored at the variable protein locus had the (FF) electromorph, and all the 74 (ff)(-) specimens which could also be scored at this locus had the (SF) electromorph, as previously observed in Scottish populations (Baker, 1977b). It is therefore probable that each of these electromorph combinations is a marker for essentially a single homogeneous clone. (sf)(-) specimens on the other hand were found with both phenotypes at the variable protein locus, so that at least two and possibly many different clones have this combination of electromorphs. Small numbers of OP resistant specimens were detected in most samples. Of the common resistant combinations, (ss)(+), 14 had (FF) at the variable protein locus and only 1 had (SF) which is consistent with a predominant single clone hypothesis, although the numbers are too small to say for certain.

If these few OP resistant specimens are disregarded, the aphids in each sample from white mustard and from swede can be divided into three kinds: (ss)(-) and (ff)(-), which are probably predominantly single homogeneous clones, and (sf)(-), which is a mixture of clones. The relative numbers of M. persicae of each kind in each sample can therefore be plotted as a point on a triangular graph. This is shown in Fig. 1 where arrowed lines link successive samples from the same site.

It is evident from this figure firstly that the three kinds of M. persicae were present in different proportions on the two different crops, and secondly that different changes took place during the cold weather. In the swede population, between Nov. 15th and Dec. 8th there was a pronounced substitution of (sf)(-) for (ff)(-). This change however, which coincided with an increase in parasitism, seems to be characteristic of swede populations as they get older and parasite numbers build up (unpublished results from three other sites). It can therefore be attributed to parasite selection rather than to frost selection. The frost killed sample collected on Dec. 8th had an intermediate composition which is to be expected if they had been dead some days and were therefore a sample of the population at an earlier date. The mustard population, on the other hand, showed little change during this period despite the frost kill and the composition of the frost killed sample on Dec. 7th was little different from that of the survivors. The data therefore indicates that despite the aphids killed the short period of frost had little selective effect on the mustard population and probably on the swede population also.

By Jan. 26/27th, it had become difficult to find aphids and only small numbers could be collected. These samples however were substantially different in composition from the previous samples from these sites, indicating that directional selection had taken place. (ss)(-) specimens appeared to have survived much better on the mustard crop and (ff)(-) specimens, much better on the swedes. Immigration or emigration could not have accounted for these changes because very few winged aphids were detected on these crops. It would therefore appear that, depending on the plant host, (ss)(-) and (ff)(-) clones survive long periods of frost relatively well and that (sf)(-) clones are selectively eliminated. Frost tolerance may be either behavioural or metabolic because frost survivors were usually found in sheltered niches in the swede and mustard leaves.

The correctness of this interpretation is supported by an examination of the composition of samples collected in the first four months of 1977 from nettles (Urtica urens) at several sites (Table 3). In each of these samples, numbers of (sf)(-) specimens were small and (ss)(-) or (ff)(-) were the predominant kinds present, as expected if selection against (sf)(-) clones had taken place. Moderate numbers of OP resistant specimens, mainly (ss)(+)(FF), were also detected on these nettle hosts.

It had been expected on the basis of examples of resistance in other species, that OP resistant forms would be less tolerant to frost than others (Baker, 1977a). This would certainly be the case if the mutation conferring OP resistance also confers lower frost tolerance, as, for example, the mutation to warfarin resistance in rats confers lowered fitness because it is lethal in homozygotes. But if this mutation does not affect frost tolerance, then the possibility that the common OP resistant clone or group of clones, (ss)(+)(FF), could have mutated directly from (ss)(-)(FF), that is, from the frost selected clone on the mustard crop, could indicate that this resistant form may also be selected by frost on mustard and perhaps on some other overwintering hosts as well. In this way, frost, despite its effectiveness in killing aphids could select for OP resistance. This would account for the somewhat larger number of OP resistant specimens in the samples collected in the first four months of 1977 from nettles, although, as these were nearly all collected near Dirleton, use of OPs in this area in the past could also be responsible for this.

The question of frost/OP cross-resistance could be settled by carrying out similar investigations on populations containing appreciable numbers of OP resistant specimens, and over a wide range of host plants. Frost is unlikely to contribute much to the OP resistance problem in East Scotland because the predominant and favoured clone on summer hosts, (ff)(-)(SF) (Baker, 1977b), appears to be a good frost survivor. In England however this clone has not been detected so that frost will probably have little selective effect on swede populations but will select for the clone (ss)(-)(FF) and possibly also for the OP resistant clone (ss)(+)(FF) on mustard and perhaps on some other crops. If frost resistance is positively associated with insecticide resistance on some crops, then this could have serious consequences for the control of M. persicae because the effect of frost would then be to increase the proportion of insecticide resistant individuals on these crops at sites where some M. persicae survive the winter. This could be assessed experimentally using the techniques described here.

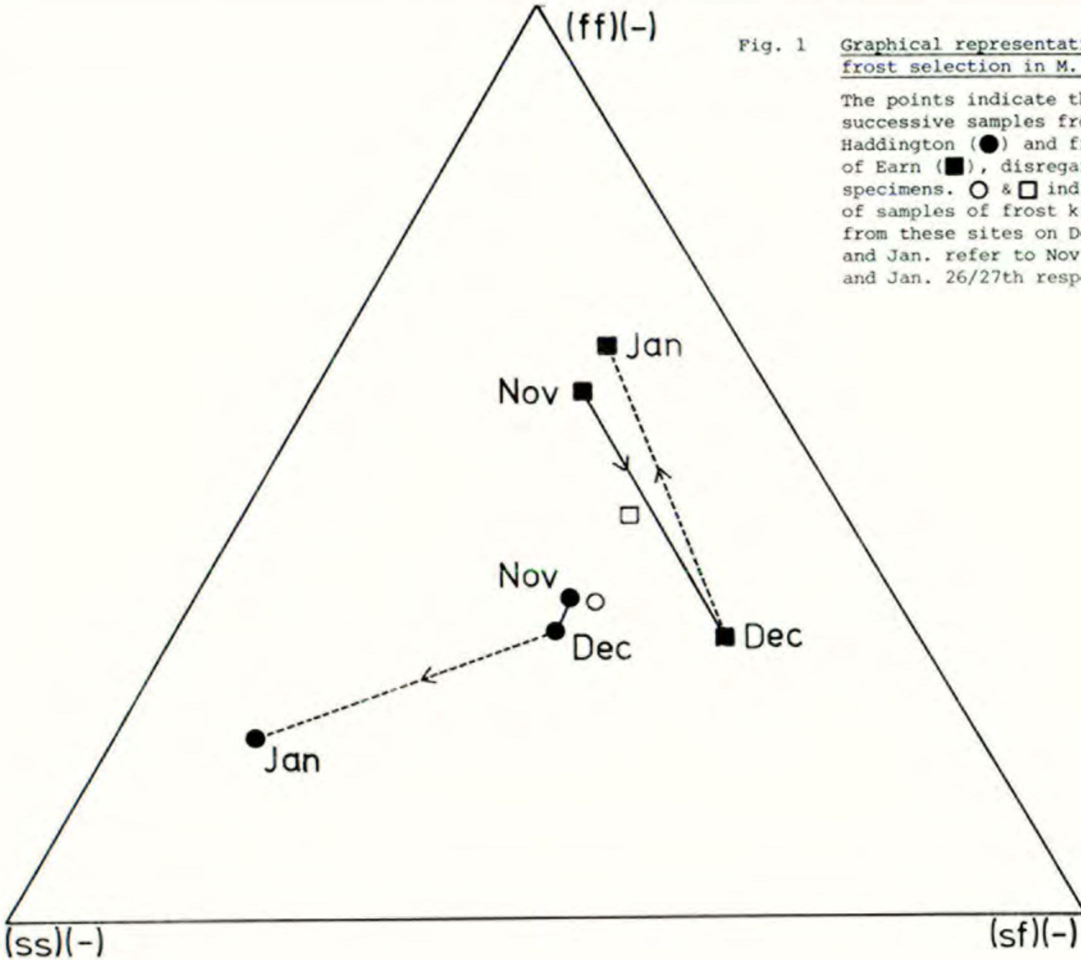


Fig. 1 Graphical representation of interclonal frost selection in *M. persicae* populations.

The points indicate the compositions of successive samples from white mustard at Haddington (●) and from swedes at Bridge of Earn (■), disregarding OP resistant specimens. ○ & □ indicate the compositions of samples of frost killed aphids, collected from these sites on Dec. 7/8th. Nov., Dec. and Jan. refer to Nov. 15/16th, Dec. 7/8th and Jan. 26/27th respectively.

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## References

BAKER, J.P. (1977a). Assessment of the potential for and development of organophosphorus resistance in field populations of *Myzus persicae*. *Annals of Applied Biology* 86, 1-9.

BAKER, J.P. (1977b). Electrophoretic studies on populations of *Myzus persicae* in Scotland from March to July, 1976. *Annals of Applied Biology* 88, in press.

BERANEK, A.P. & OPPENOORTH, F.J. (1977). Evidence that the elevated carboxylesterase (esterase 2) in OP resistant *Myzus persicae* (Sulz.) is identical with the organophosphate-hydrolyzing enzyme. *Pesticide Biochemistry and Physiology* 7, 16-20.

BROWN, A., NEVO, E. & ZOHARY, D. (1977). Association of alleles at esterase loci in wild barley *Hordeum spontaneum* L. *Nature* 268, 430-431.

DEVONSHIRE, A.L., FOSTER, G.N. & SAWICKI, R.M. (1977). Peach-potato aphid (*Myzus persicae* Sulzer) resistant to organophosphorus and carbamate insecticides on potatoes in the west of Scotland. *Plant Pathology* 26, in press.