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CHEMICAL CONTROL OF FRENCH 'FLY' TYROPHAGUS LONGIOR (ACARINA : ACARIDAE) ON CUCUMBER

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Summary Acarid mites of the genus Tyrophagus often cause serious damage to young cucumber crops grown in straw bales. To prevent attacks the straw is usually given prophylactic treatments of parathion, a pesticide of high mammalian toxicity.

Less toxic pesticides were screened in the laboratory for activity against T. longior collected from cucumber crops; and in glasshouse tests for phytoxicity to young cucumber plants. Pirimiphos-methyl, already in use against other cucumber pests, gave good control of mites and was not phytotoxic to young cucumber plants at rates below 0.05% a.i. A field trial confirmed that this compound was an effective alternative to parathion for control of French 'fly' on cucumber.

INTRODUCTION

Young cucumber plants can be attacked and seriously damaged by acarid mites of the genus Tyrophagus. These mites are more commonly associated with stored grain, hay and straw (Hughes, 1976) and several species have been recorded from straw stacks in the field (Griffiths, 1960). Of the 230 ha of cucumbers grown under glass in the United Kingdom, about 60% are grown on straw-based substrates. This use of straw provides an obvious link between the usual habitat of the mites and the cucumber crop. Straw bales are normally brought into the glasshouse after being stored for several months and are then treated with ammonium nitrate, lime and water to induce a fermentation which raises the temperature at the centre of the bale to about 35°C. This temperature is probably well above the optimum for the mites and may even be lethal. Any mites present in the bale will be forced to migrate to the periphery, where they will find easy access to the young cucumbers planted on the top of the bale.

French 'fly' damage to cucumber plants takes the form of irregular holes in the leaves which enlarge as the plant develops. In severe cases, the growing point may be attacked, resulting in distortion and blindness of the shoots. All stages of the mite, including eggs, may be found on the cucumber leaves, but infestations normally terminate within a few weeks, although serious damage may have already occurred.

Since all cucumber crops grown on straw bales are susceptible to French 'fly' damage, control measures are widely used. The most commonly practised form of treatment is the routine application of high volume sprays of parathion to the straw bales, either before planting or when damage is first seen. However,

parathion has a high mammalian toxicity, the acute oral LD50 for rats being 3.6 mg/kg (Worthing, 1979), and is no longer approved for use against cucumber pests under the Agricultural Chemicals Approval Scheme. Alternative methods of controlling T. longior involving less toxic pesticides have therefore been investigated.

MATERIALS AND METHODS

During February 1978, mites were collected from cucumber crops in the Lea Valley, Hertfordshire, showing typical French 'fly' damage and from straw bales on nurseries where damage regularly occurred. Specimens of mites were identified and a laboratory culture was set up and maintained on a mixture of dry yeast and wheatgerm at 17.5°C and 75% r.h.

Prospective pesticides were chosen from those compounds known to be effective against Tyrophagus species (Wilkin and Hope, 1973; Anon, 1978). Additionally, some other pesticides already in use for the control of glasshouse pests, were investigated. The materials used, together with their accute oral LD50 for rats are shown in Table 1.

Table 1

Candidate pesticides for control of Tyrophagus spp

Pesticide	formulation	acute oral LD50 for rats (Worthing, 1979)
amitraz	20% e.c.	800
azinphos methyl + demeton-S-methyl sulphone	25% + 7.5% w.p.	16.4 + 37.5
dicofol + tetradifon	20% + 6.25% e.c.	809 + 14,700
dinocap	25% w.p.	1,190
etrimfos	50% e.c.	1,800
fentrifanil	12.5 w.p.	94
parathion	20% e.c.	3.6
pirimiphos-methyl	25% w.p. and 50% e.c	2,050
phoxim	50%	1,976

The candidate pesticides were screened for phytotoxicity symptoms on young cucumber plants in a glasshouse experiment. Plants of cv. Farbiola were grown in 11 cm pots at 21°C until they had 5 - 6 true leaves. High volume sprays of each pesticide were then applied at three rates to three plants (Table 3). A wetter ('Agral') was added to each spray solution at the rate of 0.06 ml/l. Plants treated with water only and water + wetter acted as controls. After 1, 3 and 5 days, plants were given a score based on six visual phytotoxicity symptoms, viz 0 - severe necrosis and plant death, 1 - severe leaf spotting and marginal necrosis, 2 - mild leaf spotting and marginal necrosis, 3 - severe marginal necrosis, 4 - mild marginal necrosis, 5 - unaffected.

All pesticides shown in Table 1 except phoxim were screened for effectiveness against the laboratory culture of glasshouse mites. Commercial formulations were diluted in water and sprayed on to wheat grains. The doses were calculated in mg a.i./kg of grain and the lower dose was related, where possible, to those known to be effective against stored product mites. A higher dose was included to ensure that all chemicals were tested at concentrations likely to cover the range used in horticulture. Samples of treated grain (50 g) were placed in wide-mounted jars and approximately 0.01 g of a vigorous mite culture added. The jars were sealed with filter paper tops and stored at 17.5°C and 75% r.h. Mites were also introduced to untreated grain to act as controls. After 14 days the mites were removed from two replicates of each treatment by passing them through a 710 µm sieve. Mites were examined using a low power binocular microscope and grouped according to mortality in categories 0–4; where 0 represents less than 10% mortality and 4 represents 100% kill. Results from any test where control mortality exceeded category 0 were discarded and the test repeated.

As a result of these experiments, the most promising chemical, pirimiphos-methyl, was compared with the previous standard, parathion, when applied to a cucumber crop growing in straw bales in early March. One week after planting, the crop was artificially infested with a laboratory culture of T. longior and within two days showed typical damage symptoms. Single H.V. sprays of pirimiphos-methyl (0.025% a.i.) and parathion (0.0125% a.i.) were then applied and mite mortality was assessed 5 days later. Mites were counted on three upper leaves per plant, taken from ten plants in each treatment. The plants were then allowed to grow normally and three weeks after treatment fruit were taken for residue analysis.

RESULTS

Specimens of mites collected from the glasshouses in the Lee Valley were identified and included the species T. longior, T. palmarum, and T. similis. However, although no efforts were made to select a pure population, only T. longior could be found in the laboratory culture.

Phytotoxicity tests

The results of the phytotoxicity tests are given in Table 2. Five days after treatment no symptoms of phytotoxicity were seen on plants sprayed with azinphos-methyl + demeton-S-methyl sulphone, dinocap or parathion. In addition, plants showed no visible effects from sprays of dicofol + tetradifon, etrimfos or pirimiphos-methyl at the lowest rate. Phoxim was severely phytotoxic at all rates, and was therefore not included in subsequent laboratory screening tests.

Laboratory screening tests

The results of laboratory screening experiments on T. longior are given in Table 3 together with the dosage rates of pesticides. Four compounds gave good control of mites after 14 days exposure. Etrimfos caused 100% mortality while pirimiphos-methyl, parathion and azinphos-methyl + demeton-S-methyl sulphone killed most mites, leaving only a very few live individuals, all of which showed visible signs of acaricide toxicity (e.g. lack of co-ordination).

Table 2

Phytotoxicity of pesticides on young cucumber plants

Pesticide	Rate (% a.i.)	Phytotoxicity rating 1 day	3 days	5 days
amitraz	0.06	5	4	3
	0.03	5	4	4
	0.015	5	5	5
azinphos-methyl + demeton-S-methyl sulphone	0.05 + 0.015	5	5	5
	0.25 + 0.007	5	5	5
	0.01 + 0.003	5	5	5
dicofol + tetradifon	0.08 + 0.025	5	4	4
	0.04 + 0.012	5	5	5
	0.02 + 0.006	5	5	5
dinocap	0.012	5	5	5
	0.006	5	5	5
	0.003	5	5	5
etrimfos	0.1	3	3	3
	0.05	4	4	4
	0.025	5	5	5
fentrifanil	0.02	3	2	2
	0.01	3	2	2
	0.005	4	3	2
parathion	0.025	5	5	5
	0.0125	5	5	5
	0.006	5	5	5
pirimiphos-methyl (w.p.)	0.1	3	3	3
	0.05	5	4	4
	0.025	5	5	5
pirimiphos-methyl (e.c.)	0.1	5	4	4
	0.05	5	5	5
	0.025	5	5	5
phoxim	0.2	1	0	0
	0.1	1	0	0
	0.05	2	1	1

Table 3

The effect of pesticides on T. longior after 14 days exposure

Pesticide	Rate (mg/kg)	Mortality Category
amitraz	12	1
	2	0
azinphos-methyl + demeton-S-methyl sulphone	6.7 + 3.3	3*
	1.3 + 0.7	3*
dicofol + tetradifon	11.4 + 3.6	1
	2.3 + 0.7	1
dinocap	5	2
	1	0
etrimfos	20	4
	4	4
fentrifanil	5	2
	11	1
parathion	5	3*
	1	3*
pirimiphos-methyl (e.c.)	20	4
	4	3*

*Only a few individuals survived which were noticeabley affected by treatment.

Field trial

Both pirimiphos-methyl and parathion gave good control of T. longior on cucumber plants growing in straw bales (Table 4). No further French 'fly' damage was seen on treated plants and no signs of phytotoxicity were apparent. Untreated plants continued to suffer from leaf holing, although the shoots were not affected and the plants grew normally. Fruit taken from plants sprayed with pirimiphos-methyl showed residues of only 0.003 mg/kg.

Table 4

Number of T. longior on bed-sown cucumbers 5 days after treatment

	Mean number of mites per leaf		% Mortality
	Alive	Dead	
pirimiphos-methyl	1.0	27.0	96.4
parathion	2.5	23.0	90.2
untreated	22.5	2.0	8.2

DISCUSSION

Three species of Tyrophagus: T. longior, T. palmarum and T. similis have been found associated with typical French 'fly' damage on commercial cucumber crops. It was demonstrated that a culture of T. longior could cause these symptoms when introduced to young cucumber plants and therefore confirmed the pest status of this species. In laboratory tests, four acaricides were effective in controlling T. longior in culture. Dicofol + tetradifon, used for the control of red spider mite (Tetranychus urticae), and often suggested as a possible alternative to parathion for French 'fly' control, was ineffective. Phoxim, although known to be active against mites of stored products, was found to be severely phytotoxic and was not included in subsequent laboratory tests. Two compounds, etrimfos and pirimiphos-methyl which gave effective control of T. longior in culture, had low mammalian toxicity and low risk of phytotoxicity. Since pirimiphos-methyl already had commercial recommendations for control of other pests on cucumber, this compound was chosen for further study. The small field trial showed that pirimiphos-methyl was as effective as parathion against T. longior and the level of pirimiphos-methyl residues in fruit from treated plants was extremely low.

Biological control of red spider mite is practised on the majority of heated cucumber crops in the United Kingdom. Any insecticide applied to young cucumber plants must therefore be capable of integration into the biological control system. During these investigations the predatory mite Phytoseiulus persimilis was successfully introduced to cucumber plants two weeks after treatment and did not appear to be adversely affected by either pirimiphos-methyl or parathion at this stage.

This work has shown that pirimiphos-methyl may be used as an effective alternative to parathion for the control of T. longior on cucumber. It has a much lower mammalian toxicity and therefore provides a greater degree of operator safety.

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References

ANON (1978) Screening tests of new pesticides, Pest Infestation Control Laboratory Report, 1974-1976, 47

GRIFFITHS, D. A. (1960) Some field habitats of mites of stored grain, Annals of Applied Biology, 48,134-144

HUGHES, A. M. (1976) The mites of stored food and houses, HMSO, London, p. 58

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

WORTHING, C. R. (1979) The Pesticide Manual, British Crop Protection Council, Croydon

SESSION 4B

BEHAVIOUR AND FATE OF PESTICIDES AFTER APPLICATION

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FACTORS AFFECTING THE APOPLASTIC TRANSLOCATION OF SYSTEMIC FUNGICIDES IN PLANT STEMS

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Summary Lignin was found to be the main component of plant stems or roots which adsorbed the five systemic fungicides,carbendazim, triadimefon, nuarimol, triarimol and fenarimol. The adsorption of fungicides by fresh and dried ground-stem or root tissues of pepper, cotton and bean plants, was correlated with their lignin content. A significant correlation was found between these adsorption rates and the 1-octanol-water (log $P_{oct.}$) partition coefficients of the fungicides. The lipophilic fungicides, triarimol and fenarimol (log $P_{oct.}$ about 2.6) were adsorbed to the largest extent. Triadimefon and nuarimol (log $P_{oct.}$ about 2.0) were moderately adsorbed, whereas carbendazim with the lowest log $P_{oct.}$ (1.34) was adsorbed the least.

Labelled fungicide solutions were perfused through excised stem segments, and the fungicide concentration of exudate measured at various time intervals in order to determine break - through curves. Grouping of the fungicides according to their break - through curves were the same as indicated by their partition coefficients.

INTRODUCTION

Systemic pesticides are translocated in the transpiration stream from the roots through the stems to the leaves (Crowdy, 1977). The uptake of pesticides by roots does not depend on metabolic energy supply (Ashton and Crafts, 1973). Different compounds move at a different rate in the same plant. On the other hand, the same systemic compound may move at different rates in different plants. Translocation of pesticides is regulated by the nature and quantity of different barriers in the plant and on the chemical character of the pesticide molecule (Shephard, 1973). According to Edgington and Peterson (1977), the lipophilic character of pesticides regulates their uptake by plant roots, while their hydrophilic character determines flow rate in the transpiration stream. From studies on the translocation of organic nitro and amino compounds (Hill-Cottingham and Lloyd-Jones, 1968; Die and Vonk, 1967) it was concluded that xylem vessels' walls contain nondiffusable monovalent anionic groups. These sites can adsorb basic organic molecules or cations which are moving in the xylem sap. This exchange process resembles adsorption of moving molecules on chromatographic columns (Jacoby, 1967).

In this investigation we studied the characteristics of barriers affecting the apoplastic translocation of lipophilic systemic fungicides through plant roots and stems.

MATERIALS AND METHODS

Fungicides: Five radio-tracer labelled systemic fungicides we used: triarimol (EL-273-α-(2,4-dichlorophenyl)-α-pheny1-5-pyrimidinemethanol) and triadimefon (1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)-2-butanone) were [^{3}H] labelled. Carbendazim (MBC - methyl benzimdaz-2-ylacarbamate), nuarimol (EL-228-α-(2-chlorophenyl)-α-(4-fluorophenyl)-5-pyrimidinemethanol), and fenarimol (EL-222-α-(2-chlorophenyl)-α-(4-chlorophenyl-5-pyrimidinemethanol), were [^{14}C] labelled in the methyl group, carbinol group and pyrimidine ring, respectively.

Other chemicals used: 1-octanol, ethyl cellulose - type T-50, cellulose - Sigma - cell type 100, bovine serum albumin, and polygalacturonic acid - sodium salt, all from Sigma Chemical Division, U.S.A. Pine lignin polymer from Westvaco, Chemical Division, U.S.A.

Plants: The plants for experiments were grown in the greenhouse at 25^{c}. Bean plants (Phaseolus vulgaris L. cv. Brittle Wax) were used 7 days after sowing. Pepper plants (Capsicum annuum L. cv. Pelle California) were used 50 or 120 days after sowing, representing the herbaceous or woody state, respectively. Cotton plants (Gossypium hirsutum L. cv. Pima SJ-1) were used 21 or 90 days after sowing, representing the herbaceous or woody state, respectively.

Plant preparations: Pepper and cotton lignins were prepared from woody plants as described in AOAC (1970). Ground stems or ground roots were prepared by homogenizing fresh tissue. The homogenate was filtrated through 6 layers of muslin, and the residue washed with large amounts of distilled water, dried at about 80^{c}, and ground again to powers.

Methods: The adsorption of fungicides to different organic substrates was characterised by adsorption isotherms. The substrates were incubated in a shaking termostatic bath for 3 hr. at 25^{c} in citrate buffer (pH=5) containing labelled fungicides at various true solution concentrations. After incubation, the substrate was separated by centrifugation and aliquots of the supernatant were counted in Bray's liquid scintillator.

1-octanol - water partition coefficients were determined at 25^{c} in a manner similar to as reported by Fujita et al. (1964). The pK_a values were obtained by spectrophotometric titration (Albert and Serjeant, 1962).

Perfusion of 5.2 μM labelled fungicides solution through 6 cm long stem segments was performed in a pressure apparatus (Jacoby, 1965). The rate of exudation from the decapitated tipes and the radioactivity of the exudates were measured over 6 hr. period.

RESULTS

Identification of adsorbing components The adsorption of five labelled systemic fungicides on plant stem components was investigated. The results presented in table 1, show that there is very little, or no adsorption of fungicides on cellulose or polygalacturonic acid. Some affinity of proteins to fungicides of the pyrimidinemethanol family was found as reported previously (Wallerstein et al., 1967). However this is of no great importance in apoplastic pathways, since the amount of proteins present there is very small. Lignin is thus, probably the main apoplastic compound responsible for the adsorption of the five fungicides examined. Therefore, in further experiments we studied adsorption to lignins. The investigated fungicides can be grouped according to the intensity of their adsorption by lignin. The first group consisting of fenarimol and triarimol, these are adsorbed relatively very well by lignins. A second group comprises of triadimefon and nuarimol, which were adsorbed by lignins to about half the extent of the first group. Carbendazim showed on anomalous adsorption pattern. The data in table 1 show difference in the adsorption of the fungicides to three different types of lignins which we used. Lignins prepared from different plant sources are known to differ in their composition (Schubert, 1973).

Adsorption by stem tissues Stems or roots of several plants species at different ages were ground, washed, dried and powdered. The adsorption of the various fungicides by these was measured and isotherms were constructed. Adsorption constants for the five systemic fungicides on plant's powders are presented in table 2.

The fungicides may again be classified according to their adsorption to ground shoot tissues, into the same 3 groups as they were classified for adsorption to lignins. The group of fenarimol and triarimol had high adsorption constants compared to triadimefon and nuarimol with relatively low adsorption. Adsorption of carbendazim was low compared to the other two groups of fungicides (table 2).

Adsorption of the fungicides to ground pepper stem substrate was not significantly different from adsorption to ground root substrate of the same plant (table 2). The analyses show (table 2) that there was no difference in lignin contents of root and stem in these plants.

TABLE 1

Adsorption constants ($K=\frac{\text{nmole/gr dry tissue}}{\text{nmole/ml}}$) of five systemic fungicides for plant stem components (experimental condition: 25°C, pH=5, 3 hr. of incubation).

Adsorbing Compound	Adsorption Constants (K)				
	Carbendazim	Triadimefon	nuarimol	triarimol	fenarimol
Pepper lignin	700	1122	1288	2691	2300
Cotton lignin	800	513	537	1349	1100
Pine lignin	590	224	270	457	660
Cellulose	4	6	7	20	20
Ethyl cellulose	36	78	73	96	102
Protein (B.S.A)	7	8	125	203	211
Polygalacturonic acid	0	0	0	0	0

TABLE 2

Lignin content of various ground plant stems and root tissue and adsorption constants (K) of five systemic fungicides (experimental conditions: 25°C, pH=5, 3 hr. of incubation).

Plant Substrate	% lignin in fresh tissue	Adsorption Constants (K)				
		car--bendazim	Tri--adimefon	Nuarimol	Tri--arimol	Fen--arimol
Woody pepper stems	15	23	72	71	190	206
Fresh woody pepper stems	15	10	75	115	242	247
Woody pepper roots	15	26	69	71	183	186
Methylated woody pepper stems	15	19	139	145	478	557
Herbaceous pepper stems	7.5	16	59	60	175	170
Woody cotton stems	13	17	42	62	95	102
Herbaceous cotton stems	10	15	36	59	90	108
Herbaceous bean stems	4.5	8	23	40	65	71

Experimental measurement of adsorption isotherms with preparations of homogenized fresh tissue which were not washed and dried, so that the substrates include cytoplasmatic proteins and salts. Such preparations gave the same adsorption results with triadimefon as powdered substrates. The pyrimidinemethanol fungicides were adsorbed somewhat more by fresh tissues than by washed and dried ones, possibly because of their affinity to proteins. The carbendazim adsorption constant for fresh tissue was less than that found for powdered tissues (table 2).

Methylation of the ground stems of woody pepper by refluxing with methanolic HCl (Hance, 1965), increased the adsorption of four of the investigated fungicides, but not of carbendazim (table 2). The same general phenomenon was found by comparing the adsorption of the fungicides by cellulose and ethyl cellulose (table 1), although in this case there was also some effect on carbendazim adsorption as well. Thus, addition of alkyl groups to the organic matter increased their ability to adsorb the fungicides. Since adsorption of the fungicides by organic matter obtained from the stem was not affected by soxhlet extractions (benzene:ethanol (2:1) and chloroform:methanol (2:1)), it is not related the presence of lipids (Kates, 1972).

Physicochemical properties and adsorption mechanism Adsorption of the fungicides to lignins and ground stems was reversible. The time needed to attain equilibrium varied between 1-3 hr. according to the fungicide employed.

Adsorption of the fungicides to lignins and ground stems was determined at different pH values. The rate of adsorption of the pyrimidinemethanol fungicides and triadimefon by organic matter was not affected by the pH of the solution in the range of pH=6.5 to pH=2. The adsorption of carbendazim was increased when the pH increased above 4.0 and reached a maximum at about pH=5. The different effects of pH on the adsorption of the various fungicides could be expected from their pK_a values (table 3).

TABLE 3

Dissociation constants (pK_a) and 1-octanol - water partition coefficient ($P_{oct.}$) of the systemic fungicides investigated

Fungicides	pK_a	$P_{oct.}$	Log $P_{oct.}$
Carbendazim	4.0	21.9	1.34
Triadimefon	2.55	63.1	1.80
Nuarimol	2.6	158.5	2.20
Triarimol	2.5	363.1	2.56
Fenarimol	2.5	416.9	2.62

Briggs (1973) found that the 1-octanol - water partition coefficient was the most efficient factor for the prediction of lipophilic compound movement in an organic matter media. The $P_{oct.}$ values for the fungicides used in this investigation are presented in table 3. A significant correlation was found between log $P_{oct.}$ and the adsorption constant (log K) of the fungicides on pure lignins and ground stems (figure 1).
The regression equation for this correlation is:

$$\log K_i = 0.621(\pm 0.061) \log P_{oct.} + a_i \qquad (r=.9707)$$

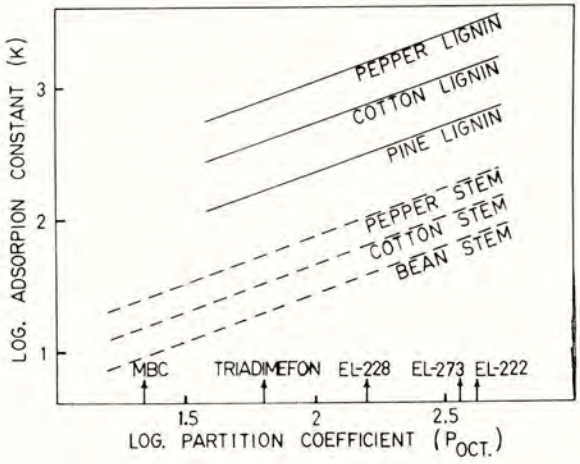
were: i - one of the types of the organic matter examined; and a_i - the intercept factor of the i organic matter.

The value for the slope of the linear regression 0.62, means that the dimension of the water envelope surrounding the fungicide molecules in the adsorption process to lignin is about half or more of that occuring during partitioning into 1-octanol

phase (Uchida and Kasai, 1980). The slope values were the same for all the lignins and the ground stems investigated. This finding indicates that the fungicides are adsorbed to the same component and by a similar mechanism to lignins as well as in all the ground stems.

FIGURE 1

Relation between logarithms of adsorption constants of systemic fungicides for organic matterial (log K) and logarithms of 1-octanol - water partition coefficients ($P_{oct.}$)



The adsorption mechanism appears to be a hydrophobic interaction between the lipophilic fungicides and hydrophobic lignin groups. This is in accordance with the characteristics of "hydrophobic binding" as summerized by Weed and Weber (1974). By increasing the lipophilic properties of fungicide, the $P_{oct.}$ increases and its adsorption to lignin increases as well. The logarithms of the partition co-efficients of fenarimol and triarimol are both about 2.6 (table 3), so their adsorption to the organic matters which include lignin were the highest in our experiments. The second group, triadimefon and nuarimol with log $P_{oct.}$ of about 2.0, adsorbed moderatly (tables 1,2). Carbendazim has the lowest partition co-efficient either for octanol - water (log $P_{oct.}$ =1.34) as well as dodecane - water (log $P_{dodec.}$ =-1.1), and relatively high pK_a. These properties responsible for it's low adsorption on the ground stem substrate.

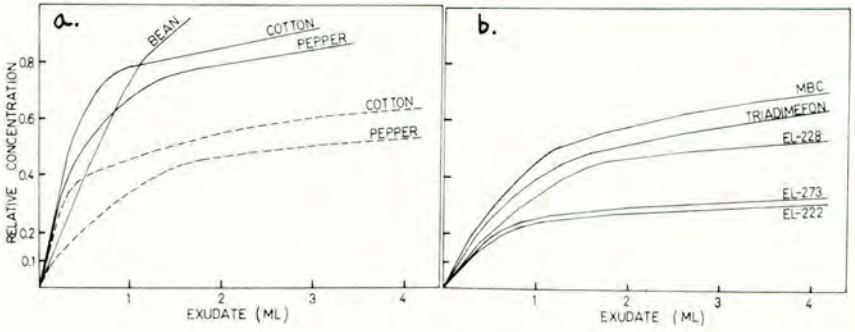
Addition of hydrophobic groups to the organic matter increased the hydrophobic interaction between the organic substrate and the lipophilic fungicides. This explain the increased adsorption of fungicides to ethyl cellulose and methylated pepper stem compared with the unsubstituted materials.

Perfusion of stems with fungicides A solution of labelled fungicide is perfused through excised 6 cm long stem segments. The relative radioactivity of the exudated solution was plotted against time (figure 2). Relatively small amounts of fungicide solution were needed for break-through in herbaceous tissue, but much more volume of fungicide solution was needed for break-through in woody tissue. Only the carbendazim break-through curves are presented in Fig. 2a, but similar relations between herbaceous and woody stems were obtained for the other examined fungicides. This experiments emphasized the difference between herbaceous and

woody plants much larger than expected from the data regarding adsorption of fungicides by ground stem (table 2). In intact woody stems most of the lignin is concentrated around the xylem vessels, therefore fungicides translocated in the stems' apoplast might be exposed to a greater concentration.

FIGURE 2

Break-through curves of systemic fungicides solution (5.2 μM) forced through isolated stems (6 cm long) under pressure at uniform flow rate. a. Carbendazim solution through woody (----) or herbaceous (———) stems. b. Five systemic fungicides through woody pepper stems.



The results of the perfusion experiment with excised stems coincided with the classification previous grouping of the fungicides according to the intensity of their adsorption by ground stems (figure 2b).

We found that the relative amount of fungicides adsorbed in the excised stems was very much affected by the flow rate of the fungicide solutions. An increased flow rate resulted in decreased retention of the fungicides in the stems per ml of perfused solution. Perfusion flow rates were about the physiological.

DISCUSSION

Transpiration is the main factor responsible for the movement of xenobiotic compounds in the apoplast (Edgington and Peterson, 1977). We found in this study that lignin, which is concentrated in xylem vessels, could be an important factor impeding the apoplastic movement of lipophilic fungicides. A good correlation was found between P_{oct} of the fungicides and their adsorption constants for ground stems and lignins. It seems that for fungicides examined but carbendazim, hydro-phobic interactions are the main factor responsible for their retention in the apoplastic pathway. This interaction is reversible and equilibrium is attained rather slowly.

Carbendazim was the least lipophilic fungicide examined ($\log P_{oct} = 1.34$). The intensity of its adsorption by excised stems and ground tissues was, according the lowest by a special affinity of carbendazim to pure lignin, much more than we

could expect, was found.

The lipophilic property of the fungicides are expressed by the P_{oct} coefficients and its increase correlates with a decrease of their movement through the xylem vessels. Uchida and Kasai (1980) reported similar results for movement of fungicides in soil which contained organic compounds. Lignins derived from different plant species differ some of their properties (Shubert, 1973). In the present work we found that pepper lignin adsorbed larger amounts of fungicides than lignins derived from cotton or pine.

Briggs et al. (1976) used octanol - water partition coefficients of pesticides for predicting their systemic properties in plants, but did not investigate the affect of plant species and age on pesticides movement in the plant.

The present investigation may indicate some practical conclusions. When a systemic fungicide is applied through the root system for the control of foliar diseases in herbaceous plants,it may be preferable to use lipophilic fungicides with log $P_{oct} > 2.5$ like fenarimol. Because of small amounts of lignin present in such herbaceous plants, fungicides with lower log P_{oct} would be too quickly translocated to the leaf margins. On the other hand, a very lipophilic fungicide like iprodione (log P_{oct} =2.89) can be excessively retained in the stem of a herbaceous plant like potato (Cayley and Hide, 1980). In moderately woody plants (like pepper or cotton) it may be preferable to use lipophilic fungicides for controlling stem or root diseases and to use less lipophilic fungicides against foliar diseases. In woody plant such as trees it seems that even fungicides like carbendazim with a low P_{oct} will not move efficiently enough, because of the large amount of lignin present in their apoplastic pathway.

Acknowledgements

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References

ALBERT, A. and E.P. SERJEANT (1962). Ionization constants of acids and bases. Methuem, London.

ASHTON, F.M. and A.S. CRAFTS (1973). Mode of action of herbicides. A. Willey Interscience Pub.

Association of Official Analytical Chemists (1970). Lignin official final action indirect method. AOAC designation 3.101 in: W. Horwitz ed. Official Methods of Analysis. AOAC Pub., Washington.

BRIGGS, G.G. (1973). A simple relationship between soil adsorption of organic chemicals and their octanol - water partition coefficients. Proc. 7th British Insectic. Fungic. Conf., 1:83-86.

BRIGGS, G.G., R.H. BROMILOW, R. EDMONDSON and M. JOHNSTON (1976). Distribution coefficient and systemic activity. Pages:129-134 in: M.R. McFarlane ed. Herbicides and Fungicides - Factors Affecting their Activity. The Chem. Soc., London.

CAYLEY, G.R. and G.A. HIDE (1980). Uptake of iprodione and control of diseases on potato stems. Pestic. Sci. 11:15-19

CROWDY, S.H. (1977). Translocation. Pages: 92-114 in: R.W. Marsh ed. Systemic fungicides. Longman, London and N.Y.

DIE, J.V. and C.R. VONK (1967). Selective and sterospecific adsorption of various amino acids during xylem translocation in tomato stems. Acta Bot. Neerl. 16:147-152.

EDGINGTON, L.V. and C.A. PETERSON (1977). Systemic fungicides, theory, uptake and translocation. Vol. 2, pages:51-89 in: M.R. Siegel and H.D. Sisler eds. Antifungal compounds. Marcel Dekker Inc., N.Y. and Basel.

FUJITA, T., J. IWASA and C.T. HANSCH (1964). A new substituent constant, π, derived from partition coefficients. Am. Chem. Soc. 86:5175.

HANCE, R.J. (1965). Observation on the relationship between the adsorption of diuron and the nature of the adsorbent. Weed Res. 5:108-114.

HILL-COTTINGHAM, D.G. and C.P. LLOYD-JONES (1968). Relative mobility of some organic nitrogenous compound in the xylem of apple shoots. Nature 220:389-390.

JACOBY, B. (1965). Sodium retention in excised bean stems. Physiologia Pl. 18:730-739.

JACOBY, B. (1967). The effect of roots on calcium ascent in bean stems. Ann. Bot. 31:725-730.

KATES, M. (1972). Techniques of lipdology. Vol. 3, pages:267-610 in: T.S. Work and E. Work eds. Laboratory Techniques in Biochemistry and Molecular Biology. North-Holland Pub. Comp., Amsterdam, London.

SHCUBERT, W.J. (1973). Lignin. Vol. 3, pages: 132-153 in: L.P. Miller ed. Phytochemistry. V.N. Reinhold Comp., N.Y., Cincinnati, Toronto, London and Melbourne.

SHEPHARD, M.C. (1973). Barriers to the uptake and translocation of chemicals in herbaceous and woody plants. Proc. 7th British Insectic. Fungic. Conf. 3:841-850.

UCHIDA, M. and T. KASAI (1980). Adsorption and mobility of fungicidal dialkyl--dithiolanylidenemalonates and their analogs in soil. J. Pestic. Sci. 5:553-558.

WALLERSTEIN, I.S., B. JACOBY and A. DINOOR (1976). Absorption, retention and translocation of the systemic fungicide triarimol in plants. Pestic. Biochem. Physiol. 6:530-537.

WEED, S.B., J.B. WEBER (1974). Pesticide-organic matter interactions. Pages:39-66 in: W.D. Guenzi ed. Pesticides in Soil and Water. Soil Sci. Soc. Amer. Pub., Madison and Wisconsin.

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UPTAKE, TRANSLOCATION AND DEGRADATION OF METALAXYL IN LETTUCE

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Summary The uptake, translocation and degradation of ^{14}C-metalaxyl by lettuce plants was investigated. One week after foliar application at the recommended concentration (200 mg/l) uptake was 85-90% and the concentration in leaf tissue was 3.7 mg/kg. Metalaxyl accumulated in the margins and distal parts of leaves resulting in wide variation in concentration within individual leaves. Metalaxyl was transported acropetally and basipetally within the plant following application to a single leaf. Shading did not increase the export of metalaxyl from the treated leaf to untreated foliage but did increase the proportion that was transported acropetally. Loss of the fungicide from the surface of leaf discs occurred rapidly and was greater the higher the temperature. Two degradation products of metalaxyl were found in lettuce seedlings 1 week after germination and growth in the fungicide.

INTRODUCTION

The acylalanine fungicide, metalaxyl, has high systemic activity against plant pathogens of the Peronosporales (Urech et al., 1977; Knauss, 1977; O'Brien, 1978; Cohen et al., 1979; Dueck and Stone, 1979; Kerkenaar and Sijpesteijn, 1981). This chemical has been shown to give effective control of lettuce downy mildew (Bremia lactucae) (Griffin and Griffin, 1977; Paulus et al., 1977; Crute, 1980; Crute and Gordon, 1980) and has had commercial clearance for use on this crop for several years in the U.K. It is currently available as a mixed formulation with mancozeb.

The uptake and translocation of metalaxyl has been studied by bioassay in grape vines and tomatoes (Staub et al., 1978; Cohen et al., 1979). Quantitative data has been produced on translocation of the chemical in tomato, avocado (Persea americana) and Persea indica using ^{14}C-metalaxyl (Zaki et al., 1981). Both upward and to a lesser extent downward movement from points of application has been demonstrated. There are no published quantitative data however on the uptake and translocation of metalaxyl when applied to lettuce.

This paper provides information on the uptake, transport and distribution of metalaxyl in lettuce, the rate of metalaxyl loss from detached leaf tissue and the effect of environment on this process. The metabolic breakdown of metalaxyl was investigated in order to estimate the concentration of the fungicide in lettuce seedlings used in a bioassay system for surveying variation for sensitivity of B. lactucae isolates (Wynn and Crute, 1981).

MATERIALS AND METHODS

Chemicals

^{14}C-metalaxyl was supplied by Ciba-Geigy Ltd (Basle, Switzerland). The

labelled fungicide (0.06 mCi, 1.33 mg) was mixed with a further quantity of 25% wettable powder formulation of metalaxyl (Ridomil 25% w.p.) and a small volume of distilled water to make a stock solution. Small quantities of this solution were later added to solutions of the 25% w.p. metalaxyl formulation to provide experimental treatments as required.

Determination of ^{14}C-metalaxyl concentration in lettuce tissue

Plant tissues were homogenised in methanol. The extracts were centrifuged and aliquots of the supernatant added to scintillation fluid (toluene-methoxy ethanol, 60:40 with 5 g/l butyl-PBD) or Brays reagent (Bray, 1960). The volume of supernatant used varied between 0.5 and 2 ml depending on chlorophyll and water content. The efficiency of extraction was determined by complete combustion of the washed and dried centrifuge pellets for each type of tissue. In all cases, less than 1% of the label remained in the dry matter.

The quantity of metalaxyl in plant tissue was calculated from the activity and volume of extract and the fresh weight of the extracted tissue. The results are expressed as mg/kg plant tissue or as a percentage of total recovered metalaxyl where appropriate. It is assumed that ^{14}C-metalaxyl was not taken up, translocated or degraded differently from unlabelled metalaxyl.

Experiment 1

Lettuce plants (cv. Hilde) were grown in 12 cm diam. pots in Levington Universal potting compost in a glasshouse maintained at 15°C ± 2°C. Two four week-old plants were each sprayed with 8 ml of metalaxyl (0.05 µCi/ml, 200 mg/l). The applications were made to run off giving a similar rate per plant to a field application. One week later, plants were harvested and each leaf rinsed 4 times with 5 ml of methanol to remove the fungicide remaining on the leaf surface. The metalaxyl concentration in the leaves and the washings was determined.

Three leaves from each plant were examined for the distribution of metalaxyl within the leaves. The leaves were cut in half at right angles to the midrib, and from the distal half five strips were cut from each leaf parallel to the edge, starting at the widest part. The proportion of total metalaxyl in the lower half of the leaf and in each strip was determined. The leaves were 6-8 cm at the widest part and all the strips had fresh weights in the range 0.7-1.0 g.

Experiment 2

Lettuce plants (cv. Hilde) grown under similar conditions as Experiment 1 were used. Metalaxyl (0.1 µCi/ml, 200 mg/l) was applied to leaves in small droplets of approximately 0.02 ml to provide a volume of 0.5 ml per leaf. Applications were made to one fully expanded leaf on each of six plants, and the fungicide was wiped over the surface using a fine brush. With the exception of the treated leaf, the foliage of three of these plants was shaded with muslin to alter the 'source-sink' relationships within the plants. One week later, the metalaxyl content in all the leaves of each plant was determined. The leaves to which the fungicide had been applied were rinsed with methanol before extraction.

Experiment 3

Loss of metalaxyl from the surface of leaf discs was investigated using leaves from plants (cv. Hilde) grown under the conditions described for Experiment 2.

Experiment 3A. A 20 µl drop of metalaxyl (0.1 µCi/ml, 200 mg/l) was placed on the adaxial surface of a 2 cm diam. disc of leaf tissue. The drops were dried in a moving air current, and the discs placed in Petri dishes on moistened filter paper. Five replicate discs were removed at 0, 24, 48, 72 and 96 hours after application and rinsed three times with 1 ml of methanol. Preliminary studies had shown that the rinses removed all the applied fungicide immediately after the spot had dried. The metalaxyl concentration of the discs was determined.

Experiment 3B. The experimental procedure used for 3A was modified as follows. After the drops of fungicide had dried, the leaf discs were placed in 5 cm Petri dishes without filter paper and the dishes floated on glycerol solutions in sealed boxes. The glycerol solutions provided relative humidities of 80% and 90% within the boxes. Discs were also placed in dry boxes and boxes with distilled water only. Each humidity treatment was incubated for 24 h at 3.5, 15, 20 and 25°C. The discs were then rinsed with methanol and loss from the disc surface was calculated.

Experiment 4

The degradation of metalaxyl was investigated in lettuce seedlings (cv. Hilde) grown in 7 cm crystallising dishes containing vermiculite and 20 ml Hewitt's nutrient solution amended with fungicide (0.02 µCi/ml, 10 mg/l). There were approximately 40 seedlings per dish. Fungicide concentrations in the seedlings were estimated 1, 2, 3 and 4 weeks after sowing. Each week all the seedlings from each dish were extracted together. The remaining supernatant from the extractions was rotary evaporated to approximately 500 µl and a 100 µl spot placed on a silica gel TLC plate (Whatman LK6DF). The plates were run in the solvent systems A: n-butanol-acetic acid-water (84:6:10), B: n-butanol-ammonium hydroxide-water (85:5:14), C: ethyl acetate and D: dichloromethane-methanol (85:15). The positions of metalaxyl and ^{14}C-metabolites were obtained by exposing the plates in a spark-chamber and by scraping the plate in segments. Silica gel removed from the plates was ground, eluted in methanol and counted. The concentrations of metalaxyl and its breakdown products present in the seedlings were determined.

RESULTS

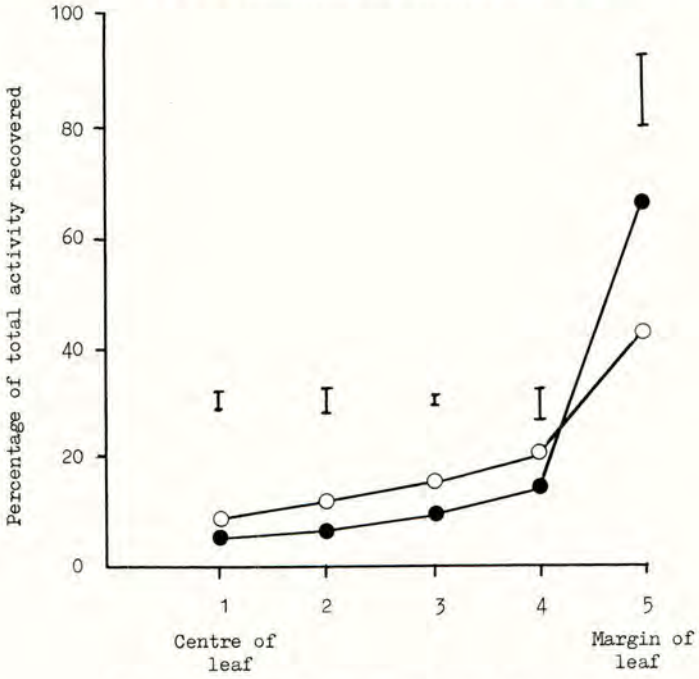
Experiment 1

The proportion of the total recovered activity found within the leaves of the two plants was 88.2 ± 7.0% and 85.8 ± 7.8% (95% confidence intervals). With an estimated fungicide concentration of 3.7 ± 1.1 mg/kg and 3.6 ± 1.1 mg/kg leaf tissue respectively. The oldest and youngest leaves generally contained the lowest concentrations of fungicide.

Metalaxyl consistently accumulated in the outermost leaf strips (Fig. 1), with concentrations ranging from 6.0 to 27.0 mg/kg; in the innermost central strips concentrations ranged from 0.5 to 2.0 mg/kg. A comparison of the overall concentrations of chemical in the portion of the leaves divided into strips (upper half) with the remaining basal parts showed that the mean concentrations were 5.9 ± 1.1 mg/kg and 0.2 ± 0.04 mg/kg respectively.

Fig. 1

The proportion of ^{14}C-metalaxyl recovered from each of five strips of leaf tissue taken from the margin to the centre of three leaves per plant



(●) and (○) refer to leaves from different plants. Bars are 95% confidence intervals for difference between means.

Experiment 2

Metalaxyl was found in untreated foliage both above and below the treated leaf in all the plants. There was no significant difference ($P = 0.05$) between the percentage of total metalaxyl recovered from the untreated leaves of shaded (6.6%) and unshaded (5.0%) plants. In unshaded plants there was no significant difference between the proportion of total exported metalaxyl found in foliage above and below the treated leaf. However, shading resulted in a significantly higher ($P = 0.05$) proportion of the total exported metalaxyl being transported acropetally (Table 1).

Table 1

Distribution of ^{14}C-metalaxyl in plants 1 week after application of the chemical to a single leaf

	% of total ^{14}C-metalaxyl exported from treated leaf in:	
	Upper leaves[a]	Lower leaves[b]
Unshaded plants	47.8(54.7)[c]	45.3(42.5)
Shaded plants	61.1(75.9)	28.9(24.1)

LSD = 22.5 (P = 0.05) (horizontal comparison only)

[a] leaves above treated leaf
[b] leaves below treated leaf
[c] figures are angular transformed percentages; figures in parentheses are actual percentages

Experiment 3

When discs were rinsed immediately after the fungicide drop had dried, all the applied fungicide was recovered from the surface. It was therefore assumed that the quantity of fungicide taken up by the discs and lost by volatilisation was the difference between the amount applied and that recovered by rinsing.

The loss from the discs was rapid; almost 50% in the first 24 hours and more than 90% after 3 days (Fig. 2).

When discs were incubated at different temperatures and relative humidities it was found following analysis of variance that humidity had no significant effect (P = 0.05) while increasing temperatures significantly (P = 0.001) increased loss. The highest loss was obtained at 90% RH and 25°C (Table 2).

Experiment 4

Apart from metalaxyl, two further areas of radio activity, designated I and II were found on TLC plates. These two areas are assumed to be the positions of the metabolic breakdown products of metalaxyl. The compounds were less mobile than metalaxyl in all the solvent systems used, and they did not separate in ethyl acetate (Table 3). Table 4 shows that the total concentration of ^{14}C-labelled compound in the seedlings increased during the period of the experiment. The proportion of label remaining in the form of metalaxyl decreased to 35% of the total residual label in the seedlings. However, the metalaxyl concentration in the seedlings remained the same throughout the experiment.

Fig. 2

The percentage of ^{14}C-metalaxyl lost over a five day period from a dried droplet of chemical applied to leaf discs

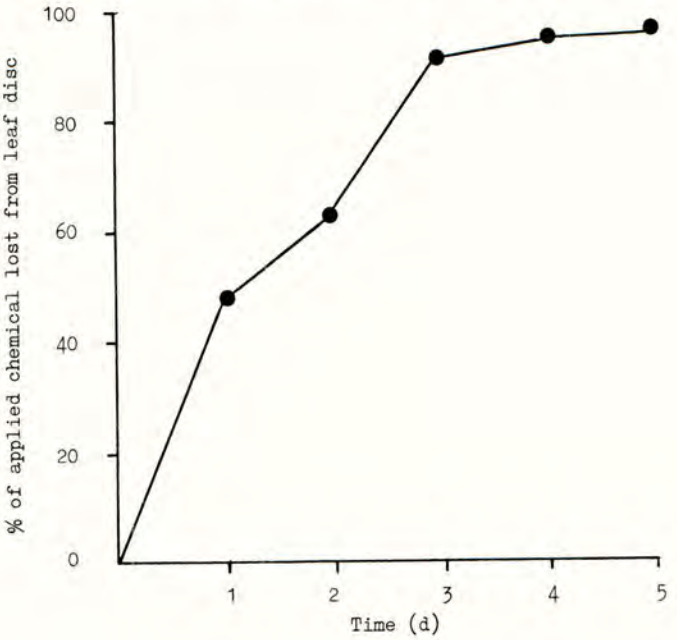


Table 2

The effect of temperature and relative humidity on the loss of metalaxyl from the surface of leaf discs

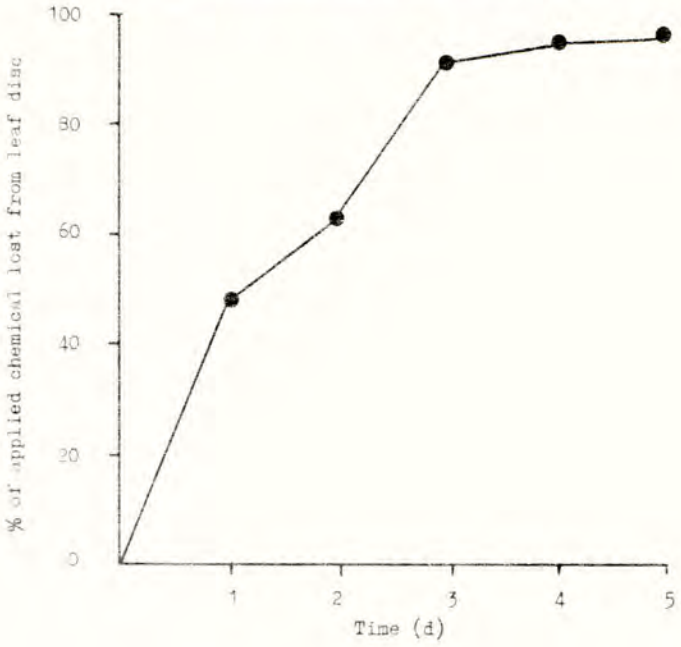
Proportion of applied chemical lost from leaf discs after 24 h				
	Temperature (°C)			
r.h. (%)	3.5	15	20	25
0	18.1(25.2)[a]	27.0(31.3)	38.8(38.5)	47.4(43.5)
80	13.3(21.4)	32.7(35.0)	43.2(41.0)	40.9(39.8)
90	20.5(26.9)	25.7(30.5)	44.5(41.8)	65.9(54.3)
100	19.4(26.1)	20.1(26.6)	43.7(41.4)	45.0(42.1)

LSD = 13.1 ($\underline{P}$ = 0.05) (horizontal comparison only)

[a] Figures are angular transformed percentages; figures in parentheses are actual percentages

Fig. 2

The percentage of ^{14}C-metalaxyl lost over a five day period from a dried droplet of chemical applied to leaf discs



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90	22.5(26.9)	25.7(30.5)	44.5(41.8)	65.9(54.3)
100	19.4(26.1)	20.1(26.6)	43.7(41.4)	45.0(42.1)

LSD = 17.1 (P = 0.05) (horizontal comparison only)

[a] Figures are angular transformed percentages; figures in parentheses are actual percentages

Table 3

The movement of metalaxyl and metalaxyl degradation products in various solvent systems

Solvent system[a]	100 x Rf values		
	Metalaxyl	I	II
A	85	60	40
B	85	10	5
C	80	0[b]	0[b]
D	90	50	10

[a] see text for details

[b] both I and II did not move in solvent C

Table 4

The appearance of ^{14}C-metalaxyl degradation products in lettuce seedlings over a 4 week period

Time (weeks)	Total residual label	% of total label in:		
		Metalaxyl	I	II
1	7.8[a]	93.5	3.8	2.7
2	10.2	62.7	36.3	1.0
3	16.1	42.9	33.5	23.6
4	19.5	34.9	37.4	27.7

a Metalaxyl equivalents in mg/kg of plant tissue

DISCUSSION

In these experiments the proportion of the applied chemical entering the leaves was high. Once within the plant metalaxyl was transported to the distal end of the leaves and across the lamina concentrating in the leaf margins. Translamina movement was demonstrated by Staub et al. (1978) in grape vines and tomato. However, autoradiographs of ^{14}C-metalaxyl in leaves of P. indica and avocado suggested a more even distribution (Zaki et al., 1981), although redistribution of metalaxyl may have occurred during preparation of the material. The movement of metalaxyl in lettuce leaves results in much variation in concentration within a single leaf. Studies on the sensitivity of some B. lactucae isolates to metalaxyl have shown that concentrations of 0.07 mg/kg prevented sporulation in lettuce seedlings while 0.007 mg/kg significantly reduced sporulation (Wynn and Crute, 1981). The lowest concentrations of metalaxyl found in the basal regions of leaves would still be sufficient to prevent disease development. However, if the quantity of metalaxyl accumulating at

the leaf margin was increased by environmental factors such as those favouring a high transpiration rate then the concentration in other parts of the leaf could conceivably fall to levels that would allow disease development. It appears that B. lactucae would need to be insensitive to 0.1 mg/kg metalaxyl before problems of control in the field situation would be likely to occur.

A small proportion of the applied fungicide moved from the site of application both up and down the plant. The proportions were similar to those obtained by Zaki et al. (1981) with tomato and P. indica. The appearance of metalaxyl in untreated foliage below the treated leaf together with an alteration in the distribution of the fungicide following shading suggests that the fungicide may be transported in the phloem as well as in the xylem (Zake et al., 1981).

In the leaf disc experiments, there was a significant effect of temperature but not relative humidity on loss from the disc surface. The vapour activity of metalaxyl has been demonstrated (Kerkenaar and Sijpesteijn, 1981) and volatilisation may account for some of the redistribution between leaves in Experiment 2 and of the loss from the discs in Experiment 3 (T. Staub, personal communication). The leaf discs may be useful for the investigation of environmental effects on uptake only where loss from the surface can be separated into components of uptake and volatilisation. This volatility may also cause problems in the interpretation of experiments with whole plants.

The results of the degradation studies show that metalaxyl was readily taken up by seedlings. The increase of labelled material in the seedlings was due to continuous uptake from a relatively large reservoir. The two areas of radioactivity observed on the TLC plates apart from ^{14}C-metalaxyl, are assumed to be degradation products of metalaxyl. Their presence increased with time until after 4 weeks only 35% of the total labelled material in the seedlings was parent compound. If the reservoir of metalaxyl had been small and had thus restricted uptake the final proportion of metalaxyl could well have been lower. The concentration of metalaxyl in the seedlings remained almost constant throughout the experiment and was approximately 70% of that applied in the nutrient solution. These results demonstrate that the seedling system provides a standard and uniform method for assaying isolates of B. lactucae for sensitivity to metalaxyl. The substantial breakdown of metalaxyl within 2 weeks indicates that experiments with ^{14}C-metalaxyl conducted over these or longer periods must take into account this degradation. There are no published data on the degradation products of metalaxyl in plants although unpublished data quoted by Zaki et al. (1981) indicated that 30-60% of ^{14}C-metalaxyl was in its original form 21-28 days after application. The data reported here agree with this finding. There are no published data on the biological activity or identity of either of the two products although their sequential appearance suggests a step-wise degradation of metalaxyl to compound I followed by compound II.

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References

BRAY, G.A. (1960) A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Analytical Biochemistry, 1, 279-285.

COHEN, Y; REUVENI, M.; EYAL, H. (1979) The systemic antifungal activity of Ridomil against Phytophthora infestans on tomato plants. Phytopathology, 69, 645.

CRUTE, I.R. (1980) Foliar applications of metalaxyl against lettuce downy mildew, 1978. Tests of Agrochemicals and Cultivars (Annals of Applied Biology 94 Supplement), No. 1, 30-31.

CRUTE, I.R.; GORDON, P.L. (1980) Foliar applications of metalaxyl to control lettuce downy mildew, 1979. Tests of Agrochemicals and Cultivars (Annals of Applied Biology 94 Supplement), No. 1, 32-33.

DUECK, J.; STONE, J.R. (1979) Evaluation of fungicides for control of Albugo candida in turniprape. Canadian Journal of Plant Science, 59, 423-428.

GRIFFIN, M.J.; GRIFFIN, G.W. (1977) Evaluation of new systemic fungicide against lettuce downy mildew. Proceedings 1977 British Crop Protection Conference, pp. 301-308.

KERKENAAR, A.; KAARS SIJPESTEIJN, A. (1981) Antifungal activity of metalaxyl and furalaxyl. Pesticide Biochemistry and Physiology, 15, 71-78.

KNAUSS, J.F. (1977) CGA48988, a new systemic fungicide for control of Phytophthora nicotianae var. parasitica on Pepperamia oblusifolia. Proceedings of the Florida State Horticulture Society, 90, 330-332.

O'BRIEN, R.G. (1978) Systemic chemicals for tobacco blue mold control. Plant Disease Reporter, 62, 277-279.

PAULUS, A.O.; NELSON, J.; SNYDER, M.; GAFNEY, J. (1977) Downy mildew of lettuce controlled by systemic fungicides. California Agriculture, 31, 9.

STAUB, T.; DAHMEN, H.; SCHWINN, F.J. (1978) Biological, characterisation of uptake and translocation of fungicidal acylalanines in grape, and tomato plants. Journal of Plant Disease Protection, 85, 162-168.

URECH, P.A.; SCHWINN, F.J.; STAUB, T. (1977) CGA 48988, a novel fungicide for the control of late blight, downy mildews and related soil-borne diseases. Proceedings 1977 British Crop Protection Conference, pp. 623-631.

WYNN, E.C.; CRUTE, I.R. (1981) Studies on the sensitivity of Bremia lactucae to metalaxyl. Netherlands Journal of Plant Pathology (in press).

ZAKI, A.I.; ZENTMYER, G.A.; LEBARON, H.M. (1981) Systemic translocation of ^{14}C-labelled in tomato avocado and Persea indica. Phytopathology, 71, 509-514.

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THE EFFECT OF FORMULATION, STRUCTURE TYPE, AND ENVIRONMENTAL CONDITIONS ON THE BEHAVIOR AND FATE OF SELECTED PESTICIDES APPLIED IN GREENHOUSES WITH PULSE-JET APPLICATORS

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Summary Permethrin 25% EC, 25% WP, benomyl 50% WP, and iprodione 50% WP were dispersed in crop-free greenhouses with a pulse-jet applicator. The effect of formulation, temperature, and structure covering on permethrin deposition and toxicity to Trichoplusia ni larvae was observed. Fungicide dispersal and deposition was measured via Botrytis sp. bioassay. The effect of air movement on upper and lower surface deposition also was measured.

Most pesticide deposition was on upper surfaces. Permethrin WP residues were heavier, more evenly distributed, and more toxic to T. ni than the EC. Temperature or structure covering did not affect results. Both fungicides settled out heavily within 4-5 m of the applicator whether applied in large or small greenhouses, but overall deposition was more even in larger greenhouses. Air movement did not improve lower surface deposition, and may have reduced upper surface deposition.

INTRODUCTION

The use of various types of low-volume (LV) pesticide application equipment in greenhouses is increasing in the U.S. because they save labor and may save pesticide. This kind of equipment has been used for many years in Europe, and a number of studies have covered the advantages and disadvantages of LV applications and equipment (Anonymous, 1976; Jarrett and Burges, 1978; Matthews, 1976, 1979; Wenner, 1979). Lindquist and Powell (1980) evaluated several LV applicators for efficacy against selected pests, and examined droplet deposition. Results indicated that the specific type of LV equipment used was secondary in importance when determining efficacy to the properties of the pesticide and/or behavior of the pests. Further, most droplets were deposited within a relatively short (10-20 m) distance from the applicator. However, many questions remained concerning the fate, behavior, and efficacy of different pesticides and pesticide formulations. The results reported here are from experiments designed to define and explain some of the variables encountered in applying pesticides in protected environments. We wished to obtain further information on the role of pesticide formulation in dispersal, deposition, and efficacy, and to examine how these properties were modified by structure type and environmental conditions within the greenhouse.

MATERIALS AND METHODS

All applications were made with a Pulsfog® K-30 (Manufactured by Dr. Stahl and Sohn GMBH, Uberlingen, West Germany), supplied by Dramm International, Manitowoc,

Wisconsin, U.S.A. This equipment was designed to apply both liquid and powder pesticide formulations, when mixed with the proper dispersal agent. The dispersal agents VK-I® (for EC) and VK-II® (for WP) were used according to the manufacturer's directions. VK-I is a mixture of aliphatic chloric hydrocarbons and hydrocarbons with a 95% unsulphonating residue, designed for use with petroleum-based pesticides, and VK-II is a mixture of multihydric alcohols and water, for use with powder formulations.

Insecticide Evaluations - The insecticide used was permethrin as a 25% EC or WP (2EC, 25WP). Application rates were 25 ml and 25 g a.i./1000 m^2 for the EC and WP formulation, respectively. Twenty-three separate applications were made in this study (11 WP, 12 EC). Not all parameters were measured in each application. Experiments were conducted in 12 x 32 m greenhouse compartments, with the pesticide applied from a stationary position at one end. There were no crops in the greenhouses and potted cabbage plants were placed in the houses for determination of permethrin toxicity to T. ni. One greenhouse was of standard glass construction, while the other was glass covered with an air-supported double layer of polyethylene.

Surface residues of permethrin were estimated by placing glass plates (124 cm^2) at 12 different positions throughout the greenhouses immediately prior to applications. Two plates were placed on top of one another at each position in several experiments, so that an indication of residues on upper and lower surfaces could be obtained. In most experiments plates were collected 1 h after treatment and washed with acetone for subsequent residue analysis by gas chromatography. Results are reported as $\mu g/cm^2$.

Short-term effects of temperature on residues and efficacy were determined by making applications during "cool" parts of the day (i.e., early morning or evening) and "hot" periods (mid to late-afternoon). Temperatures during "cool" applications averaged 22.2°C in both polyethylene and glass-covered greenhouses. Relative humidities averaged 93% in the polyethylene-covered house and 80% in the glass-covered house. During "hot" applications, temperatures averaged 49°C and relative humidities 30%. In all but the air movement studies, ventilators were closed and fans turned off during, and for 1 h post-treatment.

The effect of air movement on coverage of upper and lower surfaces of glass plates was determined in the polyethylene-covered greenhouse, which was equipped with an overhead fan-jet air curculation system. This consisted of a fan with a large polyethylene tube extending nearly the length of the greenhouse. The tube had holes (ca. 10 cm diam.) spaced along its length for air escape. This allowed air to be circulated within the greenhouse, without any ventilators being opened to the outside. The circulation system was turned on immediately prior to each application and remained on until 10-15 minutes after treatment. Glass plates were placed in several locations, washed, and residues analyzed as described previously.

Permethrin toxicity to T. ni larvae was measured by placing 12-20 potted cabbage plants in different locations within the greenhouse just prior to applications. For evaluation of "residual" deposits, plants were brought to the laboratory ca. 1 h post-treatment and 2-3 leaf discs (2.5 cm diam.) removed and placed on moist filter paper in petri dishes. Five T. ni larvae (instar 2 or 3) were then transferred to each leaf disc. After 48 h, larval mortality and feeding injury were recorded. "Contact" activity was assessed by placing 10-15 larvae on cabbage plants just prior to application and allowing the larvae and plants to remain in the greenhouse for 1 h post-treatment, before removal to the laboratory. A 2.5 cm wide barrier of sticky tape was placed around the edge of each pot to restrict larvae migration. Larval mortality was recorded 18 h after treatment.

Fungicide Evaluations - The fungicides used were benomyl 50% WP and iprodione 50% WP. Both materials were applied at 4.9 gm a.i./1000 m^2 in a mixture of VK-II

and water, according to the manufacturer's directions. Most experiments were in a standard glass greenhouse, 6 x 8 m, but several applications were made in a polyethylene greenhouse, 19 x 48 m. Applications were made from a stationary position at one end. Ventilators were closed and fans turned off for 2 h after each treatment, except when a centrally located overhead turbulator (Nivola Dutch Mill) remained on for 2 h to determine effects of air circulation on fungicide dispersal and deposition.

Fungicide deposition was bioassayed with conidia of a Botrytis sp. Uncovered petri dishes containing 10.6 ml of acidified potato dextrose agar (0.3 ml lactic acid/ℓ) were placed throughout the greenhouse just prior to each experiment. Two h after treatment, plates were seeded with 0.05 ml of a suspension of conidia adjusted to 25 spores/uℓ of distilled water. The percentage of germinated spores was determined 24 h after inoculation. For all experiments, 100 spores were counted on each dish. Six petri dishes were placed at each location (3 facing up, 3 facing down) within the greenhouse for each application, and each experiment was repeated 3 times.

The susceptibility of Botrytis spores to the fungicides was measured by dose/response curves. A X2 concentration series (µg a.i./ℓ) between .006 and .42 was prepared for each fungicide. One-half ml of fungicide suspension, with 10 ml/Tween-20® added as a wetting agent was put on each petri dish. The fungicide solution was then spread evenly with a sterilized glass bar. This resulted in a range of fungicide concentrations of between 0.052 and 3.44 µg benomyl/cm^2 and 0.052 and 3.40 µg iprodione/cm^2. Uncovered petri dishes were then left to dry in a refrigerator overnight. The following day the plates were inoculated with Botrytis as described above. Using these curves, the residues of fungicide resulting from the applications was determined.

RESULTS

Insecticides - Permethrin residues were much higher on upper glass plates for both EC and WP formulations (approx. 95% of total residues). Air circulation with the fan jet circulator did not improve lower surface residues (Table 1). Consequently, only residues on upper surfaces are presented in the other tables. When residues deposited by EC and WP formulations were compared, averaged over all locations (Table 2), the WP formulations were generally significantly higher. Allowing glass plates to remain in the greenhouse overnight (12 h) did not increase residue levels. Temperature during and immediately following application seemed to have no effect, nor did the type of greenhouse covering.

The generally higher deposition of the WP formulation is reflected in higher mortality figures (Table 3). Also, when mean residue levels at each location are compared, the WP was more evenly distributed, while the EC had highest residues in areas closest to the applicator. This also is shown in Table 3. There was a significant correlation (r = .555) between permethrin residues and T. ni mortality at all locations. The correlation between residues and feeding injury rating (r = -.757) also was significant. (A high residue level generally resulted in a low feeding injury rating). The lack of residual deposit of the EC did not affect control of T. ni larvae on plants in the greenhouse during applications (= "contact" activity). Larval mortality was 100% in all locations with both formulations, whether the greenhouse was ventilated after 1 h, or remained closed overnight.

Fungicide Applications - Figures 1 and 2 show the estimated deposition of fungicide, by Botrytis bioassay, on both upper and lower surfaces with and without air movement for benomyl and iprodione, respectively, in the smaller greenhouses. Figure 3 compares iprodione deposition in a small and larger greenhouse. When Figures 1 and 2 are compared, more benomyl apparently was deposited than iprodione. However, iprodione, at rates used, was more effective against Botrytis than benomyl, and spore germination percentages were lower. Distribution patterns were similar

Table 1

Residues ($\mu g/cm^2$) of permethrin WP and EC on upper and lower surfaces of glass plates with air circulation on or off during application with a pulse-jet applicator.

	AIR OFF[a/]		AIR ON[a/]	
	EC	WP	EC	WP
Upper surface	.018 b	.034 a	.011 b	.028 a
Lower surface	.001 c	.002 c	0 c	.002 c

a/ Means of 2 experiments each with 4 replications; means followed by a letter in common are not significantly different (P = 0.05), according to Duncan's New Multiple Range Test.

Table 2

Permethrin WP and EC residues ($\mu g/cm^2$) washed off glass plates after application with a pulse-jet applicator.

Formulation[a/]	Temp.[b/]	Cover[c/]	Mean Residue[d/]
WP	H	G	.042 a
WP	C	G	.040 a
WP	C	P	.037 ab
WP	H	P	.035 b
WP	H	P	.033 b
WP	C	G	.033 b
EC	H	P	.033 b
WP	C	P	.032 b
WP	C	G	.026 c
WP	C	P	.026 c
EC	H	P	.022 cd
EC	C	G	.022 cd
WP	H	G	.021 cd
EC	H	G	.019 de
EC	C	P	.018 de
EC	H	G	.014 e
EC	C	P	.009 f

a/ WP = permethrin 25% WP; EC = permethrin 25% EC.
b/ H = hot conditions, 49°C; C = cool conditions, 22.2°C.
c/ G = standard glasshouse; P = double-layered polyethylene over glass.
d/ Upper surface residues; means of 12 locations at each date. Means followed by a letter in common are not significantly different (P = 0.05), according to Duncan's New Multiple Range Test.

Table 3

Permethrin residues ($\mu g/cm^2$) washed from glass plates, plus Trichoplusia ni larval mortality and feeding injury rating after application with a pulse-jet applicator.

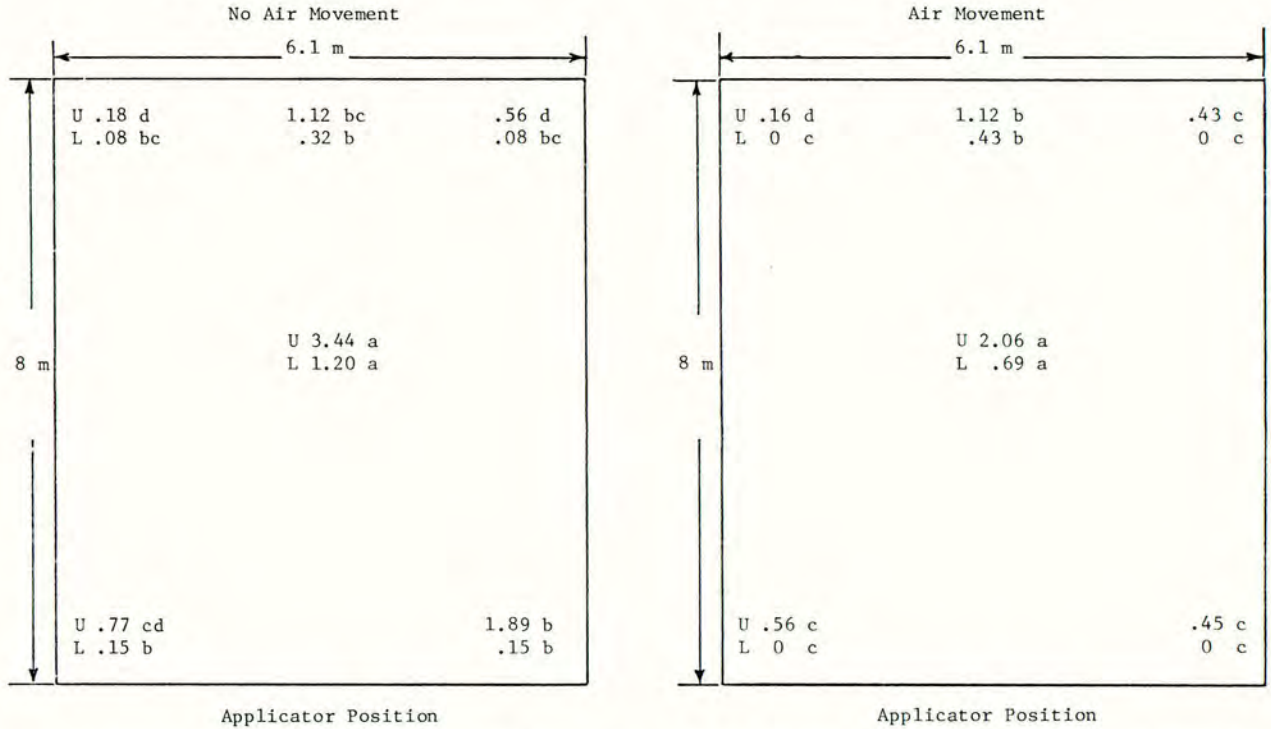
Position[a]	Residue (WP)[b]	% Mortality[c]	Feeding Injury Rating[c]	Residue (EC)[b]	% Mortality[c]	Feeding Injury Rating[c]
1	.028 abc	91	0.2	.016 d	66	1.9
2	.032 a	94	0.5	.018 c	53	1.9
3	.032 a	100	0	.018 c	70	1.5
4	.032 a	95	0.2	.018 c	63	2.1
5	.032 a	94	0.2	.016 d	63	2.0
6	.036 a	97	0.2	.020 bcd	71	1.5
7	.036 a	98	0	.018 cd	62	1.8
8	.028 abc	100	0	.033 a	86	1.0
9	.028 abc	94	0	.018 cd	60	2.0
10	.036 a	88	0.2	.020 bcd	61	1.9
11	.036 a	88	0.2	.016 d	53	2.0
12	.035 a	92	0	.014 d	51	1.9
Untreated		0	4		0	4

a/ Positions in 3 rows of 4 across the greenhouse, from back to front, 1-4, 5-8, 9-12, with position 8 in front center, closest to applicator; applications were made from a stationary position.

b/ Residues (upper surface) washed off glass plates; residue data averaged over temperatures; means of 10 replications at each location; means followed by a letter in common are not significantly different (P = 0.05), according to Duncan's New Multiple Range Test.

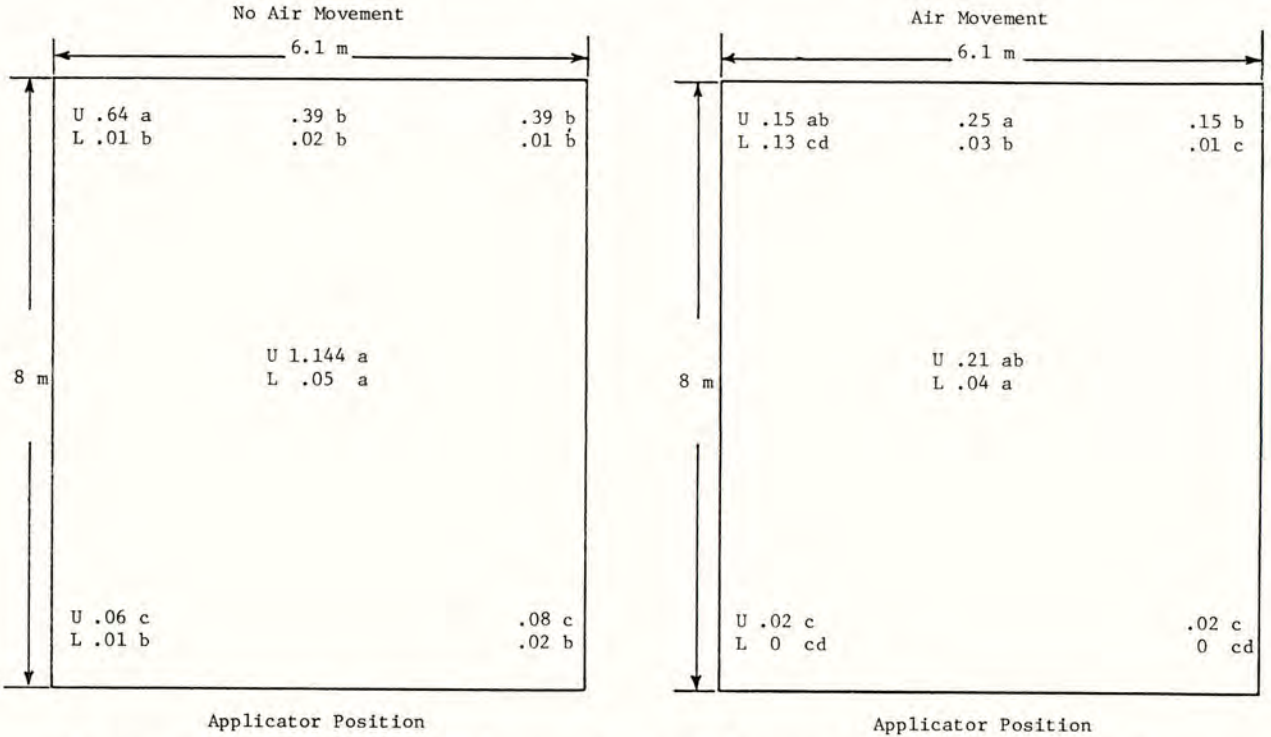
c/ Mortality and feeding injury rating means of 4 (WP) or 6 (EC) applications 48 h after larvae were confined on 2.5 cm diam. leaf discs. Injury ratings on a 0-4 scale, 0 = no feeding, 4 = leaf discs consumed. Correlation between T. ni mortality and residues, 4 = .555 and between feeding injury and residues, r = -.757.

Figure 1. Deposition of benomyl ($\mu g/cm^2$), as estimated by Botrytis bioassay, with and without air circulation.



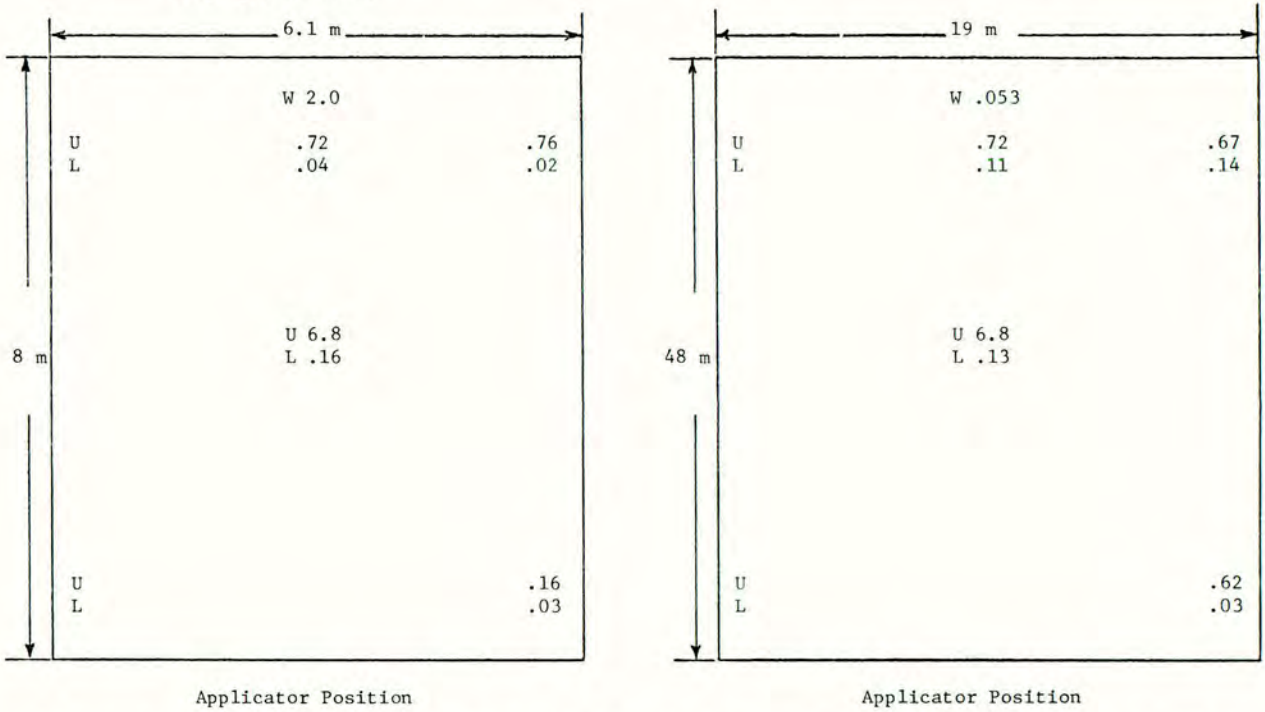
U = upper surface, L = lower surface; 3 applications, with 3 replications/position; means on each surface followed by a letter in common are not significantly different (P = 0.05), according to Duncan's New Multiple Range Test.

Figure 2. Deposition of iprodione ($\mu g/cm^2$), as estimated by Botrytis bioassay, with and without air circulation.



U = upper surface, L = lower surface; 3 applications, with 3 replications/position; means on each surface followed by a letter in common are not significantly different (P = 0.05), according to Duncan's New Multiple Range Test.

Figure 3. Deposition of iprodione ($\mu g/cm^2$), as estimated by Botrytis bioassay in small and large greenhouses.



U = upper surface, L = lower surface, W = wall; 2 applications, with 3 replications/position; "center" position ca. 5 m from applicator in both greenhouses.

for both materials. Both fungicides settled out heavily within 4-5 m of the applicator in all experiments. Iprodione deposited less heavily in the areas directly beside the applicator. Little deposition was measured on lower surfaces. When turbulators were on, less fungicide was deposited on both upper and lower surfaces. Applications of iprodione in smaller greenhouse resulted in considerable deposition of fungicide on the back wall, compared with the large greenhouse (Figure 3). Deposition in the larger greenhouse was heavier and more uniform in areas beside the applicator than in the small house.

DISCUSSION

Crop-free greenhouses were used in this study to provide information on basic deposition and distribution phenomena. If applications are made through a crop, some filtering would occur, as was shown by Jarrett and Burges (1978). Also, vertical distribution may be affected. Although glass plates or petri dishes may have resulted in different deposition patterns than with leaves (Uk, 1978), we used them to eliminate any effects of leaf type and leaf angle. Furthermore, we felt that by keeping the pulse-jet applicator in one location we would obtain better information on the behavior and fate of the pesticides.

As was shown by results with the WP formulations, the type of pulse-jet applicator used in these experiments is able to apply a residual deposit of a pesticide (permethrin) on plants sufficient to kill 90-100% of T. ni larvae placed on them 1-2 hr. post-treatment. Except for the area just in front of the applicator, where residues from the EC were statistically identical to those of the WP, much lower residues were detected after EC applications. Perhaps the droplets from the EC were too small to impinge heavily on either glass plates or leaves, but drifted throughout the greenhouse, filling the space with pesticide. This may have been the reason for the effective control of T. ni larvae when the insects were actually present during the application.

An interesting aspect of this study was the relatively uniform residue deposits obtained with permethrin WP, compared with the fungicides. An equivalent amount of dispersal material was used with both insecticide and fungicide applications, and the particle size of the dry WP formulations was similar (although benomyl appeared to have smaller, more uniform particles, when suspended in the dispersal material). The difference may have been due to using a smaller injection nozzle orifice with permethrin, which resulted in smaller particles. Further experiments are underway to investigate this.

The apparent better suspension of the benomyl was reflected in more even distribution within the small greenhouses, compared with iprodione. Another important factor to consider by both researchers and growers when making LV applications is the apparent relationship of greenhouse size to overall distribution of the pesticide. When applications are made in small greenhouses or if the application is started too close to a wall in larger greenhouses, a significant amount of pesticide may impact on this surface rather than on plants or pests.

Air movement with fan-jet circulators or overhead turbulators did not improve deposition on lower surfaces, and with the turbulator may actually have circulated the pesticide away from the petri dish targets. However, if the greenhouse contained closely-spaced plants, air movement may aid coverage because of the plants' filtering effect. We also plan to investigate this area in future experiments.

This study again illustrates the importance of the behavior of both the pesticide (and formulation) and pest when making LV applications in greenhouses. Lindquist and Powell (1980) stated that pesticides with vapor activity probably will be most effective in these situations. However, the number of such pesticides is

limited, and to be useful, LV applicators will need to successfully apply a range of pesticides with only contact or residual activity. The data presented here illustrated such results, and showed that despite the relatively poor deposition on lower surfaces, control of some pests was obtained via contact and residual effects rather than the vapor activity of the pesticide. Probably, many pesticides can be successfully applied with this type of pulse-jet application equipment, but the choice of the best pesticide for particular problems will need further study.

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References

ANONYMOUS. (1976) Fogging under glass - special review, The Grower, 22 July, 1976, 191-198.

JARRETT, P., BURGES, H. D. (1978) Penetration of controlled drop spray of Bacillus thuringiensis into chrysanthemum beds compared with high volume spray and thermal fog, Proceedings of the Symposium on Controlled Drop Application, BCPC, 75-81.

LINDQUIST, R. K., C. C. POWELL, JR. (1980) Evaluation of low-volume pesticide applicators for use on glasshouse crops, Proceedings of Symposium on Spraying Systems for the 1980's - March 1980, BCPC, 271-281.

MATTHEWS, G. A. (1976) CDA - a revolution in spray application, Big Farm Management, July, 1976, 55-56.

MATTHEWS, G. A. (1979) Pesticide Application Methods, Longman, London, 334 p.

UK, S. (1977) Droplets in air, Part 2: Capture by natural surfaces, Pestic. Sci., 8, 501-509.

WENNER, M. A. (1979) Advances in controlled droplet application. Agrichemical Age, Nov-Dec. 1979, 18-25.

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INFLUENCE OF SOME NEW APPLICATION VARIABLES ON INSECTICIDE BEHAVIOUR AND AVAILABILITY IN SOIL

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Summary The uptake of disulfoton, terbufos and thiofanox by Brussels sprout plants was compared after their incorporation into peat blocks before sowing and also after application as sub-surface bands at transplanting. In the 7 weeks between incorporation and planting, disulfoton and terbufos residues changed only slightly while thiofanox residue concentrations declined by 30-35%, with extensive oxidation to the sulphoxide and sulphone. Dose-for-dose, thiofanox was the most, and terbufos the least, readily accumulated insecticide but, with all the insecticides, residue concentrations in the upper leaves were always greatest in plants treated with the sub-surface band.

The behaviour of carbofuran, chlorfenvinphos and phorate was also compared following application of granular formulations at 50 mg a.i./m to sandy loam in mineral colloid and natural gum carrier gels and by the bow-wave method. The uniformity of insecticide distribution along the row was similar by the two application methods. All the insecticides were more persistent after application in the natural gum gel and oxidation of phorate to its sulphoxide and sulphone was slower with both gel applications than in the bow-wave treatment.

INTRODUCTION

The behaviour, availability and performance of insecticides applied to soil by conventional techniques have been comprehensively reported for many years. Such abundant data mean that the relative merits and limitations of common application variables are now reasonably predictable. However, some recent developments in plant propagation, such as the sowing and raising of plants in peat blocks and the sowing of pre-germinated seed in carrier gels, have introduced new application procedures which have been shown to be very effective. Damage by cabbage root fly (Delia radicum) on summer cabbages (Saynor & Davies, 1977; Dunne et al, 1979) and early summer cauliflowers (Thompson & Percivall, 1978), by carrot fly (Psila rosae) on celery (Emmett, 1979) and by root aphid (Pemphigus bursarius) on lettuce (Thompson & Percivall, 1979) was reduced when crops were raised in insecticide-treated peat blocks. Some granular formulations of insecticides incorporated into gels used for drilling pre-germinated seed also protected carrots against carrot fly (Thompson et al, 1980). These results, together with studies of insecticide persistence in peat blocks (Suett & Padbury, 1981), suggested that the behaviour of some insecticides was modified by their incorporation into either peat blocks or into gels. Aspects of insecticide uptake by plants and behaviour in soil following field applications by these two new application methods were therefore further investigated.

METHODS AND MATERIALS

Insecticide uptake by block-raised and band-treated Brussels sprout plants

The prolonged persistence of phorate in peat blocks (Suett & Padbury, 1981) and

its ready uptake by lettuce (Suett & Padbury, 1980) suggested that systemic insecticides incorporated into the peat may protect block-raised Brussels sprouts against cabbage aphid (Brevicoryne brassicae) for much of the season. The uptake of 3 insecticides was therefore compared after incorporation into blocks immediately before sowing and also after application as sub-surface bands at transplanting.

The insecticides studied were granular formulations of disulfoton (Disyston FE-10, 10% a.i.), terbufos (10% a.i.; J.D. Campbell & Sons Ltd.) and thiofanox (Dacamox 5G, 5% a.i.). Peat blocks, 4.3 x 4.3 x 5.2 cm, were prepared from L + K blocking compost (Lindsey & Kesteven Fertilisers Ltd.) on 3 April 1979 using an Adelphi Biocompost mixer and a Visser blocking machine.' Disulfoton and terbufos were incorporated at target doses of 25 and 50 mg a.i./block and thiofanox at 10 and 25 mg a.i./block. Brussels sprout seed, cv. Citadel, was sown immediately after blocking and plants were retained under glass until 4 May when they were hardened off before hand-planting into sandy-loam at Wellesbourne on 24 May. Despite satisfactory emergence, incorporation of 25 mg thiofanox/block subsequently induced severe phytotoxic damage and this treatment was discarded before planting.

Sub-surface band applications of the 3 insecticides at 1.4 kg a.i./ha (0.98 g a.i./10 m row) were applied to untreated 'pulled' plants on 31 May using a Super Prefer twin-unit planter fitted with a Leeds(R) coulter as described previously (Wheatley, 1971). The experimental area comprised 3 replicates of 8 treated and 3 untreated plots, each plot consisting of 3 rows of 20 plants spaced at 71 cm between rows and 60 cm within rows. Ten peat blocks were analysed individually from each treatment at blocking and planting. Duplicate 20-plant samples were taken from the disulfoton- and terbufos-treated blocks at planting but insufficient plants remained in the thiofanox treatment for representative sampling. Plants were subsequently sampled at intervals by bulking the uppermost unfurled leaves from alternate plants in each plot.

Insecticide behaviour after field applications of fluid-drilling gels and by the bow-wave method

The behaviour of 3 insecticides was compared following applications in the field in gels and by the bow-wave method (Makepeace, 1965). The insecticides studied were granular formulations of carbofuran (Yaltox, 5% a.i.) chlorfenvinphos (Birlane Granules, 10% a.i.) and phorate (Campbell's Phorate, 10% a.i.). The experimental area, established at Wellesbourne on 4 June 1980, comprised 3 replicate blocks of nine randomised treated plots, each plot consisting of 2 x 10 m row, with 30 cm between rows. The soil was a light sandy-loam of the Wick Series, some relevant properties of which have been described previously (Suett, 1971). Each insecticide was applied by the bow-wave method and in 2 different carrier gels. The bow-wave method placed the insecticide granules in bands 8-10 cm wide on the soil surface so that the seeder share incorporated them to about 1.0 cm, the soil being consolidated by the rear press-wheels. The fluid carrier gels were a mineral colloid (Laponite(R), Laporte Industries Ltd) and a natural gum (Polymer DP 433, Hercules Powder Co. Ltd) prepared at concentrations of 20 and 8 g/l water respectively and were applied using a hand-operated fluid drill (Fluid Drilling Ltd). All applications were made at 50 mg a.i./m row, insecticide concentrations in the gels being determined from the drilling extrusion rate of 21.6 ml/m row. The area remained fallow for the duration of the experiment.

Soil samples were taken from all treatments immediately after application and after a further 6 and 12 weeks. To facilitate precision sampling from band applications in the field, a soil-sampling tool was designed and constructed. This comprised 2 parallel steel plates, 15 cm wide x 10 or, for later samples 15 cm deep and 5 cm apart, rigidly mounted on the lower end of a 90 cm-long vertical tube fitted with a horizontal handle. Before each core was taken, the internal distance between the plates was measured accurately with a caliper gauge. The plates were then inserted in the soil to full depth at right-angles to the row, with the centre of the

plates aligned with the row-centre. To isolate a rectangular core of soil, two more plates were then inserted hard against the vertical edges of the parallel plates and the core was removed carefully. Although time consuming, this procedure ensured that a precisely-measured length of row was sampled in each core. On each sampling occasion, 4 cores were taken from each plot (2 cores per row), bulked, sieved and subsampled. Initial samples were taken to a depth of 10 cm and subsequent samples to 15 cm.

To compare application uniformity, duplicate samples of 10 cores were taken 8 weeks after application from chlorfenvinphos treatments applied by the bow-wave method and by fluid drilling with the natural gum gel. Each of these cores was sieved and analysed individually.

Analytical procedures

Previously described analytical procedures were used to determine residues of chlorfenvinphos and phorate in soil (Suett, 1971): chlorfenvinphos was determined as the total of the Z- and E- isomers and phorate as residues of the parent compound and its sulphoxide (PSO) and sulphone (PSO_2). Carbofuran residues were determined by on-column transesterification of methanolic extracts (Moye, 1971).

Disulfoton and terbufos residues in peat blocks and Brussels sprouts were determined largely as described previously for disulfoton (Suett, 1977). Only the parent compounds and their respective sulphoxides (DSO, TSO) and sulphones (DSO_2, TSO_2) were detected in the peat blocks and these were separated by GLC on a diethylene glycol succinate column. Plant extracts were cleaned-up on a carbon-cellulose column before fractionation of the parent and oxygen analogue sulphoxides (DOASO, TOASO) and sulphones ($DOASO_2$, $TOASO_2$) on 7.5% deactivated silica gel, followed by GLC on 4% OV-101. Reliable GLC analysis of TSO was critically dependent on an accurately-maintained injection port temperature (165°C in the Phillips PV 4000 gas chromatograph used for these analyses).

Thiofanox residues were determined by modifying substantially the method of Chin et al (1975). Soil and plant samples were extracted with acetone: dichloromethane (1:1) and an acid precipitation cleanup was introduced to remove interfering co-extractives from the sprout extracts. A measured proportion of the extract was retained for analysis of parent thiofanox, the remainder being oxidised to thiofanox sulphone ($ThSO_2$) with peracetic acid, cleaned-up on a 3 g activated Florisil column and the concentration of total sulphoxide and sulphone determined by difference. Extracts were analysed by GLC on a 7.5% Reoplex 400/Chromosorb W-HP column using a flame photometric detector in the sulphur mode. Response was optimised by injecting on to non-silanised Pyrex glass wool within 2-3 mm of the start of the column packing.

Analytical efficiencies, assessed by fortifying untreated samples at 0.1-1.0 mg/kg, exceeded 90% and results were not corrected.

RESULTS

Uptake by block-raised and band-treated Brussels sprouts

Residue concentrations in the peat blocks are summarised in Table 1 as means of analyses of 10 individual blocks. Thiofanox was the least stable insecticide so that, although the mean doses achieved at blocking were closest to the target doses, these declined by 30-35% during the 7-week period prior to planting. In contrast, the achieved doses of disulfoton and terbufos were all substantially below the target dose but subsequently changed only slightly. During this period, all the insecticides were partially oxidised to their sulphoxides and sulphones, oxidation in all instances being more extensive in the blocks containing the lower doses. Thiofanox was the most readily oxidised insecticide, the residues in the lower dose blocks being comprised of almost 80% sulphoxide and sulphone. At the other extreme residues in the terbufos-treated blocks still comprised more than 85% parent

Table 1

Changes in concentration (mg a.i./block) and composition of residues of disulfoton, terbofus and thiofanox in peat blocks between sowing and planting block-raised Brussels sprouts

Weeks after sowing	Dose (a)	disulfoton				terbufos				thiofanox		
		D	DSO	DSO_2	total ±se	T	TSO	TSO_2	total ±se	Th	ThSO + $ThSO_2$	total ±se
0	H	38.3	3.9	*	42.1 ±6.6	38.2	*	*	38.2 ±3.3	19.7	4.4	24.1 ±1.9
	L	14.8	3.7	*	18.5 ±2.4	17.1	*	*	17.1 ±2.3	6.8	1.9	8.7 ±0.9
7	H	20.2	19.4	1.7	41.3 ±2.6	31.0	3.0	*	34.0 ±1.7	6.8	10.3	17.1 ±0.9
	L	5.0	10.6	1.2	16.8 ±2.0	14.2	1.8	0.1	16.1 ±1.1	1.2	4.3	5.5 ±1.0

(a) target doses disulfoton + terbufos = 50 (H) + 25 (L), thiofanox = 25 (H) and 10 (L) mg a.i./block
D, T, Th = parent insecticides; ( )SO = parent sulphoxide; ( )SO_2 = parent sulphone; * = not detected

insecticide 7 weeks after treatment, with only a trace of the sulphone (<1% of the total residue) in the lower-dose blocks.

Table 2 shows the residue levels in the whole block-raised plants at planting and in the upper leaves in plants from all treatments on subsequent sampling occasions, the results at planting being expressed as the means of two replicate samples and all subsequent results as the means of three replicates.

In all treatments the residue concentrations in the upper leaves declined as the plants grew and, on each sampling occasion, they were always greatest in the plants treated with the sub-surface band. Terbufos was the only parent insecticide detected and this was present in only trace amounts in the block-raised plants at planting and in the first band sample, taken 4 weeks after planting. Neither the oxygen analogues nor the oxygen analogue sulphoxides of disulfoton and terbufos were detected (<0.01 mg/kg). Whole plant analyses of these two treatments generally reflected the different levels but, dose for dose, residues from disulfoton-treated blocks were always 5-10 times higher than in plants raised in terbufos-treated blocks. The slower uptake of terbufos was further evident in subsequent leaf samples and residues could not be detected in block-raised plants 4 wk after planting, or in those from the band treatment after 7 wk. The composition of the residues of the two organophosphorus insecticides also differed markedly. The disulfoton residues changed gradually from predominantly DSO_2 (in the whole plants and the band treatment after 4 wk) to only $DOASO_2$ after 10 wk while the proportions of $TOASO_2$ in the total residue declined.

Dose-for-dose, thiofanox was the most readily-accumulated insecticide and its uptake into the upper leaves was also the most prolonged. Despite a dose of only 5.5 mg a.i./block at planting (Table 1), thiofanox residues were, except for the 4-wk sampling, always greater than disulfoton residues in plants from blocks containing 8 times as much insecticide. In the band treatment, where similar doses were applied, thiofanox residues were consistently 5-10 times greater than those of disulfoton.

Table 2

Changes in residues (mg/kg) of disulfoton, terbufos and thiofanox in whole plants and upper leaves of Brussels sprouts

Weeks after planting	Plant part	treatment (a)	disulfoton				terbufos					thiofanox
			DSO	DSO_2	$DOASO_2$	total	T	TSO	TSO_2	$TOASO_2$	total	$(ThSO + ThSO_2)$
0	whole plant	block H	17.8	55.4	8.3	81.5	0.03	4.0	3.0	1.8	8.8	-
		block L	7.7	35.1	8.8	51.6	0.04	3.0	1.1	1.0	5.1	-
4	upper leaf	block H	0.09	0.21	0.27	0.57	*	0.05	0.02	*	0.07	-
		block L	0.03	0.03	0.14	0.20	*	0.01	*	*	0.01	0.26
		band	1.4	4.0	0.86	6.3	0.01	0.61	0.36	0.03	1.0	21.0
7	upper leaf	block H	*	0.02	0.05	0.07	*	*	*	*	*	-
		block L	*	0.03	0.01	0.04	*	*	*	*	*	0.15
		band	*	0.06	0.37	0.43	*	0.03	0.05	*	0.08	2.5
10	upper leaf	block H	*	*	0.04	0.04	*	*	*	*	*	-
		block L	*	*	0.01	0.01	*	*	*	*	*	0.03
		band	*	*	0.11	0.11	*	*	*	*	*	0.96
13	upper leaf	block H	*	*	0.03	0.03	*	*	*	*	*	-
		block L	*	*	0.01	0.01	*	*	*	*	*	*
		band	*	*	0.03	0.03	*	*	*	*	*	0.26
17	upper leaf	band	*	*	*	*	*	*	*	*	*	0.06

(a) see footnote, Table 1, for explanation of all symbols ( )$OASO_2$ = oxygen analogue sulphone
* = not detected

Application in gels and by the bow-wave method

Soil residues in Table 3 are expressed as the means of three replicate plots. Unless otherwise stated, all significant sample mean differences have been established at the 95% confidence level.

Table 3

Residues of carbofuran, chlorfenvinphos and phorate (mg a.i./m) in sandy-loam after application at 50 mg a.i./m in fluid-drilling gels and by the bow-wave method

Weeks after applic^{n}	Method of applic^{n}	Carbofuran	Chlorfenvinphos	Phorate			
				P	PSO	PSO_2	total
0	bow-wave	41.05	44.97	7.32	11.17	5.79	24.28
	Laponite	40.04	35.43	8.29	5.66	3.46	17.41
	DP 433	44.17	46.33	7.39	7.64	4.25	19.28
6	bow-wave	11.70	25.56	0.38	3.89	9.76	14.03
	Laponite	10.88	29.97	1.63	4.42	6.38	12.43
	DP 433	18.40	35.06	2.98	6.47	7.59	17.04
12	bow-wave	5.77	18.39	9.17	1.81	9.66	11.64
	Laponite	5.68	26.88	0.53	3.50	8.95	12.98
	DP 433	15.02	30.15	1.23	6.13	13.40	20.76
SED (16 df)		7.29	5.24				2.79

There were no significant differences in the rates of degradation of carbofuran in the bow-wave and Laponite(R) treatments but application of the insecticide in the natural gum Polymer DP 433 prolonged its persistence. After 6 and 12 weeks, 42% and 34% respectively remained of the initial concentration in DP 433 compared with 28% and 14% respectively in the bow-wave treatment although high variability between replicates limited this significance to the 80% confidence level. There was less variability in the chlorfenvinphos and phorate treatments and both these insecticides were significantly more persistent after application in the DP 433.

Although total phorate residues were significantly more persistent in only DP 433, oxidation of parent phorate to PSO and PSO to PSO_2 was slower with both gel applications than in the bow-wave treatment. After 6 weeks, parent phorate comprised only 3% of the total residue from the bow-wave treatment compared with 13% and 17% of the residues in the Laponite(R) and DP 433 respectively. This trend continued until the end of the experiment, when only traces of parent phorate remained in all treatments but the proportions of PSO in the two gel treatments were almost twice that which remained in the bow-wave treatment.

Table 4 summarises the individual analyses of duplicate 10-core samples from

Table 4

Means of chlorfenvinphos residues (mg a.i./m) in 10 individual samples from duplicate rows treated by the bow-wave method and by fluid drilling

Replicate	Fluid drilling		Bow-wave	
	A	B	A	B
Mean	27.66	36.63	31.73	41.96
Range	17.82-36.13	23.84-76.52	16.35-76.80	26.16-74.67
$\pm$s.d.	6.23	15.70	16.84	13.70

the chlorfenvinphos bow-wave and gel treatments. There was no significant

difference in the uniformity of insecticide distribution along the row with the two methods, the standard deviations being uniformly high in 3 of the 4 rows sampled and the dose-range varying between 200 and 450%.

DISCUSSION

Incorporation into peat blocks prolongs the persistence and stability of insecticides (Suett & Padbury, 1981). In the present experiments, the minimal loss and limited oxidation of disulfoton and terbufos in the blocks during the 7-wk period prior to planting suggested that availability to the plants was unlikely to be limited by loss of insecticide from the block treatments. Nevertheless, uptake of the 3 insecticides was always greater from the sub-surface band application. Qualitative differences in residue composition can induce quantitative differences in uptake and translocation by influencing the proportions of residue components of differing water solubilities. While this may have enhanced differences between the insecticides in the present experiment, the difference between the treatments was more likely due to limited availability of insecticide in the block in relation to the root zone of optimum insecticide uptake. There may also have been substantially less movement of residue out of the block, where insecticide is strongly adsorbed (Suett & Padbury, 1981), than out of the sub-surface band; extensive downward movement of thiofanox residues has occurred following a sub-surface band application to sandy-loam (Suett & Padbury, 1979).

Thus, although incorporation of systemic insecticides into peat blocks leads to the accumulation of large residue concentrations in plants prior to planting out, which should protect them against some seedling pests, they seem less likely to provide reliable long-term protection to a crop like Brussels sprouts than the sub-surface band application. Short-term protection may be adequate for some crops, e.g. lettuce and cauliflower. However, the block incorporation of systemic insecticides is more likely to result in excessive terminal residues in lettuce, since most of the foliage is consumed, than with cauliflower, only the inflorescence of which is eaten.

An undoubted merit of the block incorporation is achieving a high degree of treatment uniformity between individual blocks (Suett & Padbury, 1981). The good uniformity in the present experiment (Table 1) was in marked contrast to the variability between individual cores from the fluid drilling and bow-wave applications (Table 4). In the fluid drilled application, this variability was possibly enhanced by the use of a hand-operated drill and may be less with a tractor-mounted drill. Nevertheless, the poor distribution in the bow-wave application illustrates amply one treatment variable which needs to be substantially improved before dosage transfer can be optimised.

An opportunity for such an improvement is presented by the incorporation of insecticide treatments into peat blocks and fluid-drilling gels and it is this aspect of their use which affords as much, if not more, promise as the prolonged stability occurring in these media. Both techniques lead to rapid release of a.i. from granular and other bases (Suett & Padbury, 1981; Suett *et al*, 1981) and create, in effect, new slow-release mechanisms. The reduced availability of the insecticides in the present peat block experiment, however, illustrated that these methods have their own limitations and, until they are more fully understood, their undoubted potential will not be fully realised.

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References

CHIN, W.T.; DUANE, W.C.; SZALKOWSKI, M.B.; STALLARD, D.E. (1975) Gas chromatographic determination of thiofanox residues in soil, plants and water, Journal of Agricultural and Food Chemistry, 23, 963-966

DUNNE, R.; DONOVAN, M.; COFFEY, J. (1979) Control of cabbage root fly (Delia brassicae) by incorporation of insecticides in peat blocks, Proceedings 1979 British Crop Protection Conference - Pests and Diseases, 1, 239-244

EMMETT, B.J. (1979) Celery: chemical control of carrot fly, 1978, Experiments and Development in the Eastern Region. M.A.F.F. (A.D.A.S.), Cambridge, pp 268-274

MAKEPEACE, R.J. (1965) The application of granular pesticides, Proceedings 3rd British Insecticide and Fungicide Conference, 1, 389-395

MOYE, H.A. (1971) Reaction gas chromatographic analysis of pesticides: on-column transesterification of N-methylcarbamates by methanol, Journal of Agricultural and Food Chemistry, 19, 452-455

SAYNOR, M.; DAVIES, M.H. (1977) Applications of insecticides to peat blocks for the control of pests of brassicas and lettuce, Proceedings 1977 British Crop Protection Conference - Pests and Diseases, 1, 279-287

SUETT, D.L. (1971) Persistence and degradation of chlorfenvinphos, diazinon, fonofos and phorate in soils and their uptake by carrots, Pesticide Science, 2, 105-112

SUETT, D.L. (1977) Influence of placement depth on the behaviour of disulfoton in soil and Brussels sprouts and on cabbage aphid control, Proceedings 1977 British Crop Protection Conference - Pests and Diseases, 2, 427-434

SUETT, D.L.; PADBURY, C.E. (1979) Cabbage aphid: control on drilled Brussels sprouts, Report of the National Vegetable Research Station for 1978, p.35

SUETT, D.L.; PADBURY, C.E. (1980) Uptake of phorate by lettuce, Pesticide Science, 11, 351-360

SUETT, D.L.; PADBURY, C.E. (1981) Incorporation and stability of chlorfenvinphos, chlorpyrifos, diazinon and phorate in peat blocks, Pesticide Science, (in the press)

SUETT, D.L.; PADBURY, C.E.; FARMER, C.J. (1981) Incorporation of insecticides into fluid-drilling gels - residues in soil and in carrots, Report of the National Vegetable Research Station for 1980, p.34

THOMPSON, A.R.; PERCIVALL, A.L. (1978) Cabbage root fly - evaluation of insecticides on summer cauliflowers, Report of the National Vegetable Research Station for 1977, pp 70-71

THOMPSON, A.R.; PERCIVALL, A.L. (1979) Lettuce foliage and root aphids, Report of the National Vegetable Research Station for 1978, p.36

THOMPSON, A.R.; PERCIVALL, A.L.; EDMONDS, G.H. (1980) Carrot fly-evaluation of insecticides, Report of the National Vegetable Research Station for 1979, pp pp 29-30

WHEATLEY, G.A. (1971) Pest control in vegetables: some further limitations in insecticides for cabbage root fly and carrot fly control, Proceedings 6th British Insecticide and Fungicide Conference, 2, 386-395

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OVIPOSITION AND DEVELOPMENT OF SITOPHILUS GRANARIUS L. IN WHEAT GRAINS TREATED WITH KNOWN AMOUNTS OF PIRIMIPHOS-METHYL

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Summary Using a novel method, known amounts of pirimiphos-methyl were applied with a microcapillary to wheat grains containing each developmental stage of S. granarius. Adult insects were also allowed to oviposit on grains containing pirimiphos-methyl deposits aged over different periods. Live S. granarius emerged from grains treated with 8 ppm (µg/g) of pirimiphos-methyl. This was the case irrespective of which stage in development was treated. It was also the case when the insects were allowed to oviposit on the treated grains, whether or not the grains were stored between treatment and oviposition. Inconsistencies between two experiments limited the conclusions that could be drawn about the relative susceptibilities of the different developmental stages.

INTRODUCTION

The grain weevil, Sitophilus granarius L. is an important pest of stored grain in many countries, including the U.K. It is difficult to detect and control because the immature stages develop inside individual grains. The female bites a hole in the grain, pushes an egg into it, and seals the cavity with a plug. The larva and pupa remain completely hidden, and the damage becomes obvious only when the adult emerges by chewing an exit hole. At favourable temperatures and humidities the life history is quite rapid; there is a minimum period of 49 days from egg to adult at 21°C, 80% r.h. (Richards, 1947).

Insecticides applied to grain need to disinfest it and also confer protection against re-infestation during the storage period. The persistence of treatments applied in farm grain stores can be tested by bringing grain samples to the laboratory and exposing subsamples to adult S. granarius. The mortality of these adults and the subsequent emergence of the F_1 and F_2 generations (if any) provide biological assessments of the treatment and parallel chemical analyses can be undertaken on other subsamples.

Such studies have shown that gamma-HCH applied at 1 ppm (µg/g seed) or more completely prevented the emergence of progeny from barley grains (Pinniger et al., 1975). By contrast treatments with pirimiphos-methyl, which is very effective against adult S. granarius compared to gamma-HCH (Tyler and Binns, 1977), always permitted some F_1 progeny to emerge, even with doses as high as 6.9 ppm (Anon., 1978). It appears that these F_1 progeny frequently die shortly after emergence and that they do not give rise to an F_2 generation. Nevertheless their presence is disturbing because they leave obvious 'holed' grains. Moreover the maximum dose of pirimiphos-methyl recommended for application to raw cereal grains in the U.K. is 4 ppm.

Several explanations for the emergence of F_1 progeny from grain treated, in commercial grain stores, with pirimiphos-methyl are possible. Treatment with malathion can result in enormous variation between the doses received by individual grains (Rowlands, 1975). A similar variation is likely to arise from practical treatments with pirimiphos-methyl, and it may be that the F_1 progeny emerge from individual grains receiving little pesticide. Alternatively the immature stages of S. granarius may be intrinsically difficult to kill with pirimiphos-methyl, or it may be that insufficient insecticide penetrates the grain and reaches them.

We report here two laboratory experiments designed to test some of these possibilities. In these tests the problems arising from unevenness in practival field applications were avoided by treating individual wheat grains in the laboratory.

METHODS AND MATERIALS

All culturing and experiments except the determination of pesticide residues took place in rooms maintained by air-conditioning units at 25°C, 70% r.h. The grain used was unsterilised insecticide-free English wheat (variety Hobbit) and the insects were a fully insecticide-susceptible strain of S. granarius.

Oviposition and development

Adult insects between two and three weeks old were sexed using characters described by Halstead (1963). Each female was confined singly in a glass tube (50 x 12 mm) for two hours, after which a single grain of wheat was placed in the tube. The grain and insect were then left together for 24 h, after which the insect was removed. The grains invariably became infested.

In order to determine the length of each developmental stage, grains infested as described were incubated, and every three days, up to 49 days after oviposition, 15 grains were dissected. The developmental stages of live insects found were noted. The different larval instars were distinguished by measuring the width of the head capsules (Soderstrom, 1960). One day after oviposition, infestation of the grains was in the form of eggs. The numbers of days after oviposition when most insects were at the given developmental stages were:

7 days - first instar; 14 days - second instar; 20 days - third instar; 24 days - fourth instar; 33 days - pupa; 40 days - unemerged adult.

Preparation and treatment of the grain

Grains selected for the experiments each weighed between 55.1 and 62.5 mg. An analytical sample of pirimiphos-methyl dissolved in hexane (0.47 µg/0.2 µl), was applied to the crease area of individual grains, with a glass capillary tube which had been calibrated for consistent delivery using a radiotracer. This gave 8 ppm on a grain weighing 58.5 mg, with a maximum error of 10% in insecticide dose.

Experiment A

The insecticide was applied to 100 grains at 1, 7, 14, 20, 24, 33 and 40 days after oviposition to correspond to the times when most insects were at each of the seven developmental stages. In addition 100 grains were treated with insecticide immediately before oviposition (the 'immediately pre-oviposition' test). On each of these occasions, 50 infested grains were treated with 8 ppm of pirimiphos-methyl and 50 were treated with 0.2 µl of hexane alone as a control. After treatment each grain was returned to the tube in which it had been exposed

to an insect (see 'oviposition and development' above). The grains were stored in these unsealed tubes until after emergence. For the 'immediately pre-oviposition' test, 100 grains were similarly treated immediately before the oviposition period, and subsequent mortality amongst the applied insects was recorded. These grains were stored in the same way as the other grains.

Emergence of progeny

On either the 42nd, 43rd or 44th day after oviposition, the grains were examined and any insects that had emerged were removed. This process was repeated at intervals of two or three days until no insects had emerged for two successive observations.

Intact grains were then dissected, and the numbers of insects at each stage in development found within were noted.

Experiment B

To provide data on the persistence of the pirimiphos-methyl residues to complement the biological results of experiment A, grains (a living substrate) and Whatman No. 1 filter paper 5 mm in diameter (a non-living substrate) were treated with insecticide as described above, and stored in open glass tubes 50 x 12 mm in a room conditioned at 25°C, 70% r.h. After 0, 1, 7, 14, 21, 42 or 70 days storage, five grains and three filter papers were analysed for pirimiphos-methyl by gas chromatography. Each grain or paper was placed in another 50 x 12 mm glass tube, then crushed and macerated with 1 ml hexane. The tubes were stoppered and left for 15 min at room temperature. Two 5 µl aliquots from each tube were injected on to the chromatograph. Grain samples more than 7 days old were enzymically digested to extract bound pirimiphos-methyl, as described by Rowlands (1981). The gas chromatograph used was a Perkin-Elmer F-33, fitted with electron-capture detection; column temperature was 220°C (nominal) and the packing was OV-17.

In an attempt to relate persistence to the biological data recorded in experiment A, the 'immediately pre-oviposition', 'day 1' and 'day 33' tests of expermiment A were repeated. In addition the opportunity was taken to assay the deposits on grains against adult S. granarius. Thus for each day on which a sample of grains and filter papers were analysed for residues, 50 similar grains were exposed to insects as described for experiment A, and processed as for the 'immediately pre-oviposition' test.

RESULTS

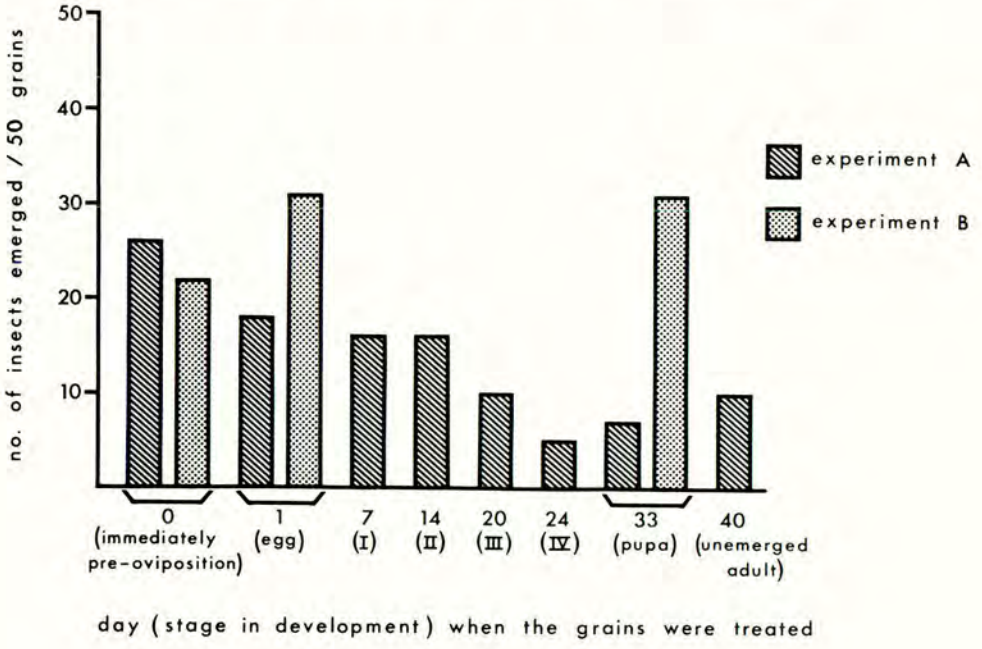
Experiment A

Fig. 1 shows that between 5 and 26 S. granarius emerged from 50 grains treated with 8 ppm of pirimiphos-methyl, whereas 48 emerged from untreated grain. Some emergence occurred whether the grains were treated before oviposition, or at any stage during development.

Dissection of grains from which no insects emerged revealed that, typically, 75% had died at the stage they had reached when treated, and 25% died as pre-emergent adults. There were two notable exceptions to this pattern. Firstly, in the 'immediately pre-oviposition' test, no pre-emergent adults were found (although because of the high emergence fewer grains were available for dissection). The second exception was in the 'day 33' test, where no pupae were found, only unemerged adults.

Fig. 1

Number of Sitophilus granarius emerged from grains treated with 8 ppm pirimiphos-methyl at various stages in the insect's development



emergence from grains treated with hexane alone:

experiment A: 47.8 ± 1.3 (p = 0.05) insects/50 grains

experiment B: 48.3 ± 5.7 (p = 0.05) insects/50 grains

Experiment B

Table 1 shows that the pirimiphos-methyl persisted well considering the method of application used and the storage conditions. Mortality of the adults after exposure was high for days 0 and 1, but low thereafter. However, no data are available for comparison with experiment A, except for the 'immediately pre-oviposition' test, where all adult insects were dead after the 24 h oviposition period.

In Fig. 1, the emergence for the 'immediately pre-oviposition', 'day 1' and 'day 33' tests of experiment B are shown beside the equivalent data from experiment A. Although the numbers of insects emerged for the two 'immediately pre-oviposition' tests are very similar the emergence for the 'day 1' test, and especially the 'day 33' test, of experiment B are higher than those of experiment A.

Fig. 2 shows the emergence from grains infested 1, 7, 14, 21, 42 or 70 days after treatment. Apart from the first of these tests, emergence was almost as high as in the control. This was a totally unexpected result because of the reasonable persistence of the insecticide.

This high emergence from all the tests of experiment B limited the numbers of grains available for dissection. Nevertheless it is notable that in the 'immediately pre-oviposition' and 'day 1' tests very few unemerged insects were adults.

DISCUSSION

The results of both experiments A and B confirm that S. granarius can emerge from grains treated with 8 ppm of pirimiphos-methyl, although the numbers emerging were decreased in both experiments. Other experiments (Anon., 1978) carried out in the same way as experiment A, but in which grains were treated with 4 or 2 ppm, showed that at 4 ppm there was significantly greater emergence than at 8 ppm; and that at 2 ppm emergence did not differ significantly from that of the controls. These results suggest that insects emerging from bulk-treated grain may come from grains containing 8 ppm, but are more likely to emerge from low-dosed grains.

An interesting observation concerned the pupal stage. Very few dead pupae were ever found in the intact grains, and none were found in those of the 'day 33' test which were treated at a time when an estimated 93% of the insects in the grains were pupae, (this was also true for experiment B). However, Fig. 1 shows that emergence from the 'day 33' test was comparatively low in experiment A. Reynolds et al. (1967), exposing grains containing S. granarius pupae to a fumigant, and thus knowing approximately when the fatal dose was contracted, noted that treated pupae almost always became adults before dying. This, as well as a possible high level of tolerance of insecticide at this stage in development, may account for the absence of dead pupae among the grains from which no insects emerged.

The observation that some insects died as unemerged adults, irrespective of their stage in development at the time of treatment, is interesting in connection with the work of Rowlands and Bramhall (1977). They established that a grain actively accumulated malathion in the aleurone layer, immediately beneath the testa, and that because of this S. granarius developing in the grain contracts a lethal dose of the insecticide only when the pre-emergent adult actually chews into the aleurone layer prior to emergence. Rowlands (1981) has shown that pirimiphos-methyl residues also accumulate chiefly in the aleurone layer, 77% and 62% of the dose applied being found in the testa and aleurone layer 1 h and 7 days after application

Table 1

Residues of pirimiphos-methyl, with 95% confidence limits, and mortality of adult Sitophilus granarius at the time of oviposition (experiment B)

No. of days after treatment	mean ppm of insecticide persisting on:		% mortality after 24 h exposure to:	
	Filter papers[1]	grains[2]	Control grains[3]	treated grains
0	8.3 ± 0.3	7.4 ± 0.3	0	98
1	8.3 ± 0.3	7.7 ± 0.3	0	98
7	8.3 ± 0.3	6.7 ± 0.5	0	14
14	7.7 ± 1.3	5.3 ± 0.4	0	0
21	6.5 ± 1.6	5.2 ± 0.5	0	0
42	4.4 ± 0.7	2.9 ± 0.2	0	0
70	2.2 ± 1.3	0.9 ± 0.2	0	0

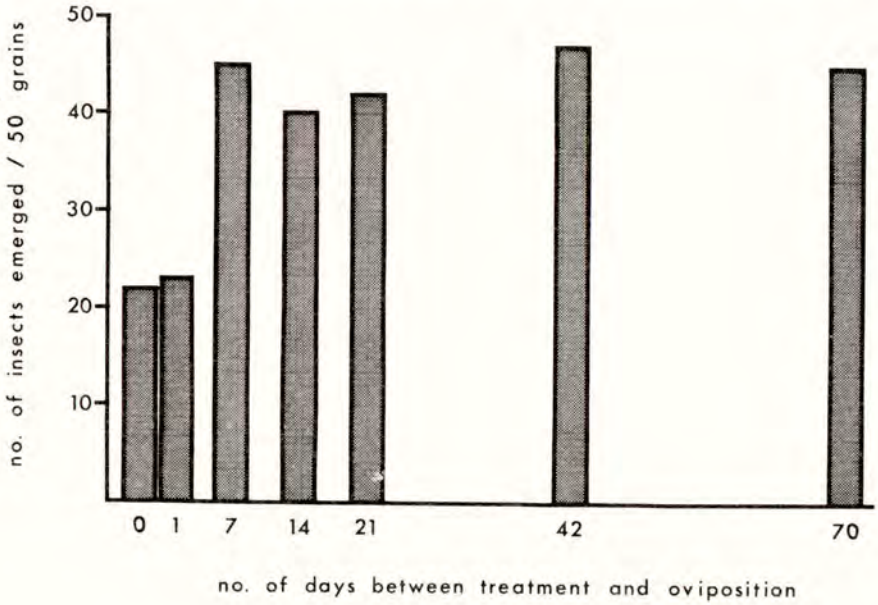
[1] mean of 3 replicates

[2] mean of 5 replicates

[3] control grains were treated with hexane only

Fig. 2

Number of Sitophilus granarius emerged from grains treated with 8 ppm pirimiphos-methyl at various times before oviposition (experiment B)



emergence from grains treated with hexane alone:
48.2 ± 1.2 (p = 0.05) insects/50 grains

respectively. However, further work would be required to establish whether this is a factor in the mortality at the pre-emergent adult stage, or whether the increased susceptibility of the adult alone accounts for it.

Why did the biological tests in experiment B produce results unexpectedly different from experiment A and from previous experiments? The chemical results suggested that correct amounts were applied, and that there was a considerable degree of persistence. Separate tests showed that this was not due to the development of insecticide resistance in the interval between experiments A and B. Another possibility is that, for some reason, more eggs were laid in experiment B, such that despite pirimiphos-methyl killing the same proportion of insects, the population in experiment B was still greater. The control emergence for experiments A and B were the same (see Figs 1 and 2) but numbers of insects emerged is not directly related to numbers of eggs laid in the circumstances of these experiments, since it is rare for two insects to emerge from a single grain (Richards, 1947).

A further possibility is that the insecticide used had altered to some form that would still respond as pirimiphos-methyl to the GLC detector. A comparison between the insecticide solution used in experiment B and a new standard dissolved in hexane using GLC as described in the methods section, showed no difference between the two. However, when a phosphorus-specific detector was used, breakdown of 18% in the original solution was indicated. This suggests that some of the pirimiphos-methyl in the original solution might have isomerised to the S-methyl isomer which still responds as pirimiphos-methyl to the electron-capture detector. Thin-layer chromatography using a sensitive anti-cholinesterase detection technique indicated that a more responsive compound of similar Rf (perhaps the S-methyl isomer?) was present in the original solution. Little is known, however, about the toxicity to insects of the S-methyl isomer of pirimiphos-methyl in vivo especially if mixed with pirimiphos-methyl. The amount of the possible isomerism was small, however, and it is unlikely that it could account for such widely differing biological results.

Yet another explanation is that in experiment B the pirimiphos-methyl, demonstrably present, was not available to the insects to the same extent as in the first; perhaps it became bound into the grain tissues. Binding of pirimiphos-methyl to lipoprotein in grain regularly accounts for at least 10% of the applied dose (Rowlands, 1975) and this needs to be digested out for full assessment of the residue. However, there is no obvious reason why the 'availability' of the residue should be different, when the grain used and method of treatment were identical.

Clearly there are some interesting problems here and it is hoped that further work will explore the complex relationships between the grain, the insect and the insecticide.

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References

ANON. (1978) Control of S. granarius Pest Infestation Control Laboratory Report 1974-76 p 93-95.

HALSTEAD, D.G.H. (1963) External sex differences in stored-products Coleoptera Bulletin of Entomological Research 54, 119-134.

PINNIGER, D.B.; TEJANI, B.; ROWLANDS, D.G.; CLARK, J.S. (1975) Bioassay using Sitophilus granarius Pest Infestation Control Laboratory Report 1971-73 p 48-49.

REYNOLDS, E.M.; ROBINSON, J.M.; HOWELLS, C. (1967) The effect on Sitophilus granarius (L.) (Coleoptera, Curculionidae) of exposure to low concentrations of phosphine Journal of Stored Products Research 2, 177-186.

RICHARDS, O.W. (1947) Observations on grain weevils, Calandra (Coleoptera, Curculionidae). I General biology and oviposition Proceedings of the Zoological Society of London 117, 1-43.

ROWLANDS, D.G. (1975) The metabolism of contact insecticides in stored grain. Part III - 1970-4 Residue Reviews. 58, 113-153.

ROWLANDS, D.G. (1981) The metabolism of pirimiphos-methyl by stored wheat grains under laboratory conditions ADAS Pest Control Chemistry Department Report No. 54 9pp (Copies available on request to D.G.R.).

ROWLANDS, D.G.; BRAMHALL, J.S. (1977) The uptake and translocation of malathion by the stored wheat grain Journal of Stored Products Research 13, 13-22.

SODERSTROM, E.L. (1960) Recognition and duration of larval instars of the rice weevil and the granary weevil Journal of the Kansas Entomological Society 33, 157-161.

TYLER, P.S.; BINNS, T.J. (1977) The toxicity of seven organophosphorus insecticides and lindane to eighteen species of stored-product beetles Journal of Stored Products Research 13, 39-43.

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TOXICITY OF SOIL INSECTICIDES AGAINST A SURFACE WALKING INSECT IN RELATION TO PHYSICO-CHEMICAL PROPERTIES

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Summary The toxicities of six organo-phosporus insecticides (carbophenothion, chlorfenvinphos, dimethoate, phorate, tetrachlorvinphos, thionazin), with a wide range of physical properties, to vestigial winged Drosophila melanogaster, were measured by topical application, after uptake from a glass surface, after uptake from soils and by fumigant action above soils. Eight soils with organic matter content in the range 0.5-57% were used. Results of bioassays are discussed in relation to the insecticide water solubility, vapour pressure, soil adsorption coefficient and octanol-water, air-water and air-wet soil partition coefficients, leading to recognition of suitable values for efficient uptake of lethal doses from soil.

INTRODUCTION

Many studies have been made of factors influencing the toxicity of chemicals in soil to insects but none has been sufficiently comprehensive to enable prediction of efficacy from physical properties, intrinsic toxicity, and persistence. Lord et al (1980) were successful in measuring and predicting uptake in a simpler case, dealing with earthworms, where only partition of the chemical between soil solids, soil water and the worm is important. The analysis becomes more complex for insects because effective soil insecticides have both contact and fumigant action (Harris, 1972) so partition into and out of the soil air phase must also be considered.

The work reported here constitutes the frist stage in developing a more general approach applicable to insects and uses insecticides with a wide range of physical properties, in soils of different organic matter content. Existing models (Nicholls and Addiscott, 1980) which simulate the movement and persistence of chemicals in soils may be extended to include predictions of uptake of lethal doses by insects once the important processes have been identified.

MATERIALS AND METHODS

Technical grade samples of the insecticides were used for all tests: these are listed together with their physical properties in Table 1.

Soils were taken from the top 15cm at the different sites, air dried and passed through a 2mm sieve. Moist soil was prepared by wetting air dried soil to a water content corresponding to a water retention pressure of -0.1 bar, determined by the methods of Hall et al, 1977. Wet soil was prepared by saturating air dried soil with water and allowing it to drain for 12h. Soil properties including pH measured in 0.01M $CaCl_2$ solution are given in Table 2.

Bioassays

Four-day old adults of a vestigial winged strain of the fruit-fly , Drosoplila melanogaster were used throughout.

small octanol-water partition coefficient	small adsorption coefficient	large air-wet soil partition coefficient
large vapour pressure small water solubility	large air-water partition coefficient	

Although thionazin has a larger vapour pressure and a smaller adsorption coefficient than phorate, its greater water solubility gives it a smaller air-wet soil partition coefficient. Carbophenothion has an air-wet soil partition coefficient fifty times smaller than that of thionazin and it had no fumigant activity from soil. The other insecticides with small vapour pressures likewise showed no significant fumigant action, except dimethoate from wet soils. This slight activity was possibly due to degradation, in the soil, to omethoate, a more volatile insecticide.

The mean toxicities for the insecticides (Table 5) from the air dried soils were not obviously dependent on organic carbon content. This was also true for the moist or wet peat soils which have organic carbon contents of 7% (Table 2). However, mean toxicities from either moist or wet mineral soils which have an organic content of 2.5% were negatively correlated with carbon content. Linear regressions of LD50 values against percentage organic carbon content for all eight soils were calculated and the mean slopes for the six insecticides are 0.2, 1.6 and 5.0 for air dried, moist and wet soils respectively. This illustrates that the sensitivity of toxicity to organic carbon content increased with soil water content, i.e. toxicity is influenced both by soil organic carbon content and by soil moisture, two further properties to be included in a model. Similar effects have been reported by Harris (1972).

An estimate of the effectiveness of dosage transfer from the soil is indicated (Table 6) by the logarithm of the ratio of the intrinsic toxicity to the LD50 from soil. In dry soils the effectiveness of dosage transfer for all compounds was poor and was presumably limited by adsorption (Harris 1972). In moist soils the order of effectiveness was phorate>thionazin>carbophenothion>tetrachlorvinphos> chlorfenvinphos>dimethoate which is the same as the order of the air-wet soil partition coefficients. However, in wet soils, dosage transfer of carbophenothion was slightly more effective than that of thionazin.

The properties of carbophenothion and dimethoate provide an interesting contrast. Neither undegraded compound had significant fumigant action so their insecticidal activity must have relied on partitioning from soil solids and soil water. Intrinsically, dimethoate was the most toxic of all compounds and its small adsorption coefficient and large solubility gave it the greatest calculated concentration in soil water. However, its large polarity, indicated by its octanol-water partition coefficient, probably limited its ability to penetrate the insect from soil water (Briggs, 1981), making dosage transfer poorly effective. Carbophenothion is very lipophilic and has a large adsorption coefficient but its large octanol-water partition coefficient probably favours partition from soil water into the insect sufficiently to overcome the effects of strong adsorption by soil solids. This effect may occur with other lipophilic compounds strongly adsorbed by soils.

The similarity in dosage transfer effectiveness and sensitivity to soil water content between carbophenothion and thionazin was examined in more detail and compared with data for phorate. Figure 1 shows that phorate and thionazin, compounds with fumigant action, reached almost maximum effectiveness at a soil water content of about 12% then increased very slightly with increasing water content but

In topical application tests, 0.04µl drops of solutions of insecticide in 2-butanone were applied to flies anaesthetised with carbon dioxide, using a Burkard Arnold Microapplicator Type LV65 and a Hamilton 250ul gas-tight syringe. The treated flies (15 per vial) were held in 80 x 30mm shell-necked vials closed with muslin gauze and fed with 10% sucrose solution from a cotton wool wick.

In uptake from glass tests, 1ml of solution of insecticide in 40-60°C petroleum ether was run into horizontally revolving 80 x 30mm shell-necked vials and the solvent allowed to evaporate. Twenty flies were introduced into each vial and held as before. For each insecticide in both techniques, up to 5 doses were used with two vials per dose plus an untreated control.

For contact on soil tests, approximately 20ml (35g) of soil was put into a 60mm crystallizing dish the internal surfaces of which had been treated with Fluon. The insecticide was introduced by running 1ml of a solution in 40°-60°C petroleum ether over the surface. Once the solvent had evaporated, the soil was gently stirred then tamped level. Again 20 flies were put onto the surface and fed on sucrose agar (5mm x 5mm x 2mm) on a small piece of aluminium foil.

In tests designed to assay the fumigant action of insecticides from soil surfaces, a similar technique for preparing treated soil to that described above was used. After tamping the soil flat, two glass rods 50mm long, 3.5mm diameter were placed 35mm apart on the surface. A glass tube 20mm tall, 45mm diameter was coated inside and outside with Fluon and closed at the lower end with muslin gauze. 20 flies were put onto the soil surface and 20 put into the tube which rested on the glass rods. The flies were fed on sucrose agar gel. For both treated-soil techniques, a plastic cover was put over the crystallizing dish. For each insecticide up to five doses were used with no replication.

The post-treatment temperature for all assays was 20°C. Food was replenished after 24 h and mortality assessments made 48 h after treatment, a sufficiently short interval to prevent insecticide degradation from having a large influence on toxicity measurements.

RESULTS AND DISCUSSION

The toxicities of all the candidate compounds by a topical application technique to adult Drosophila were similar but the most toxic was dimethoate, and the least toxic carbophenothion (Table 3). Bioassays by the contact on glass technique may have been influenced by evaporation of the more volatile compounds (thionazin and phorate) which were least toxic (Tables 1 and 3) although they were moderately toxic when topically applied. Intrinsic toxicity was therefore better represented by the topical application results.

The most toxic compounds after soil application were the volatile compounds, phorate and thionazin, suggesting significant fumigant action in this test. This was clearly confirmed in bioassays on flies held above the surface of the treated soil (Table 4). Harris and Chapman (1980) demonstrated that fumigant action and uptake from soil solids and soil water contributed to the transfer of lethal doses of phorate, but in our experiments all the insecticidal activity of both phorate and thionazin could be accounted for by their fumigant action. The physicochemical properties and partition coefficients which produce a high concentration of insecticide in the soil vapour phase are given in the following diagram.

carbophenothion increased steadily above 4%, implying that phorate and thionazin would be more effective under field conditions in drier soils. Some soil insecticides give erratic control of the target pest from year to year (Harris 1972). This may be because although they have good intrinsic toxicities they have poor dosage transfer properties under conditions sometimes prevailing in the field.

The physical properties of a compound that lead to efficient dosage transfer are an air-wet soil partition coefficient greater than 1×10^{-7} or an octanol-water partition coefficient ($\log_{10}K_{ow}$) greater than 4.0. Conversely compounds with air-wet soil partition coefficients of less than 1.0×10^{-7} and an octanol-water partition coefficient of less than 4.0 are likely to have poor dosage transfer efficiency. Compounds that have high intrinsic toxicities against the target organism and suitable persistence, both of which can be measured, as well as efficient dosage transfer properties, which can be calculated, will be good candidates for the control of surface-walking insect pests.

An understanding of the behaviour of pesticides in soil should lead to more efficient control of target pests and help in exploiting other features of pesticides such as their selective activity for the protection of beneficial insects. Principles which apply to the adult Drosophila used in these experiments may be extrapolated to other insects that live on the soil surface, although future studies will be done using larvae that migrate below the soil surface and are more typical of soil pests.

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References

BRIGGS, G.G. (1976) Degradation in soils. Proceedings BCPC Symposium: Persistence of Insecticides and Herbicides, 41-54.

BRIGGS, G.G. (1981) Theoretical and experimental relationships between soil adsorption, octanol-water partition coefficients, water solubilities, bioconcentration factors and the parachor. Journal of Agricultural and Food Chemistry (in press).

GUTH, J.A. BURKHARD, N., EBERLE, D.O. (1976) Experimental models for studying the persistence of pesticides in soils. Proceedings BCPC Symposium: Persistence of Insecticides and Herbicides, 137-157.

HALL, D.G.M., REEVE, M.J., THOMASSON, A.J., WRIGHT, V.F. (1977) Water retention, porosity and density of field soils. Soil Survey Technical Monograph No. 9, Rothamsted Experimental Station, Harpenden, Herts.

HARRIS, C.R. (1972) Factors influencing the effectiveness of soil insecticides. Annual Review of Entomology, 17, 177-198.

HARRIS, C.R., CHAPMAN, R.A. (1980) Insecticidal activity and persistence of phorate, phorate sulphoxide and phorate sulphone in soils. Canadian Entomologist, 112, 641-653.

HARRIS, C.R., BOWMAN, B.T. (1981) The relationship of insecticide solubility in water to toxicity in soil. Journal of Economic Entomology, 74, 210-212.

LORD, K.A., BRIGGS, G.G., NEALE, M.C., MANLOVE, R. (1980) Uptake of pesticides from water and soil by earthworms. Pesticide Science, 11, 401-408.

NICHOLLS, P., ADDISCOTT, T.M. (1980) Computer simulation of herbicide leaching in a structured soil. Proceedings 1980 British Crop Protection Conference - Weeds, 617-620.

WORTHING, C.R. (1979) The Pesticide Manual 6th Edition, British Crop Protection Council, England.

Table 1

Physical properties of insecticides

Compound	Water sol[a] WS (mg l^{-1})	Octanol-water partition coeff.[b] ($log_{10}K_{ow}$)	Adsorption coeff. for Begbroke soil[d] (calc)	Vapour press.[a] V (mm Hg)	Air-water partition coeff.[e] (calc)	Air-wet soil partition coeff.[f] (calc)
Carbophenothion	0.29[b]	6.0[c]	170	3.0×10^{-7}	1.9×10^{-5}	1.2×10^{-7}
Tetrachlorvinphos	11	2.9[c]	4.1	4.2×10^{-8}	7.6×10^{-8}	1.8×10^{-8}
Phorate	50	4.3	22	8.4×10^{-4}	2.4×10^{-4}	1.1×10^{-5}
Chlorfenvinphos	145	3.2	5.9	4.0×10^{-6}	5.4×10^{-7}	9.0×10^{-8}
Thionazin	1140	3.2	5.9	3.0×10^{-3}	3.6×10^{-5}	5.9×10^{-6}
Dimethoate	25000	0.96	0.4	8.5×10^{-6}	4.3×10^{-9}	7.5×10^{-9}

(a) Worthing, 1979
(b) Briggs, 1981
(c) Calculated using equation of Briggs 1981
(d) Briggs, 1976
(e) $C_{air}/C_{water}=(V \times M \times 10^6)/(WS \times 82.06 \times 760 \times (273 + 20))$ where M is molecular weight
(f) Guth et al, 1976

Table 2

Soil properties

Site	Type	Series	Organic carbon %	pH[a]	C.E.C.[b] m.e./100g
Woburn	Sandy loam	Cottenham	0.5	7.3	4.5
Broadbalk nil	Clay	Batcombe	0.8	7.7	13.0
Begbroke	Sand	Sutton	1.7	7.3	17.3
Broadbalk F.Y.M.	Clay	Batcombe	2.4	7.6	19.2
Warboys	Sandy peat	Downholland	7.5	7.7	42.1
Arthur Rickwood 1	Peat	Downholland	19.0	5.6	94.5
Arthur Rickwood 2	Peat	Adventurers	28.0	5.7	122.2
Connington Fen	Peat	Adventurers	33.0	5.8	143.2

[a]Measured in 0.01M $CaCl_2$
[b]C.E.C. = cation exchange capacity

Table 3

Toxicity of compounds to Drosophila melanogaster

Compound	LD50 after topical application[a] (ng fly^{-1})	LD50 after uptake from a glass surface[a] µg vial^{-1}	LD50 after uptake from soil[b] (µg dish^{-1}) air dry	moist	wet
Carbophenothion	4.1	0.41	480	84	27
Tetrachlorinphos	0.8	0.13	240	130	83
Phorate	0.95	1.4	19	2.1	1.7
Chlorfenvinphos	1.1	0.23	710	210	81
Thionazin	0.7	12.0	94	9.2	6.4
Dimethoate	0.4	0.061	170	140	110

[a] Standard errors of LD_{50}s were all less than 10%
[b] Each value represents the geometric mean of the 8 different soils tested.

Table 4

Toxicity after uptake from Begbroke soil illustrating compounds with fumigant action

Compound	LD50 (µg dish^{-1}) air dry on soil surface	air dry above soil surface	wet on soil surface	wet above soil surface
Carbophenothion	240	5000	79	1000
Tetrachlorvinphos	470	8000	140	2000
Phorate	20	18	3.6	3.2
Chlorfenvinphos	320	3000	120	320
Thionazin	150	160	5.5	2.9
Dimethoate	160	4000	220	1400

Table 5

Toxicity after uptake from soil[a]

Soil	LD50[b] ($\mu g\ dish^{-1}$)		
	air dry	moist	wet
Woburn	68	22	4.7
Broadbalk nil	140	31	6.8
Begbroke	140	33	12
Broadbalk F.Y.M.	260	69	22
Warboys	240	33	32
Arthur Rickwood 1	270	69	106
Arthur Rickwood 2	180	50	66
Connington Fen	180	68	61

[a]Insects on soil surface
[b]Geometric mean of the six compounds tested.

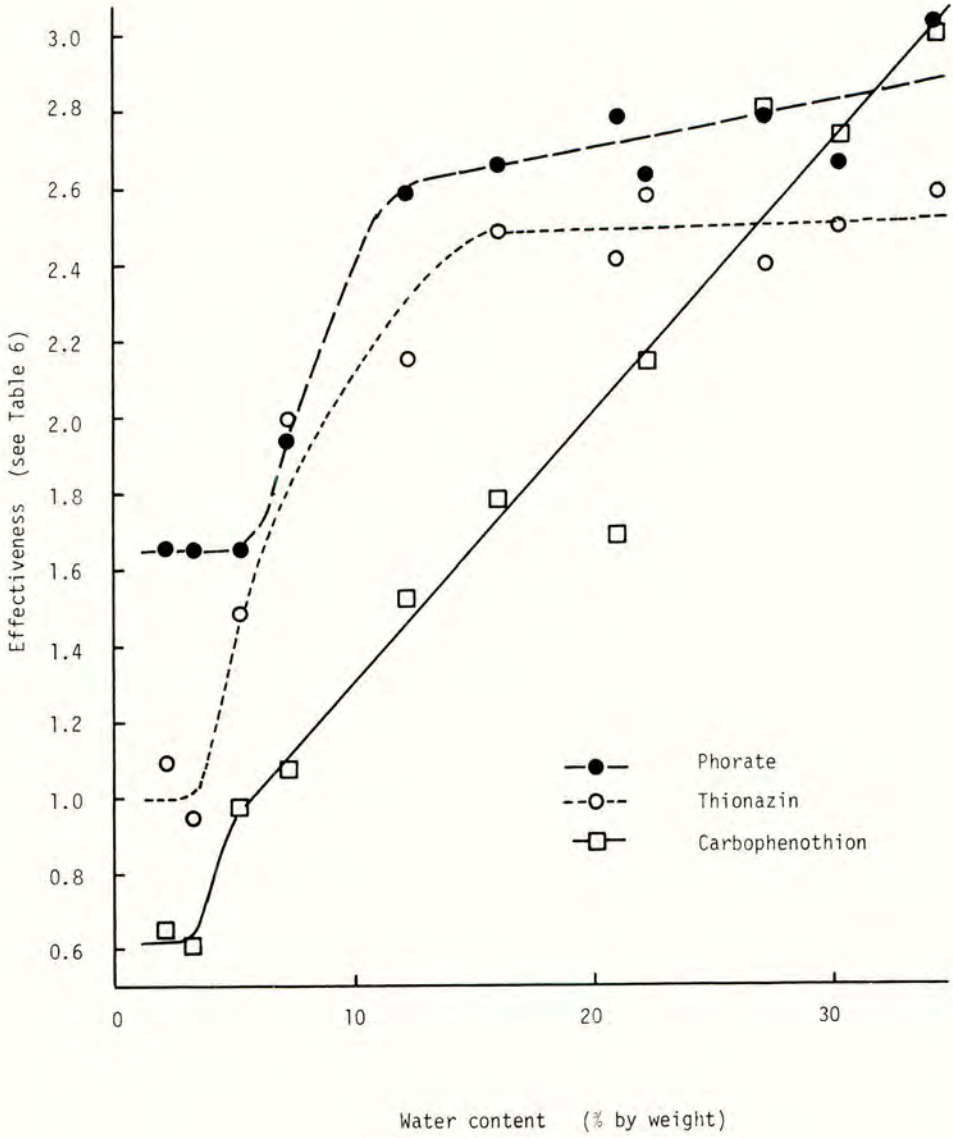
Table 6

The effectiveness of dosage transfer from soil

Compound	Effectiveness[a]		
	air dry	moist	wet
Carbophenothion	1.2	1.7	2.2
Tetrachlorvinphos	0.52	0.79	0.98
Phorate	1.7	2.7	2.8
Chlorfenvinphos	0.19	0.72	1.1
Thionazin	0.87	1.9	2.0
Dimethoate	0.37	0.44	0.58

[a]Effectiveness = $\log_{10}$(LD50 after topical application/LD50 after uptake from soil)+6 ; where LD50 after uptake from soil is the geometric mean of the eight soils tested.

Fig. 1 The influence of soil water content on the effectiveness of dosage transfer from soil



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RESIDUES OF ORGANOPHOSPHORUS PESTICIDES IN WHOLEMEAL FLOUR AND BREAD PRODUCED FROM TREATED WHEAT

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Summary - Organophosphorus pesticides are commonly used to protect cereal grains during storage. The interval between treatment and the milling/baking process can vary widely. In these experiments wheat treated with one of 6 organophosphorus pesticides was milled and baked to produce wholemeal flour and bread. Approximately 50% of pesticide present in the whole wheat flour was shown to survive the baking process. In certain instances the Maximum Recommended Level (MRL) can be exceeded in bread baked from whole wheat flour containing a level of pesticide below the MRL for whole grain.

INTRODUCTION

The routine application of pesticides to cereals before storage to prevent losses caused by infestation, has been common practice in Australia and France for some years. The practice is now being increasingly used in the United Kingdom to ensure that quality standards can be met following extended storage periods.

Organophosphorus pesticides are used almost exclusively to protect grain in store. In general they have low mammalian toxicities and degrade on the grain during storage. The rate of degradation varies with the nature of the compound and is affected by a number of factors, including moisture content and temperature of the grain. The Ministry of Agriculture, Fisheries and Food, Pesticide Safety Precautions Scheme publishes recommendations for maximum dosage rates for all pesticides cleared for admixture with grain but does not specify minimum storage periods between treatment and use.Hence, there is always the possibility that a significant proportion of the initial dose may still be present on grain about to be processed or consumed.

The amount of published data relating to residues of organophosphorus pesticides in bread is surprisingly small. Bindra and Sidhu (1972) reported that 73% of malathion present in maize flour was dissipated during processing into chapatties. Mukherjee et al (1973) studied the fate of pesticide residues in raw materials during processing and showed inter alia that 14-15% of malathion in wholewheat flour survived the preparation into chapatties. Cerna et al (1978) studied the fate of pirimiphos-methyl applied to wheat at 4 mg/kg. After 9.5 months storage the residues of pirimiphos-methyl had fallen to 2.2 mg/kg most of which (80%) was found after milling to be present in the bran. Approximately 50% of the pirimiphos-methyl present in the white flour remained in the bread following experimental baking. Hascoet (1978) found that 8-16% of residual malathion and 50-60% tetrachlorvinphos in flour (type not specified) were still present in the bread after baking.

Pirimiphos-methyl has been shown by Rowlands (1976) to accumulate preferentially in the bran. Mensah et al (1979) reported a similar situation in

which little or no residual pirimiphos-methyl occurred in white flour produced from treated wheat. Bullock (1974) found that the proportion of pirimiphos-methyl in flour which survived the baking process was approximately 50% in the case of wholemeal flour and 56% for white flour. However with white flour there was an increase in the proportion of pirimiphos-methyl surviving the baking process as the storage period of the treated grain used to produce the flour increased. For any organophosphorus pesticide which preferentially accumulates in the bran it is reasonable to expect that residues in white bread will be relatively small as a consequence of the low levels of bran in white flour. However, the consumption of brown bread, produced from wholemeal flour or low extraction flour, is increasing and the pesticide residues in the food may be considerably greater.

In addition to the studies cited above, a considerable amount of unpublished residue data will have been supplied to national regulatory authorities and international agencies such as the Codex Alimentarius Commission. One report published jointly by FAO/WHO (Anon 1979),refers to studies carried out by Australian workers who are reported as having demonstrated that, in the case of fenitrothion, approximately 50% of the residues survived baking irrespective of the level of residues in the flour and independent of the type of bread, the recipe or baking process.

Experimental programmes carried out at the Ministry of Agriculture, Fisheries and Food, Slough Laboratory, concerned with the control of infestation in stored grain now include milling and baking tests to determine the proportion of residual pesticide which is retained during baking. Studies so far have been restricted to determining the levels of intact pesticides in wheat and comparing these with the levels found in wholemeal flour and bread. Data are presented here for residues of malathion, fenitrothion, etrimfos, methacrifos, pirimiphos-methyl and chlorpyrifos-methyl.

MATERIALS AND METHODS

Treatment of grain:

"Flanders" milling quality wheat was purchased from a farm in Suffolk and delivered to the Slough Laboratory in 20 tonne batches. The grain had been stored on the farm for almost 5 months prior to delivery and its moisture content ranged between 13.5 and 15.1%. As part of a large scale assessment of grain protectants, 20 tonne batches of wheat were treated with etrimfos, methacrifos or pirimiphos-methyl. The pesticides were applied as water-based emulsions using a commercial grain sprayer. The batches treated with etrimfos and with pirimiphos-methyl were stored for 36 weeks but the grain treated with methacrifos was stored for only 24 weeks. All storage was under ambient conditions in 20 tonne capacity metal bins. At the end of the storage period 5 kg samples were withdrawn from the centre of each bin and used for the milling and baking trials.

Small-scale treatments with chlorpyrifos-methyl, fenitrothion or malathion were carried out using grain from the same batch of "Flanders" wheat used in the large-scale assessment. Diluted emulsions of the pesticides were sprayed onto separate 50 kg batches of wheat whilst the grain was being tumbled in a concrete mixer. The treated grain was then aged for 4 weeks under ambient conditions and a 5 kg sample was removed for milling and baking.

The residues of each pesticide in the grain were determined by analysis immediately before the samples were sent to the Flour Milling and Baking Research Association for milling and baking (see below).

Milling and baking:

The wheat from each treatment was ground on a hammer mill fitted with a 0.8 mm screen and the water absorption of the resultant wholemeal flour was calculated using a "Simon extrusion meter". A dough was produced using 300 g wholemeal flour, 5.35 g sodium chloride, 5.9 g fresh yeast, 2.2 g fat and the previously determined amount of water. The doughs were mixed at 11 watt hours per kg and 454 g dough pieces were cut off, moulded and then remoulded after 10 minutes at 27°C. The doughs were then proved to 10 cm height at 43°C and baked at 218°C for thirty minutes. Some doughs had insufficient gas-holding capacity to enable them to reach 10 cm and these were baked after 45 minutes proving. Three loaves were baked from each sample of treated grain.

Analysis:

Bread from treated wheat was sliced and reduced to crumbs in a bottom drive macerator. Weighed portions (20 g) were transferred to Soxhet extraction units and extracted with n-hexane for not less than four hours. Flour and whole grain were blended with methanol according to a collaboratively tested method (Anon 1973) later found to be applicable to a wider range of organophosphorus pesticides in grain (Anon,1980).

Extracts were analysed without clean-up by gas liquid chromatography using 5% OV-17-0.2% Epikote on 80-100 mesh Gas. Chrom. C as column packing. A flame photometric detector was used throughout. The minimum quantifiable level of each pesticide was less than 0.2 mg/kg based on a recorder response of 10% full scale deflection (FSD) with a noise level less than 1% FSD. Percentage recoveries of pesticides from bread were not determined because of the practical difficulties in spiking bread at known levels.

RESULTS

The levels of intact pesticide residues in the freshly treated grain (actual dose), aged grain, wholemeal flour and wholemeal bread are given in Table 1. In the case of bread, a representative sample was taken from each of the three loaves and analysed. Results are expressed as the mean $\pm$ standard error for the three determinations. The doses achieved were generally close to the recommended dose where applicable but the residues in the aged wheat were always lower.

The milling process generally had little effect on pesticide residue levels except in the case of malathion and pirimiphos-methyl where a 25% loss occurred. The largest loss of pesticide occurred during the baking process but even so between 39 and 57% of the pesticide present on the wheat before milling was detected in the bread.

DISCUSSION

There is a generally held opinion that the breakdown of organophosphorus pesticides in flour during the baking process ensures that acceptable levels in bread are not exceeded. The results from these experiments with wholemeal flour showed that at least 40% and often more than half of the pesticide survived the milling and baking process intact. Bread is about 20% heavier than the flour from which it is baked, resulting in an apparent 17% loss of pesticide. Hence the proportion of pesticide seemingly lost during the baking process is due only in part to breakdown or loss through volatilization. Maximum residue levels (MRL) in bread or in flour or in both have been set by the Codex Alimentarius Committee

TABLE 1 PESTICIDE RESIDUES (mg kg^{-1}) IN WHOLEMEAL FLOUR AND BREAD PRODUCED FROM TREATED WHEAT

	Recommended dose	Actual dose	Weeks stored	Residues			Percentage pesticide surviving baking
				Aged wheat	Flour	Bread/	
Chlorpyrifos-methyl	5	3.7	4	3.8	3.6 (2.0)*	1.5 ± .033 (2.0)	43
Etrimfos	5	5.0	36	4.4	4.6	2.6 ± .058	57
Fenitrothion	6	6.8	4	3.3	3.0 (5.0)	1.6 ± .033	53
Malathion	10	8.2	4	5.4	4.1 (2.0)	1.6 ± .088	40
Methacrifos	4	2.6	24	1.4	1.3	0.51 ± .013	39
Pirimiphos-methyl	4	3.4	36	3.1	2.3 (5.0)	1.2 ± .020 (1.0)	52

*Figures in parentheses are the Maximum Recommended Levels.
/ Results for bread are mean ± standard error for three determinations.

for 4 of the pesticides tested. The residues of 3 of these exceeded the MRL in bread or flour. No MRL has, so far, been set for fentitrothion in bread.

The surprisingly high residue levels found in wholemeal bread and flour suggest that the admixture of pesticides with grain could readily result in the present MRL's being exceeded. This, in turn, suggests that the maximum recommended doses for application to grain may be too high or that the MRL's are too low. However, there is little scope for reducing application rates of many pesticides as this would seriously limit their biological effectiveness. The levels of residues found probably do not represent a toxicological hazard but could lead to problems in grain or flour exported from the United Kingdom to other countries. Some reappraisal of the MRL's for wholemeal flour and bread seems desirable.

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References

ANON (1973) The determination of malathion and dichlorvos in grain. Analyst. 98, 19-24.

ANON (1979) Pesticide residues in food. 1979 evaluations. Food and Agriculture Organisation of the United Nations, Rome 1980.

ANON (1980) Determination of a range of organophosphorus pesticide residues in grain. Analyst.105, 515-517.

BINDRA, O.S. and SIDHU, T.S. (1972) Dissipation of malathion residues on maize grain in relation to dosage, storage conditions and baking. Journal of Food Science and Technology (Mysore). 9, 29-32.

BULLOCK, D.J.W. (1974) Pirimiphos-methyl : Residues in stored grain, bread, flour and milled products. ICI Plant Protection Limited. Report No AR 2537A.

CERNA, U., BENES, U. and HORAK, E. (1978) Dynamics of pirimiphos-methyl residues in crops. II. Residues after application to stored grain. Cesk. Hyg. 23, 321-5.

HASCOET, M. (1978) Presence of pesticides in wheat. Meunerie Francaise. 388, 29-32.

MENSAH, G.W.K., WATTERS, F.L. and WEBSTER, R.B. (1979). Insecticide residues in milled fractions of dry or tough wheat treated with malathion, bromophos, iodofenphos and pirimiphos-methyl. Journal of Economic Entomology. 72, 728-731.

MUKHERJEE, G., BANERJEE, A., MUKHERJEE, A., and MATHEW, T.V. (1973). Loss of pesticide residues from rice and flour during baking and cooking. Research and Industry, India. 18, 85.

ROWLANDS, D.G. (1976) Metabolism of insecticides in grains. Residue Reviews. 58, 113-153.

SESSIONS 4C AND 5A

CEREAL PEST AND DISEASE CONTROL (I)

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CARABID BEETLES AS PREDATORS OF CEREAL APHIDS

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Summary Populations of carabid beetles, and other polyphagous predators, were manipulated in field experiments using plots surrounded by polyethythene barriers. Carabid beetles were trapped out or killed by an insecticide (fonofos) and predation on aphids by these beetle populations was compared with that of control populations. Experiments on winter wheat in 1979 and 1980 showed strong inverse correlations between the different populations of carabid beetles (particularly Agonum dorsale), and other polyphagous predators and those of cereal aphids. It was concluded that carabid beetles are important in controlling populations of cereal aphids.

INTRODUCTION

There is a considerable literature on specific predators of aphids such as coccinellids, syrphids, chrysopids and braconids. Much less is known about the importance of polyphagous predators such as carabid and staphylinid beetles, spiders, harvestmen and earwigs. Remains of aphids in the guts of carabid beetles have been reported by Skuhravy (1959), Penney (1966), Luff (1974) and Vickerman and Sunderland (1975).

The manipulation of field populations of polyphagous predators to study their effects on numbers of cereal aphids, was described first by Edwards and George (1977). In these studies and later ones (Edwards et al, 1978) they reported strong inverse correlations between populations of cereal aphids and those of Harpalus rufipes, Pterostichus madidus and Agonum dorsale. In later work (Edwards et al, 1979), this was confirmed in two field experiments, one with a large population of cereal aphids in Sussex and one with small numbers at Rothamsted, and the importance of predation by carabid beetles was compared with that of other polyphagous predators.

Populations of cereal aphids differ from year to year both in terms of numbers and dominant species and the aim of the experiments reported here is to confirm the importance of carabids in controlling populations of different species of cereal aphids in different years.

METHODS AND MATERIALS

1979 Experiment

Three plots (each 12 m square) were marked out in a crop of winter wheat (Flanders) in Stackyard field at Rothamsted on April 24th 1979. Each plot was sur-

rounded by a double layer of polyethylene, 60 cm high buried to a depth of 15 cm and supported by wooden posts between the two polyethylene layers. These barriers were erected with the aid of a special machine with a single tine pulled by a winch on the power take-off of a Land Rover. The machine designed at the National Institute of Agricultural Engineering, Silsoe, fed the polythene sheet into the slot cut by the tine, with little soil disturbance.

In two plots, 50 plastic beakers 7 cm diameter and 9 cm deep were buried to soil level randomly over the plot except for the central 2 m x 2 m area. In the other plot eight similar beakers were placed. One of the plots was treated with 2 kg fonofos ha^{-1}. In all plots, eight beaker pitfall traps were kept one quarter full of 50% alcohol, and in the two plots with 50 traps the remaining 42 pitfall traps were kept dry. In the fonofos-treated plot, all dead or live predatory arthropods trapped were counted and removed every Monday, Wednesday and Friday. In the other plot with 50 pitfall traps, the traps were examined on the same days, carabid beetles counted and removed, and the other arthropods that had been trapped were released back into the plot. The carabid beetles and other arthropods caught in the alcohol traps in all plots were identified to species for carabids and as far as possible for other polyphagous predators. Aphids were counted weekly on tillers and/or on 25 ears taken at random. Three similar plots were set up on 23rd May 1979 and treated and sampled in an identical manner to the first block.

1980 Experiment

A duplicate experiment was set up in 1980 on a commercial farm (Thrales End) in Harpenden with barriers set up on April 29th and May 29th.

RESULTS

The total numbers of carabid beetles in eight traps trapped three times a week are summarised in Figs.1,2,3 & 4a respectively. The numbers of aphids per 25 tillers are shown in Figs. 1,2,3 & 4b. Metopolophium dirhodum dominated the aphid population in 1979 and Sitobion avenae in 1980.

The total numbers of polyphagous predators trapped are summarised in Table 1.

Table 1.

Total numbers of polyphagous predators trapped in Rothamsted experiment - 1979

	Control	Carabids removed	Fonofos treated
April barriers			
Araneae	1153	426	366
Carabidae	509	127	140
Staphylinidae	949	338	298
Other Coleoptera	199	114	87
Total arthropods	2810	1005	891
May barriers			
Araneae	779	483	221
Carabidae	319	148	70
Staphylinidae	209	144	61
Other Coleoptera	188	146	68
Total arthropods	1495	921	420

Total numbers of polyphagous predators trapped in Thrales End experiment - 1980

	Control	Carabids removed	Fonofos treated
April barriers			
Araneae	601	336	106
Carabidae	368	158	103
Staphylinidae	254	238	167
Other Coleoptera	74	12	0
Total arthropods	1297	744	376
May barriers			
Araneae	472	216	127
Carabidae	268	126	58
Staphylinidae	273	142	52
Other Coleoptera	63	25	7
Total arthropods	1076	509	244

The correlation between the total numbers of aphids counted in each plot in 1979 and 1980 and the total numbers of carabid beetles in the same plots is given in Fig. 5.

Figure 5. Correlation between numbers of aphids and carabid beetles in 1979 and 1980 experiments

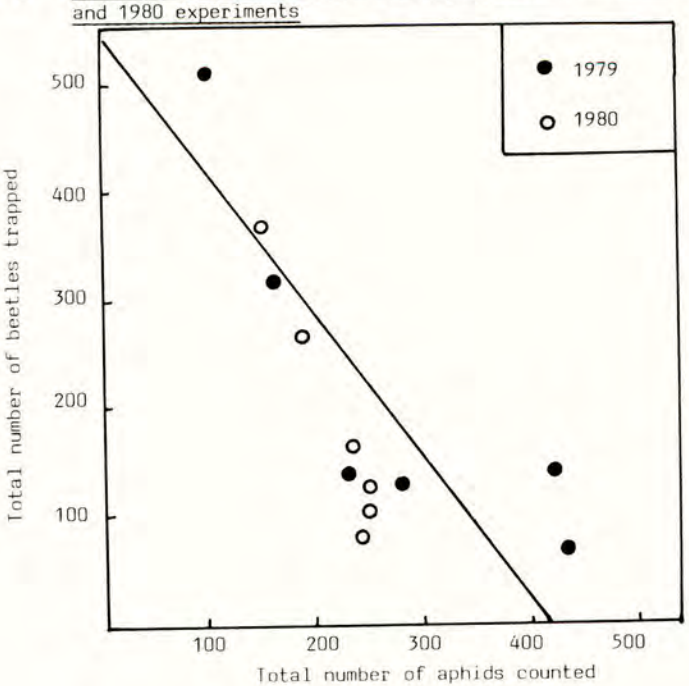


Figure 1 Carabid beetle and cereal aphid populations
April 1979 experiment

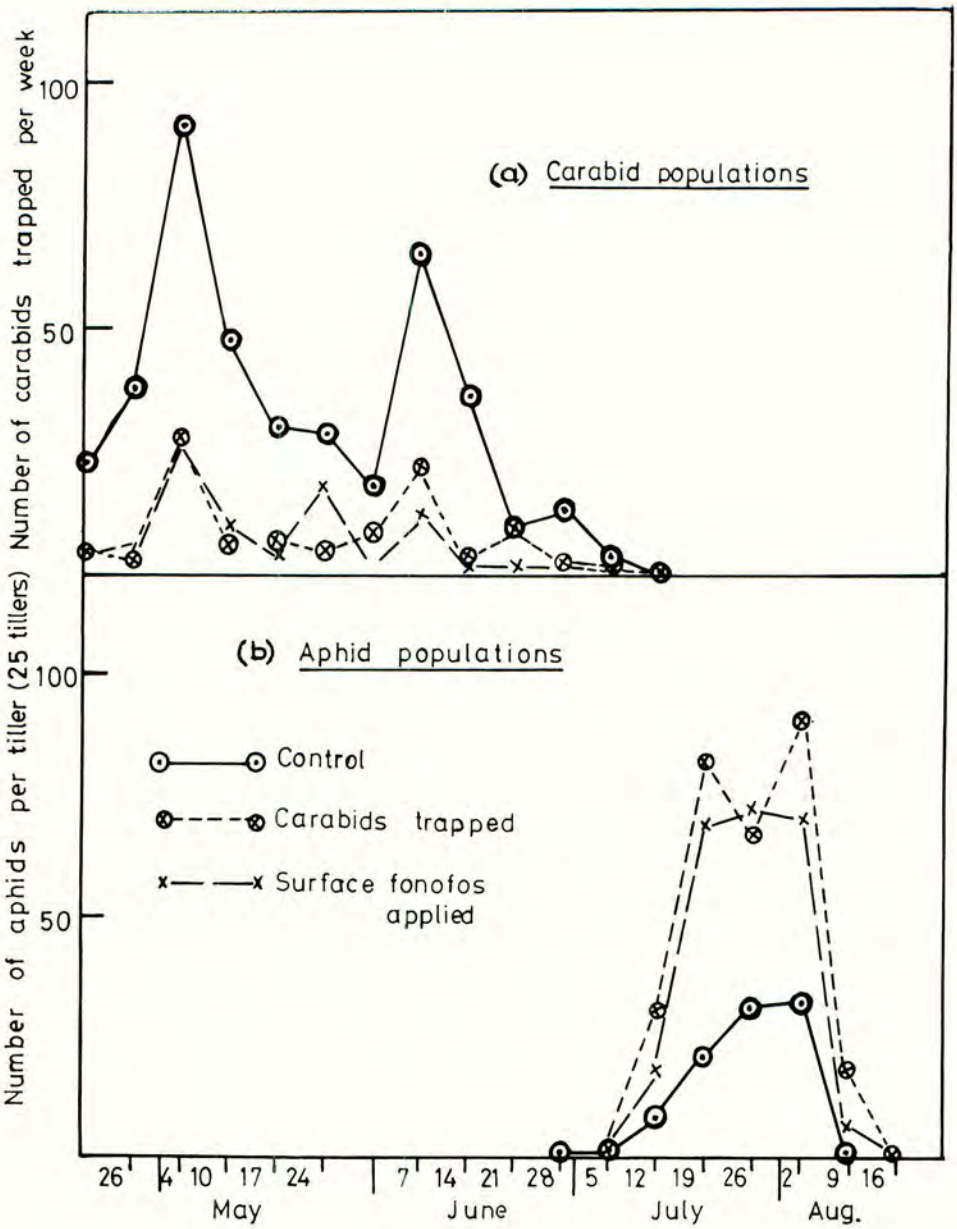


Figure 2 Carabid beetle and cereal aphid populations May 1979 experiment

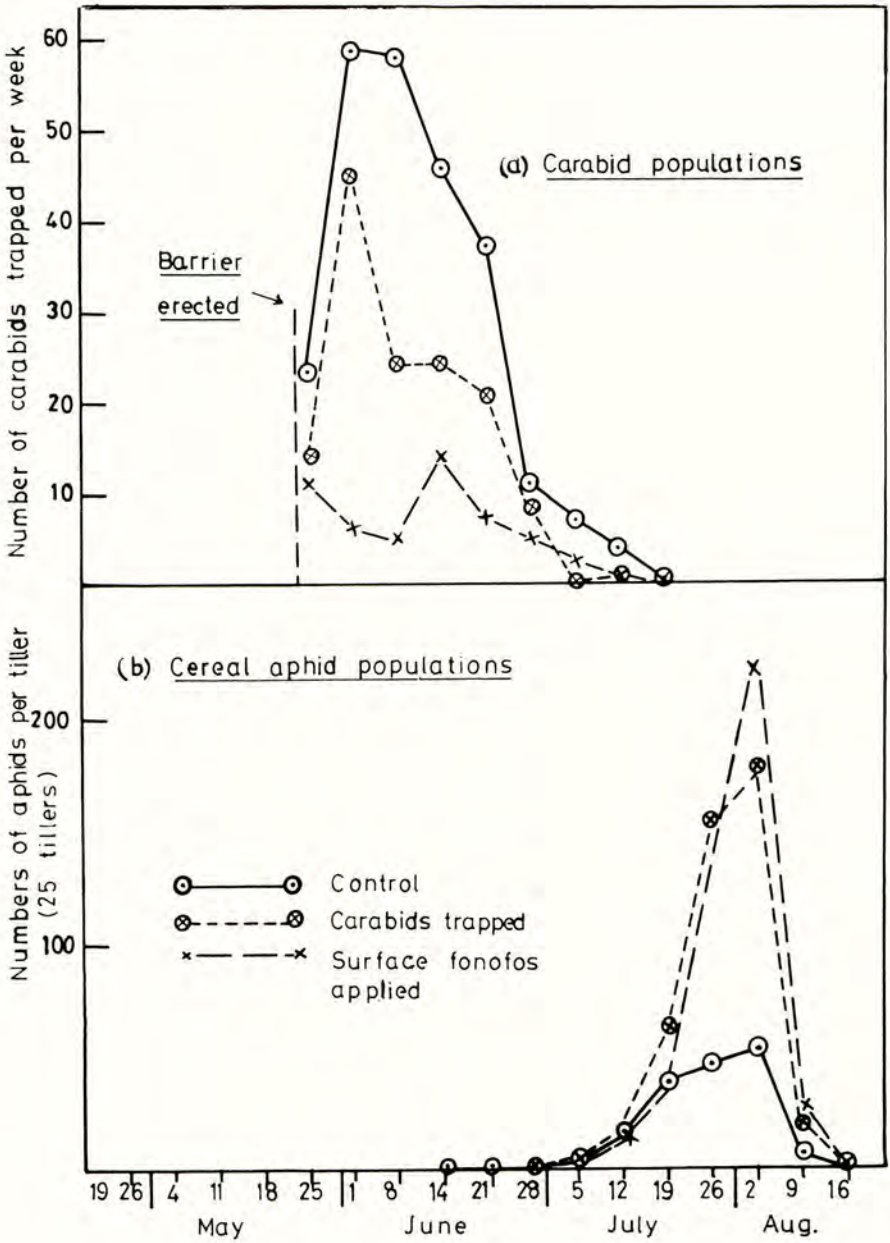


Figure 3 Carabid beetle and cereal aphid populations
April 1980 experiment

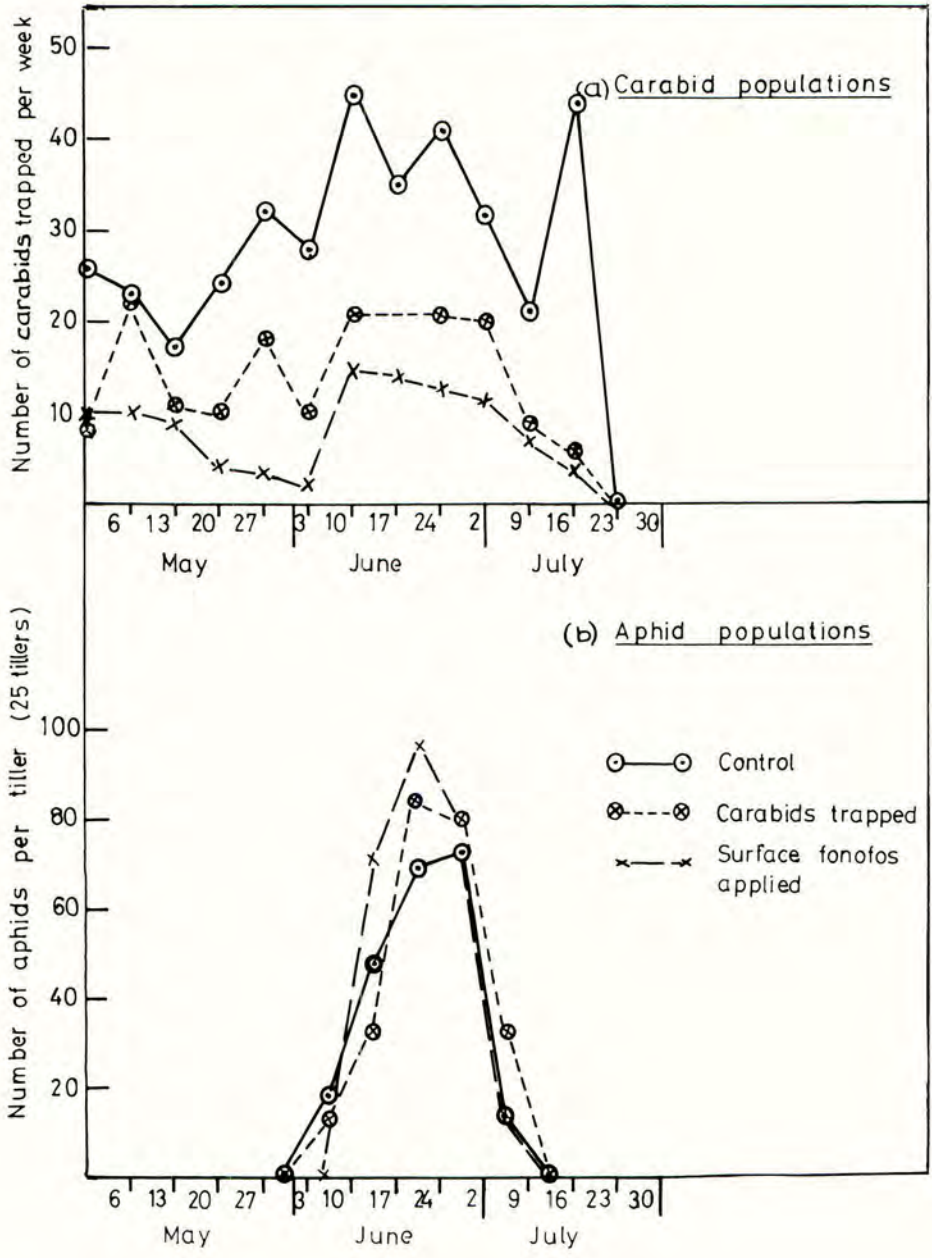
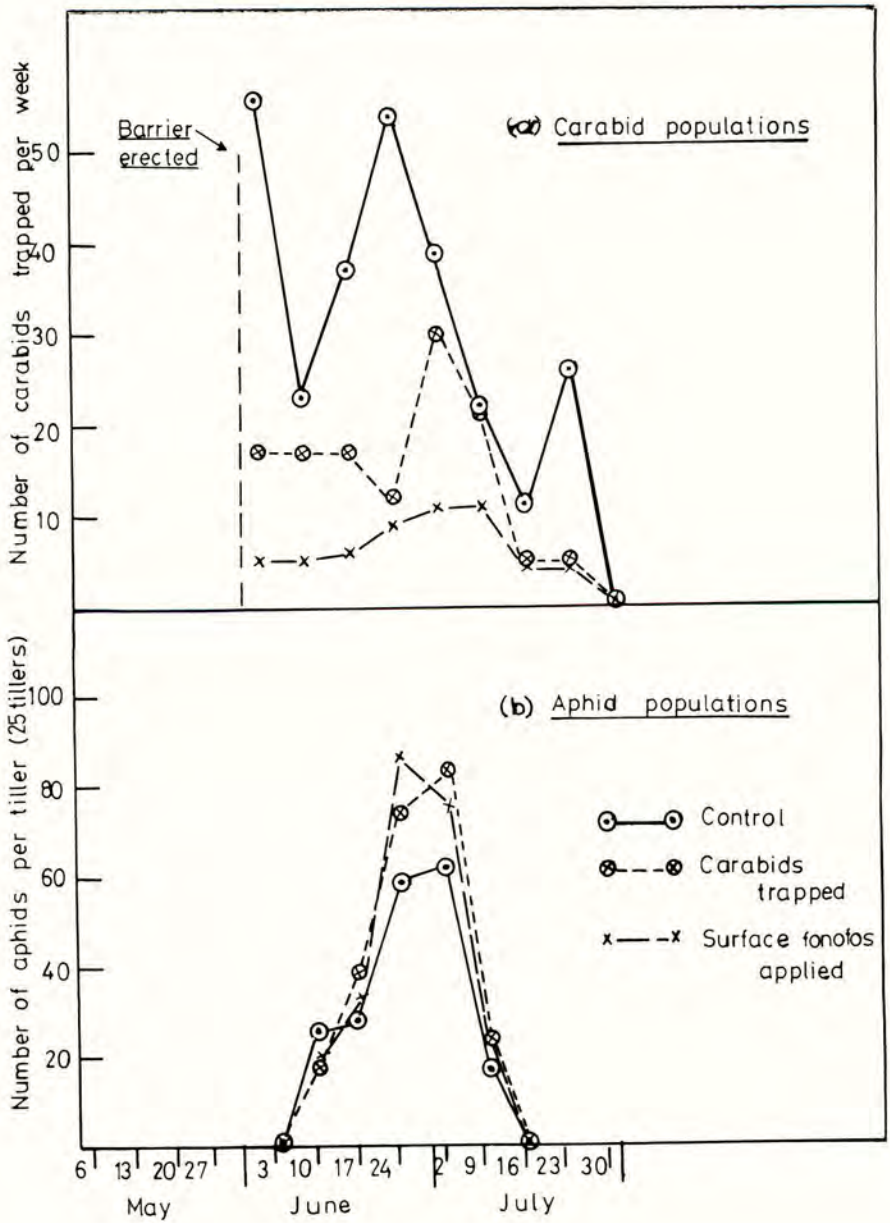


Figure 4 Carabid beetle and cereal aphid populations
May 1980 experiment



DISCUSSION

The manipulation of carabid beetle populations was successful in both years achieving three distinct levels ranging from high in the control plots to low in those treated with fonofos. However, the technique of removing carabid beetles only, with the aim of leaving other polyphagous predators untouched, was not entirely successful (Table 1) because the traps caught many of the other predators and these were dying before they could be released back into the plot. This makes it more difficult to identify which were the most important of the polyphagous predators, because populations of aphids were quite strongly inversely correlated with populations of most of the polyphagous predators (Table 1 and Fig. 3). It is interesting that the correlation coefficients between carabid beetles and aphids were so similar between 1979 and 1980 when the main aphid prey was different. The species of carabids present in the five experiments did not differ greatly either in species present or populations (Fig. 3). Bembidion lampros, Bembidion obtusum, A. dorsale, H. rufipes and Nebria brevicollis were the dominant species in Stackyard in 1979 and B. lampros, A. dorsale and P. madidus were most numerous at Thrales End in 1980. A. dorsale was numerous at both sites and was active throughout the spring as the cereal aphid populations were building up. B. lampros, the only other species that was numerous at both sites was active too late in the season to account for the changes in cereal aphid populations.

Our results confirm earlier conclusions that A. dorsale was the most important species (Edwards et al, 1979), and support Sunderland (1975) who reported finding large amounts of aphid remains in the guts of A. dorsale.

These and other studies suggest strongly that predation early in spring when the cereal plants were very small was responsible for the changed aphid populations later in the season both on the tillers and on the ears.

There was further evidence in this study that numbers of various polyphagous predators are inversely related to those of cereal aphids (Table 2) as first suggested by Edwards et al (1979). The inverse relationship implies that the polyphagous predators are less dependent upon aphids as a source of food but can consume large numbers when they are available.

Table 2

Correlation coefficients between populations of cereal aphids and polyphagous predators

Year	Group	Correlation coefficient
1979	Araneae	r = -0.814
	Staphylinidae	r = -0.862
	Carabidae	r = -0.824

From these and earlier results, it seems clear that carabid beetles and other polyphagous predators can exert an important influence upon cereal aphid populations. For this reason, the developing practice of spraying winter cereals, sometimes with broad spectrum insecticides, is to be viewed with some concern. If the overwintering polyphagous predator populations are killed the result may be increased populations of cereal aphids.

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References

EDWARDS, C.A. and GEORGE, K.S. (1977) Carabids and cereal aphids. Report Rothamsted Experimental Station for 1976, 131

EDWARDS, C.A., PARSONS, N., GEORGE, K.S. and HEILBRONN, T. (1978) Carabids as predators of cereal aphids. Report Rothamsted Experimental Station for 1977, 101

EDWARDS, C.A., SUNDERLAND, K.D. and GEORGE, K.S. (1979) Studies on Polyphagous Predators of Cereal Aphids. Journal of Applied Ecology 16, 811-823

PENNEY, M.M. (1966) Studies on certain aspects of the ecology of Nebria brevicollis (F) Coleoptera, Carabidae. Journal of Animal Ecology 35, 505-512

SKUHRAVY, (1959) Potrava Polnich Strevtikovitych Acta Entomologicae Cechosloveniae 56, 1-18

SUNDERLAND, K.D. (1975) The diet of some predatory arthropods in cereal crops. Journal of Applied Ecology 12, 755-766

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TOWARDS INTEGRATED CONTROL OF CEREAL APHIDS

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Summary Changes in cereal farming practice over the last 15 years include increased insecticide use against cereal aphids, increased herbicide application and earlier sowing of winter cereals (Edwards, 1977; Graham-Bryce et al, 1979). In small plot experiments, pirimicarb application reduced aphid parasitoid numbers, and dimethoate reduced parasitoids and polyphagous predators. Polyphagous predators were influenced by weed removal, different species being affected in different ways. More cereal aphids were infected with fungal pathogens in weedy than in clean plots. Overwintering Sitobion avenae populations on early sown winter wheat supported high parasitoid populations, and both aphid and parasitoid numbers increased earlier in the season on early- than on late-sown crops. The importance of monitoring the effects of changing farming practice on the cereal fauna in order to predict future problems and devise integrated control procedures is stressed.

INTRODUCTION

Since the late 1960s, a number of severe outbreaks of the cereal aphids Sitobion avenae and Metopolophium dirhodum in Europe, and recognition of their direct effects on cereal yields, have led to a substantial increase in the use of insecticides on cereal crops (Vickerman & Wratten, 1979). Over the same period there have been other changes in cereal farming practice, for example, increased use of fungicides and herbicides, direct drilling, straw burning and earlier sowing of winter crops (Edwards, 1977; Graham-Bryce et al, 1979). It has been suggested that changes in agricultural practices may have contributed to the increased frequency and intensity of aphid outbreaks experienced in recent years (Kolbe, 1973; Way, 1978).

Widespread use of insecticides on cereal crops, especially as a routine prophylactic measure, carries a strong risk of aggravating pest problems in the long term by damaging natural enemy populations and, particularly in the case of aphids, by promoting the development of pesticide-resistant strains. There is, therefore, an urgent need for the development of effective systems of supervised or, preferably, integrated control in cereal crops.

Integrated control systems should make full use of natural mortality factors. Cereal aphids are attacked by a variety of natural enemies - predators, parasitoids and fungal pathogens (Vickerman & Wratten, 1979; Carter et al, 1980). In order to assess the role these natural enemies could play in any proposed integrated control programme, it is important to investigate the effects of changing agricultural practices on their population size and efficiency in controlling pests. This poster demonstrates the range of work being done at Rothamsted designed to gather such information, and to illustrate the potential impact of current trends in cereal farming.

METHODS AND MATERIALS

In 1980 and 1981, three plots of winter wheat (19m x 13.5m) were treated with pirimicarb (0.14kg a.i./ha), three with dimethoate (0.35 l a.i./ha) and three were untreated. The plots were arranged as a randomised block. Treatments were applied at G.S. 61 (Zadoks et al, 1974). In 1981, polyethylene barriers, extending 15cm below the soil surface and 45cm above it, were erected around each plot.

In a separate experiment in 1980, three plots of winter wheat (19m x 19m) were treated with chlortoluron four weeks after sowing and with a mixture of isoproturon and mecoprop at G.S. 30, while three plots were untreated. The plots were arranged as a randomised block. In 1981, the experiment was repeated without the autumn herbicide treatment and with six replicates of each treatment arranged as a randomised block. Pitfall traps and an insect suction-net were used to sample the insect fauna in all the experiments. In the herbicide experiment, samples of S. avenae and, in 1981, of the aphids Brachycaudus helichrysi from Tripleurospermum maritimum, Veronica arvensis and Myosotis arvensis, and Cryptomyzus galeopsidis from V. arvensis and Lamium amplexicaule, were collected and reared in the laboratory to monitor fungal pathogens.

Towards the end of March 1980, and in early April 1981, overwintering S. avenae were collected from early-sown (before mid-October) winter wheat in Sussex, and reared in the laboratory to detect the presence of parasitoids. In 1980, the suction-net was used to sample S. avenae populations in early-sown and late-sown fields in the same area, when populations were increasing in early summer. Adult aphid parasitoids in the same suction-net samples were counted.

RESULTS

Insecticide experiments

No differences between pitfall samples from treated and untreated plots were obtained in 1980, but in 1981, when polyethylene barriers were used, treatment effects were recorded (Table 1). Dimethoate greatly reduced numbers of all three major groups of polyphagous predators, Carabidae, Staphylinidae and spiders, but pirimicarb had no significant effect. There were fewer aphid parasitoids in suction-net samples from treated than from untreated plots (Table 2).

Table 1

Polyphagous predators caught in pitfall traps in winter wheat after pirimicarb and dimethoate treatments (1981)

	Days after treatment	Untreated	Pirimicarb	Dimethoate
Carabidae	1-3	45	34	1 ***
	8-10	59	74	26 **
Staphylinidae	1-3	29	23	9 *
	8-10	24	24	6 *
Araneae	1-3	34	32	9 **
	8-10	104	81	49 *

* -p < 0.05 ** -p < 0.01 *** -p < 0.001

Table 2

Aphid parasitoids caught in suction-net samples in winter wheat after pirimicarb and dimethoate treatments

Year	Days after treatment	Untreated	Pirimicarb	Dimethoate
1980	1	89	20 **	12 **
	13	57	24 *	16 *
1981	3	14	8	2 *

* -p < 0.05 ** -p < 0.01

Herbicide experiments

High populations of broad-leaved weeds occurred in the untreated plots in both years. The dominant species were Stellaria media, T. maritimum, V. arvensis and Galium aparine. The effects on pitfall captures of carabid beetles varied according to beetle species (Table 3).

Table 3

The effects of high weed densities on the numbers of some carabid beetles caught in pitfall traps in winter wheat

Species	Year	Weedy Plots	Clean Plots
Pterostichus melanarius	1980	492	1148 ***
	1981	405	713 *
P. madidus	1980	17	97 **
	1981	103	190 *
Laricera pilicornis	1980	61	35 *
	1981	120	63 *
Agonum dorsale	1980	21	13
	1981	185	129 *
Amara spp.	1980	67	5 **
	1981	12	1 **

* -p < 0.05 ** -p < 0.01 *** -p < 0.001

Pterostichus species were caught in greater numbers in the clean plots, while L. pilicornis, A. dorsale and Amara spp. were caught more in the weedy plots. Staphylinid species were eight caught in similar numbers in weedy and clean plots or were caught predominantly in weedy plots (Table 4). The largest species, Philonthus cognatus, was greatly reduced by weed removal, whilst Tachyporus species, which may be important aphid predators (Vickerman & Sunderland, 1975) appeared to be unaffected.

Table 4

The effects of high weed densities on the numbers of some staphylinid beetles caught in pitfall traps in winter wheat

Species	Year	Weedy Plots	Clean Plots
Philonthus cognatus	1980	235	21 ***
	1981	96	16 ***
Tachyporus spp.	1980	80	59
	1981	44	57
Tachinus signatus	1980	12	0
	1981	57	17 *
Aleocharinae	1980	325	248 *
	1981	545	494

* -p < 0.05 *** -p < 0.001

In 1980, more S. avenae were infected with entomophthoraceous fungi in weedy plots than in clean plots (Table 5). This may have resulted from a higher relative humidity in the weedy plots which favoured the action of the fungi, or the presence on the weeds of infected aphids from which the fungi spread to the cereal aphids.

Table 5

Numbers of S. avenae infected with fungal pathogens in samples collected from weedy and clean plots of winter wheat June/July 1980 (n=600)

Fungus	Weedy Plots	Clean Plots
Entomophthora planchoniana	60	34 *
Condiobolus obscurus	18	6

* -p < 0.05

In 1981, populations of B. helichrysi and C. galeopsidis occurred in the weedy plots and both were attacked by entomophthoraceous fungi. On B. helichrysi, which was the more heavily attacked, Erynia neoaphidis and Entomophthora planchoniana were the dominant pathogens. Unfortunately, cereal aphid populations were too small to be sampled adequately in June when infected weed aphids were at their most abundant. Small numbers of S. avenae were sampled in July but there was no difference in infection between treatments.

Sowing date

During the mild winters of 1979/80 and 1980/81, overwintering populations of cereal aphids survived on early-sown winter wheat and barley. 36% and 30% parasitism was recorded in overwintering populations of S. avenae on winter wheat in late March 1980 and early April 1981, respectively.

S. avenae populations, with their attendant parasitoid populations, developed

earlier on early-sown than on late-sown winter wheat (Table 6).

Table 6

Densities (No./m^2) of S. avenae and aphid parasitoids in suction-net samples from early-sown and late-sown fields of winter wheat in early summer, 1980

Date	S. avenae		Parasitoids	
	Early	Late	Early	Late
13 May	14.4	2.4	0.8	1.0
20 May	40.7	7.0	3.4	0.0
3 June	375.6	62.4	30.8	12.0
9 June	585.6	177.2	32.4	25.2

DISCUSSION

The results show that insecticidal control of cereal aphids by pirimicarb and dimethoate can seriously reduce populations of beneficial non-target, polyphagous predators and aphid parasitoids. The potential of polyphagous predators, particularly carabid beetles, in helping to reduce cereal aphid numbers has been demonstrated by Edwards et al (1979).

The use of insecticides during aphid outbreak years is necessary, but it is important to reduce the risk of creating future problems by avoiding applications when aphid numbers remain below the economic thresholds recommended by ADAS and by using the more selective chemicals such as pirimicarb.

Extensive herbicide usage on cereal crops dramatically changes the nature of the cereal field as an insect habitat, and can seriously affect some species including carabid and staphylinid beetles. The availability of key food items and the degree of ground cover may both contribute to these effects. More work is needed on the relative importance of individual species of polyphagous predators as aphid consumers. Speight and Lawton (1976) recorded increased predation of fruit fly pupae attached to cards by carabid and staphylinid beetles with increased grass-weed cover in winter wheat.

Another contribution to aphid control could be the presence of other aphid species on wild plants within the crop. Their potential role in improving the efficiency of aphid natural enemies, such as fungal pathogens, by acting as alternative hosts early in the season needs to be assessed.

The trend towards early sowing of winter cereals will lead to an increase in the number of fields which become colonised by cereal aphids in the autumn. During mild winters, such as those of 1979/80 and 1980/81, overwintering aphid populations survive in early-sown fields. Despite these overwintering populations, there were no serious aphid outbreaks during 1980 or 1981. A possible explanation is that natural enemies exploited these populations early in the year, preventing a rapid increase of aphid numbers. The high rates of parasitism recorded in overwintering S. avenae populations in late spring in 1980 and 1981 support this explanation. Though early sowing followed by mild winter weather may help to prevent summer aphid outbreaks, it may increase barley yellow dwarf virus infections following colonisation by aphids in the autumn. It is thus important to investigate the consequences of an

increased autumn spraying programme.

These examples of the effects of recent trends in cereal growing illustrate the importance of monitoring accompanying changes in the cereal fauna, particularly with regard to pest natural enemies. The information gathered will be useful in identifying future problems and in helping to formulate integrated control procedures.

References

CARTER, N.; McLEAN, I.F.G.; WATT, A.; DIXON, A.F.G. (1980) Cereal aphids: a case study and review, Applied Biology, 5, 272-348

EDWARDS, C.A. (1977) Investigations into the influence of agricultural practice on soil invertebrates, Annals of Applied Biology, 87, 515-520

EDWARDS, C.A.; SUNDERLAND, K.D.; GEORGE, K.S. (1979) Studies on polyphagous predators of cereal aphids, Journal of Applied Ecology, 16, 811-823

GRAHAM-BRYCE, I.J.; HOLLOMAN, D.W.; LEWIS, T. (1979) Pest and disease control in cereals: a research viewpoint, Journal of the Royal Agricultural Society of England, 140, 131-139

KOLBE, W. (1973) Studies on the occurrence of cereal aphids and the effect of feeding damage on yields in relation to infestation density levels and control, Pflanzenschutz-Nachr. Bayer, 26, 396-410

SPEIGHT, M.R.; LAWTON, J.H. (1976) The influence of weed cover on the mortality imposed on artificial prey by predatory ground beetles in cereal fields, Oecologia, 23, 211-223

VICKERMAN, G.P.; SUNDERLAND, K.D. (1975) Arthropods in cereal crops : nocturnal activity, vertical distribution and aphid predation, Journal of Applied Ecology, 12, 755-765

VICKERMAN, G.P.; WRATTEN, S.D. (1979) The biology and pest status of cereal aphids in Europe: a review, Bulletin of Entomological Research, 69, 1-23

WAY, M.J. (1978) Integrated pest control with special references to orchard problems, Report. East Malling Research Station for 1977, 187-204

ZADOKS, J.C.; CHANG, T.T.; KONZAK, C.F. (1974) A decimal code for the growth stage of cereals, Weed Research, 14, 415-421

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SETTLING BEHAVIOUR OF CEREAL APHIDS AND FORECASTING OUTBREAKS

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Summary Alates of the grain aphid (Sitobion avenae) settled and reproduced more readily on wheat plants in the flowering and watery ripe stages than in the booting and ear emergence stages. In the field, immigration after flowering had little effect on the peak density achieved. Halving the density of tillers did not affect the number of aphids settling per unit area. The predictions of a simulation model showed that the timing and size of the aphid immigration relative to the crop development stage affect the peak aphid density achieved. These results are discussed in relation to the development of forecasting schemes for grain aphid outbreaks.

Recit Sommaire Des ailées de Sitobion avenae se sont établis et se sont multipliés plus facilement sur les plantes de blé froment dans les points 65 et 71 sur le code décimal, que dans les points 45 et 55. Peu d'ailées se sont établis et se sont multipliés sur les plantes après le point 71. Les résultats d'études sur le terrain ont indiqué que l'immigration, après le point 65, n'a pas fait grand'chose sur la densité maximum qui a été obtenue. S. avenae n'a pas montré une préférence pour s'établir dans les terrains de la densité différente de plante mais S. avenae était déposé au hasard. Les prédictions d'un appareil de simulation ont indiqué que le temps, relativement au moment de développement de la récolte, et la grandeur de l'immigration ont influé aussi sur la densité maximum des aphides. Ces résultats sont discutés relativement au développement des projets pour prévoir les éruptions des aphides de grain.

INTRODUCTION

The grain aphid (Sitobion avenae) is one of the major pest species on cereals in England. It can cause yield losses exceeding 10% (George, 1974, 1975; George and Gair, 1979) but its abundance and distribution vary from year to year (George and Gair, 1979; Carter et al., 1980). The causes of these variations are still not fully understood.

The size and timing of the spring migration are important in determining the likelihood of a cereal aphid outbreak, especially where aphids do not overwinter on cereals. The predictions of a simulation model have shown that the size of the immigration has a one-to-one effect on the peak aphid density (e.g. a 20% change in the size leads to a 20% change in the peak) (Carter and Dixon, 1981) and that its timing in relation to the crop development stage is also important in determining the peak aphid density (Carter and Rabbinge, 1980). Field studies have demonstrated that the peak densities of the bird cherry aphid (Rhopalosiphum padi) are higher the earlier the fields are infested (Henderson and Perry, 1978).

Few studies of the settling behaviour of cereal aphids have been made. Host plants of different species vary in their attractiveness to aphids (Vickerman and Wratten, 1979; Carter et al., 1980), but preferences for different development stages of host plants are less well known. Little work has been done on the effect of tiller density on the attractiveness of cereals to flying aphids.

This paper considers the effects of the size and timing of the spring migration, and the subsequent settling behaviour in different conditions, on the peak densities of aphids achieved on cereals. Their relevance to the prediction of cereal aphid outbreaks is discussed.

METHODS AND MATERIALS

Development stage of host plant

Wheat plants (cv. Maris Huntsman) at development stages booting (45) (Zadoks, Chang and Konzak, 1974), ear emergence (55), flowering (65), watery (71), milky (75) and doughy ripe (85) were used. In each experiment four plants, each with the same number of tillers and leaves, were arranged equidistant from a take-off platform which was surrounded by water. Forty alate aphids, which had been reared in isolation in clip cages from the fourth instar, were placed on the platform and the plants and platform were then covered with a muslin cage. Twenty-four hours later, the number of aphids that had settled on the plants and the number of nymphs that had been born were recorded. Each development stage was tested on its own and was compared with every other stage using a replacement series approach (de Wit, 1960). A full description of this experiment will be published elsewhere (Walters and Dixon, in prep.).

Sequential spraying

Two blocks, each 15 × 15 m, were set out in a winter wheat field, cv. Maris Huntsman, at the beginning of May. Each block was divided into nine plots, each 5 × 5 m. Three replicates of three treatments, unsprayed controls, one and two sprays, were allocated at random to the plots in a block. On 27 May at crop development stage 43 six plots in each block were sprayed with Pirimor (50% pirimicarb) 10 g/20 ℓ water, at 5 ℓ/plot using a knapsack sprayer. The second spray application on three of the original six sprayed plots was carried out on 16 June at development stage 69.

At the time of the peak aphid density, which was determined by observations in the rest of the field, the aphids were sampled by inspection of fifty tillers in each plot. Aphids were identified, and assigned to instar classes first-third, fourth apteriform, fourth alatiform, adult apterae and adult alatae.

Tiller density

Two blocks, each 5 × 20 m, were set out at the beginning of May in the winter wheat field described above. Each block was divided into four plots of 5 × 5 m. Two treatments, tiller density unaltered, and tiller density reduced by half, replicated twice, were allocated at random to the plots in each block.

Aphid sampling was carried out in each plot on 29 May (45), 23 June (71) and 7 July (75). The aphids on 100 tillers/plot were identified and assigned to instar classes as before. Tiller densities were determined after flowering using 0.25 m^2 quadrats placed at random in each plot.

A yellow water trap was positioned at crop height in the centre of each plot. The traps were emptied weekly and the number of grain aphids counted.

Simulation

A listing and detailed account of the model have been presented elsewhere (Carter et al., 1981) and only a brief description is given here.

The model used the number of alate grain aphids caught in the Rothamsted Insect Survey 12.2 m suction trap at Brooms Barn as an index of the number of aphids colonising crops (Taylor, 1979). Carter et al. (1980) have shown that the catches of this suction trap are significantly correlated with catches in water traps located in study fields near Norwich.

Subsequent development and reproduction of the aphids are dependent on daily minimum and maximum temperatures and the crop development stage. The majority of the first generation nymphs developed into adult apterae. These in turn produced more nymphs, an increased proportion of which developed into adult alatae, which emigrated. Much of the information regarding development, survival and reproductive rates used in the model comes from Dean (1974), Wratten (1977) and Watt (1979). Mortality inflicted by natural enemies was incorporated in the simulation.

The model was used to investigate the effect of changing the time of migration in relation to the crop development stage, and the number of immigrant alates on the peak density achieved. Field results from 1980, a year when there were high numbers of the grain aphid in the Norwich region, were used to initialise the model on the first simulation. Subsequent simulations were carried out using either different initial crop development stages or different numbers of immigrant alates (± 20%).

RESULTS

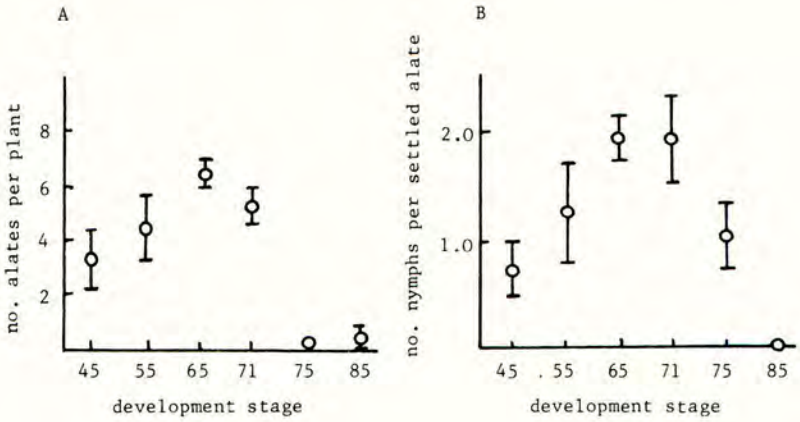
Development stage of host plant

Plants at flowering and watery ripe stages of development were colonised by more aphids ($t = 2.45$, $d.f. = 4$, $P < 0.05$) which produced more offspring ($t = 3.0$, $d.f. = 14$, $P < 0.01$) than plants at ear emergence and booting (Fig. 1). Milky and doughy ripe plants were not colonised.

The results of the comparisons of pairs of development stages by the replacement series experiment also revealed significant differences between both the number of alates settling and the number of nymphs born. The comparisons reflected the preferences shown by colonising alates in the earlier experiment (Walters and Dixon, in prep.).

Fig. 1

The number of alates settling and the number of nymphs born on plants of different growth stages



Sequential spraying

The control and single spray plots had similar densities of grain aphid, the commonest species, but the plots which received two sprays had very much lower aphid densities (Table 1). Block A at the top of the field has consistently higher densities than Block B at the bottom of the field. The difference between the logarithm of the aphid densities in the different treatments was significant ($F = 8.69$, d.f. = 2/12 , $P < 0.01$) as was that between blocks ($F = 12.37$, d.f. = 1/12 , $P < 0.01$).

Table 1

Peak densities of grain aphids/50 tillers ± S.E. in plots receiving zero, one and two sprays of Pirimor

Site	Control	One spray	Two sprays
Block A	89.0 ± 43.6	75.7 ± 13.7	16.3 ± 5.0
Block B	20.0 ± 2.1	25.3 ± 11.1	6.3 ± 4.4

Tiller density

On all three sampling occasions there were twice as many grain aphids/tiller in those plots where the tiller density had been reduced (Table 2). There were no significant differences between the number of aphids caught in yellow water traps in the high and low density plots or between the two blocks. However, the weekly catches were always very small, never exceeding five aphids/individual trap.

Table 2

The effect on mean aphid numbers/tiller on 7 July of reducing tiller density by half at two sites in a winter wheat field

Site	$323/m^2$	$147/m^2$
Block A	1.79 ± 0.13	3.38 ± 0.22
Block B	0.31 ± 0.19	0.69 ± 0.11

Two-way analysis of variance:

Treatments: $F = 36.52$, d.f. = 1/4 , $P < 0.01$
Blocks: $F = 176.41$, d.f. = 1/4 , $P < 0.001$

Simulation

The initial simulation, with no changes in parameter values, gave predictions (peak fifty-four aphids/tiller) similar to those observed in the field (peak fifty/tiller) (Fig. 2a). Changing the initial crop development stage from 43 to either 41 or 45 resulted in changes in the size of the predicted peak population but not its timing. With the earlier crop development stage, equivalent to a later sown crop, the peak aphid density was above seventy/tiller, an increase of 35%. With the later crop development stage, equivalent to an earlier sown crop, the peak aphid density was less than forty-five/tiller, a decrease of 18%.

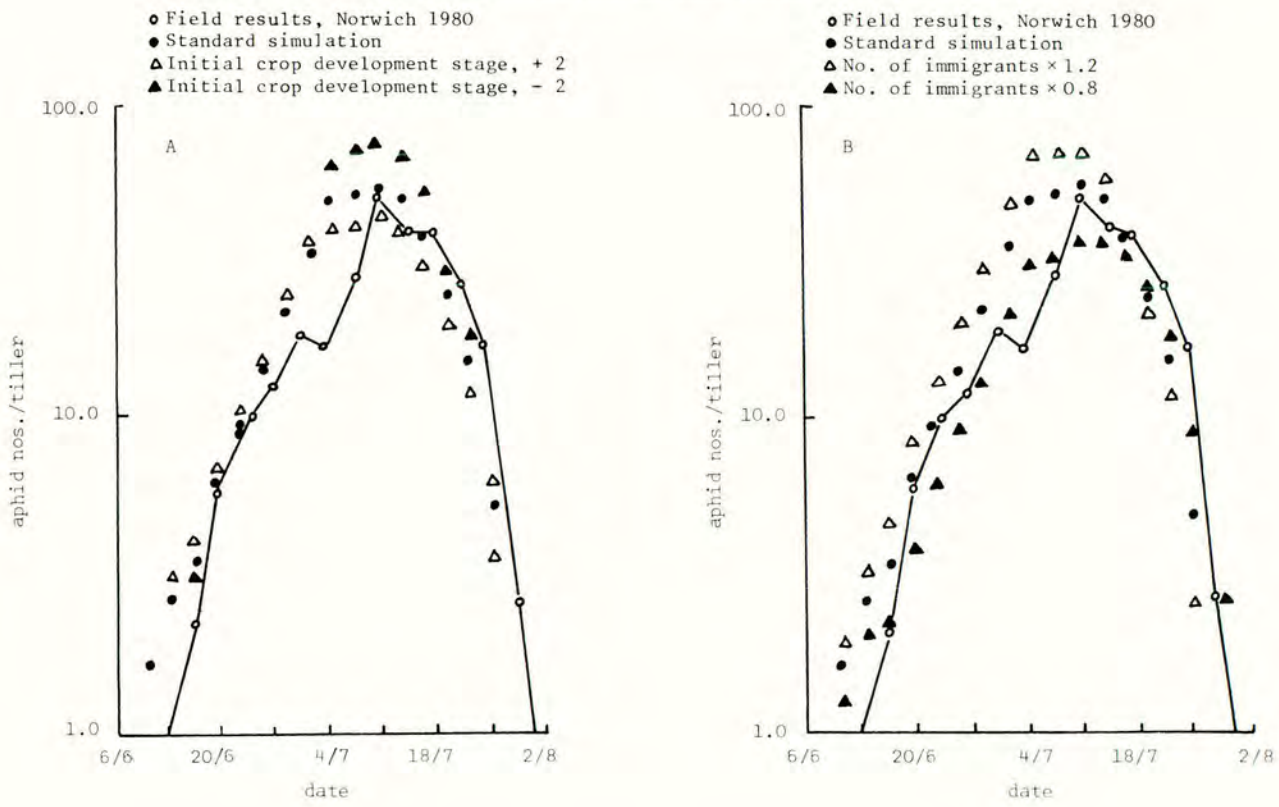
Increasing or decreasing the number of immigrant alates also changed the size of the peak aphid density but not its timing (Fig. 2b). An increase of 20% in the number of alates resulted in an increase of the peak density by 26%. The corresponding decrease in the number of alates led to a drop in the peak density of 33% to less than forty/tiller.

DISCUSSION

Different crop development stages varied in their attractiveness to alate grain aphids. The aphids readily settled and reproduced on wheat, until the watery ripe stage (71), but milky (73-77) and doughy ripe plants (83-87) were not suitable for colonisation. The field experiments complemented these results and showed that any immigration of grain aphids after the flowering stage had little effect on the peak aphid density achieved. Delaying the crop development stage at which immigration occurred in the simulation model decreased the size of the predicted peak population on the crop by giving less time for the population to develop before ripening and emigration. This is similar to the conclusions of earlier studies (Rabbinge et al., 1979; Carter and Rabbinge, 1980; Carter et al., 1980). These observations on the grain aphid reinforce the conclusions of George and Gair (1979) who suggest that aphicides should be applied to cereal crops at flowering if the economic threshold of five aphids/tiller by an increasing population is exceeded.

Fig. 2

Simulation of the effect of changes in the initial crop development stage (A) and the number of immigrant alate aphids (B) on aphid population development



The greenbug (Schizaphis graminum) showed no preference for young against mature wheat (Brown, 1972). However, the aphids had previously undergone tethered flight on pins for periods up to 1 h, a process which induces premature settling in the black bean aphid (Aphis fabae) (Kennedy and Booth, 1963). It is interesting to note that after a few days there was a tendency for more aphids to depart from mature leaves than from young leaves (Brown, 1972).

Tiller density did not affect the settling response of the grain aphid which is surprising as some species fly towards plants surrounded by bare earth (A'Brook, 1968; Smith, 1969; A'Brook, 1973). Compared with control plots there were double the number of grain aphids per tiller throughout the season in those plots from which half the tillers had been removed. Although the tiller density in the field was lower than that normally found in commercial fields, the results supported the view that crops should be planted densely to reduce aphid damage (A'Brook, 1968).

The predictions of the simulation model using 1980 data showed that an increase in the number of immigrant grain aphids resulted in an increase (slightly larger than that reported for previous years (Carter and Dixon, 1981)) in the size of the peak aphid population on the crop. These results are supported by field evidence as the number of aphids found on the crop before the end of flowering can often be related to the final peak densities (Carter et al., 1981). The relationship is, however, not a simple one. A high input of aphids does not always lead to a high peak density on the crop (Carter et al., in prep.), due to various factors such as weather and natural enemies affecting the population after immigration.

In the development of forecasting schemes for the grain aphid the timing of the migration with respect to the crop development stage is important for two reasons. It determines how long the aphid population has to develop before deteriorating crop conditions cause the crash in numbers at ripening and, as the different crop development stages are not equally attractive to aphids, it determines the settling rate on the crop.

The size of the spring migration will affect the size of the initial population, particularly on crops where the aphids have not overwintered, which will influence the peak density. The tiller density in individual fields will also be important in determining the aphid densities on a more local scale, the lower the number of tillers per unit area the larger the number of aphids per tiller.

Thus the timing and size of the spring migration, and growth stage and tiller density of the crop, are important in determining cereal aphid outbreaks.

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References

A'BROOK, J. (1968) The effect of plant spacing on the number of aphids trapped over the groundnut crop, Annals of Applied Biology, 61, 289-294

A'BROOK, J. (1973) The effect of plant spacing on the number of aphids trapped over cocksfoot and kale crops, Annals of Applied Biology, 74, 279-285

BROWN, H.D. (1972) The suitability of some crops to infestation by the wheat aphid, Schizaphis graminum (Homoptera: Aphididae), Entomologia experimentalis et applicata, 15, 128-138

CARTER, N. (1978) A simulation study of English grain aphid populations, unpublished Ph.D. Thesis, University of East Anglia

CARTER, N. & DIXON, A.F.G. (1981) The use of insect population simulation models in breeding for resistance, I.O.B.C. Bulletin (W.P.R.S.), III (I), 21-24

CARTER, N., McLEAN, I.F.G., WATT, A.D. & DIXON, A.F.G. (1980) Cereal aphids: a case study and review, Applied Biology, V, 271-348

CARTER, N. & RABBINGE, R. (1980) Simulation models of population development of Sitobion avenae, I.O.B.C. Bulletin (W.P.R.S.), III (IV), 93-98

CARTER, N., RABBINGE, R. & DIXON, A.F.G. (1981) Cereal aphid populations: biology, simulation and prediction. Simulation Monograph Series, PUDOC, The Netherlands

DEAN, G.J. (1974) Effect of temperature on the cereal aphids Metopolophium dirhodum (Wlk.), Rhopalosiphum padi (L.) and Macrosiphum avenae (F.), Bulletin of Entomological Research, 63, 401-409

GEORGE, K.S. (1974) Damage assessment aspects of cereal aphid attack in autumn- and spring-sown cereals, Annals of Applied Biology, 77, 67-111

GEORGE, K.S. (1975) The establishment of economic damage thresholds with particular reference to cereal aphids, Proceedings of the Eighth Insecticide and Fungicide Conference, 79-85

GEORGE, K.S. & GAIR, R. (1979) Crop loss assessment on winter wheat attacked by the grain aphid, Sitobion avenae (F.), 1974-77, Plant Pathology, 28, 143-149

HENDERSON, I.F. & PERRY, J.N. (1978) Some factors affecting the build-up of cereal aphid infestation in winter wheat, Annals of Applied Biology, 89, 177-183

KENNEDY, J.S. & BOOTH, C.O. (1963) Free flight of aphids in the laboratory, Journal of Experimental Biology, 40, 67-85

RABBINGE, R., ANKERSMIT, G.W. & PAK, G.A. (1979) Epidemiology and simulation of population development of Sitobion avenae in winter wheat, Netherlands Journal of Plant Pathology, 85, 197-220

SMITH, J.G. (1969) Some effects of crop background on populations of aphids and their natural enemies on brussels sprouts, Annals of Applied Biology, 63, 326-330

TAYLOR, L.R. (1979) The Rothamsted Insect Survey - an approach to the theory and practice of synoptic pest forecasting in agriculture, In Movement of highly mobile insects: concepts and methodology in research (Ed. R.L. Rabb & G.G. Kennedy). North Carolina State University. 148-185

VICKERMAN, G.P. & WRATTEN, S.D. (1979) The biology and pest status of cereal aphids in Europe: a review, Bulletin of Entomological Research, 69, 1-32

WATT, A.D. (1979) The effect of cereal growth stages on the reproductive activity of Sitobion avenae and Metopolophium dirhodum, Annals of Applied Biology, 91, 147-157

de WIT, C.T. (1960) On competition, Verslagen landbouwkundig Onderzoek, 66.8, 1-82.

WRATTEN, S.D. (1977) Reproductive strategy of winged and wingless morphs of the aphids Sitobion avenae and Metopolophium dirhodum, Annals of Applied Biology, 85, 319-331

ZADOKS, J.C., CHANG, T.T. & KONZAK, C.F. (1974) A decimal code for the growth stages of cereals, Eucarpia Bulletin, 7, 11 pp.