Danish Research Service for Plant and Soil Science
Research Centre for Plant Protection
Laboratory for Pesticide Analysis, Flakkebjerg
DK-4200 Slagelse

Report no. 1808

Correlation between residual analysis of 3 soil-incorporated insecticides and effect on the parasitization capacity of the rove beetle *Aleochara bilineata* (Gyll.)

Sammenhænge imellem restanalyse af 3 jordinsekticider og rovbillen Aleochara bilineata's *parasiteringskapacitet*

Erik Kirknel

Summary

Under field conditions, fine sand and silt loam were treated with 1, 4 and 8 kg a.i./ha respectively of diazinon, chlorfenvinphos and isofenphos in an 8 cm deep layer. After 0, 32 and 127 days, soil samples were taken from 0–4 cm and 4–8 cm depths and 1) analysed for chemical residues and 2) investigated for effect on the parasitization capacity by the rove beetle *Aleochara bilineata.* A correlation between the 2 parameters was established.

The results of the chemical analysis of the soil samples did not enable generalizations to be made on the degradation of the pesticides. The degradation was influenced by dosage, depth of placement and the soil type. Half-lives for diazinon varied between 41 and 88 days, for chlorfenvinphos between 35 and 167 days and for isofenphos between 86 and 454 days. In general, it was concluded that half-life increased with increased dosage. The influence of the soil type was pronounced especially with chlorfenvinphos where the half-life was 2 to 4 times longer in fine sand than in silt loam. For all 3 pesticides in fine sand, half-life was longest in the deeper layer 4–8 cm. This was also applicable for isofenphos in silt loam.

The bioassay clearly demonstrated differences between the effect of the individual pesticides. Also dosage, soil types and depth of placement significantly influenced the toxicity of the pesticides. This was especially true for diazinon and isofenphos and to a lesser degree for chlorfenvinphos. Chlorfenvinphos was the least toxic to the rove beetle, followed by diazinon as an intermediate and isofenphos as the most toxic.

When coupling the chemical analysis and the bioassay, it is demonstrated that on basis of concentration of the pesticide, diazinon is the most toxic to the rove beetle followed by isofenphos with chlorfenvinphos as the least toxic. Soil type and depth of placement influenced the biological effect measured at the same concentration of pesticide.

It is concluded that residue of the 3 pesticides is correlated to biological effect, but parameters such as soil type, and depth of placement of the pesticides must be considered when the total effect of the pesticides on *A. bilineata* is to be estimated.

Key words: *Aleochara bilineata,* staphylinid, rove beetle, diazinon, chlorfenvinphos, isofenphos, pesticides, soil types, parasitization capacity, bioassay, residue, interactions.

Resumé

Fin sandjord og lerjord blev behandlet under markforhold med 1, 4 og 8 kg akt. st./ha af diazinon, chlorfenvinphos og isofenphos, i et 8 cm tykt jordlag. Jordprøverne blev udtaget i dybderne 0–4 cm og 4–8 cm efter 0, 32 og 127 dage, og analyseret for restindhold ved kemisk analyse og for biologisk effekt ved måling på rovbillen *Aleochara bilineata's* parasiteringskapacitet.

Ved den kemiske analyse af jordprøverne var det ikke muligt generelt at karakterisere pesticidernes nedbrydning. Nedbrydningen var signifikant afhængig af dosis, dybden og jordtypen. Halveringstiden for diazinon varierede fra 41–88 dage, for chlorfenvinphos fra 35–167 dage og isofenphos fra 86–454 dage.

Generelt øgedes halveringstiden med stigende dosis. Jordtypens indflydelse var tydeligst for chlorfenvinphos, hvor halveringstiden var 2–4 gange større i sandjord end i lerjord. For alle 3 insekticider i sandjord var halveringstiden længst i det dybe jordlag 4–8 cm. Kun for isofenphos gjaldt det også for lerjorden.

Resultaterne fra de biologiske undersøgelser viste, at der var stor forskel imellem effekterne af de enkelte insekticider. Dosis, jordtyper og dybder, hvor insekticiderne var placeret, påvirkede ligeledes signifikant pesticidernes effekt på parasiteringskapaciteten. Dette var mest udpræget for diazinon og isofenphos i mindre grad for chlorfenvinphos. Chlorfenvinphos viste sig som det mest skånsomme insekticid mod rovbillen dernæst diazinon og sidst isofenphos som det mindst skånsomme.

Ved at sammenholde resultaterne fra de biologiske undersøgelser med resultaterne fra den kemiske analyse er det vist, at med samme dosis er diazinon det giftigste mod rovbillen dernæst isofenphos og med chlorfenvinphos som det mindst giftige. Jordtype og dybde har desuden indflydelse på den biologiske virkning ved samme koncentration.

Det konkluderes, at restindhold bestemt ved kemisk analyse har sammenhæng med biologisk virkning, men faktorer som jordtype og dybde må tages i betragtning, når effekten af insekticider på *A. bilineata* skal vurderes.

Nøgleord: *Aleochara bilineata,* staphylinider, rovbiller, diazinon, chlorfenvinphos, isofenphos, pesticider, jordtyper, parasiteringskapacitet, biotest, kemisk analyse, interaktioner.

Introduction

Correlations between pesticide, dosage and biological effect are basic ingredients in chemical plant protection and considerable variations are often registered in these correlations. The reasons for these variations are often found to be due to temperature, precipitation and humidity, composition of the soil and agricultural management of the soil, all of which can influence the living organisms in the soil and the degradation of pesticides.

Lichtenstein et al. (6) extracted phorate-treated soil with an organic solvent. The treated soil was not as poisonous against insects as was the extract after evaporation of the solvent. On the other hand, *Khan et al.* (2) and *Khan* (3) demonstrated the presence of biologically active residues in the soil after extraction.

There are 2 areas of importance connected with this. The first is the availability of the pesticides, the second, the effect of the pesticides on the organism.

Availability of the pesticide involves factors which are able to block or promote contact with a target such as insects or fungi. Important factors to be considered are binding and degradation, oxygen availability, humidity and temperature which influence vapour pressure of the pesticide. It is very difficult to determine the available amount of pesticide because it also depends on the nature of the target organism. The pesticide residue is determined most often by chemical analysis. On the basis of samples taken at different times it is possible to produce a degradation curve, which shows the degradation speed. By plotting the logarithm of the pesticide concentration against elapsed time one obtains a straight line on the graph. In these cases it is possible to calculate a time interval in which the concentration of the pesticide is reduced to half the amount. This time interval is called the half-life of the pesticide. Often the symbol $t_{1/2}$ is used for half-life.

The second area to be considered is the effect of the pesticide when it comes into contact with the target, for example the insect. The more pesticide the greater the effect. This correlation is called the dosage-response correlation. The dosage-response correlation is S-shaped when plotted or so called sigmoid-like shape. Because the pesticides are often degraded at a constant speed and the dosage-response correlation is sigmoid, it must be concluded that degradation speed does not always express the loss of biological activity. For example if the $t_{1/2}$ of the pesticide is 2 days, 50% is degraded every 2 days. After 4 days 25% is still left and so on. But the biological effect is not necessarily reduced to 25% after 4 days. It depends on the dosage-response correlation which is not linear.

The present report investigate the effect of 3 soil insecticides on the parasitization capacity of the rove-beetle *A. bilineata,* in 2 soil types at 3 dosages and in 2 depths. The aim being to investigate the degradation patterns by chemical analysis as well as to correlate the bioassay with the chemical analysis to clarify which effect can be measured from the same dosage of the 3 pesticides and to which degree soil type and depth of placement influences the effect of the pesticides.

Materials and methods

The experiment started 6 June. Plastic cups with a diameter of 6.7 cm, cross section area being 35.2 cm^2 and 10 cm high, were filled with soil containing the pesticides and constructed with drain holes in the bottom. The cups were placed in the field at The Research Centre for Plant Protection in Lyngby. The location is, with respect to wind shelter, more open than a fruit plantation but more sheltered than an open field.

Treatment of the soil

10% of the soil was air-dried at room temperature and wetted with a solution containing the exact dosage of the pesticide. The wet soil was again dried at room temperature and mixed with the remaining 90% of the soil. The dosage in the cup was calculated on basis of the surface area i.e. kg per ha, and mixed up in the total volume of the cup. The cup was filled with the soil up to 2 cm from the edge, hereby having an 8 cm deep layer of pesticide-containing soil. The cup was placed in the soil in such a way that 2 cm of the edge was above the ground. There were no replications.

Insecticides

Basudin emulsion	
(diazinon 25%)	1, 4 and 8 kg a.i. per ha
Shell Birlane 24 EC	
(chlorfenvinphos 24%)	» » »
Oftanol	
(isofenphos 50%)	» » »

Soil types

The 2 soil types were characterized as fine sand and silt loam. Texture of the soils is shown in Table 1.

Table 1. Texture of the soils.
Teksturanalyse af jordene.

	pHw *pHv*	Clay *Ler* 2 μ	Silt *Silt* 2–20 μ	Fine sand *Fin sand* 20–200 μ	Coarse sand *Grov sand* 200–2000 μ	Organic matter *Humus*
Fine sand *Fin sand*	5,6	2,1	4,7	80,5	9,4	3,3
Silt loam *Ler*	6,8	18,3	25,2	37,7	15,9	2,9

Registration of temperature and precipitation
Fig. 1 illustrates registered temperature and precipitation 2 m above ground level.

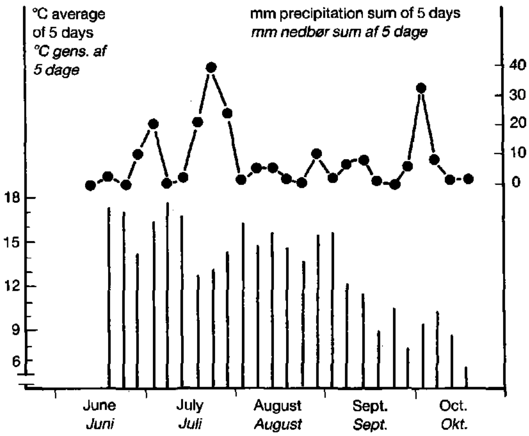

Fig. 1. 5 days registration of temperature and precipitation. Temperature is calculated as (sum min. in 5 days + sum max. in 5 days)/10. Precipitation indicated as sum of the same 5 days precipitation.
5 døgns opgørelse for temperatur og nedbør. Temperaturen er (min. i 5 døgn + sum max. i 5 døgn)/10. Nedbør er målt som sum af samme 5 døgns nedbør.

Sampling
Soil samples were taken using a steel tube, 10 mm inside diameter, from the surface to the bottom of the cup i.e. an 8 cm deep sample. The sample was divided into 2 sub samples, 0–4 cm and 4–8 cm. The samples were either stored at –30°C, in plastic bags, or used directly in the experiments.

Bioassay
The rove beetle *Aleochara bilineata* is both a parasite and a predator of the turnip root fly, *Hylemya floralis*. In the present investigations the rove beetle was used as a parasite. The rove beetle lays its eggs in the vicinity of the turnip root fly pupa. The eggs hatch and the newly hatched larva find the root fly pupa, penetrate the puparium, move inside and close the hole in the puparium. The parasite develops into an adult inside the pupae by eating the content. The principle in the bioassay, is to let the parasite larva hatch from the egg, move through a pesticide-treated soil and try to penetrate the puparium. Even if the larva do not succeed in closing the entrance hole or develop into an adult insect, the root fly will die shortly after the puparium is punctured. A punctured puparium will allow micro organisms to enter the pupa and destroy the root fly. Therefore as a criterion of succesful attack by the rove beetle, the presence of entrance hole, closed or open, is used. The bioassay was performed in a micro climate chamber, described in details by *Kirknel* (4). Briefly, a Pyrex glass ring, 15 mm high, 25 mm inside diameter, is placed on a glass plate. At the bottom of the ring is placed 20 turnip root fly pupae covered with soil sample 10 mm high. The sample is lightly compressed and a filter paper (Whatmann AA disc, 13 mm diameter) is placed on top of the sample. 20 rovebeetle eggs are placed on the filter paper. The eggs will hatch and the larvae will penetrate the soil sample in their search after the root fly pupae, and try to penetrate the pupae. No replications.

The micro climate chamber is thermostatically controlled at 25°C and a relative humidity greater than 99% without condensation. The chamber is equipped with forced ventilation in order to avoid non-intended accumulated pesticide vapours.

After 6 days the chamber is opened and the pupae are examined.

$$X\%\ \text{parasitization in treated soil} = \frac{V \times 100}{20\ \text{parasite eggs-v}}$$

$$Y\%\ \text{parasitization in untreated soil} = \frac{U \times 100}{20\ \text{parasite eggs-u}}$$

$$\text{Relative parasitization: } \frac{X \times 100}{Y}$$

V and U: Number of entrance holes in the puparium.
v and u: Number of eggs not hatched.

Relative parasitization is therefore, the effect of the pesticide in relation to a control adjusted for eggs not hatched on the filter paper. There was no statistically significant difference between eggs not hatched in control and the pesticide-treated soils.

Chemical analysis

25 g soil was extracted on Soxhlet, 3 hours with 100 ml acetone. The acetone was evaporated and the sample was dissolved in 25 ml ethyl acetate.

The sample was GLC-detected. The column was a 180 cm glass – column 2 mm inside diameter filled with Chromosorb W, AW, DMSC, 80/100 mesh, coated with 4% silicone OV-17. Carrier gas was 45 ml He per min, AFID detector, 40 ml H_2 per min. 350 ml air per min. The oven temperature was 230°C, injector temperature 245°C and detector temperature 245°C.

Recovery for all 3 insecticides was 90–100% when extracting humid soil immediately after spiking.

Results

Bioassay

The figures 2, 3 and 4 show the results from the bioassay, and expressed as parasitization relative to control. The parasitization in the control was on average 71%.

The positive correlation between relative parasitization and elapsed time is in accordance with earlier results in experiments with diazinon, trichloronate, carbofuran and chlorfenvinphos (4).

Diazinon, and especially isofenphos, prevent parasitization to a high degree, even after 127 days, with 1 kg a.i. per ha. An exception is diazinon in silt loam, 1 kg a.i. per ha, where parasitization is higher than the control. Chlorfenvinphos is more moderate in its effect, and at 1 kg a.i. per ha 0–4 and 4–8 cm depth the parasitization is stimulated compared to the control. This phenomenon was observed earlier (4). The reason for this is either a direct stimulation with subtoxic doses on the rove beetle or the effects on the microorganisms of the rove beetle larva.

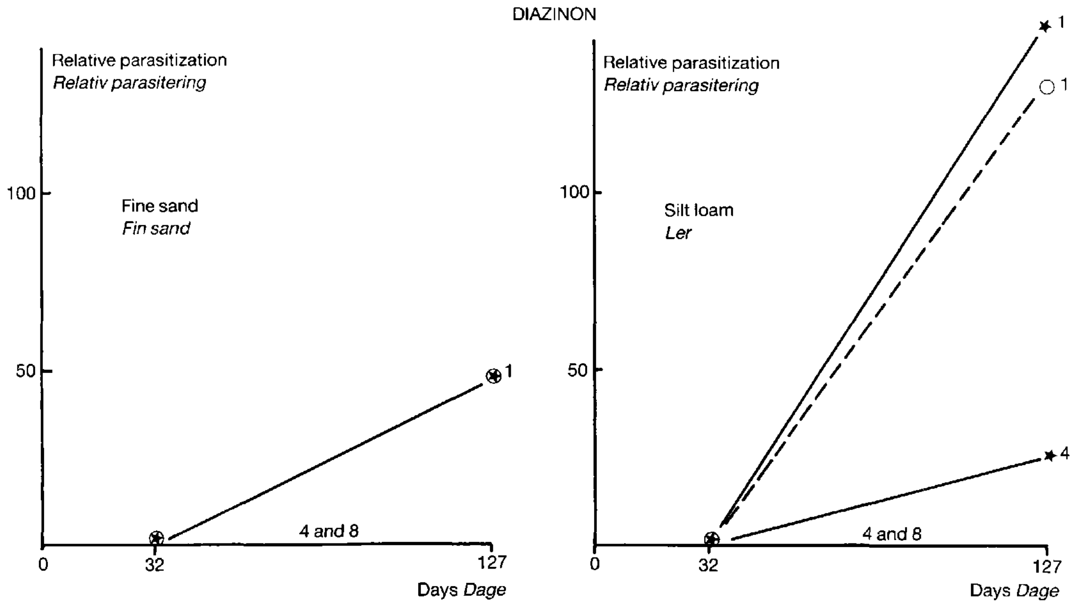

Fig. 2. The influence of diazinon on the parasitization capacity of *A. bilineata* in relation to control. 2 soil types, samples taken after 0, 32 and 127 days in 2 depths.

★—★ 0–4 cm   1 = 1 kg a.i./ha
○--○ 4–8 cm   4 = 4 kg a.i./ha
8 = 8 kg a.i./ha

Diazinons indflydelse på A. bilineatas *parasiteringskapacitet i forhold til ubehandlet. Diazinon opblandet i 2 jordtyper og herfra udtaget efter 0, 32 og 127 dage i to dybder.*

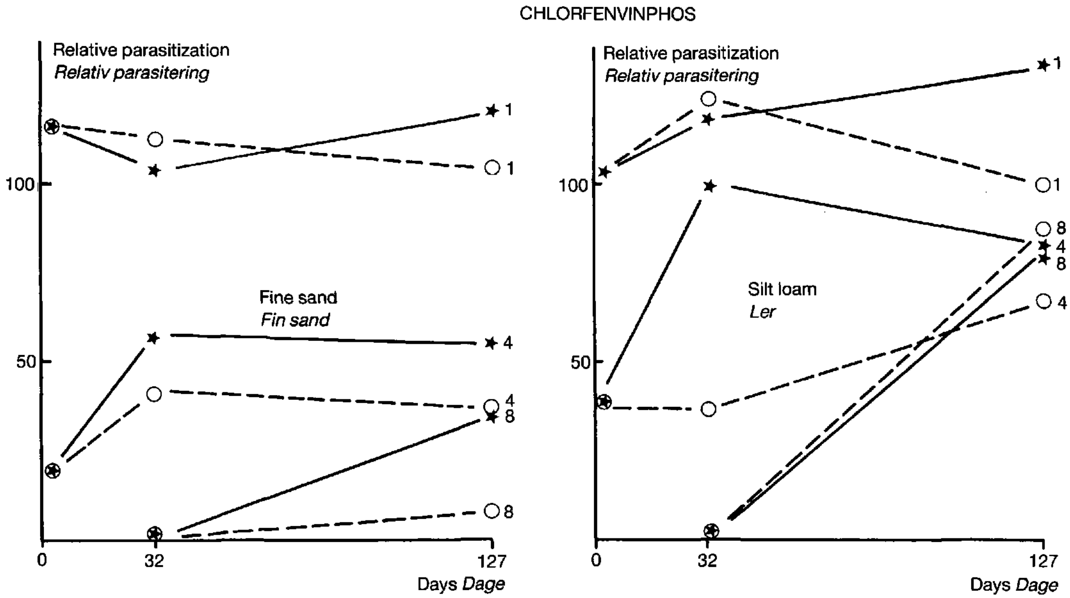

Fig. 3. The influence of chlorfenvinphos on parasitization capacity of *A. bilineata* in relation to control. 2 soil types, samples taken after 0, 32 and 127 days in 2 depths.
★—★ 0–4 cm 1 = 1 kg a.i./ha
○--○ 4–8 cm 4 = 4 kg a.i./ha
8 = 8 kg a.i./ha

Chlorfenvinphos' indflydelse på A. bilineatas *parasiteringskapacitet i forhold til ubehandlet. Chlorfenvinphos opblandet i 2 jordtyper og herfra udtaget efter 0, 32 og 127 dage i 2 dybder.*

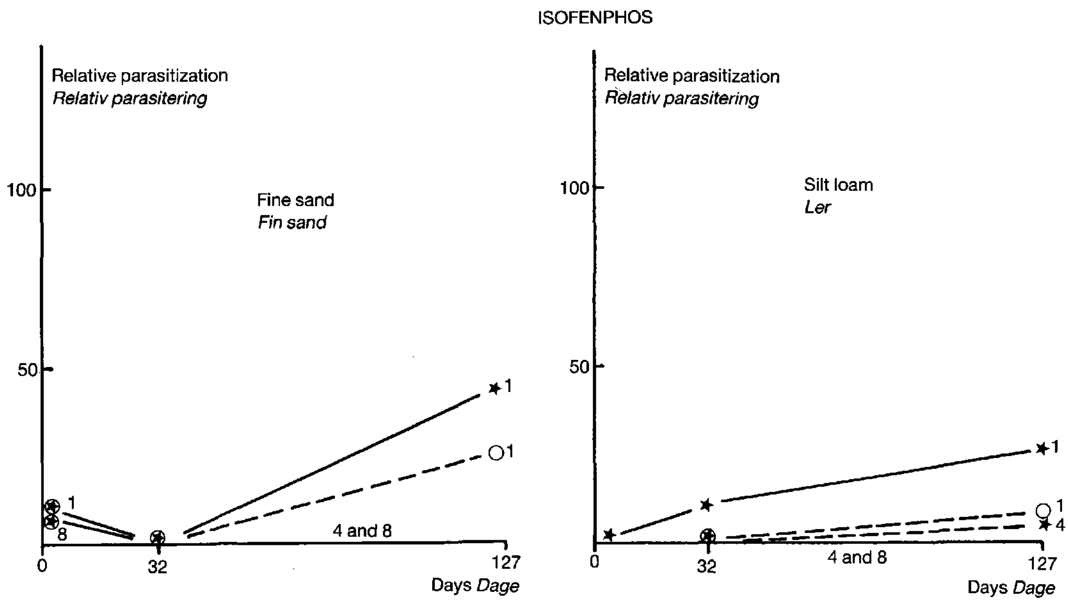

Fig. 4. The influence of isofenphos on the parasitization capacity of *A. bilineata* in relation to control. 2 soil types, samples taken after 0, 32 and 127 days in 2 depths.
★—★ 0–4 cm 1 = 1 kg a.i./ha
○--○ 4–8 cm 4 = 4 kg a.i./ha
8 = 8 kg a.i./ha

Isofenphos' indflydelse på A. bilineatas *parasiteringskapacitet i forhold til ubehandlet. Isofenphos opblandet i 2 jordtyper, udtaget efter 0, 32 og 127 dage i 2 dybder.*

Diazinon (1 kg a.i./ha) and chlorfenvinphos (4 kg a.i./ha) are approved in Denmark for the control of carrot and onion fly as well as the cabbage root fly.

Isofenphos must not be used for this purpose but for treating all cruciferae seeds except radish.

The Figures 2, 3 and 4 show clearly that chlorfenvinphos is the most lenient against the rove beetle, followed by diazinon and finally isofenphos.

Finlayson et al. (1) arrived at the same conclusion for chlorfenvinphos and isofenphos in field trials using *A. bilineata* and *Hylemya brassicae*, the cabbage root fly.

Statistical processing of results

Table 2 shows the result of a variance analysis executed on results from 127 days after the start of the trial. In the upper part of the table is the total analysis and in the lower part the analysis of individual pesticides.

The variance analysis shows which factors affect parasitization; in addition the importance of the interaction is shown.

There are significant differences between different doses, soils, pesticides (99,9%) and depths (99%). That is to say that the results show that parasitization varies depending on the pesticide, soil type and dosage at the 99,9% level. The interaction between pesticides × doses, soil type × pesticides, and doses × soil type × pesticides is significant at 99,9%.

This means, for example, that the 2 soil types have a different influence on the effect of the 3 pesticides on parasitization (soil type × pesticide).

Analysis of the individual pesticides shows that the 3 doses have a greatly varying effect when diazinon and isofenphos (99%) and chlorfenvinphos (95%) are used. The effect of diazinon is very dependent upon soil type (99%) chlorfenvinphos and isofenphos less dependent (95%).

The depths have some relevance with isofenphos (95%). The interactions dose × soil type is significant for diazinon (95%), doses × depths and soil type × depths (95%) for isofenphos.

Table 2. Analysis of variance of bioassay after 127 days.
Variansanalyse of bioassay efter 127 dage.

Total analysis. *Total analyse.*

1. Dosage *Doser*	xxx
2. Soil types *Jordtyper*	xxx
3. Depths *Dybder*	xx
4. Pesticides *Pesticider*	xxx
1x2	
1x3	
1x4	xx
2x3	
2x4	xx
3x4	
1x2x3	
1x2x4	xx
1x3x4	
2x3x4	

Analysis of the individual pesticides.
Analyse af de enkelte pesticider.

	Diazinon	Chlorfen-vinphos	Isofen-phos
1. Dosages *Doser*	xx	x	xx
2. Soil Types *Jordtyper*	xx	x	x
3. Depths *Dybder*			x
1x2	x		
1x3			x
2x3			x

Significance *Signifikans:* x = 95%, xx = 99%, xxx = 99.9%.

Chemical analysis

The figures 5, 6 and 7 show the results from the chemical analysis. The insecticide concentration is given on a dry matter basis of the soils. The course of degradation for the pesticides occurs in various ways and at different speeds. Often the sequence may be described as a first order reaction of the type: $\log y = a + bx$, where:

y = concentration.
b = slope.
x = time in days.
a = concentration at day o.

Degradation after this course is characterised by a constant fraction of the remaining amount of pesticide being degraded per unit of time.

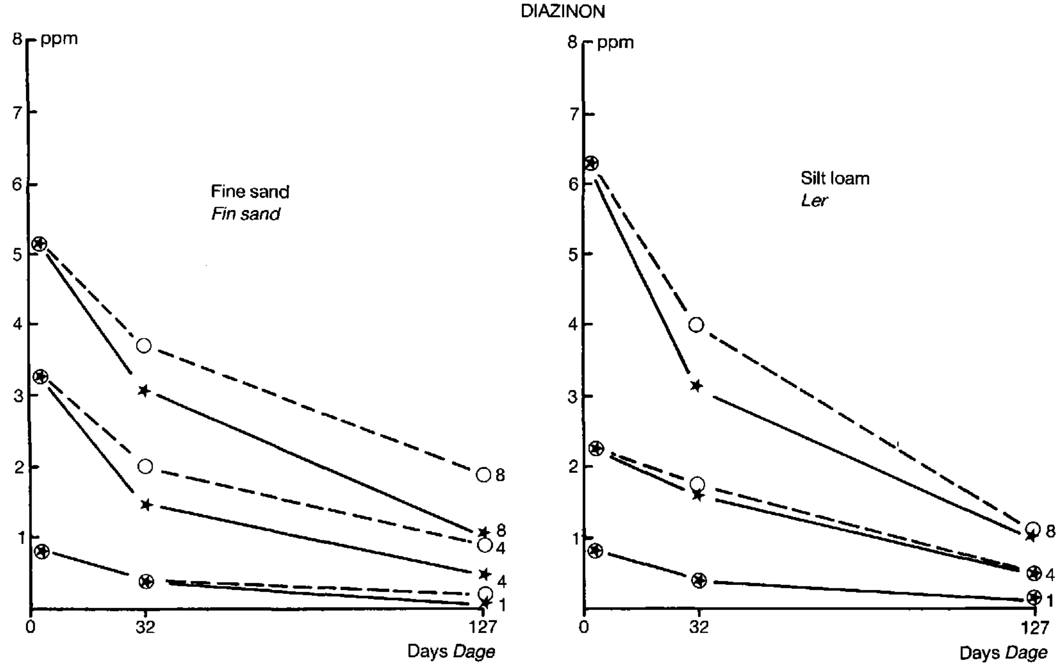

Fig. 5. Residual analysis of diazinon in 2 soil types after 0, 32 and 127 days. Samples taken in 2 depths.

★—★ 0–4 cm   1 = 1 kg a.i./ha
○--○ 4–8 cm   4 = 4 kg a.i./ha
8 = 8 kg a.i./ha

Restindholdet af diazinon i 2 jordtyper efter 0, 32 og 127 dages forløb i 2 jorddybder.

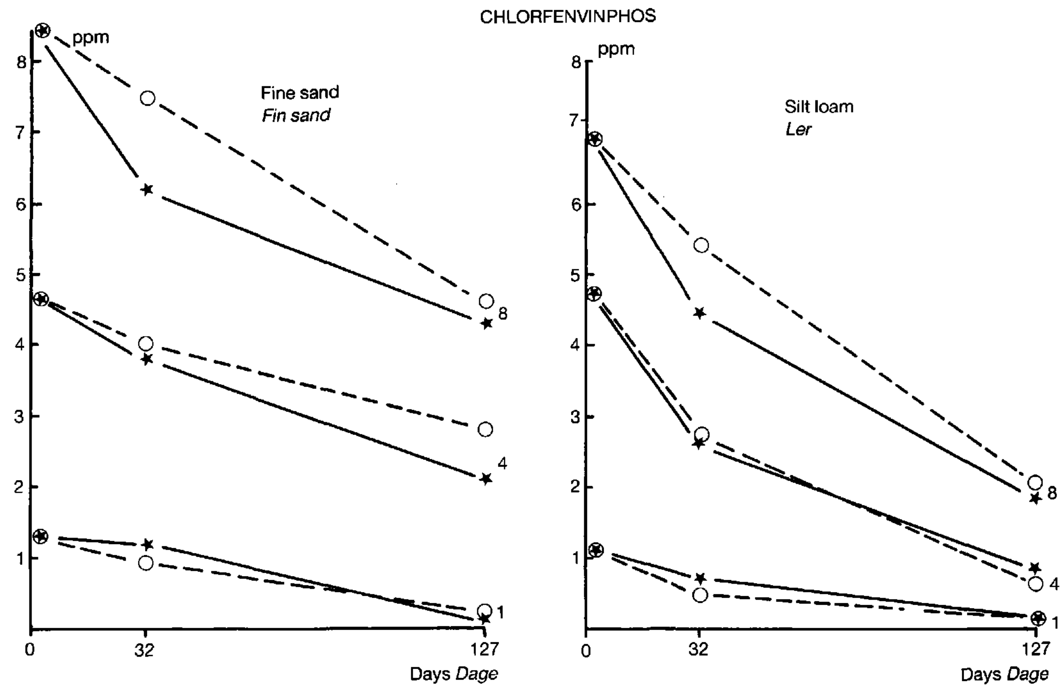

Fig. 6. Residual analysis of chlorfenvinphos in 2 soil types after 0, 32 and 127 days. Samples taken in 2 depths.

★—★ 0–4 cm   1 = 1 kg a.i./ha
○--○ 4–8 cm   4 = 4 kg a.i./ha
8 = 8 kg a.i./ha

Restindhold af chlorfenvinphos i 2 jordtyper efter 0, 32 og 127 dages forløb i 2 jorddybder.

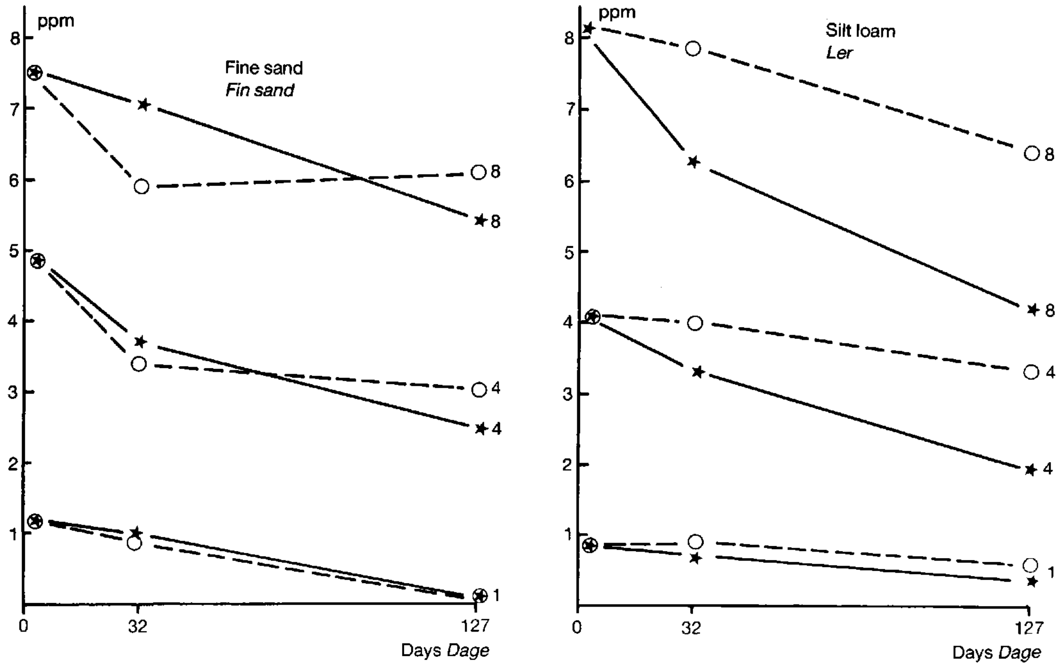

Fig. 7. Residual analysis of isofenphos in 2 soil types after 0, 32 and 127 days. Samples taken in 2 depths.

★—★ 0–4 cm 1 = 1 kg a.i./ha
○--○ 4–8 cm 4 = 4 kg a.i./ha
8 = 8 kg a.i./ha

Restindhold af isofenphos i 2 jordtyper efter 0, 32 og 127 dages forløb i 2 jorddybder.

If 50% of the pesticide is degraded over 10 days then the half-life ($t_{1/2}$) is 10 days.

In Table 5 the $t_{1/2}$ is shown for the 3 insecticides, 3 doses, 2 soil types as well as 2 depths. This number is to the left of the slash. Number to the right of the slash is $t_{1/2}$ calculated as described below. Values from 41–454 days, dependent on insecticides, doses, soil types and depths, are shown.

Statistical processing of the results

In order to characterize the meaning of the individual factors for $t_{1/2}$, an analysis of variance was carried out using a multiplicative model. 95% was chosen as significance level.

In Tables 3, 4 and 5 the results of the variance analysis are shown.

Presentation of the results begins in Table 3 which deals with 1 kg a.i./ha. Table 4 gives, using

Table 3. Results of a multiple analysis of variance. Half-lives in days at 1 kg a.i./ha.
Resultater af en multipel variansanalyse. Halveringstider i dage når der tilføres 1 kg v.st./ha.

Depth *Dybde*	Diazinon		Chlorfenvinphos		Isofenphos	
	fine sand *fin sandjord*	silt loam *lerjord*	fine sand *fin sandjord*	silt loam *lerjord*	fine sand *fin sandjord*	silt loam *lerjord*
0–4 cm	41 a	41 a	115	36 b	77 c	98 cd
4–8 cm	64	41 a	152	36 b	113 d	251

Half-lives with same letter do not differ significantly at the 95% level.
Halveringstider med samme bogstav er ikke signifikant forskellige på 95%-niveauet.

Table 4. Results of a multiple analysis of variance. Relative change of half-life at 4 and 8 kg a.i./ha.
Resultat af en multipel variansanalyse. Relativ ændring af halveringstiden ved 4 og 8 kg akt. st./ha.

Dosage *Dosis*	Diazinon fine sand *fin sandjord*	Diazinon silt loam *lerjord*	Chlorfenvinphos fine sand *fin sandjord*	Chlorfenvinphos silt loam *lerjord*	Isofenphos fine sand *fin sandjord*	Isofenphos silt loam *lerjord*
4 kg a.i./ha *4 kg v.st./ha*	+ 9%	+39% e	+2% f–	+29% f–	+ 78% g	+31% g–
8 kg a.i./ha *8 kg v.st./ha*	+34% e	+18%	+5% f–	+85%	+269%	+39% g–

Values with the same letter do not differ significantly at the 95% level. Values followed by – (minus) do not differ significantly from 1 kg a.i./ha at the 95% level.
Procentuelle ændringer med samme bogstav er ikke signifikant forskellige på 95%-niveauet.
Ændringer efterfulgt af et – (minustegn) er ikke signifikant forskellige fra 1 kg v.st./ha på 95%-niveauet.

Table 3 as a basis, the percentage change in $t_{1/2}$ for 4 and 8 kg a.i./ha.

For example $t_{1/2}$ for diazinon 1 kg a.i./ha 0–4 cm, fine sand is 41 days (Table 3). In Table 5 may be seen an increase in dosage from 1–8 kg a.i./ha resulting in a $t_{1/2}$ in 41 days + 34% = 55 days. In Table 5 the 55 are placed on the right handside of the slash. In other words the 55 days are attained by calculating the effect of the individual factors on $t_{1/2}$. The 54 days are, as named earlier, $t_{1/2}$ calculated on all data used to produce the curve (Fig. 5, fine sand, 1 kg a.i./ha 0–4 cm). It is seen in Table 5 that the variance analysis figure in Table 3 and 4 lies very close to the values which are calculated from the actual curves. This means that there is reasonable ground to rely on the variance analysis.

Effect of dose on $t_{1/2}$ (Table 5)

For *diazinon* dosage causes a significant difference, in both fine sand soil and silt loam soil, on $t_{1/2}$. In fine sand $t_{1/2}$ increases with dosage, in silt loam soil it is lowest at 1 kg longest at 4 kg and decreases slightly at 8 kg a.i./ha.

For *chlorfenvinphos* there is no significant difference caused by dosages in fine sand soil. In silt loam soil there is no difference between 1 and 4 kg a.i./ha but between 4 and 8 and 1 and 8 kg a.i./ha, $t_{1/2}$ is longer at the highest dose.

Isofenphos degrades in the soil quickest at lower doses and slowest at the high doses. In silt loam soil there is no difference. Therefore generally, the greater the dosage the longer the $t_{1/2}$.

Lichtenstein et al. (5) found for chlorinated insecticides that higher dosages had a longer $t_{1/2}$ than lower ones.

Table 5. Half-lives in days and/calculated half-lives according to Table 3 and 4.
Halveringstider i dage samt/beregnede halveringstider ifølge tabel 3 og 4.

Dose *Dosis*	Depth *Dybde*	Diazinon fine sand *fin sandjord*	Diazinon silt loam *lerjord*	Chlorfenvinphos fine sand *fin sandjord*	Chlorfenvinphos silt loam *lerjord*	Isofenphos fine sand *fin sandjord*	Isofenphos silt loam *lerjord*
1	0–4	41/41	41/41	110/115	35/36	86/77	110/98
1	4–8	64/64	41/41	159/152	36/36	101/113	223/251
4	0–4	46/45	58/58	109/118	50/46	135/137	116/129
4	4–8	69/70	57/58	167/155	42/46	205/201	366/329
8	0–4	54/55	49/49	137/121	65/66	259/283	135/136
8	4–8	88/86	49/49	141/159	67/66	454/415	352/348

Effect of soil depth on $t_{1/2}$ (Table 3)
For diazinon and chlorfenvinphos $t_{1/2}$ is significantly longer in fine sand soil 4–8 cm than 0–4 cm. There is no difference in silt loam soil.

For isofenphos $t_{1/2}$, in both soil types, is longest in the deeper soil layers.

Effect of soil types on $t_{1/2}$ (Table 3, 4 and 5)
For diazinon 1 kg a.i./ha there is no difference on $t_{1/2}$, between fine sand soil and silt loam soil at 0–4 cm. At 4–8 cm depth $t_{1/2}$ is longest in fine sand soil. At 4 kg a.i./ha $t_{1/2}$ is longest in silt loam soil 0–4 cm. In 4–8 cm it is longest in fine sand soil. At 8 kg a.i./ha 0–4 cm and 4–8 cm $t_{1/2}$ is longest in fine sand soil.

For *chlorfenvinphos* $t_{1/2}$ is for all dosages longest in fine sand soil.

For *isofenphos* there is no difference on $t_{1/2}$ at 1 kg a.i./ha in 0–4 cm. In 4–8 cm $t_{1/2}$ is longest in silt loam soil. At 4 kg a.i./ha the only difference (very close to 95% level) between the soils is at 4–8 cm depth where $t_{1/2}$ is longest in silt loam soil. At 8 kg a.i./ha the only difference is 0–4 cm depth. Here $t_{1/2}$ is longest in fine sand soil.

The effect of soil type is most apparent for chlorfenvinphos, to a lesser degree and less reliable for diazinon and isofenphos.

The significant differences between soil types at 1 kg a.i./ha can be read in Table 3.

The following smallest quotients are used to calculate significant difference for 4 and 8 kg a.i./ha in Table 5, the figures to the left of the slash.

Smallest quotients at 95% level for 4 and 8 kg a.i./ha

Diazinon	1.09
Chlorfenvinphos	1.63
Isofenphos	1.81

The influence of dosage, depth and soil type on $t_{1/2}$, should be accepted with reservation. Leaching of pesticides, from the upper soil layer 0–4 cm, to the lower 4–8 cm depth can give a false impression of a more rapid degradation in the upper layer. The results should be seen from a practical point of view where pesticides are mixed up homogeneously in an 8 cm deep soil layer and exposed to climatic influence in field conditions. Half-life cannot directly be used to evaluate biological effects without obtaining a dose-response curve. However if half-life is used for evaluation, an account must be made for the factors which influence it. Soil type, dosage and depth have, for diazinon involved variations in half-life from 41–88 days, chlorfenvinphos from 35–167 days and for isofenphos from 86–454 days.

Correlation between parasitization and insecticide concentration for the period 0–127 days
In Fig. 8, for the period 0–127 days, is shown the correlation between pesticide concentration and parasitization.

$\log y = a + bx$, where
y = pesticide concentration
x = relative parasitization
b = slope
a = y when x approximates zero.

The correlation coefficient r, is indicated on each curve.

Number from the 2 depths are pooled for each insecticide in each soil type.

It can be seen in Fig. 8 that the insecticides clearly differentiate themselves from each other in both soil types. I.e. the toxic effect is clearly different. It is noted that diazinon is the most poisonous, the next is isofenphos and lastly chlorfenvinphos as the least poisonous.

It has not been possible to separate soil depths, probably because of, not only statistical uncertainty (this arises from both the bioassay and the chemical analysis), but also the insecticides different bondings to the soil which are also dependent on time.

The slope and position (also called the function) was for both 2 soils, fairly similar for chlorfenvinphos the 2 b values are not significantly different (95% level).

The b values for isofenphos are only different at a significance level between 90–95%.

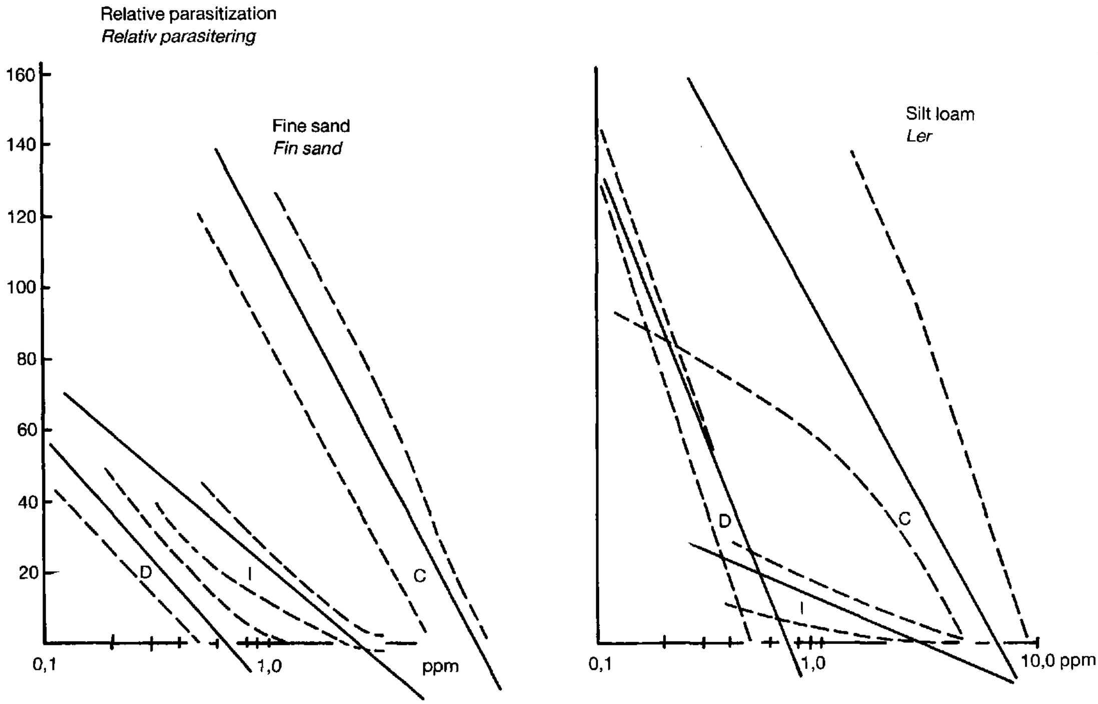

Fig. 8. Correlation between ppm in the soils and relative parasitization. The different concentrations in the soil obtained by decaying the insecticides up to 127 days in the field.

Sammenhænge mellem ppm og relativ parasitering. De forskellige koncentrationer i jordene er opnået ved at nedbryde insekticiderne i marken op til 127 dage.

Diazinon, however, has b values which are significantly different at the 99% level.

The aim of the project has not been to produce a correlation between pesticide dosage and biological response. This could be done more easily by producing a series of different concentrations of pesticides in the soil and thereby test the parasite-host system. The idea was to let the practical conditions influence degradation of pesticides, measure the degradation and compare the residue concentrations with biological response. The dosages chosen have had a practical connection. Therefore, the dose-response curves in Fig. 8 are not complete for diazinon and isofenphos.

The curves for diazinon describe the progress from 0% parasitization to 100% and above. For chlorfenvinphos in silt loam soil, it is apparent that the spread of the dose-response curve, increases greatly over 100% parasitization.

The depths are not shown in Fig. 8 but there is a tendency for the stimulations in parasitization of over 100% to be greatest at 0–4 cm depth and least at 4–8 cm for the same dose.

At day 127

In Figs 9, 10 and 11 is shown the correlation between parasitization and residue concentration after 127 days. Where possible, a correlation coefficient has been given (with data pair of only 2 it has no relevance and therefore not given).

Common for the 3 pesticides is an inverse proportionality between concentration and parasitization.

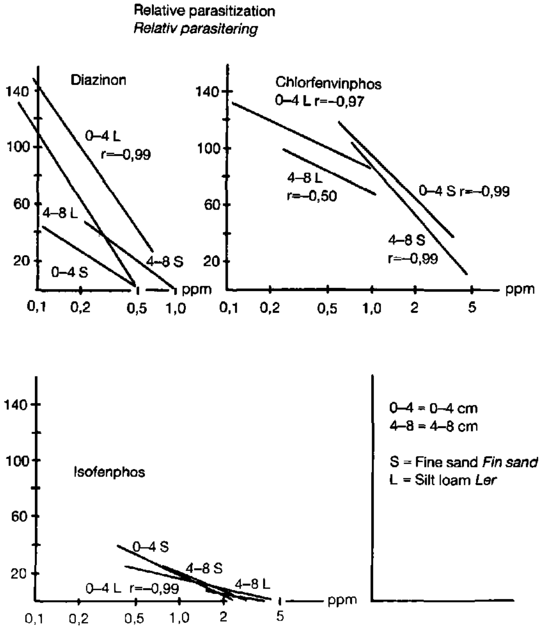

Fig. 9, 10 and 11. Correlation between residues of diazinon, chlorfenvinphos, isofenphos and relative parasitization. The different concentrations in the soils is obtained by decaying the insecticides 127 days in the field. *Sammenhænge mellem rester af diazinon, chlorfenvinphos, isofenphos og relativ parasitering. De forskellige koncentrationer i jordene er opnået ved nedbrydning af insekticiderne 127 dage under markforhold.*

For isofenphos the correlation between residue concentration and relative parasitization is not apparently affected by soil type or depth. Parasitization has been very low here from 0–30%, which can perhaps explain this.

For both chlorfenvinphos and diazinon the slope is not dependent on depth, but of soil type. For diazinon the curve is steeper for fine sand soil than silt loam soil, for chlorfenvinphos the opposite is the case. In addition there seems to be a difference between the effect of the soil depth.

Results available are few so no significant statistics can be produced. But the results imply that the correlation between residue concentration and relative parasitization for diazinon and chlorfenvinphos is dependent on soil type and depth.

Conclusion

The rove beetle *Aleochara bilineata's* capacity for parasitization is reduced especially by isofenphos followed by diazinon and to a lesser degree by chlorfenvinphos. However, effect is dependent on dosage, soil type and soil depth in which the insecticide is placed.

Generally it was not possible, with the chemical analysis of the soil type, to assess the degradation of the pesticides. Degradation was significantly dependent on dosage, soil type and depth.

$t_{1/2}$ for diazinon varied from 41–88 days, for chlorfenvinphos from 35–167 days and isofenphos from 86–454 days.

Generally the $t_{1/2}$ increased with increasing dosage. The influence of soil type was most apparent for chlorfenvinphos where the $t_{1/2}$ was 2–4 times greater in fine sand soil than in silt loam soil.

$t_{1/2}$ was longest in the 4–8 cm soil layer in sandy soil, for all 3 insecticides.

This was also valid for isofenphos in silt loam soil.

By comparing results from the bioassay with rove beetle and those from the chemical analysis for the 127 days in which the examination lasted, it can be seen that diazinon is the most poisonous for the rove beetle, followed by isofenphos with chlorenvinphos as the least poisonous.

For chlorfenvinphos the soil type had no apparent influence, but in silt loam soil there was a clear tendency for the parasitization to be greatest at the 0–4 cm depth and least at 4–8 cm for the same concentration.

For isofenphos there is a little but significantly greater toxicity in silt loam soil at the same concentration.

For diazinon there is, conversely, a significantly greater toxicity in fine sand soil at the same concentration.

It has not been possible to draw any general conclusions concerning the difference between 0–4 cm and 4–8 cm depths.

If results after 127 days from the rove beetle bioassay and chemical analysis, alone are compared, a slightly clearer picture is obtained, though, because of sparse evidence, it cannot be supported by statistical tests.

For diazinon toxicity, i.e. the biological effect at the same concentration, was greatest in fine sand soil and in fine sand soil greatest at a depth of 0–4 cm. In silt loam soil toxicity was greatest at 4–8 cm.

For chlorfenvinphos the influence of the soil type was not clear. It seems like the 4 functions belongs to only one function.

For isofenphos the effect was independent of soil type and depth.

Generally, it can be concluded that there is a reasonably good correlation between residue concentrations in the soils and biological effect. However, results suggest that if insecticides are to be used in a plant protection programme where the effect on beneficial insects is evaluated, allowances must be made for factors which can affect the biological effect of the insecticides, (e.g. soil type and soil depth).

In addition, consideration must be made for the factors which affect degradation of the insecticides.

Literature

1. *Finlayson, D. G., Mackenzie, J. R. & Campbell, C. J.* 1980. Interactions of insecticides, a carabid predator, a staphylinid parasite, and cabbage maggots in cauliflower. Envir. Ent. 9, 789–794.
2. *Khan, S. U. & Iversen, Karl, C.* 1981. Microbiological release of unextracted (bound) residues from an organic soil treated with prometryn. J. Agric. Food Chem. 29, 1301–1303.
3. *Khan, S. U.* 1982. Distribution and characteristic of bound residues of prometryn in an organic soil. J. Agric. Food Chem. 30, 175–179.
4. *Kirknel, E.* 1978. The influence of diazinon, trichloronate, carbofuran and chlorfenvinphos on the parasitization capacity of the rove beetle *Aleochara bilineata* (Gyll.). Tidsskr. Planteavl 82, 117–129.
5. *Lichtenstein, E. P. & Schultz, K. R.* 1959. Persistence of some chlorinated hydrocarbon insecticides as influenced by soil types, rate of application and temperature. J. Econ. Ent. 52, 124–131.
6. *Lichtenstein, E. P., Fuhremann, T. W., Schultz, K. R. & Liang, T. T.* 1973. Effect of field application methods on the persistence and metabolism of phorate in soils and its translocation in crops. J. Econ. Ent. 66, 863–866.

Manuscript received 19 July 1985.