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1388. beretning

The influence of diazinon, trichloronate, carbofuran and chlorfenvinphos on the parasitization capacity of the rove-beetle *Aleochara bilineata* (Gyll.)

Indvirkning af diazinon, trichloronat, carbofuran og chlorfenvinphos på rovbillen Aleochara bilineata's *parasiteringskapacitet*

Erik Kirknel

Abstract

The parasitization capacity of the rove-beetle *Aleochara bilineata* (Gyll.) has been investigated in both sandy loam and sand, treated with 1, 2, 4 or 16 kg active ingredient per ha of the insecticides diazinon, trichloronate, carbofuran and chlorfenvinphos. Plastic jars were filled with soil treated with insecticide and placed in the field at ground level. After 7, 60, 120 and 305 days soil samples were taken at depths of 0–4 cm and 4–8 cm. It has been investigated under laboratory conditions to which degree the rove-beetle larvae were capable of parasitizing the turnip root fly *Hylemya floralis* Fall.

It has been shown that five factors are important when evaluating the insecticides concerning the effect on the rove-beetle. These five factors are arranged in decreasing importance as follows: 1) soil type, 2) compounds, 3) exposure time in the field, 4) dosage and 5) location depth of placing the insecticides.

With the exception of carbofuran the insecticides lost their activity faster in sandy loam than in sand.

According to increasing toxicity to the rove-beetle larvae the insecticides may be arranged in the following order: Chlorfenvinphos, carbofuran, diazinon and trichloronate.

Proportional to the time of exposure in the field, the insecticides lost toxicity (i.e. increased parasitization). But after some periode of time the parasitization in treated soil exceeded the parasitization registered in control except of trichloronate.

The insecticides lost toxicity inversely proportional to the dosage.

The toxicity decreased more quickly in soil samples taken at a depth of 0–4 cm than 4–8 cm.

Key words: *Aleochara bilineata,* diazinon, trichloronate, carbofuran, chlorfenvinphos, beneficial capacity, soil factors influencing toxicity.

Resume

Rovbillen *Aleochara bilineatas* parasiteringskapacitet er undersøgt i fin lerblandet sand og i sand behandlet med 1, 2, 4 eller 16 kg aktivt stof pr. ha af diazinon, trichloronat, carbofuran eller chlorfenvinphos. Den behandlede jord blev fyldt i plastpotter og anbragt på friland med overkant i niveau med jordoverfladen. Efter 7, 60, 120 og 305 dages forløb blev prøver udtaget fra 0–4 cm og 4–8 cm dybde. I laboratoriet undersøgtes, i hvilken grad rovbillelarver i disse jordprøver var i stand til at parasitere den store kålflue *Hylemya floralis* Fall.

5 faktorer viste sig at være af betydning, når disse insekticiders effekt på rovbillen skal vurderes. Med faldende betydning kan de opstilles som følger: »Jordtype«, insekticid, opbevaringstid i jorden, dosis og jorddybde.

Med undtagelse af carbofuran mistede insekticiderne hurtiger deres virkning i fin lerblandet sandjord end i sand.

Med stigende giftighed mod rovbillelarver kan insekticiderne opstilles som følger: Chlorfenvinphos, carbofuran, diazinon og trichloronat.

Insekticiderne mistede deres giftighed proportionalt med opbevaringstiden på friland. Det blev for alle midler undtagen trichloronat vist, at den med tiden aftagende giftighed (udtryk som øget parasitering) på et tidspunkt ændredes til en større parasitering end i ubehandlet. Om denne forøgede parasitering var et resultat af sublethale dosers stimulering af rovbillelarven til større aktivitet eller en følge af at mikroorganismer som parasiterede på rovbillelarven blev undertrykt af insekticiderne eller helt andre årsager vides ikke.

Jordene med de højeste insekticiddoser bevarede giftigheden længst.

Insekticidernes giftighed aftog hurtigere i jordlaget 0–4 cm dybde end i 4–8 cm dybde.

Nøgleord: *Aleochara bilineata,* diazinon, trichloronat, carbofuran, chlorfenvinphos, parasiteringskapacitet, giftighed påvirket af jordbundsfaktorer.

Introduction

Investigations of the effect of pesticides on beneficial insects have been performed in numerous ways. A critical review of the different test methods has been published by *Franz* in 1974. It has been tried in a publication by *Kirknel* (1974) to extract the results from similar type of investigations and characterize some newer insecticides with respect to their toxicity against groups of beneficial insects. But the experimental conditions under which the tests were made were different from one publication to another and with varying results.

An international cooperation concerning development of testmethods was started in 1973 under the guidance of IOBC (The International Organization for Biological Control of Noxious Animals). Further information regarding this cooperation has been reported by *Kirknel* (1975).

The test method used in the present report has been developed by the author in cooperation with the above mentioned working group under IOBC. One of the demands of the working group on a test method is that it should be possible to observe the insect being tested for a full generation. This aim has been difficult to achieve, as the hatch of the parasite from the host was percentually low. Efforts in solving this problem continues. The author has considered it appropriate to publish some of the results despite of a not yet finished test method, allowing practical conclusions to be drawn.

The rove-beetle *Aleochara bilineata* is a predator and a parasite of the cabbage root fly *Hylemya brassicae* (Bouché) and the turnip root fly *Hylemya floralis* Fall.

After the egg stage the rove-beetle first instar larvae penetrates the fly puparium by means of the mandibles. If the puparium is penetrated, only few percent will hatch. The parasite will either enter the pupae, digest the content and try to carry out its normal development or, if the parasite does not enter the pupae, the pupae will be attacked and be destroyed by micro organisms.

It has been shown in Danish experiments that the density of predators (carabids) in cabbage fields are negatively correlated to the damage caused by theturnip root fly. Furthermore it was pointed out the damage was often increased by improperly planned use of insecticides against the turnip root fly. Time of application of the insecticides was more important than the type of pesticide (*Kirknel* 1971).

Hassan (1969) showed the importance of placing the insecticide so that interference with the rove-beetle was avoided. Mixing of granules of diazinon and chlorfenvinphos in the soil spared the rove-beetles more than if the insecticides were distributed on the soil surface.

This should not lead to the conclusion that insecticides always are more active on the soil surface than when admixed to the soil. It has been demonstrated that persistence is positively correlated with the depth of placement, e.g. *Lichtenstein* et al (1973) using chemical analysis and by *Read* (1974 and 1976) using a bioassay with the cabbage root fly. Some of the explanations may probably be found in the fact that the insects are exposed to a much higher concentration of insecticide on the soil surface than in the soil, where the same dosage is distributed into a volume of the soil. Furthermore the insects of interest, the rove-beetles and carabids, are living mainly on the soil surface.

In Danish investigations *Bro-Rasmussen* et al. (1968) have found that the soil type influences the rate of degradation of diazinon. Diazinon showed faster degradation in loam than in sandy loam.

It is important to use selective insecticides in chemical control of the turnip root fly to secure constant results. Investigations with Aldrin (a broad spectrum insecticide with a half life of several years now prohibited in Denmark) confirm that it is not so important to find the most poisonous insecticide. Results from these investigations were random (*Jørgensen* 1957). It is particularly important to use selective insecticides in these cases where it is intended to use reduced amounts of insecticides.

On the basis of the above mentioned causes it was decided to investigate the insecticidal effect from the following four factors: 1) depth of placement of the insecticide, 2) soil type, 3) exposure time in the field and 4) dosage. Effects should be measured on the rove-beetle *Aleochara bilineata* Gyll. which is an important predator and parasite of the cabbage root fly *(H. floralis) and the turnip root fly (H. brassicae).*

Materials and methods

Pupae of the turnip root fly *(H. floralis)* are placed in sandy loam or sand containing different concentrations of insecticides. Eggs of the rove-beetle *A. bilineata* are placed on the soil surface. The hatched rove-beetle larvae tries to reach the pupa by moving through the soil in order to parasitize it. Depending on the activity of the insecticide and its metabolites, parasitization will take place. The activity of the insecticide and its metabolites is expressed as deviation in parasitization from control. Concentration of the insecticides are calculated as kg active ingredient per hectare in an eight cm soil layer.

The plastic jars containing the soil have a diameter of 6.7 cm, the cross-section area being 35.2 cm^2. They are 10 cm high and have drain at the bottom. The jars are filled with soil until 2 cm under the upper edge. The weight of sandy loam is 340 g per jar and of sand 470 g per jar. The analysis of texture is shown in table 2. The treatment rate expressed in kg a.i. per ha may be converted to soil content measured as ppm (or mg per kg) by multiplication with the following numbers:

$$\text{Sandy loam} \quad \frac{0.352 \cdot 10^6}{340 \cdot 10^3} = 1.035$$

$$\text{Sand} \quad \frac{0.352 \cdot 10^6}{470 \cdot 10^3} = 0.749$$

The treatment of soil with insecticides is performed in the following way: 10 per cent of the soil is air dried at room temperature and wetted with the exact amount of emulsified insecticide. The wetted soil is dried at room temperature and thereafter mixed ten times with the remaining 90 per cent in a funnel. The soil is filled in the plastic jars and placed in the field at ground level. No replications. The jars are wetted to field capacity at the start of the experiment and at the time of each sampling. Registration of temperature at two depths, namely in 2 cm and 6 cm below surface are shown in fig. 1. The first two weeks after the start of the experiment on June 22nd. 1976, continuous registration is performed.

After 7, 60, 120 and 305 days soil samples are taken with a soil auger (10 mm inside diameter). The samples are either stored at ÷ 30°C in plastic bags or used immediately in the test.

A pyrex glass ring (15 mm high, 25 mm inside diameter) is placed on a micro slide. 20 turnip root fly pupae (collected in the field and brought out of diapause) are arranged on the bottom of the glass ring in one single layer. The soil samples are

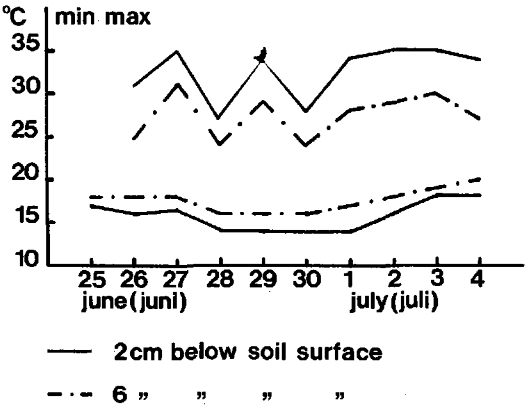

Fig. 1. Daily minimum and maximum temperature registered 2 and 6 cm's below soil surface in plastic jars in the field 1976. Experiment started on 22nd June 1976. (Daglig minimum og maximum temperatur registreret i 2 og 6 cm dybde i plastpotter på friland 1976. Forsøget påbegyndt den 22/6 1976).

slightly wetted and placed on the puape in a 1 cm lightly pressed layer.

20 eggs of the rove-beetle, three or four days old, are transferred with a camels hair brush onto a filter paper disc (Whatmann AA disc. 13 mm diameter). The disc is placed on the surface of the soil samples.

Micro slide, glass ring with sample, pupae and eggs are placed in a 1.5 litre humidity chamber made of plexi glass (29 cm × 6.5 cm × 8 cm). The box is ventilated with humid air (> 99% R.H.). This is done by dipping the box in a thermostated water jacket. An Erlenmeyer flask is filled with destilled water, turned upside down and placed in the same water jacket. Air is pumped through this flask into the humidity chamber. The humidity is registered with a dry and a wet thermistor (Figure 2). The temperature is kept at 23°C.

After 6 days the humidity chamber is opened. The eggs are examined and the number of hat-

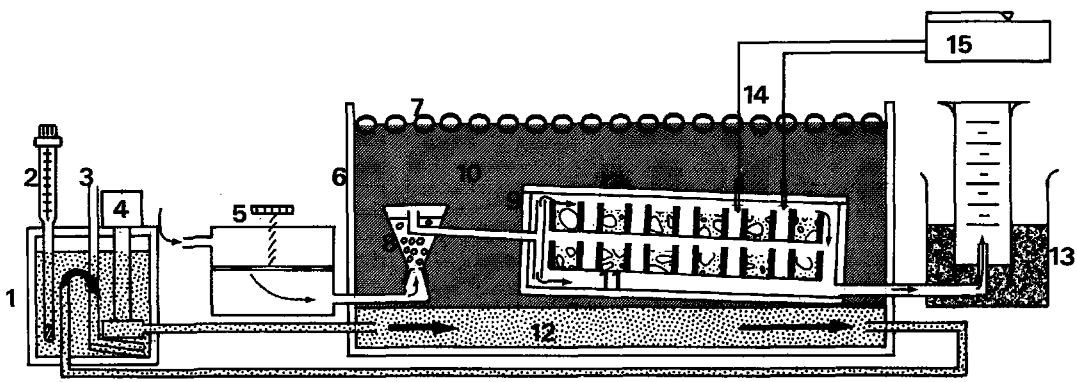

Fig. 2. Air ventilated humidity chamber for testing of the effect of insecticides on the rove-beetle *Aleochara bilineata*. (Ventileret fugtkammer til afprøvning af insekticiders effekt mod rovbillen *Aleochara bilineata*).

1. thermostatic unit (varmebad)
2. contact thermometer (kontakttermometer)
3. heating unit (varmelegeme)
4. circulating pump (cirkulationspumpe)
5. adjustable air pump (justerbar luftpumpe)
6. water jacket box (vandkappe kasse)
7. insulating plastic balls (isolerende plastkugler)
8. humidifier (befugter)
9. test chamber (fugtkammer)
10. water jacket (vandkappe)
11. glass tubes with soil (glasrør med jord)
12. thermostat-controlled water jacket (termostateret vandkappe)
13. »air flow meter« (luftmængdemåler)
14. wet and dry glass bulb thermistors (våd og tør glastermistorer)
15. recorder (skriver)

ched eggs are noted. The pupae are sifted from the soil samples and examined. The numbers of the typical entrance holes are noted.

Per cent parasitization in treated soil:

$$\frac{v}{20\ (\text{eggs}) - a} \times 100 = X$$

Per cent parasitization in control:

$$\frac{u}{20\ (\text{eggs}) - b} \times 100 = Y$$

Per cent deviation in parasization from control

$$100 - \frac{x}{y} \times 100$$

a and b: numbers not hatched

v and u: numbers of entrance holes

The per cent deviation is plotted against the numbers of days during which the insecticide has been exposed to field conditions.

Results and discussion

Evaluation of the results is done best by observing the graphic representation in the figures 3, 4, 5 and 6.

Every statistical test assumes that the data are following well known mathematical laws. This is seldom the case. Neither is the demand of stocastical variables net. Nevertheless an analysis of variance is often supporting the evaluation which is valid for the present report too.

Separate analysis have been made for sandy loam and sand thereby reducing the error. Statistical tests are not made between sandy loam and sand. The difference between the two is best evaluated by observing the figures. The analysis of variance is presented in table 3 and 4.

The results in the present report cannot always be compared directly with other investigations on the influence of insecticides on beneficial insects. Comparison is only possible in relative terms, as the effects reported in other investigations is often registered on derived functions. This is for example reported as numbers of insects caught in traps (e.g. pit-fall traps). The surviving insects of a treatment could either have been stimulated or retarded and in this way give a wrong picture of

Table 1. Insecticides used in the experiments
(Insecticider anvendt i forsøgene)

Trade mark (Handelspræparat)	active ingredient (aktivt stof)	kg a.i. per ha (kg akt.st. pr. ha)
Basudin Emulsion	diazinon 50%	1, 2, 4 and 16
Agritox emulsion 50	trichloronat 50%	» » » » »
Furadan 75	carbofuran 75%	» » » » »
Shell Birlane 24 EC	chlorfenvinphos 24%	» » » » »

Table 2. Analysis of the soils (Analyse af jordene)
Per cent

	pH_w (pH_v)	Clay (Ler) $<2\mu$	Silt (Silt) $2–20\mu$	Fine sand (Finsand) $20–200\mu$	Coarse sand (Grovsand) $200–2000\mu$	Organic matter (Humus) –
Sandy loam .... (Fin lerblandet sandjord)	5.6	8.5	16.1	46.9	25.7	2.8
Sand .... (Sand)	6.3	0.7	0.2	30.8	68.2	0.1

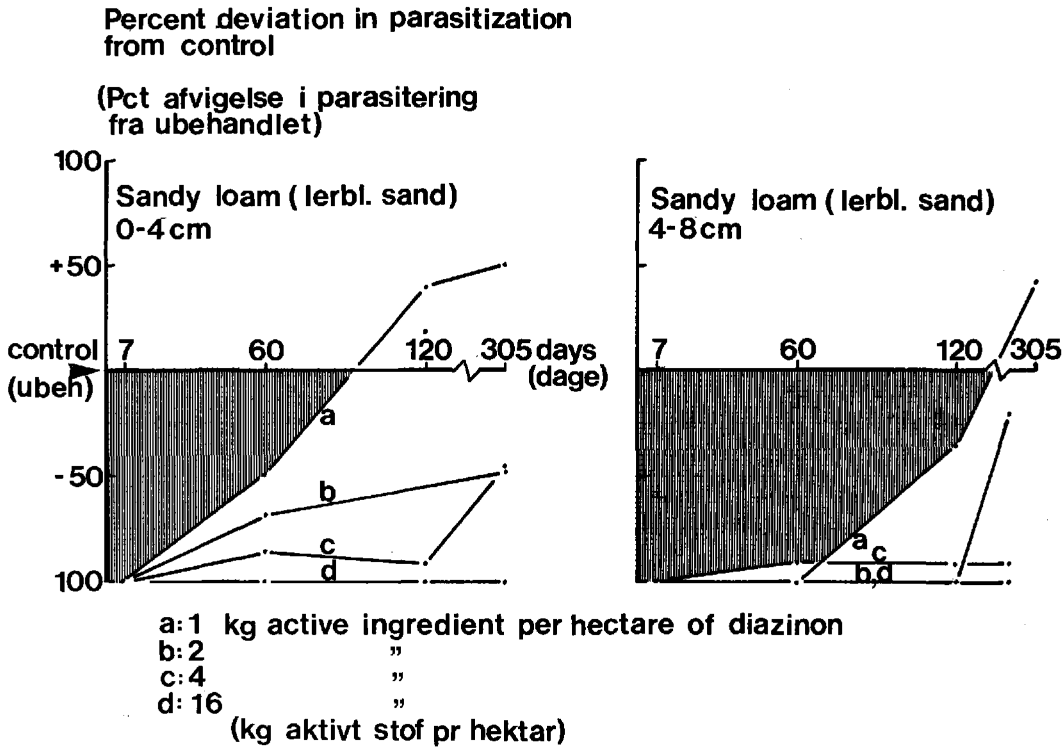

Fig. 3. Parasitization capacity of *A. bilineata* in 2 different soil types (sandy loam and sand) containing 4 concentrations (1,2,4 and 16 kg a.i. per ha) of diazinon originating from 2 depths (0–4 cm and 4–8 cm). Sampling after 7, 60, 120 and 305 days.

(Rovbillen *A. bilineata's* parasiteringskapacitet ved ophold i 2 jordtyper (fin lerblandet sandjord og sand), der indeholder 4 ioncentrationer (1,2,4 og 16 kg akt.st. pr. ha) af diazinon udtaget i forskellige dybder (0–4 cm og 4–8 cm) til forskellige tider (7, 60, 120 og 305 dage)).

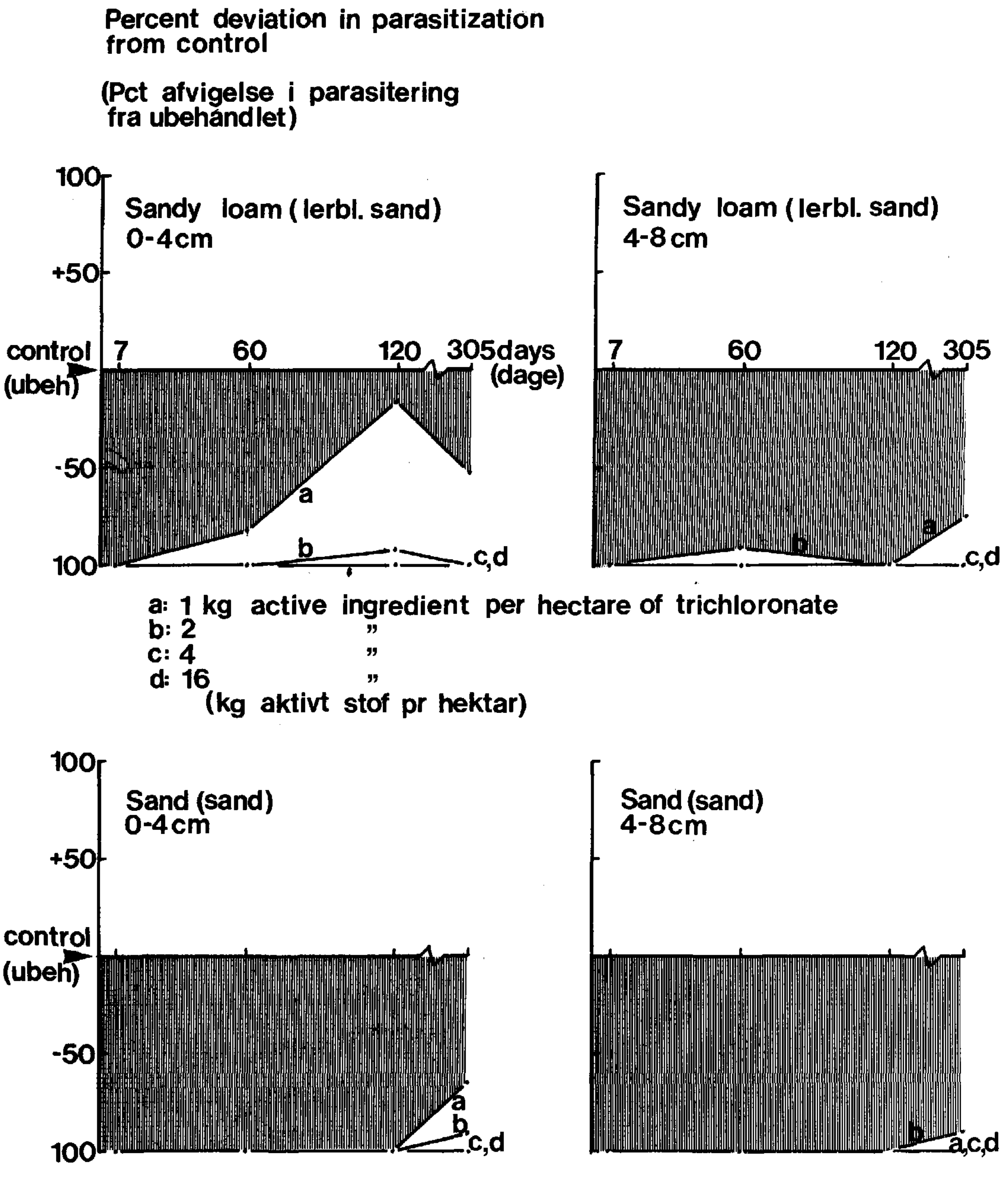

Fig. 4. Parasitization capacity of *A. bilineata* in 2 different soil types (sandy loam and sand) containing 4 concentrations (1,2,4 and 16 kg a.i. per ha) of trichloronate originating from 2 depths (0–4 cm and 4–8 cm). Sampling after 7, 60, 120 and 305 days.

(Rovbillen *A. bilineata's* parasiteringskapacitet ved ophold i 2 jordtyper (fin lerblandet sandjord og sand), der indeholder 4 koncentrationer (1,2,4 og 16 kg akt.st. pr. ha) af trichloronat udtaget i forskellige dybder (0–4 cm og 4–8 cm) til forskellige tider (7, 60, 120 og 305 dage)).

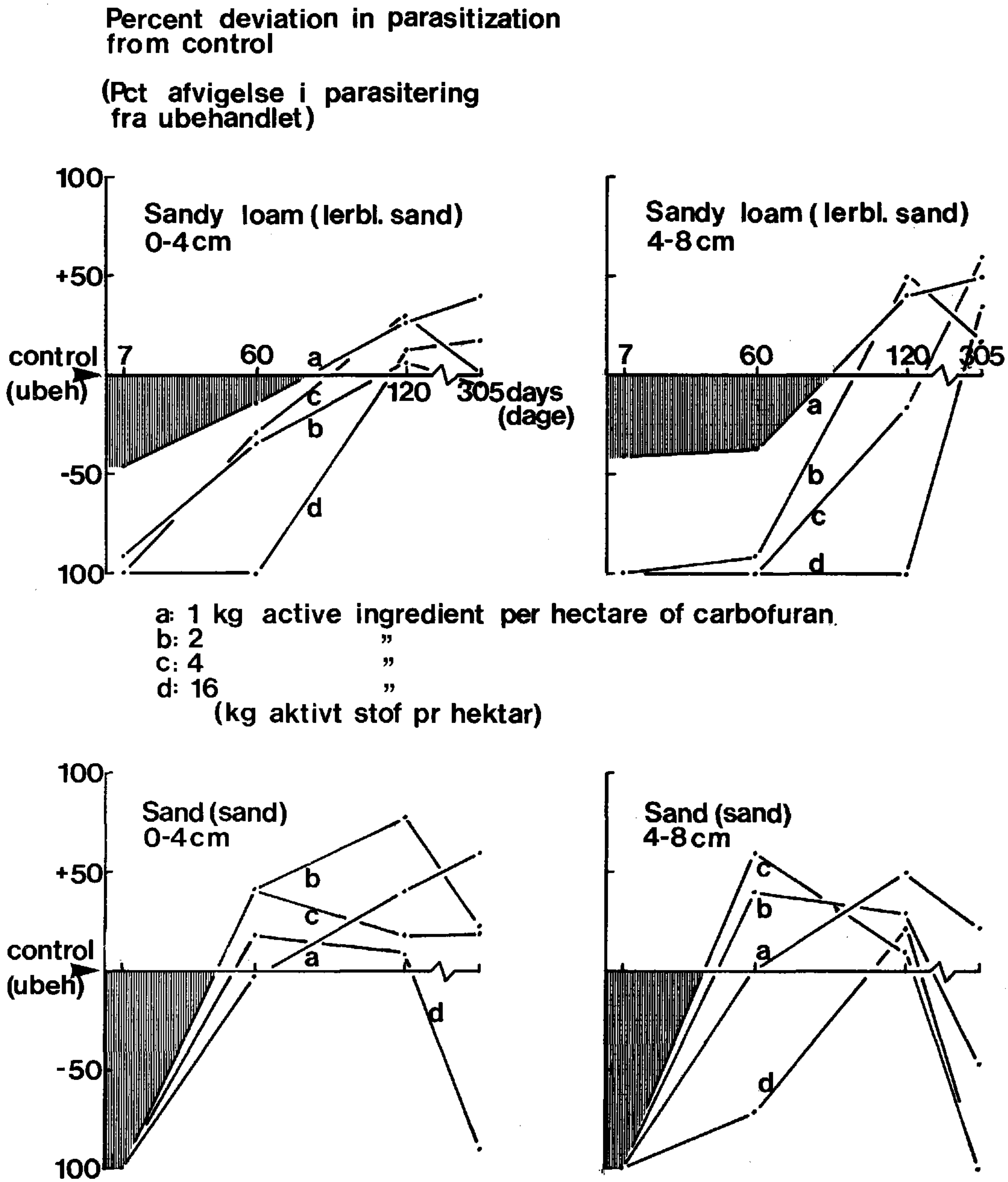

Fig. 5. Parasitization capacity of *A. bilineata* in 2 different soil types (sandy loam and sand) containing 4 concentrations (1,2,4 and 16 kg a.i. per ha) of carbofuran originating from 2 depths (0–4 cm and 4–8 cm). Sampling after 7, 60, 120 and 305 days.
(Rovbillen *A. bilineata's* parasiteringskapacitet ved ophold i 2 jordtyper (fin lerblandet sandjord og sand), der indeholder 4 koncentrationer (1,2,4 og 16 kg akt.st. pr. ha) af carbofuran udtaget i forskellige dybder (0–4 cm og 4–8 cm) til forskellige tider (7, 60, 120 og 305 dage)).

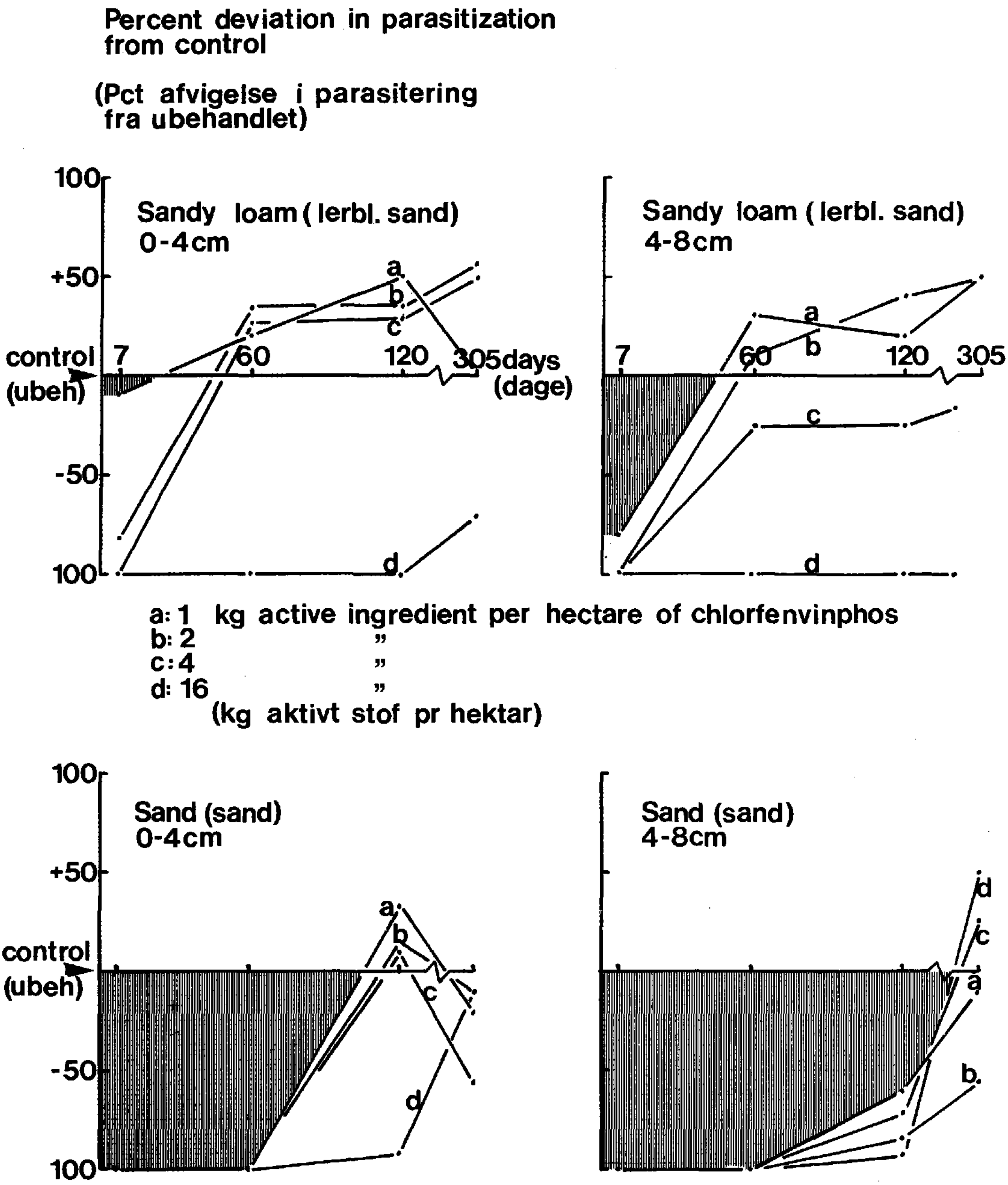

Fig. 6. Parasitization capacity of *A. bilineata* in 2 different soil types (sandy loam and sand) containing 4 concentrations (1,2,4 and 16 kg a.i. per ha) of chlorfenvinphos originating from 2 depths (0–4 cm and 4–8 cm). Sampling after 7, 60, 120 and 305 days.

(Rovbillen *A. bilineata's* parasiteringskapacitet ved ophold i 2 jordtyper (fin lerblandet sandjord og sand), der indeholder 4 koncentrationer (1,2,4 og 16 kg akt.st. pr. ha) af chlorfenvinphos udtaget i forskellige dybder (0–4 cm og 4–8 cm) til forskellige tider (7, 60, 120 og 305 dage)).

Table 3. Analysis of variance for sand after 305 days
(Variansanalyse for sandjord efter 305 dage)

Source of vatiation (variationsårsag)	DF (df)	meansquare (s^2)	F (F)	significance (signifikans) P	signific. after 120 days (signifik. efter 120 dage) P
Total	127	2742.14			
1. Dosages (doser)	3	2854.51	6.25	0.01	0.05
2. Depths (dybder)	1	4947.80	10.84	0.01	0.05
3. Times (tider)	3	17839.63	39.08	0.001	0.001
4. Compounds (midler)	3	38735.81	84.86	0.001	0.001
1 × 2	3	657.63			
1 × 3	9	969.94			0.05
1 × 4	9	1064.43	2.33	0.05	
2 × 3	3	1163.19			0.05
2 × 4	3	700.68			
3 × 4	9	8840.98	19.37	0.001	0.001
1 × 2 × 3	9	596.70			
1 × 2 × 4	9	488.05			
1 × 3 × 4	27	959.52			
2 × 3 × 4	9	1286.41			
1 × 2 × 3 × 4	27	456.45			

Standard deviation = 19.26. Standard deviation in per cent of the average = 71.1.
(Spredning = 19.26. Spredning i % af gennemsnit = 71.1%)

the density of insects at the locality concerned. Investigations where mortality is used as criterion, have also limited value because survivors might have their beneficial capacity reduced.

Read (1960) investigated the influence of heptachlor and parathion on the parasititzation of the cabbage root fly in field experiments and found that spraying heptachlor and parathion along the plant rows have reducing effects on the parasitization by 80% and 25% respectively.

In the present report it is demonstrated that the toxicity remains for a longer period of time in sand than in sandy loam except for carbofuran where there is a tendency to act conversely. *Bhirud* and *Pitre* (1972) report best initial effect in light soil for carbofuran. This is in agreement with the present results. *Bro-Rasmussen* et al. (1968) report from chemical analysis that the degradation of diazinon was faster in loam than in sandy loam.

The relatively persistent trichloronate is shown to have great effect on the larvae, while chlorfenvinphos and carbofuran must be graded as being relatively harmless. Carbofuran and chlorfenvinphos probably are selective insecticides in proportion to the larvae. It seems not only to be a matter of fast degradation. *Caro* et al. (1973) report 46–117 days for a 50% degradation in outdoor experiments with carbofuran. *Bro-Rasmussen* et al. (1970) report trichloronate and chlorfenvinphos being degraded at approximately the same rate namely with 37% and 33% of residues remaining respectively after 10 months. Diazinon was degraded relatively fast showing only 5% of residues after 10 months. In the present report diazinon must be graded as being fairly toxic to the rove-beetle larvae. The presence of toxic metabolites probably is of importance here. *Read* (1969) demonstrates in a bioassay that cabbage root flies were sensitive against carbofuran and chlorfen-

Table 4. Analysis of variance for sandy loam after 305 days
(Variansanalyse for fin lerblandet sandjord efter 305 dage)

Source of vatiation (variationsårsag)	DF (df)	meansquare (s^2)	F (F)	significance (signifikans) P	signific. after 120 days (signifik. efter 120 dage) P
Total	127	3198.77			
1. Dosages (doser)	3	21846.55	29.22	0.001	0.001
2. Depths (dybder)	1	6634.23	8.88	0.01	0.01
3. Times (tider)	3	29173.98	39.04	0.001	0.001
4. Compounds (midler)	3	35008.52	46.85	0.001	0.001
1 × 2	3	243.02			
1 × 3	9	1488.56			0.05
1 × 4	9	3023.42	4.05	0.01	0.05
2 × 3	3	1334.71			
2 × 4	3	280.69			
3 × 4	9	4481.32	6.00	0.001	0.01
1 × 2 × 3	9	237.17			
1 × 2 × 4	9	487.15			
1 × 3 × 4	27	884.52			
2 × 3 × 4	9	495.77			
1 × 2 × 3 × 4	27	747.19			

Standard deviation = 27.36. Standard deviation in per cent of the average = 77.0.
(Spredning = 27.36. Spredning i % af gennemsnit = 77%)

vinphos even after the compounds have been exposed to mineral soil in out door experiments for 90 days. Contrary, diazinon lost its activity very fast. Chemical analysis of the original compound only seldom gives sufficient information for evaluation of the biological effect. The importance of chemical analysis coupled onto bioassay is pointed out by *Read* (1976).

The influence of the exposure time in the field is significant both in sandy loam and sand. The general pattern is that low dosage looses its effect before higher dosage does, with the exception of carbofuran in sand, which has been unexplained so far. The average parasitization in control was 62.7%. As shown in figures 3, 4, 5 and 6 the per cent parasitization was smaller at the start of the experiment in treated soils than parasitization registered in control. But after some period of time this situation was reversed to a higher parasitization in treated soils than that found in control except for trichloronate and the lower layer of diazinon in sand.

This period of time varied considerably. It was shorter for chlorfenvinphos, then carbofuran and diazinon, it was shorter in sandy loam except for carbofuran, it was shorter in the 0–4 cm layer than in the 4–8 cm layer and it was finally smaller in low than in high dosages. The reason for this increase in parasitization to a higher level than control is not known. It is suggested that the reason could either be a direct stimulation of the rove-beetle larvae with sublethal dosages or a suppression of microorganisms parasitizing the larvae.

The dosage was an important factor too, but the highest significance was found in experiments with sandy loam (Table 4). Within one growing season only 95% significance was registered in sand. But after 305 days the significance raised to 99% (table 3).

The toxicity disappeared faster in samples taken at 0–4 cm than from 4–8 cm depth. This is registered with high significance (99%) only in sandy loam within one growing season. But after 305 days the same significance was also found in sand. The results obtained within one growing season (120 days) has the highest interest in integrated control of pest insects in agricultural crops. The factors determining the fast disappearance of toxicity in the upper layer are not known with certainty, but at high temperatures evaporation might be of great importance. The temperature has been registered the first two weeks after starting the experiment and as expected, the average temperature and daily variation from the average temperature was higher in the upper than in the lower layer (Fig. 1).

The most significant interactions are dosages × compounds and times × compounds. This means the toxicity is different from compound to compound when compared at fixed time and dosage.

Conclusions

Several factors are influencing the toxicity of diazinon, trichloronate, carbofuran and chlorfenvinphos to the rove-beetle *Aleochara bilineata* Gyll.

The factors investigated are arranged in decreasing importance as follows: Soil type, insecticides, exposure time in the field, dosage and location depth of placement of the insecticides.

It is demonstrated that the toxicity remains for a longer period in sand than in sandy loam except for carbofuran.

The insecticides were found with increasing toxicity in the following order: Chlorfenvinphos, carbofuran, diazinon and trichloronate.

The insecticide lost toxicity (i.e. increased parasitization) proportional to the time of exposure in the field. But after some period of time the parasitization in treated soil exceeded the parasitization registered in control except for trichloronate. This period of time was shortest for chlorfenvinphos, followed by carbofuran and diazinon, shorter in sandy loam than in sand except for carbofuran, shorter in the 0–4 cm layer than in the 4–8 cm layer and finally shorter in low dosages than in high.

The insecticides lost toxicity inversely proportional to the dosages.

The toxicity disappeared more quickly in soil samples taken at the depht of 0–4 cm than at 4–8 cm.

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