Side effects of the herbicide 2,4,5-T affecting mobility and mortality of the springtail Onychiurus quadriocellatus Gisin (Collembola)

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With 19 figures

Abstract

The herbicide 2,4,5-T is widely used in forestry and nature reserves against black cherry *(Prunus serotina)* and birch (*Betula* spp.). When adult specimens of the springtail *Onychiurus quadriocellatus* Gisin (taken as a representative of the important group of the soil fauna, the Collemboles) in laboratory experiments were continuous exposed to the herbicide, a dose of 0.003 ml/dm² already caused a significant increase in mortality.

Not only the active ingredient of the herbicide (2,4,5-T isooctylester), but also the formulation fluid itself proved to be toxic, be it to a lower extent.

The toxic action of the herbicide is mainly caused by direct contact; there was only a slight effect after application as a vapour.

Juvenile springtails proved to be more susceptible than adults. The herbicide also influenced the mobility of the springtails. A high dose (0.025 ml/dm²) decreased mobility, whereas a low dose (0.003 ml/dm²) gave an increased mobility.

Moreover the herbicide excercised a repellent action on springtails; these were able to avoid treated sections of the substrate, as well as to escape from them.

In spite of this repellency a considerable proportion of the springtails tested died, although given the opportunity to escape. They died on the treated substrate or after escape from it.

The amounts of herbicide found on the soil under and between black cherry shrubs after practical applications, varied from 0.0005 to 0.017 ml/dm². From this and the above mentioned results of laboratory experiments, in the field side effects of the herbicide 2,4,5-T are to be expected.

1 Introduction

The herbicide 2,4,5-T is widely used in forestry. In recent years there has been growing concern about its toxicity for organisms other than the plants that are to be controlled, but little information is available (ERNE 1974) on the fauna which can come into contact with this herbicide in the forests and the effects of this exposure. Nevertheless, a number of countries have restricted or prohibited the application of 2,4,5-T ester.

In The Netherlands the herbicide 2,4,5-T is used on a large scale in forests and nature reserves to control birch (*Betula* spec.) and black cherry (*Prunus serotina).* This practice can also affect other plants and animals, adversely, and herbicide falling on the soil may have an effect on life in the soil.

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To obtain information about the effect of the herbicide 2,4,5-T on soil fauna, a laboratory investigation was performed on its impact on the mortality and activity pattern of the springtail *Onychiurus quadriocellatus* Gisin. Springtails are abundant in the soil of forests and nature reserves. This particular species was chosen because it is present in large numbers and the specimens are easy to collect and handle.

Mortality can be used as a parameter to assess the toxic effect of the herbicide during a specified period of time. In the present case, the mortality rate depends on the springtails' sensitivity to 2,4,5-T and on their chances of coming into contact with it. The likelihood of contact is determined to a large extent by the insects' activity, a high degree of activity enhancing the risk of contact with the herbicide. This factor has been put forward as an argument to explain the high sensitivity to pesticides of predacious soil arthropods (EDWARDS 1969). On the other hand, the springtails' activity may also enable them to escape the effects of a toxic substance. This holds especially for cases where the herbicide has been spread unevenly over the substrate and when its action remains confined to the spots on which the herbicide has settled. Such uneven spreading is to be expected in the field because of the topical application to the black cherry bushes, which leads to high quantities of herbicide under the bushes, spot concentrations caused by dripping spray liquid, and low quantities between the bushes resulting from drift of the spray.

2 Material and methods

2.1 Material

Onychiurus quadriocellatus is a white springtail, one to three millimetres long, with a reduced furcula and very short antennae (see fig. 1).

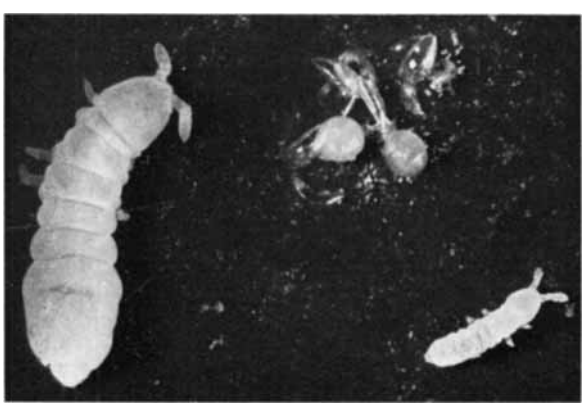

Fig. 1. Onychiurus quadriocellatus Gisin, eggs, empty chorions, and a juvenile specimen

Little is known about its biology. During collection of the insects needed for our experiments, they were found just under the upper litter layer when moist conditions prevailed, but in dry soil they tended to occur further down (down to 5 cm). Changes in the juvenile population in the litter samples collected in the course of the year indicated

that *Onychiurus quadriocellatus* reproduces in the spring and early summer. No quantitative observations on this species have been made, although data are available on related species, viz. *O. absoloni*, *O. armatus*, *O. furcifer*, *O. justi*, *O. latus*, *O. procampatus*, *O. tricampatus* (sources: GLASGOW 1939; AGRELL 1941; DHILLON and GIBSON 1962; MILNE 1962; HALE 1965a, 1965b; ALJENIKOVA and MARTYNOVA 1966; HEALY 1967; SINGH 1969; USHER 1970; PETERSEN 1971; SNIDER 1971). These data make it possible to form a general picture of the genus.

Onychiurus species are distributed throughout the world and occur in various biotopes. They are usually found in the upper centimetres of the soil profile. Only in dry, unfavourable conditions – which prevail in the summer in the temperate zone – do they tend to go deeper. Under such unfavourable conditions the populations decrease. The largest numbers are found in the spring and late autumn, which is related to the period in which ovipositon has been observed (May - October), the increased availability of food (falling leaves), and favourable weather conditions.

The food of *Onychiurus* is very diverse, consisting mainly of fungi, bacteria and decomposing wood and litter, but also of roots and seedlings. Laboratory tests (PETERSEN 1971; SINGH 1969) have demonstrated a distinct preference for fungi. According to SINGH (1969), wood and litter must be infected with micro-organisms before they are consumed.

The growth of *Onychiuridae* is continuous (PETERSEN 1971) and moulting continues throughout the lifespan.

The springtails for the experiments were collected by drying out litter in Tullgren funnels (for collection methods, see MURPHY 1962). The insects fell from the litter into small pots provided with water or moist filter paper, from which they were transferred to Perspex dishes on a base of plaster of Paris and activated carbon. The activated carbon served to enhance the visibility of the springtails and to neutralize any remaining quicklime. The base was kept moist and the insects were fed regularly with germinated seed of red clover (*Trifolium pratense*) or fine litter particles. The springtails were kept in the dark at 15° C in closed dishes with maximum humidity for a period of at most 3–4 weeks before they were used in experiments.

2.2 Methods

In the experiments two substrates were used: a soil/agar substrate containing the mixed herbicide and a soil substrate onto which the herbicide was sprayed. The sandy soil (with a content of 2.9 % organic matter and 4.6 % lutum) used for both substrates was sifted through a fine sieve (4 mm and/or 1 mm mesh) and stored air-dry. For the soil/agar substrate (method based on SANOCKA-WOLOSZYN and WOLOSZYN 1970), glass petri dishes (∅ 9 cm) were provided with 20 g of soil, over which 20 ml 2 % agar solution into which 0.67 ml diluted herbicide (see also section 2.3) had been mixed, was poured, producing a smooth, dark substrate on which the springtails were clearly visible. The substrate afforded a sufficiently high degree of humidity, which, according to CHRISTIANSEN (1964), is important for the springtails' survival.

The experiments were carried out in glass petri dishes, which for some experiments were partitioned into separate sections and in some cases had a specially polished edge and cover to ensure optimum closure. A preliminary test to investigate variation in the moisture content of the substrate, had shown that in ordinary petri dishes there was a decrease of 5 % per 10-day period and in specially polished dishes of 1 %.

A possible adverse influence of the temperature of the agar before pouring as well as of plastic dishes, the quantity of soil added, and the initial density of the springtail population was also investigated.

The findings may be summed up as follows:

The temperature of the agar before pouring should not be higher than about 60° C to prevent premature evaporation of the herbicide. On the other hand, the temperature must not be too low, because otherwise the agar solodifies too soon, making it impossible to pour smooth substrates.

In the preliminary experiments in which both plastic and glass dishes were used, the effect of the herbicide was smaller in plastic than in glass dishes, probably owing to a difference in adsorption; for this reason, only glass dishes were used for the study.

A reduction of the quantity of soil in the dishes led to an increased mortality rate and a higher overall mortality.

The initial density (from 25 to 225 specimens per dish) did not affect mortality during

a period just exceeding 60 days, but when there were more than 25 springtails in a dish, it became difficult to count the moving insects accurately.

The experiments with soil substrate were performed in specially polished petri dishes (∅ 9 cm) containing 20 g sifted sand (1 mm mesh). The sand was moistened and compacted to eliminate air bubbles, thus preventing the formation of minute cavities into which the springtails could settle. Excess water was drained off. The springtails were placed on the soil base and sprayed with an aqueous solution of the herbicide. For this purpose, a spraying apparatus equipped with an adjustable nozzle (TEN HOUTEN and KRAAK 1949) was used. The nozzle assured homogeneous distribution of the herbicide, the size of the drops corresponding to that produced by apparatus used in actual practice.

At the start of the experiments the springtails were transferred to the substrate with the aid of a small brush. During the experiment the dishes were held in a climate room at 15° C, a minimum of 90 % relative humidity (RH), and 8 hours of light (1200 lux). Checks were carried out by eye and, where necessary, with a binocular microscope (x 16).

2.3 Dosage and gas chromatographical analysis of 2,4,5-T

Depending on the method used for spraying in forests 500 to 800 l/ha of a 1.5 or 1 % solution of the herbicide is applied for the control of *Prunus serotina*. Of this quantity, 0.001 to 0.017 ml/dm² of the herbicide ends up on the ground between and under the bushes (EIJSACKERS, in prep. c.), mainly by dripping down from leaves. This leads to local concentrations with much larger quantities of herbicide per surface unit.

The herbicide used in the experiments was AAnetos L, produced by the AAgrunol Company (Groningen, The Netherlands), further to be referred to as the herbicide. It contains 40 % of the isooctylester of 2,4,5-T-trichlorophenoxyacetic acid, which will be referred to as „2,4,5-T". In addition, the compound used for formulating 2,4,5-T (the "formulation agent") was used (made available by AAgrunol, composition: 91 % petroleum derivative as a carrier and 9 % emulgator).

For each petri dish, 0.67 ml of the solution was added to the soil/agar substrate, corresponding to 1.05 ml/dm². The quantitity of the solution deposited in the dishes with the soil substrate by spraying was 0.95 ml, corresponding to 1.49 ml/dm². A series of solutions with 5, 2.5, 1.25, 0.6, 0.3, and 0.15 vol.% of the herbicide was used. Table 1 shows the quantities of herbicide from these values.

Table 1. Concentrations of the herbicide in the solutions applied and the resulting amounts (ml/dm²) on the soil substrate and the soil/agar substrate

Concentration of herbicide solution in substrate	0.15 %	0.31 %	0.62 %	1.25 %	2.5 %	5.0 %	
dosage							
soil-agar	0.0016	0.0032	0.0065	0.0131	0.0262	0.0525	ml/dm²
soil	0.0022	0.0046	0.0092	0.0186	0.0372	0.0745	ml/dm²

To assess the persistence of the 2,4,5-T ester in the soil/agar substrate, the substrate was ground and extracted at 10° C with ethanol for 10 minutes, after which the extract was filtered and methylated according to CLARK (1969).

The soil substrate was directly extracted and methylated. The quantity of 2,4,5-T ester was measured with a BECKER gas chromatograph (column 3 % OV-17 at gaschrom Q 200° C detector ECD Ni^{63} 300° C, gas N_2 30 ml/min over column and 30 ml/min over detector).

A strongly diluted solution (1:5000) was injected into the gas chromatograph. The criterion used for the measurements was the difference between the peaks of the herbicide and of the separately injected formulation agent. Peaks 1, 2, and 3 (fig. 2b) did not occur in the chromatogram of the formulation agent (fig. 2c), but because of the limited heights of peaks 1 and 2 they could not be used for measuring purposes. Peaks 1 and 2 could be measured after the injection of concentrated solution (fig. 2a). Further analysis by thin layer chromatography showed that peak 3 was the 2,4,5-T isooctylester and peaks 1 and 2

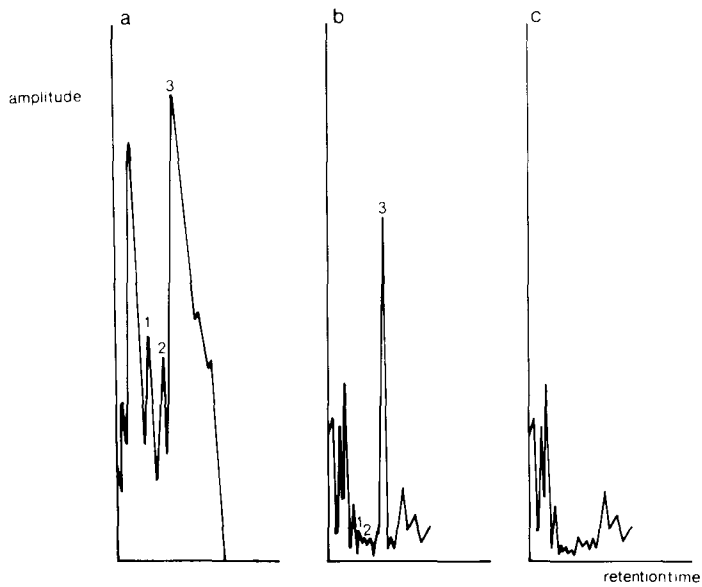

Fig. 2. Chromatograms of AAnetos (2,4,5-T isooctylester) made after injection of concentrated solution (a) and of strongly diluted solution (b). Peaks 1 and 2 represent contaminants in the herbicide and peak 3 the 2,4,5-T isooctylester. Chromatogram C was obtained after the injection of "formulation agent" alone

were caused by contaminants which were also present in the 2,4,5-T isooctylester solution without the formulation agent.

3 Results

3.1 Mortality

3.1.1 Persistence of 2,4,5-T

With this gas chromatographical detection method it was found that the amount of 2,4,5-T isooctylester in the soil substrate did not show a clear decrease within 14 weeks after application (fig. 3). Immediately after application, 35 ml of the 45 ml applied was recovered. In the soil/agar substrate no decrease in the 2,4,5-T content had occurred after more than 14 weeks. From the 1st to the 6th week, however, an increase was detected, probably due to a decrease in the absorption capacity of the substrate. Immediately after the application of 35 ml 2,4,5-T, only 10 ml was recovered.

In the soil substrate, the contaminants showed an almost immediate reduction of 30 %. After 150 hours, only 7 % remained. In the soil/agar substrate, about 20 % of the quantity of contaminants traced immediately after pouring was found after more than 250 hours.

3.1.2 Effect of the herbicide

To assess mortality, 25 springtails were placed in "polished" dishes containing the soil/agar substrate to which a 0.3, 0.6, 1.25, 2.5 or 5 % solution

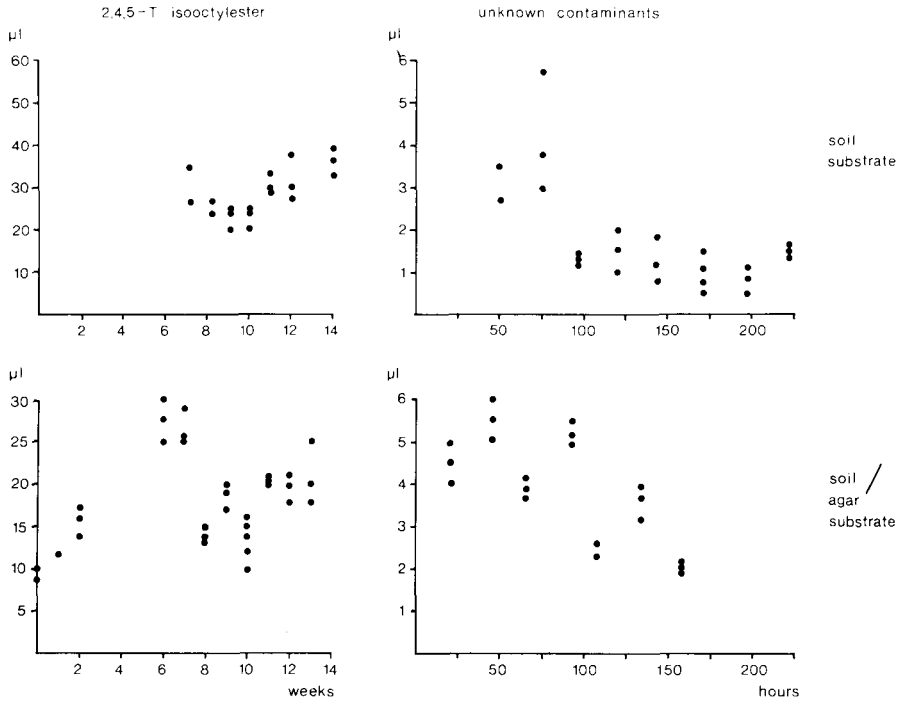

Fig. 3. Persistence of 2,4,5-T isooctylester and unknown contaminants in the soil and soil/agar substrates. Note the difference in scale on the ordinates

of the herbicide had been applied. Ten dishes were used per dose. Over a 40-day period, the dead springtails were counted and removed daily.

An experiment was also carried out with soil substrate to which 0.3, 1.25, and 5 % solutions had been applied. For each dosage, 4 dishes with 25 springtails each were used. These dishes were also checked every day.

Fig. 4 shows the mortality trend for the insects on the soil/agar substrate. The herbicide had a distinct influence, starting with the 1.25 % concentration. The differences between 1.25 % and 2.5 % and between 1.25 % and 5 % were found to be significant from the first day of the experiment onward (t-test, $p < 0.05$), and the difference between 2.5 % and 5 % from the second day onward. The difference between 1.25 % and the untreated substrate became significant after 6 days. No differences were detected between the untreated substrate and the 0.6 and 0.3 % concentrations.

After spraying of the soil substrate (fig. 5), the mortality was substantially higher than for the soil/agar substrate and the mortality trend became much steeper. This might be explained by the larger quantities of herbicide on the soil substrate at the same concentration (see table 1). Moreover, the determination showed that more 2,4,5-T was extractable from the soil substrate (about 80 %) than from the soil/agar substrate (about 30 %).

These observations concerning mortality clearly demonstrate that AAnetos is toxic for the springtail, but the question remains whether only the 2,4,5-T isooctylester is toxic or also certain other components of the herbi-

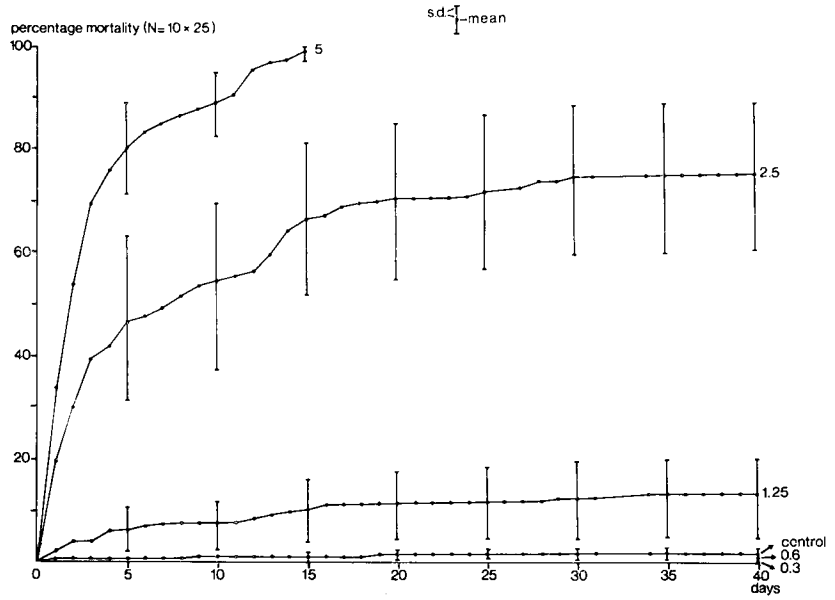

Fig. 4. Cumulative mortality under continuous exposure to the soil/agar substrate (mean + standard deviation)

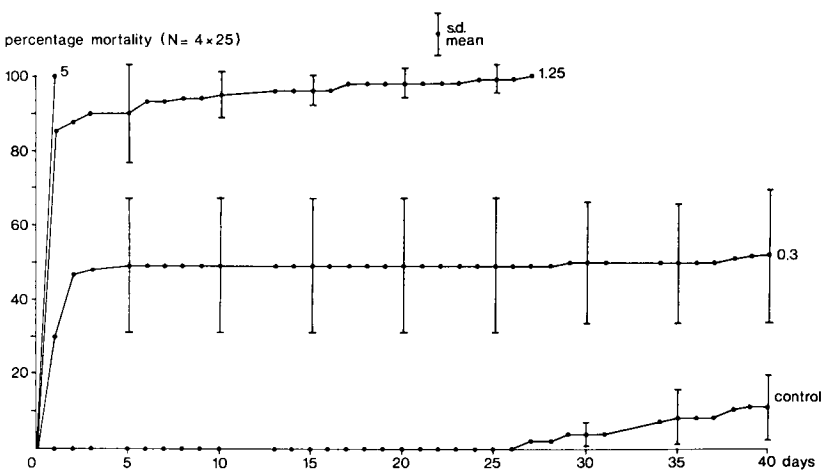

Fig. 5. Cumulative mortality under continuous exposure to the soil substrate (mean + standard deviation)

cide. Other questions concern exactly how the herbicide affects and is assimilated by the springtail and whether all springtails are equally sensitive to the herbicide. In the following sections of this paper, a number of tests and experiments carried out to find answers to these and other questions are discussed.

3.1.3 Herbicide uptake

Since the springtail can come into contact with the herbicide in various ways, the herbicide might act on the springtail in different ways. There may be direct contact when the springtail walks across the substrate. The herbicide could diffuse through the skin (possibly in the gaseous phase) or be taken in through the mouth while the insect is cleaning its legs. Unfortunately, no direct observations that might have provided information on the latter possibility have been made.

Furthermore, the herbicide might be taken in together with food (EIJSACKERS, in press a.).

Uptake of the herbicide in the gaseous phase may be of importance because of the faster penetration into the springtail and deeper penetration into the soil. Although the vapour pressure of the isooctylester itself is rather low (MAIER-BODE 1971), this does not necessarily hold for the formulation agent. HARTLEY (1976) has warned, moreover, that considerable quantities of herbicides with a low vapour pressure may evaporate in moist soil (codistillation).

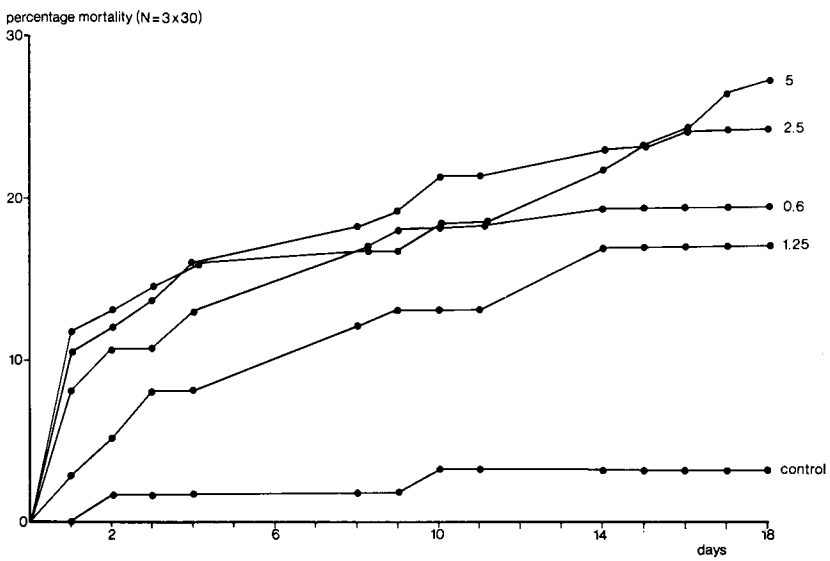

Fig. 6. Cumulative mortality for herbicide application in vapour form

To study its effect in vapour form, the herbicide was applied to a disc of filter paper (⌀ 5 cm) which was fixed to the lid of the petri dishes. To prevent the springtails from reaching the paper, "fluon" was smeared on the edges. The experiment was carried out with 0.6 %, 1.25 %, 2.5 %, and 5 % concentration of the herbicide and a control. For each dose, 3 dishes were used and 30 springtails were placed in each dish on a base of untreated soil/agar substrate.

As fig. 6 illustrates, the herbicide is active in vapour. For all concentrations the mortality was distinctly higher than in the control. However, there is no clear relationship between the concentration of the herbicide and the

effect measured. The effect on mortality was lower than in the case of direct contact. The only possible conclusion to be drawn from these findings is therefore that an influence of the herbicide in the gas phase on the springtail is possible.

3.1.4 Effect on the formulation agent

Apart from 2,4,5-T itself, contaminants of this compound or the formulation agent could also be active. A test was therefore carried out in which the same quantities of the formulation agent were applied to the soil/agar substrate as had been applied in the first mortality experiment. For each dose, 6 dishes with 25 springtails each were used. The dead springtails were counted and removed daily.

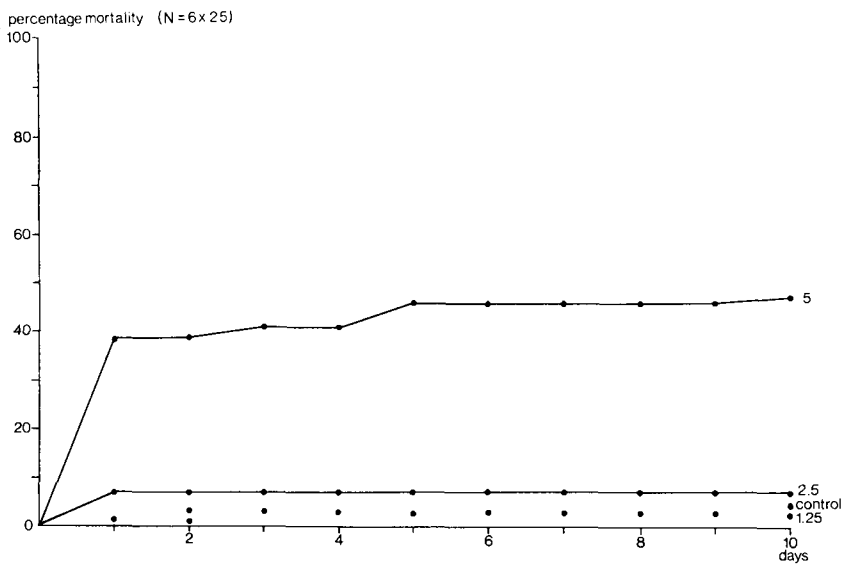

Fig. 7. Cumulative mortality under continuous exposure to the formulation agent of the herbicide AAnetos

Only the 5 % and, to some extent, the 2.5 % concentration led to an increase in mortality, and this mortality was almost completely restricted to the first day (fig. 7). At the 5 % concentration, a wide variation in mortality was seen; in some dishes the mortality percentage was virtually 100, but in others it was very low. Moreover, a substantial number of springtails remained motionless during the first few days but then recovered and survived the test period. These results differ from those obtained with the herbicide itself (fig. 4). The mortality percentage is lower and the increase in mortality lasted for a much shorter period. Judged from these findings, the high mortality during the first few days in the tests in which the complete product was used could have been partially caused by the formulation agent.

To determine whether the toxic influence was exerted by AAnetos alone, a small test was carried out with the herbicide Orga 2,4,5-T (which contains the same quantity of 2,4,5-T isooctylester but a formulation agent with a different composition). In this test, mortality after 10 days at 0.3, 0.6, 1.25,

2.5, and 5 % was, 1, 8, 23, 79, and 81 % respectively, and no mortality was observed in the untreated dishes. It may therefore be concluded that, although the formulation agent also has a certain toxic effect, it is the 2,4,5-T ester and/or the contaminants that are particularly toxic. On the basis of the period in which mortality occurred (predominantly during the first ten days) and the limited persistence of the contaminants compared with the 2,4,5-T ester (see section 2.3), it seems likely that these contaminants have a toxic effect. The very small quantities present made it impossible to extract enough of the substance to permit experimentation. The nature of the impurity is not known, but it may be that the dioxin present in the herbicide is to blame. We did not determine the dioxin content of the samples used, but it was at any rate less than 0.05 ppm, and probably less than 0.01 ppm (BESEMER, pers. comm.). Because dioxin is highly poisonous, no research was done on this point.

3.1.5 Influence of the age of the springtails

The extent to which the age of the springtails effects their susceptibility to the herbicide may be important, because in the spring, when the herbicide may be still persistent in soil, young springtails are abundant. If they were particularly sensitive, the mortality of the population in the field would be higher than would be inferred from fig. 4, which is based on adult insects.

To investigate this point, the springtails were classified according to length (see PETERSEN 1971) into a number of arbitrary groups. The mean length ± standard deviation per group was as follows: group I: 1.24 ± 0.23 mm, group II: 1.71 ± 0.18 mm, group III: 2.22 ± 0.18 mm, and group IV: 2.82 ± 0.16 mm.

The springtails were placed on a soil/agar substrate containing 2.5 % herbicide. For each group, 3 dishes with 25 springtails each were used. For the determination of mortality on untreated substrate, only 1 or 2 dishes could be used for each length category because of the limited number of insects available. The dead springtails were counted and removed daily. The results are shown in fig. 8.

Group I is the most striking of the four, because of the rapid rise of the number of deaths during the first few days. In this group the mortality on

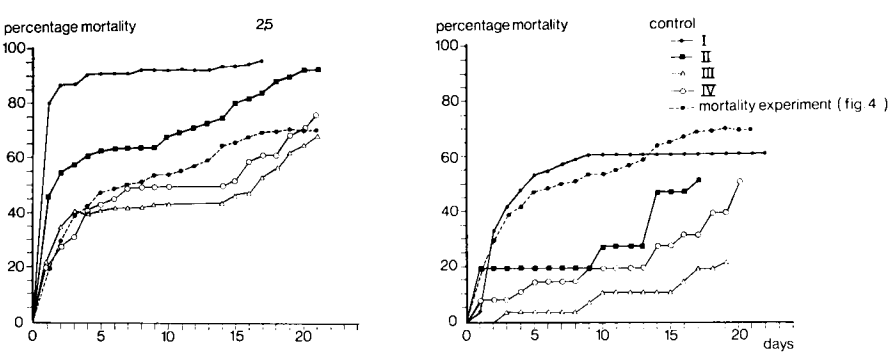

Fig. 8. Cumulative mortality in groups with different body length (I: 1.24 mm, II: 1.71 mm, III: 2.22 mm, IV: 2.82 mm) on the soil/agar substrate. For comparison, the dotted line indicates the mortality trend for the same dosage for adult specimens (see fig. 4)

the untreated substrate was also considerable. Mechanical damage caused during the transfer of these tiny insects may have played a role in these experiments. Overall mortality – attributable to various factors including the herbicide – is very high in these young springtails. Juvenile specimens also proved to be susceptible to smaller doses (see below). In group II, III, and IV the relative mortality was roughly the same. When the mortality figures for these groups on the treated substrate are adjusted for mortality on the untreated substrate, as recommended by HEALY (1952), the mortality after 10 days of exposure to the herbicide was 58 % in group II, 35 % in group III, and 35 % in group IV.

A second experiment was carried out with juveniles which had just emerged from the egg (length 1.0–1.2 mm). The concentrations were 0.6 and 0.3 (control: soil/agar substrate). The dishes were checked daily and the number of juveniles counted.

The decrease in the number of live animals slowed down during the first 10 days, after which the number declined more rapidly. The decrease from the 10th day onward is plotted in fig. 9 according to KUENEN (1957). The angle which a line through the points obtained with the 0.3 and 0.6 % solutions makes with the abscissa is wider than in the case of the insects in the control dishes. In these concentrations the mortality in large springtails hardly differed from that in the control (see section 3.1.2). These results justify the conclusion that young (small) springtails are susceptible to the herbicide even at low concentrations. This susceptibility persists up to a certain age (size). In the specimens used in the other experiments, this difference in sensitivity played no part.

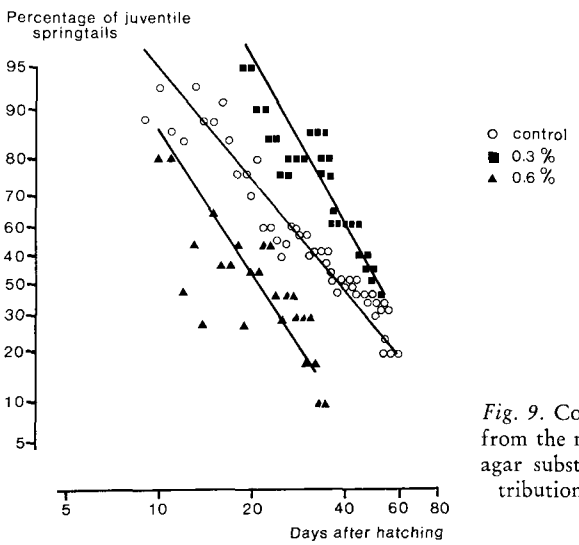

Fig. 9. Course of the number of juveniles from the moment of hatching, on the soil/agar substrate. Abscissa: logarithmic distribution ordinate: normal distribution

3.1.6 Onset and duration of the toxic action

In all of the experiments discussed so far, the springtails were continuously exposed to the action of the herbicide. In the field, however, the mobility

of the insects is not restricted, which means that the following questions should be taken in consideration.

I. How long must a springtail stay on a treated spot before becoming irreversibly affected?

II. How long after a spraying operation can a springtail arrive at a treated spot without running the risk of being affected?

To gather information in connection with question I, springtails were placed for 6, 12, or 24 hours on a soil/agar substrate containing a 1.25, 2.5, or 5 % herbicide solution, after which they were transferred to an untreated substrate. The dead springtails were counted and removed every day. For each period and dosage, 4 dishes with 30 or 35 springtails each were used. Concurrently, 5 dishes with 30 springtails each on untreated substrate served as controls.

Table 2. Mortality 10 d after exposure for 6, 12, and 24 h (the percentages for 240 h of exposure are derived from fig. 4)

Exposure (in h)		6	12	24	240
concentration	1.25 %	4 ± 2	3 ± 1	9 ± 3	7 ± 8
	2.5 %	7 ± 2	7 ± 1	11 ± 6	52 ± 41
	5 %	9 ± 3	28 ± 7	21 ± 4	88 ± 16
	control	no mortality			

The percentage of springtails that died after 10 days (table 2) show that mortality increased with increasing concentrations, even after brief exposure. No clear relationship was found between the duration of exposure and mortality during the first 24 hours. After this period, the toxic action causes a significant difference in mortality, as shown by comparison of these data with the mortality data obtained under continuous exposure for 10 days. After 24 hours of exposure, the mortality after the 10th day was lower for 2.5 and 5 % than for continuous exposure during the same 10-day period.

To answer question II we had to know how long the herbicide remained active in the substrate. This was determined by placing springtails in dishes with the soil/agar substrate 1, 3, 5, and 7 days after the herbicide had been poured into it, and assessing the mortality after 10 days had elapsed. For each concentration (1.25, 2.5, and 5 %) and for each period, 3 dishes with 25 specimens each were used and after the first day 3 control dishes were added.

Table 3. Mortality after 10 d of continuous exposure to a soil/agar substrate prepared 1, 3, 5, and 7 d before the insects were placed on it

Interval after preparation of substrate (d)		1	3	5	7
concentration	1.25 %	68	11	15	17
	2.5 %	91	51	36	61
	5 %	100	79	71	95
	control	11			

The activity of the herbicide declined after the first day in all three concentrations (table 3). Nevertheless, the herbicide can also harm springtails after the first day. In the following period (3 + 10, 5 + 10, and 7 + 10 days), however, there was no further drop in mortality at these three concentrations.

3.1.7 Discussion

The mortality of *Onychiurus quadriocellatus* increases after direct contact with AAnetos. This is due not only to the active ingredient of this herbicide (2,4,5-T isooctylester) but also to the formulation agent and possibly also to at least one impurity. Since corresponding results were obtained in experiments with Orga 2,4,5-T, it must be concluded that these side effects do not apply exclusively to the AAnetos used in our experiments.

Although the dosages at which mortality was observed in the laboratory (from 1.25 % ~ 0.012 ml/dm²) correspond to quantities found in woods under *Prunus serotina* shrubs (0.006 to 0.017 ml/dm²), the laboratory conditions described above cannot be directly compared with the situation in the field. For one thing, in the experiments the springtails were placed in closed dishes containing a homogeneous substrate and kept in a climate room and under these conditions the springtails were continuously exposed to the influence of the herbicide. Moreover, the herbicide could evaporate freely, and displayed strong persistence.

However, evaporation of the herbicide is not of great importance, as borne out by the weak influence of the herbicide in the gaseous phase on springtail mortality. Similarly, in a number of experiments in which open dishes were used (not discussed in this paper), the mortality was only very slightly less than in comparable experiments done in closed dishes. The persistence of chlorophenoxy herbicides also appears to be substantial in coniferous litter (TORSTENSSON 1974), plants, toadstools (WELLENSTEIN et al. 1975), and fallen, sprayed leaves of black cherry (EIJSACKERS in prep. b.).

The experimental conditions in the climate rooms were adjusted such that they were in line with the average values in the litter layer in autumn (VAN DER DRIFT, pers. comm.). The above-mentioned differences between the laboratory and field situations therefore do not seem to have any significant influence.

Young springtails seem to be more sensitive to AAnetos than older ones are. SANOCKA-WOLOSZYN and WOLOSZYN (1970) observed a considerable reduction in the number of young springtails *(Folsomia candida)* after exposure of the insects to the herbicides prometryne, aphalon, and chlorpropham. This implies that the effect on the population as a whole may be greater than might be inferred from the present mortality tests (with adult insects).

Because the herbicide affects the insects mainly through direct contact, the intensity of the effect depends on the duration of the contact and on the interval between the application of the herbicide and the occurrence of the first contact. In the case of brief contact (up to 6 hours), mortality is low and the springtails proved to be capable of recovery. More prolonged contact (up to 24 hours) caused higher mortality. If the springtails only come into contact with the herbicide after 24 hours, mortality is low. Consequently it is important for springtails not to come into direct contact with the herbicide

during the first 24 hours after spraying and that they quickly leave or avoid soil on which the herbicide has been deposited.

To find out whether a role is played by the insect's avoiding or leaving treated soil, the influence of the herbicide on the activity (mobility) of the springtail was studied, the aim being in particular to determine the extent of the influence of the herbicide in concentrations which under permanent exposure tend to increase the mortality rate in springtails.

3.2 Activity

3.2.1 Mobility

A number of direct observations were made to determine the influence of the herbicide on springtail mobility. In a climate room (1200 lux, reduced to 45 lux between 6 p.m. and 6 a.m. hours), 25 springtails were placed in petri dishes (⌀ 9 cm) containing a soil substrate incorporating the herbicide in concentrations of 0.15, 0.6, and 2.5 %. Four dishes were used for each concentration. During the first 24 hours, with a 3-hour interruption, the number

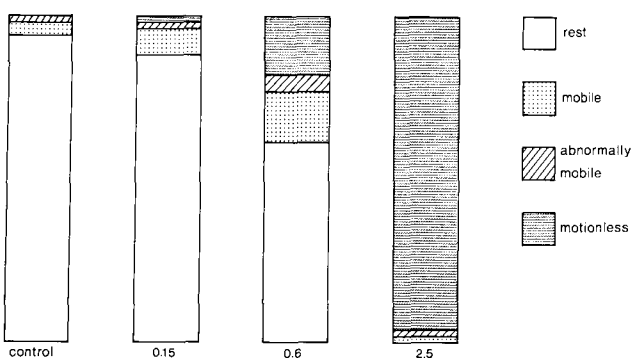

Fig. 10. Breakdown of various reactions to direct contact with the herbicide during the first 12 hours of exposure to the soil substrate, expressed as percentages

of insects displaying a particular form of mobility was recorded every half hour. The following criteria were adopted for this purpose:

At rest: no noticeable displacement, only slight vibration of antennae;

Walking: the individual displays a regular rhythm of motion and generally walks in a straight line;

Abnormally mobile: virtually no noticeable displacement; the individual displays violent spasms with strong arching of the body;

Motionless: no noticeable movement; here, a distinction was made between insects lying in an arched or a stretched position. The distinction between "motionless" and "rest" was made on the basis of a difference in habitus, the "motionless" specimens being more stretched as well as thinner.

The results of the observations were initially quantified for the first 12 h, because in this period the reaction pattern showed the most variation. Fig. 10 gives the percentages of the various reactions.

In the treated dishes the springtails were "at rest" most of the time. A low dose of the herbicide (0.15 %) caused mobility to increase. At the 0.6 % concentration, 5 % of the springtails became abnormally mobile, whereas others were inactivated (18 % arched and motionless). At the 2.5 % concentration inactivation was so strong that only a few of the insects still showed movements during the first 12 h. As can be seen from fig. 11, the mobility showed a clear trend as time progressed. In the control, the walking activity

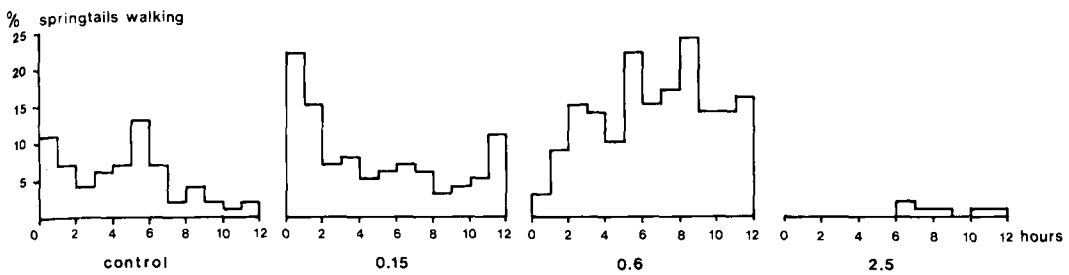

Fig. 11. Trend of the percentage of walking springtails during the first 12 hours of exposure to the herbicide on the soil substrate

showed, probably as a result of a disturbance at the start of the test, a clear peak at the beginning and after 6 h, but then gradually declined, reaching the lowest level after 12 h. At a low dosage (0.15 %), which caused only activation, the first few hours were characterized by considerable walking activity, followed by a rapid decrease after 2 h and remaining at the level seen in the controls from 3 to 8 h, again showing a distinct increase. The 0.6 % dose, which caused both activation and inactivation, produced a gradual shift to reactions indicative of a diminishing influence of the herbicide. During the first few hours, most of the springtails were motionless whereas others showed arching spasmodically. These reactions then diminished and walking activity increased. This increase continued during the first 12 h, exceeding that in the control and the 0.15 % concentration. A similar shift was observed in a higher concentration (2.5 %) although only a few springtails displayed any activity as this dose.

The most important conclusions to be drawn from these data are that the herbicide has an activating effect in dosages of 0.15 % and 0.6 % and an inactivating effect in a dosage of 2.5 %.

In further experimentation, activation proved to continue for the first 24 h, with on average 12 % of the springtails walking at the 0.6 % concentration, 8 % at the 0.15 % concentration, and 5 % in the untreated dishes. The differences between the control and both doses (Student, $p < 0.01$) and between the two doses ($p < 0.05$) are significant. To find out whether activation is the result of continuous action of the herbicide on the springtails or a single stimulus springtails were transferred to the untreated substrate after 8 h of continuous exposure (fig. 12). The activity increase, which was probably caused by the disturbance associated with the transfer (see control in fig. 11), diminished rapidly. The activation level in the control and at 0.15 % was roughly the same as in the preceding experiment but was lower at 0.6 % which suggests that the increased activity diminishes on withdrawal of the stimulus arising from direct contact with the herbicide.

The influence of the formulation agent on activity was determined by direct observation of springtails treated with a dosage corresponding with 0.6 % herbicide. After the insects were sprayed, the percentage of active springtails did not exceed that of the controls during the first 8 h.

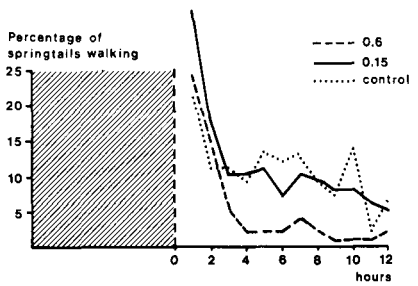

Fig. 12. Course of the numbers of walking springtails on untreated soil substrate after 8 hours of exposure to the herbicide on the soil substrate

The enhanced activity (mobility) may lead to more frequent contacts with the herbicide. If the herbicide possesses repellent properties the springtail would avoid it sooner or after contact would leave the sprayed spot faster than it would an unsprayed spot. In the latter case, if the springtail is to survive, the speed with which it leaves the spot with the herbicide would have to be greater than the speed with which the substance acts. The avoidance of or departure from spots where the herbicide is present may be the result of continued activity while contact lasts, in contrast with a quieter form of behaviour on untreated spots. Alternatively, the reaction may result from repellency, the springtail making a deliberate choice.

3.2.2 Repellency and escape

3.2.2.1 Choice observations

The springtails' reaction on coming into contact with the herbicide was investigated in direct observations. For these observations use was made of petri dishes (⌀ 9 cm) divided by low partitions into four compartments. Two opposite sections were filled with soil/agar substrate only and the other two with soil/agar substrate containing the herbicide (1.25, 2.5, and 5 %). The sections were filled up to the edge of the partitions, so that the latter did

Table 4. First choice, total number of changes, and mobile period of springtails with access to both untreated and treated substrate

	Control		1.25 %		2.5 %		5 %	
	—	—	—	+	—	+	—	+
first choice	8	12	14	6	17	3	16	4
total number of changes	40	40	38	22	29	9	20	6
period of stay (in s)	237	271	455	60	523	46	442	83
total mobile period (in s)	237	271	355	49	369	24	198	16
percentage of time mobile in each section of dish	100	100	78	82	71	53	45	19

— = without 2,4,5-T
\+ = with 2,4,5-T

not form an obstacle for the springtails. At the beginning of each experiment, the insects were placed one by one in the centre where the partitions intersected. In the direct observation experiments, this was done with one springtail at a time. The activity of these individual springtails was recorded by tape recorder for 10 min. For each herbicide dosage, 20 springtails were tested (4 series of 5 observations).

In the control dishes (table 4) the springtails showed no clear preference for or rejection of any of the compartments, either in their first or subsequent choices, but after the herbicide was applied, most of the insects entered the untreated sections. This difference is significant ($p = 0.055$). The differences between the two treated sections and between the two untreated sections are not significant for any of the (separate) experiments.

A number of springtails gave the impression of making a clear choice. At first, these specimens spent some time turning round and round at the intersection of the partitions, swinging their head above a treated section for a few seconds and then turning away. After having done this repeatedly, they invariably entered the untreated section. From this behaviour and the findings from the "first choice" observations it may be concluded that avoidance of the herbicide is partially based on repellency leading to an explicit choice. Variations of the dosage did not produce a distinct difference in the springtails' first reaction.

A second criterion for avoidance of the herbicide by the springtails is the length of time spent in the treated and untreated sections. The duration of the visits to treated sections decreased as the dosage increased. At a 5 % concentration the duration of the stay increased. As already mentioned in connection with activity, a high dosage led to inactivation. This is shown by the data in table 4 indicating the mobile period of the insects and the percentage of time spent actively in each section of the dishes. Such inactivation means that some of the insects would find it difficult or impossible to leave a high-dose section. At the higher concentrations the number of times that insects entered a section diminished.

The fact that the number of entries was much higher than the number of first entries shows that the repellency is not absolute. Indeed, springtails that had first entered an untreated section sometimes went on to a treated section, but they only stayed there very briefly (for duration of the stay, see above) and almost without exception returned to the untreated substrate.

3.2.2.2 Distribution pattern with free choice

With a view to determining the way in which the springtails' reaction on treated substrate affects their ultimate distribution, the distribution pattern was studied in 25 springtails released in the centre of a dish. For each dosage of 5, 2.5, or 1.25 %, 4 dishes were used. During the first 10 h, the number of insects found in each section was recorded every hour. After that they were counted once a day, and the number of dead insects was also recorded. The latter were not removed so as not to disturb the others, which would have distorted the distribution pattern. Fig. 13 shows distribution during the first 10 h. The null hypothesis in this experiment was that the springtails had no clear preference or aversion and that 50 % of the insects would therefore end up in the sections having the same position as the treated sections. After the first hour a small number of springtails was found in the treated sections,

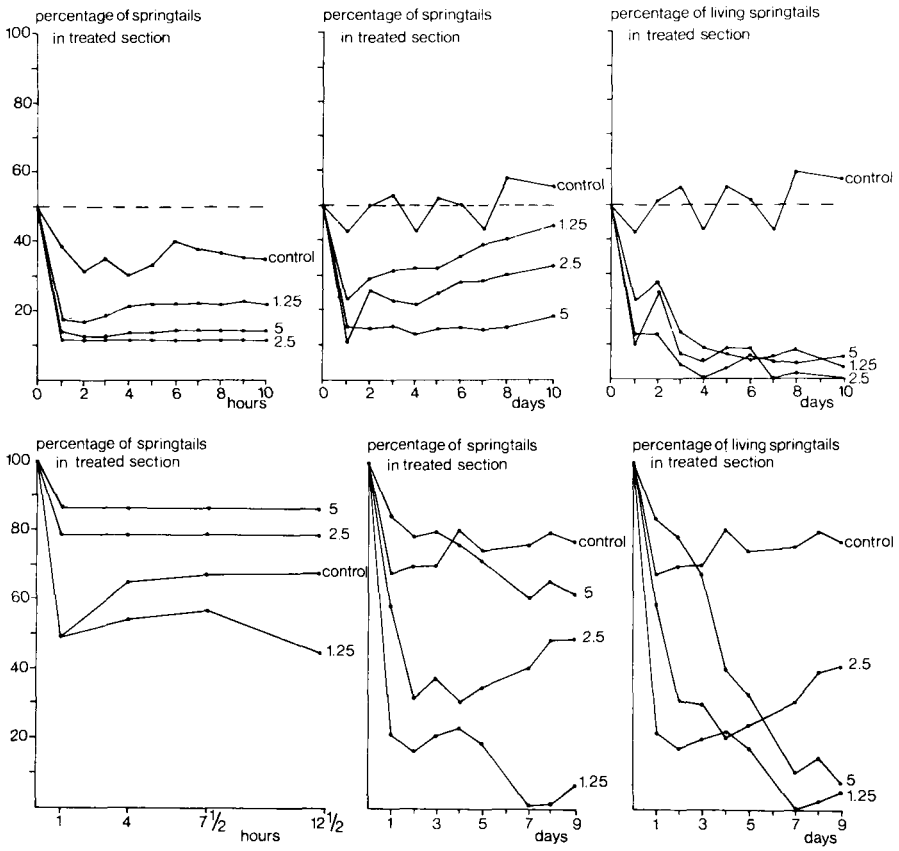

Fig. 13 (top left). Course of the percentage of springtails on compartments with treated substrate during the first 10 hours after being placed in the centre of a dish with treated and untreated compartments. – *Fig. 14 (bottom left).* Percentage of springtails on compartments with treated substrate during the first 12½ hours after being placed on treated compartments in a dish with an equal number of treated and untreated sections. – *Fig. 15 (top center).* Percentage of all springtails on treated substrate during the 10 days after being placed in the centre of a dish with treated and untreated sections. – *Fig. 16 (bottom center).* Percentage of all springtails on treated substrate during the 9 days after being placed on the treated parts of a dish in which half of the sections had been treated. – *Fig. 17 (top right).* As fig. 15, but percentage of living springtails only. – *Fig. 18 (bottom right).* As fig. 16, but percentage of living springtails only

the rest having proceeded to the untreated sections. The distribution showed very little variation for the different dosages, and in all cases clearly deviated from distribution in the control dishes.

3.2.2.3 Distribution pattern on treated substrate

In conjunction with the foregoing experiment, the springtails' reaction after being placed on treated substrate was followed. Twentyfive springtails were placed in the two sections with treated substrate, and 4 dishes were

used for each concentration. Data were collected during the first 12 hours and after that once a day for 9 days.

Table 5. Percentage of springtails on treated sections of the substrate with free choice or after being placed on the treated substrate after 1 h, 1 d, and 8 d

	Free choice between treated and untreated substrate				After being placed directly on treated substrate			
	control	1.25 %	2.5 %	5 %	control	1.25 %	2.5 %	5 %
% springtails in treated sections after								
1 h	38	12	14	16	49	49	78	86
1 d	43	23	11	15	66	22	57	84
8 d	58	40	30	15	78	7	49	62

In all cases some of the springtails managed to leave the treated part of the substrate during the first hour (fig. 14 and table 5). The percentage of insects that escaped from the treated sections decreased as the concentration increased, and was lower at 2.5 and 5 % than in the preceding free-choice experiment. In the control dishes, too, some of the springtails escaped from the sections in which they had been placed.

After the first hour, no further change occurred for the two higher concentrations, whereas the percentage of insects in the treated sections at 1.25 % continued to decline until there was an even distribution over the treated and untreated parts of the substrate. In the control dishes the number of springtails in the sections having the same position as the treated sections showed a slight increase. This may possibly be due to the pheromones produced by the insects themselves which, as shown by VERHOEF et al. (1978), may influence springtail distribution.

3.2.2.4 Distribution pattern over 10 days

In both of the two experiments described above the distribution patterns were recorded for 10 days.

After one day the differences had not changed for the 5 % concentration (see table 5). In the dishes with 1.25 and 2.5 % the percentage of escaping insects increased. This increase continued during the following days, but after the eighth day the percentage of springtails in the treated sections was still higher than under the condition of free choice between treated and untreated sections except at the 1.25 % concentration. Under free choice the percentage of insects in the treated section gradually increased. This is illustrated in figs. 15–18.

When the springtails had a free choice (fig. 15), after the first day 10 till 20 % were found on the treated parts of the substrate, at all doses. When the springtails were placed on the treated substrate (fig. 16), the percentage still present there after one day increased with increasing concentration; in other words, at a higher dosage fewer springtails succeeded in escaping to the untreated parts of the substrate. This difference is the result of inactivation caused by the herbicide (see section 3.2.1). Thus inactivated, a number of insects will fail to escape and subsequently die. This holds not only for the

early stages of the experiment but also for springtails reaching the treated substrate later. Similar to the findings made in the earlier experiments with respect to the onset and duration of the toxic action, the risk of death was lower for springtails that managed to escape during the first 24 h. Furthermore, the springtails that arrived on the treated substrate after the first 24 h ran a lower, though still appreciable risk of dying. If we consider only distribution of the living springtails (figs. 17 and 18), we find for all doses a very distinct avoidance of the treated parts of the substrate, both in the case of free choice and after placement on the treated substrate. From the even distribution in the control dishes of the free-choice experiment, there do not seem to have been other factors apart from the herbicide that influenced distribution. In the second experiment there was no change in distribution after the first day either. It is not clear why the distribution in this experiment was not even. It is not very likely that the amount of light played a role, since opposite sections had been treated and left untreated.

3.2.2.5 Effect of activity on mortality

In addition to the above experiments, the springtails' reaction after having them placed on treated substrate was also investigated in such a way that a distinction could be made between the mortality of escaped and not escaped springtails. In this experiment the springtails could not return after escaping. Ordinary unpartitioned petri dishes (⌀ 9 cm) provided with soil/agar substrate were employed, but space was left between the lid and the dish (unlike the situation in the partitioned and the polished dishes) to enable the springtails to leave the dish. Escaping insects were caught in a larger waterfilled petri dish (⌀ 12 cm) in which the experimental dish had been placed. The springtails floated and could thus be counted, as were the living and dead insects remaining in the experimental dish. The escaped springtails from some of the dishes were placed in small dishes (⌀ 4 cm) provided with moist sand, and the living and dead specimens were counted after 10 days.

The experiment was carried out with herbicide concentrations of 5, 2.5, and 1.25 % and a control. For each dosage, 12 dishes with 25 springtails each were used. Mortality among the escaped springtails was determined in 4 dishes at 1.25 % and 2.5 % and in the control and in 6 dishes at 5 %. Fig. 19 shows the percentage of escaped insects, the percentage that remained and survived on the substrate, the percentage that remained and died, and the percentage of dead insects eaten by the others. Eating of the dead within the same species (necrophagy) is frequently found in *Onychiurus quadriocellatus*. The percentage of springtails that died after escaping is shown in table 6.

Because in this experiment it was possible to determine accurately how many insects escaped and what occurred afterward, we will discuss this aspect first.

The percentage of insects that escaped on the first day was very substantial, even in the control dishes and at 1.25 %, and later rose to 70–80 %. At 2.5 and 5 % the escape trend was slower. After 10 days the percentage of escaped insects at 2.5 % was also about 80, but at the 5 % concentration this percentage was distinctly lower. For all doses, 6–12 % of the escaped insects died but only 1 % at the control (table 6).

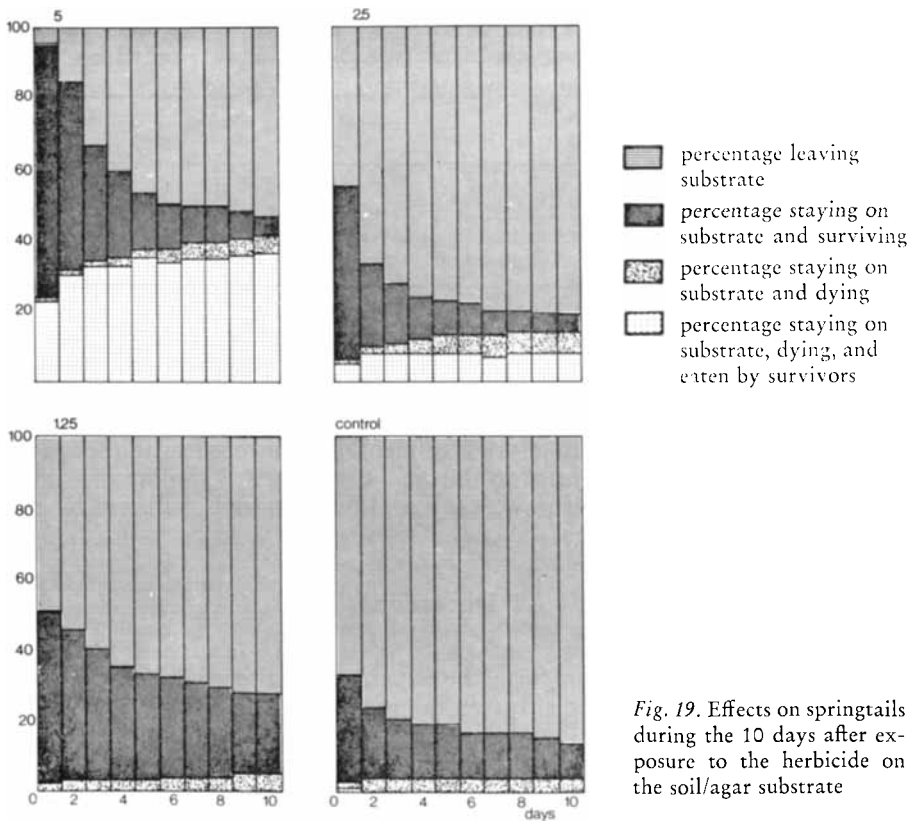

Fig. 19. Effects on springtails during the 10 days after exposure to the herbicide on the soil/agar substrate

Table 6. Number of springtails that escaped from the treated soil/agar substrate during 10 d after exposure and number and percentage of escaped insects that died within 10 d of escaping

	Total number of springtails	Number escaped	Number dying after escape	Percentage mortality
control	100	86	1	1 %
1.25 %	100	47	3	6 %
2.5 %	100	85	10	12 %
5 %	150	79	7	9 %

The percentage of living springtails that remained on the substrate declined rapidly and was distinctly lower for the concentrations of 5 and 2.5 % than for 1.25 % and the control. The difference between 1.25 % and the control after 10 days was not significant.

The percentage that remained on the substrate and died rose to 40 % for 5 % concentration, reached 14 % for 2.5 % concentration, and at the lowest dosage (1.25 %) equalled the mortality in the control dishes (less than 5 %).

If the mortality level after escape is assumed to have been equal in all dishes, the distribution after 10 days would be as illustrated in table 7.

The mortality percentage after 10 days where escape was possible, was distinctly lower than in the experiment in which the springtails were con-

Table 7. Survey of the fate of a group of springtails 10 d after being placed on treated substrate, expressed as percentage derived from the data in fig. 19 and table 6

	Remained on the substrate		Escaped from the substrate		Total mortality
	alive	dead	alive	dead	
control	10	5	85	0	5
1.25 %	25	5	65	5	10
2.5 %	5	15	70	10	25
5 %	5	40	45	10	50

stantly exposed to the herbicide and had no way of escaping (cf. fig. 4). Contact with the herbicide does not promote the escape of the insect – probably due to the immobilizing action of the compound – as indicated by the lower escape percentages in the various concentrations as compared with the control. Under these particular experimental conditions it was impossible for escaped springtails to return to the dish. Consequently, unlike the situation in the free-choice experiment, they could no longer be affected by the herbicide.

4 Discussion

The conclusion to be drawn form the experiments discussed above is that the herbicide has a repellent effect on the springtail. This repellency can be observed at both high and low doses. The repellency acted on not only the springtails on the treated substrate but also those which never entered the treated compartments.

The repellent effect of the herbicide is very rapid: during the first hour after exposure the majority of the springtails avoided or left the treated substrate. Nevertheless, this effect is not absolute. Not all of the insects will be able to avoid contact with the herbicide or continue to avoid subsequent contacts.

Moreover, there is a second factor to be taken into consideration for the insects on the treated substrate. The herbicide also affects the springtails' mobility. In low concentrations they are activated, but in higher concentrations (2.5 and 5 %) they are inactivated. Only a very small proportion of the insects thus inactivated managed to leave the treated substrate during the first hour after exposure. The others remained on the treated substrate and consequently continued to be exposed to its action, which is strongest during the first 24 hours. After this initial period some of the insects recover, becoming active again and leaving the substrate after some time; nevertheless, for all of the springtails in this category the mortality risk is higher.

In the field the repellency process will occur mainly on a limited surface (viz. in the local concentrations under the bushes). Here it should be added that repellency is not absolute in the field either. After a few days some of the springtails will return to the treated substrate. But the duration of the herbicide's action is such that the springtails can still be affected by it, with fatal consequences for a number of them. It must therefore be concluded that the herbicide, despite its repellent effect, may be lethal for springtails.

It should be borne in mind that these experiments concerned short-

term effects. It is possible that certain effects are not discernible in the insects' behaviour or mortality but that they nevertheless affect other processes, e.g. reproduction, after continuous exposure to non-lethal doses.

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Zusammenfassung

Nebenwirkungen des Herbizids 2,4,5-T auf den Springschwanz Onychiurus quadriocellatus Gisin (Collembola)

Die Anwendung des Mittels 2,4,5-T zur Bekämpfung von *Prunus serotina* und *Betula* spp. hat in den Niederlanden sowohl in Wäldern als auch in Naturreservaten in der letzten Zeit mehr und mehr zugenommen.

Es stellte sich heraus, daß in Laborversuchen, wo erwachsene Tiere von *Onychiurus quadriocellatus* Gisin – als Vertreter der Collembolengruppe – ständig dem Einfluß des Mittels ausgesetzt waren, eine Dosis von 0,003 ml/dm² schon eine ziemlich hohe Mortalität verursachte. Die schädliche Wirkung war dabei nicht nur der wirksamen Substanz (2,4,5-T isooctylester) des Mittels zuzuschreiben, sondern auch den Formulierungsbestandteilen.

Die Toxizität wurde hauptsächlich durch direkte Berührung mit dem Mittel verursacht, jedoch nahm bei Ausbringung in Dampf-Form die toxische Wirkung ab.

Die Jungtiere zeigten größere Empfindlichkeit als die Adulten. Das Unkrautbekämpfungsmittel beeinflußte weiterhin die Mobilität der Springschwänze. Bei hoher Dosis (0,025 ml/dm²) kam es zu einer Abnahme der Mobilität, bei geringer Dosis (0,003 ml/dm²) zu einer Zunahme. Außerdem zeigte das Herbizid eine abschreckende Wirkung auf die Springschwänze. Dennoch starben die meisten Versuchstiere, weil es ihnen nicht gelang, den vergifteten Stellen zu entrinnen. Die Herbizid-Akkumulationen, die in der Praxis in Waldböden gefunden wurden, wechselten wie in den Laborversuchen zwischen 0,0005 und 0,017 ml/dm². Deshalb ist es sehr wahrscheinlich, daß bei der Verwendung des Unkrautbekämpfungsmittels 2,4,5-T in unseren Böden ebenfalls schädliche Nebenwirkungen auftreten.

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