Physical conditions affecting pyrethroid toxicity in arthropods

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Physical conditions affecting pyrethroid toxicity in arthropods

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Stellingen

1. Met deltamethrin vergiftigde spinnen sterven door uitdroging.
   - dit proefschrift.

2. Immobilisatie en wateruitscheiding als gevolg van vergiftiging met deltamethrin zijn van elkaar onafhankelijke processen.
   - dit proefschrift

3. De tegenstrijdigheid in de literatuur over de invloed van temperatuur op de toxiciteit van pyrethroïden heeft de volgende oorzaken:
   a. In experimenten worden immobilisatie en mortaliteit zelden onderscheiden.
   b. Immobilisatie vertoont een negatieve temperatuur-effect relatie, wateruitscheiding daarentegen een positieve.
   c. Pyrethroïden verschillen in de mate waarin ze immobilisatie en/of wateruitscheiding veroorzaken.
   - dit proefschrift.
   - G. Scott and F. Matsumura 1983. Pestic. Biochem. Physiol. 19: 141-150.

4. Het effect van toxische stoffen op de waterhuishouding van arthropoden verdient uit ecotoxicologisch oogpunt meer aandacht.
   - Sun Y.-P., Johnson E.R. 1972. J. Econ. Entomol. 65.
   - Kramer R.D. *et al.* 1989. J. Econ. Entomol. 82.
   - dit proefschrift.

5. 'No one would now dream of testing the response to a treatment by comparing two plots, one treated and the other untreated'.
   - R.A. Fisher (1930).

6. Omdat de theorieën van Lamarck wel van toepassing zijn op cultuur, gaan wetenschap en kunst een steeds snellere ontwikkeling tegemoet.

7. De levenskwaliteit van toekomstige generaties wordt sterker bepaald door hun cultuur dan door hun aantal.

8. Het ecologisch verantwoord beleggen van de Nederlandse pensioengelden zou een steun van 170 miljard gulden betekenen voor het milieu.

9. Er dient voor te worden gewaakt dat langdurige toediening van antibiotica bij imuundeficiëntie de resistentieontwikkeling van besmettelijke humane bacterie- en schimmelziekten niet onaanvaardbaar vergroot.

10. Naar 't Twents: 'Ak ow geliek gewe', zee de boer,'dan zi'w doaluks oetkuiert'.

Stellingen behorende tot het proefschrift:
'Physical conditions affecting pyrethroid toxicity in arthropods'
Gerard Jagers op Akkerhuis, Wageningen, 17 september 1993

Voor mijn ouders

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VOORWOORD

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HET IS KLAAR

ABSTRACT

The aim of this thesis was to obtain mechanistic information about how the toxicity of pesticides in the field is affected by physical factors, pesticide bioavailability and arthropod behaviour. The pyrethroid insecticide deltamethrin and linyphiid spiders were selected as pesticide-effect model. In Part I of this thesis the relationships between the toxic action of deltamethrin, physical factors and spider behaviour were studied in field experiments, and attention was focused on the contribution of walking behaviour to pesticide exposure. In Part II laboratory experiments were carried out where special attention was payed to residual bioavailability of deltamethrin and the effects of this compound on the behaviour and hygrothermal physiology of female *O. apicatus*.

Part I: Field studies

Field studies have shown that high walking activity in spiders after deltamethrin application leads to a strong reduction of the trapping success (Chapter 6). This supports the hypothesis that walking activity is an important means of exposure to residues of deltamethrin for spiders on the soil surface. The testing of the above hypothesis was made possible by the spraying of the pesticide under conditions of low and high spider walking activity, the use of small plots that still allowed for independent replication, and the use of a low level of toxicant application rate. The low application rate was chosen so that trapping success would then depend both on physical factors and the effect of the toxicant. The prediction of daily spider walking activity in relation to physical factors was based on observations of the effects of physical factors and internal activity rhythms on trapping success. Factors showing a very high positive or negative relationship with trapping success were identified in an experiment in which the number of spiders trapped daily in 30 pitfall traps for a period of 72 days was correlated with a number of easily measurable physical factors using multivariate analysis (Chapter 2). Another experiment demonstrated that spiders posess highly predictable, species dependent nicthemeral activity rhythms which are little affected by varying physical conditions, the latter implying an internal mechanism, i.e. some physiological factor (Chapter 3). It was shown that the independence of treatments for at least one week could be assured when traps in a certain field are surrounded by a similarly treated boundery of 7 meters, as spider migration into a sprayed plot was found to be relatively slow; however, the spatial distribution of spider activity showed rapid changes in relation to microclimatic conditions in the crop (Chapter 4). An appropriate low dose rate for deltamethrin was determined to be between 0.2 and 0.5 g ai ha^{-1} deltamethrin (Chapter 5).

Part II: Laboratory studies

Laboratory experiments were used to study the bioavailability of deltamethrin in substrate, and the effect of deltamethrin on the hygrothermal physiology and behaviour of *O. apicatus*.

Using [^{14}C]deltamethrin, it was shown that residual bioavailability was more than 100 times higher for moss or fungi covering the soil, than for the clay soil used in the experiments. Bioavailability showed an exponential decline over time with a halflife of 157 min and was raised by increasing water content of the soil only at 63% (dwt), at which humidity the soil was water-logged. The rate of pesticide uptake by *O. apicatus* decreased with distance walked (Chapter 7).

Experiments on the hygrothermal physiology of female *O. apicatus*, indicated that water loss is probably the main cause of mortality following deltamethrin poisoning. Water loss in *O. apicatus* is the sum of evaporation and deltamethrin induced water excretion. Evaporation was related linearly to the vapour pressure deficit of the air, and showed an exponential increase with temperature in relation to water conductivity of the cuticle. Deltamethrin induced water excretion was independent of the vapour pressure deficit, and showed an increase with dose and temperature (Chapter 8).

As long as spiders are able to walk, they can compensate for water loss by drinking. Immobile spiders loose the capacity to drink free water and are therefore vulnerable to lethal water loss. In this respect it should be noted that the results in chapter 8 indicated that the immobilisation rate of spiders poisoned by deltamethrin depends on the humidity of the air. A simulation model combining quantitative measurements of the independent effects of deltamethrin on immobilisation and water excretion is presented in Chapter 9. The model simulates mortality in a laboratory population of female *O. apicatus* at different air humidities and temperatures.

CHAPTER 1

General Introduction

In spite of numerous publications on the effects of pesticides on arthropods in the laboratory and field (see Newsom 1967, Croft and Brown 1975, Bostrom and Lofs-Holmin 1982, Croft and Whalon 1982, Basedow 1985, Smith and Stratton 1986, Thieling and Croft 1988, Inglesfield 1989), little attention has been paid to the influence of physical factors on pesticide toxicity. Yet these factors govern the bioavailability of pesticides, and due to the poikilotherm nature of arthropods, may cause changes in the physiology and behaviour of these animals. The less homogeneous the environment, the more pronounced this influence will be. As physical factors at the soil surface of arable fields show large temporal and spatial variation, these factors are likely to affect pesticide poisoning of arthropods in the field.

The relevance of taking into account the role of physical factors in pesticide poisoning may be deduced from the following and other studies. A shift in temperature from 15 to 35 °C, increases the penetration rate of permethrin throught the cuticle of the house fly *Musca domestica* by nearly 200 % (Ahn *et al.* 1987). The inhibitory effect of pyrethroids on the closing of the sodium channel gating in neurons shows an exponential decrease with temperature (Vijverberg *et al.* 1982). In analogy to the positive temperature effect relationship of metabolic activity in arthropods, walking activity, and the metabolisation and excretion of a pesticide increase considerably with temperature. Effects of air humidity on pesticide toxicity have also been reported. High air humidity reduced toxic effects in carabid beetles (*Demetrias atricapillus*) exposed to cypermethrin (Jepson *et al.* 1987), and in a linyphiid spider (*Oedothorax apicatus*) exposed to deltamethrin (Everts 1991).

The observation that a multitude of laboratory and field studies about pesticide effects in arhropods has not as yet resulted in adequate predictions of effects under field conditions, may reflect the limited understanding of how physical factors affect the following factors:

a. bioavailability of the compound,
b. behaviour and physiology of arthropods.

The bioavailability of a compound depends on the physico-chemical characteristics of the compound, the substrate, and the cuticular contact area (Salt and Ford 1984, Ford and Salt 1987, Jepson 1989, Mullie and Everts 1990). Bioavailability from substrate normally shows a decline with time (Salt and Ford 1984, Cilgi *et al.* 1988, Mullie and Everts 1990, Wiles 1992).

Arthropod behaviour determines exposure in the field. Arthropods living at the soil surface may be exposured to pesticides by direct contact with spray droplets (topical exposure)(Everts 1991, Jepson 1987), by contact with the substrate during walking activity (residual exposure), or from eating contaminated food (oral exposure)(Salt and Ford 1984, Mullie and Everts 1990, Wiles 1992). Following uptake, pesticide poisoning may affect physiology as well as the behaviour of an arthropod (Salt and Ford 1984).

It is likely that the importance of physical factors in field trials with pesticides has often been underestimated, because the relationships between toxicity and the above factors are obscured by the use of the advised high application rates. A few unpublished results exist on the effects of ambient factors on pesticide toxicity, even at advised application rates. Tobacco budworm control in Imperial Valley, California, failed when temperatures exceeded 40 °C during the night (see Miller and Salgado 1985) and different effects between years have been reported for linyphiid spiders, as non-target organisms, in winter wheat, whilst all other treatment parameters, except the physical conditions, remained the same (Inglesfield 1989).

It should be noted that insight into the modification of pesticide effects by physical factors in the field is of particular importance, when the use of a reduced application rate is required for environmental reasons.

Practical approach

Understanding pesticide effects in the field requires insight into many different relationships. In order to reduce the complexity of the problem to a manageable situation, a decision was made to work with one taxon, with one pesticide, in one crop and to study in the field only the short term effects, excluding population recovery. Laboratory experiments were used to supply quantitative information about subjects for which the field was too complex an environment.

- The taxon of linyphiid spiders, notably *Oedothorax apicatus*, was chosen for its stable temporal and spatial abundance in arable crops in temperate regions, and for its sensitivity to agricultural insecticides (Everts 1990). Linyphiid spiders are easy to collect, to rear, and to identify, and can withstand the gentle treatment required in experiments.
- The insecticide deltamethrin was selected as a commonly used representative of the economically important group of synthetic pyrethroids, which includes many highly insecticidal compounds.

- Winter wheat was chosen as it was thought to have a relatively stable crop microclimate, with a stable species composition. In addition, many relevant field trials using linyphiid spiders have been conducted in winter wheat.
- Only short term effects were studied because this allowed for the reduction of the temporal and spatial scale of the field experiments, which would otherwise have had to have been much larger to prevent long-term effects of immigration.

Linyphiid spiders

Money spiders or linyphiids (*Aranaea, Linyphiidae*) inhabit a wide range of habitats including arable land. Many species show ballooning behaviour which allows them to invade new habitats rapidly (van Wingerden and Vughts 1979, van Wingerden 1980, Thomas *et al.* 1990). The dominant species in arable fields in the Netherlands and surrounding countries are *Erigone atra, E. dentipalpis, Oedothorax apicatus, O. fuscus, O. retusus, Bathyphantes gracilis, Lepthyphantes tenuis* and *Meioneta rurestris* (Cottenie and De Clercq 1977, De Keer *et al.* 1989, Everts *et al.* 1989).

These species dwell in cracks in the soil and on the soil surface, or in the crop canopy just above the soil surface. Some species rely on their webs to catch prey, others hunt. The prey consists mainly of collembola such as *Isotomuridae* or *Sminthuridae*, but also includes mites, flies and aphids (Nyffeler 1982, De Keer and Maelfait 1987). The contribution of linyphiid spiders to the biological control of aphids depends on species and the time of the year (Sunderland *et al.* 1985).

Linyphiid spiders inhabit arable land in densities of 20 to more than 100 per m^{-2} (De Keer *et al.* 1989, Heydemann 1961). In grassland, densities of *O. fuscus* were reasonably constant throughout the year at a level of ± 30 m^{-2}. This species preferred low vegetation from February to June and high vegetation from July to January (De Keer and Maelfait 1987).

In *O. fuscus* seasonal variations in activity were related to the occurrence of new generations, to the walking activity of males searching for females to mate, and for females, to the deposition of egg-batches (De Keer and Maelfait 1987).

Nicthemeral or 'diel[1]' cycles in activity in linyphiidae show no general pattern when comparing species or sex. Dondale (1972) has shown that, in *Erigone autumnalis*, male activity peaked strongly between 11.00 and 16.00 h, whereas females spread their activity throughout the afternoon and night. In both sexes of *O. fuscus* walking activity in the field was lowest from 06.00 to 08.00 and from 14.00 to 16.00 hours (De Keer and Maelfait 1987a). Male *O. fuscus* showed shifts in microhabitat conditions, with a preference for high vegetation during the night (De Keer *et al.* 1989).

1: *The word 'diel' is used according to Cloudsley-Thompson In: Ed. Nentwig, W. (1987).*

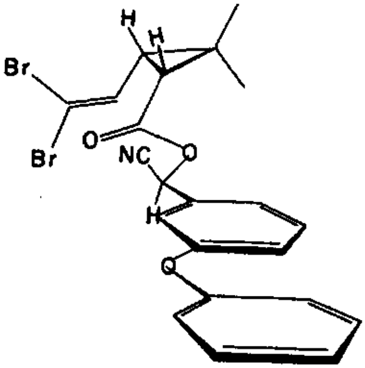

Figure 1.1: The molecular structure of deltamethrin.

Depending on species and the availability of prey, a female linyphiid spider produces 2-17 egg-batches during her life, which may contain up to 25 eggs. There is little variation between linyphiid spider species in the development of the eggs, which hatch after 11 to 14 days at 20 °C (De Keer mandaat 1985). The development rate of the instars shows a species dependent increase with temperature as has been demonstrated for O. fuscus, *O. apicatus*, *B. gracilis* and *E. atra* (Schaeffer 1976, De Keer and Maelfait 1987b, Lissens 1987). The rate of development may depend on day-length as has been demonstrated for *O. fuscus* (Schaeffer 1976).

Deltamethrin and pyrethroid effects on arthropods

Synthetic analogues of pyrethrins, the pyrethroids, have been developed, some of which show increased insecticidal activity and light-stability. At present, deltamethrin (synonym: decamethrin), S-α-cyano-3-phenoxybenzyl (1R,3R)-cis-3-(2,2-dibromophenyl)-2,2-dimethylcyclopropane-carboxylate (Fig. 1.1), is one of the most potent pyrethroids. At room temperature deltamethrin is a white solid. It has a molecular weight of 507.21, a melting point of 101-102 °C, a very low vapour pressure of 3 10^{-10} mm Hg (at 25 °C), a $\log K_{ow}$ value of 6.2, indicating high lipophylicity, a water solubility of less than 0.1 ppm at 20 °C, and high thermal stability below 150 °C. It can show photodegradation both as a solid and in aqueous solution. It is toxic to arthropods in concentrations below 0.01 mg kg^{-1}, which is a factor 10.000 less than in mammals (Roussel Uclaf, 1982). It is commercially available in an emulsifiable concentrate formulation (Decis®) for crop spraying.

The effects of pyrethroids on arthropods have been reviewed in two excellent papers written by Miller and Salgado (1985) and Ruigt (1985). The extent to which these reviews cover the subject, means that it is sufficient at present to give only a short outline of the effects of pyrethroids on arthropods.

Pyrethroid poisoning in arthropods includes contact with and the bioavailability of the compound, spread over the cuticle and penetration through the integument into the haemolymph, metabolisation and excretion of the compound, effects on target organs, disturbance of behaviour and physiology, and finally recovery or mortality.

There is ample evidence that, at the molecular level, pyrethroids inhibit the closing of sodium channel gating (review by Satelle and Yamamoto 1988), which causes prolonged signalling in axons. This inhibition is less severe at high temperature (Van den Bercken *et al.* 1973, Vijverberg *et al.* 1983). It should be noted that pyrethroids and DDT produce very similar effects on invertebrate axons (review by Narahashi 1969) which suggests a similar mode of action at the molecular level. The synthesis of insecticidally active DDT-pyrethroid hybrid molecules (Holan *et al.* 1978) has strengthened this suggestion.

Pyrethroids disturb activity in a range of neurocells (review by Soderlund and Bloomquist 1989), including the central nervous system (i.e. the ganglia, connectives, and commissures from the brain to the terminal abdominal ganglion), and peripheral neurons such as neurosecretory cells, motory and sensory axons. Sensory structures are particularly sensitive to pyrethroids (Clements and May 1977, Gammon *et al.* 1981). Neural effects may, in turn, affect spider behaviour and physiology in different ways. Effects on sensory or motory neurons will certainly impose a stress on the animal but as arthropods may survive a prolonged period of shut down of respiration and circulation, this is not necessarily a cause of mortality (i.e. Sun and Johnson, 1972).

With respect to the lethal effect of pyrethroids, it may also be of marked importance that pyrethroids affect the neurosecretory cells (Orchard 1980, Greenwood *et al.* 1985), which regulate the production of many different hormones including a diuretic hormone. Increased water loss in relation to pyrethroid treatment has been shown by different authors (Casida and Maddrell 1970, Sun and Johnson 1972, Gerolt 1976). Water loss has also been observed for many other insecticides (review by Gerolt 1983). This water loss, in relation to ambient conditions, may contribute to mortality in arthropods poisoned by insecticides.

Quantitative models in studying pesticide effects on terrestrial arthropods

Poisoning of arthropods by pesticides involves many steps. Each step in the pesticide-effect chain is confined to a specific temporal and spatial scale. For instance, physiological effects are related to the individual level and to relatively short periods. A framework for arranging pesticide effects on arthropods, according to temporal and spatial scales ranging from small and shortlived to large and longlasting, has been proposed by Jepson (1988, 1989). The desire to extrapolate within these scales, i.e. between species, fields or pesticides, or to extrapolate between scales, such as from laboratory results to the field situation, requires qualitative and quantitative insight into the most important relationships involved, from the largest scale included downwards. As quantitative simulation models allow for the calculation of many simultaneously acting relationships, this makes modelling an attractive approach for extrapolation studies. Quantitative models have been developed for different aspects of the poisoning process and for different arthropod species and compounds. Modelling studies have dealt with pesticide uptake from substrate in relation to the behaviour of arthropods, and the bioavailability and spatial distribution of a pesticide (Salt and Ford 1984). Toxicokinetic models have been developed which describe pesticide penetration through the cuticle, distribution in the haemolymph and internal tissues, and excretion or metabolism of the compound (Welling and Paterson 1985, Welling 1977, Ford *et al.* 1981, Hadgraft and Brain 1990, Greenwood *et al.* 1990). On the large temporal and spatial scale, quantitative modelling has been used to study the effects of pesticide use on arthropod populations in areas of more than one field, in relation to arthropod migration (Jepson 1993).

Outline of this thesis

The aim of this thesis is to obtain mechanistic insight in how physical factors and arthropod behaviour may affect pesticide toxicity for arthropods in the field. Therefore, the effects of physical factors on pesticide bioavailability, uptake, and toxicity for arthropods had to be identified and, as much as possible, quantified. The experiments included field-trials and laboratory observations.

Part I. Field trials

Walking activity determines the exposure of arthropods to a sprayed substrate which implies that it is an important cause of residual pesticide uptake. The walking behaviour of linyphiid spiders was, therefore, investigated under field conditions in relation to physical factors (Chapter 2) and nicthemeral activity rhythms (Chapter 3). These autecological parameters were studied in experiments in which no pesticides were applied.

Given restricted availability of time, field facilities and manpower, a spatial and temporal scale was sought for the experiments that was as small as possible without risking the loss of relevant information (Chapter 4). To allow for effects of physical factors on arthropod mortality following field application of deltamethrin, an appropriate application rate below the advised, had to be determined (Chapter 5). Chapter 5 also introduces 'short time series analysis', a regression method used to obtain an accurate description of the pesticide effects in relation to an untreated control situation.

The combined effect of arthropod activity and physical factors was studied in an experiment presented in Chapter 6.

Part II. Laboratory studies

In laboratory studies emphasis was put on pesticide bioavailability and spider water loss. The bioavailability of deltamethrin from substrate was studied in relation to soil moisture, substrate type, deltamethrin deposition, time after spraying and the distance spiders walk on a sprayed substrate (Chapter 7). Measurements of the effect of deltamethrin on behaviour and water loss, in relation to temperature and air humidity (Chapter 8), were combined in a quantitative deterministic model, which explains the mortality of linyphiid spiders on a basis of underlaying water relationships (Chapter 9).

A summary and concluding remarks are provided in Chapter 10.

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PART I: FIELD STUDIES

CHAPTER 2

Predicting the surface activity of epigeal money-spiders in arable crops using relationships between the trapping success of pitfall traps and ambient physical factors

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Summary

This study aimed at the identification of relationships between the walking activity at the soil surface of a field population of money-spiders (*Linyphiidae*) and the, simultaneous, action of physical factors. These relationships were to be used in later experiments, as, for prediction of linyphiid activity under field conditions, the underlaying causal relationships and their interactions are not yet identified or quantified to any extent. Spider activity was monitored on a daily basis, for 72 days, in a winter wheat and a sugar beet crop using pitfall traps. The species composition was determined at weekly intervals. Physical factors were measured on a daily basis at a nearby weather station and in the crop. Correlations between physical factors and trapping success were studied using multifactorial analysis.

The results showed that the walking behaviour of linyphiid spiders is closely related to the simultaneous influence of physical conditions, mainly net energy gain of the crop microclimate by radiation, and the humidity of the soil surface. The calculated relationships reflected the microclimatic differences between the sugar beet and the winter wheat crop. The data allowed the identification of optimum conditions for walking activity in relation to various physical factors. The analysis indicated that spiders compensate for high or low walking activity on one day by low or high activity the next day. Species composition in sugar beet seems to be more stable than in winter wheat.

Introduction

In agroecosystems, linyphiid spiders form a group of very abundant, small, predators of collemboles and other arthropods such as aphids (Sunderland *et al.* 1985). Linyphiid spiders have been shown to suffer severe reductions in numbers following application of agricultural insecticides (Inglesfield 1989, Chambon 1987, Everts 1989). In this respect, the exposure of these spiders to an insecticide by walking over residues of the sprayed pesticides is generally believed to contribute markedly to pesticide uptake and effect (Jepson 1989, Mullié and Everts 1991). The testing of this hypothesis in future field studies should involve the spraying of pesticide at moments of low and high spider activity. Instead of spraying at random dates with unknown spider activity, it is more convenient to use insights into relationships which determine the presence and activity of these spiders at the soil surface under field conditions for the selection of appropriate days for pesticide application. Therefore this paper aims at identifying relationships between physical factors and spider activity at the soil surface. Temperature near the soil surface naturally shows a close correlation with spider activity. Yet this variable could not be used in the present regression calculations because the prediction of trapping success from a regression equation including the soil temperature, would imply an improper use of temperature as an independent variable. The temperature near the soil surface could only be predicted itself from the other variables already in the regression equation, which would imply a shifting from predicting of spider behaviour to predicting of a temperature. A broad range of physical factors was examined by sampling in a winter wheat and a sugar beet crop.

With respect to the relationship between crop conditions in the field and trapping success of arthropods, it has been shown that for different carabid, lycosid and staphylinid species, trapping success was best predicted by a combination of temperatures measured at selected moments over the 24 hours of trapping, or the sunshine hours during the trapping period. Species dependent catches often parallel the trends observed for families (Honek 1988). It has often been shown that trapping success of arthropods in the field increases with temperature (Baars 1978, Ericson 1979). Desender (1983) calculated separate correlations between the activity of *Clivina fossor* (Coleoptera) and seven physical factors. The highest significance was found for a positive correlation with global solar radiation.

In most studies, the trapping success of arthropods has been studied in relation to a separate physical factor. In order not to disregard the simultaneous action of physical factors, it was chosen to analyse, in the present study, the correlations between physical factors and trapping success using multivariate analysis.

Materials and methods

The study was carried out on arable land from 1 June to 8 August 1988. Linyphiid spiders were captured in a winter wheat and a sugar beet crop using pitfall traps.

The fields were situated at the Institute for Plant Protection Research (IPO) at Wageningen. The soil is a heavy river clay soil ('medium high Nude silty clay loam', lutum content of 59%, 5.3% organic matter). Each field measured one hectare, and the crop was subject to standard agricultural practice.

At the start of the experiment the winter wheat crop had almost reached its final height. On 8 June the cereal was sprayed with a mixture of prochloraz (Sportak^R):propiconazol (Tilt^R):pirimicarb (Pirimor^R), (11 : 11 : 0.25l) in 400 l water ha^{-1}. The crop flowered from 14 to 30 June and was harvested on the 9th of August.

Ground cover for the sugar beet crop was estimated visually, and ranged from 50% on 6 juli, 60% on 15 July to 90% on 5 August. The crop was sprayed with mecoprop (Betanal^R):methamitron (Goltix^R), (31:31) on 1 June, and was hoed manually from 17 to 20 June, and from 14 to 20 July.

Arthropods were trapped using pitfall traps i.e., plastic cups with a diameter of 10 cm, and 6 cm deep, filled with an aqueous solution of 4% formaldehyde in tap-water with a few drops of detergent (teepol). The trapping fluid was renewed weekly. The traps were shielded from rain by a transparant round cover of 15 centimeter diameter, attached at 7 to 10 cm above the trap. To account for variability inherent in measurements of activity, 30 pitfall traps were used in each crop. These were positioned in three rows of 10 traps with 5 m between traps and 15 m between rows. All traps were situated at least 20 m from the edge of the field. Every morning between 08.00 and 10.30 local time (06.00 to 08.30 Universal Time Constant), trapped arthropods were collected in the field, and stored in 70% alcohol for later identification using Locket and Millidge (1951, 1953, 1974).

The physical factors used in the regression calculations were selected on the basis of expected relationships with the microclimate at the soil surface.

Soil humidity of the top 2 cm of the soil, being the substrate epigeal arthropods walk on, was measured in the crop. Samples were taken every morning before emptying the pitfall traps, and stored in plastic bags. Soil humidity was determined as percentage dry-weight, by oven-drying overnight at 105 °C.

Information from the weather station of the Agricultural University Wageningen, situated 300 m south-west of the plots, included: positive net radiation (J cm^2 24h^{-1}), negative net radiation (J cm^2 24h^{-1}), sunshine hours (h 24h^{-1}), daily mean of hourly measured relative humidity (%) at 1.5 m, daily mean of the windspeed measured during the last 5 minutes of every hour (m s^{-1}), minimum and maximum temperature at 1.5 m (°C), time (min 24h^{-1}) and quantity (mm 24h^{-1}) of rain. As the occurrence of rain was thought more important than correlations with the highly variable duration or quantity of

Table 2.1. Mean values and statistical information for the catches and the physical variable measurements (units are given in the text).

variable	abbreviation	mean	standard deviation	minimum	maximum
measured in the crop:					
Linyphiidae in winter wheat	-	114	58.6	20	260
Linyphiidae in sugar beet	-	158	88.5	21	430
soil humidity winter wheat	SH	27.8	8.4	11.0	41.4
soil humidity sugar beet	SH	21.6	11.2	6.4	41.7
SH winter wheat previous day	SHX	27.8			
SH sugar beet previous day	SHX	21.7			
measured at the weather station:					
air humidity	RH	82.5	8.6	65.0	98.0
positive net radiation	PNE	783.9	283.9	273.0	1459.0
negative net radiation	NNE	-92.2	47.4	-200.5	- 16.8
sunshine hours per day	SUN	3.9	3.7	0.0	15.3
daily minimum temperature	MIT	11.2	2.2	6.9	18.3
daily maximum temperature	MAT	19.3	2.6	14.0	26.8
wind speed	WS	2.6	1.0	1.1	6.0
incidence of rain	DEP	(42 days with rain)			
RH previous day	RHX	82.7			
PNE previous day	PNEX	776.7			
NNE previous day	NNEX	- 90.7			
SUN previous day	SUNX	3.8			
MIT previous day	MITX	11.1			
MAT previous day	MATX	19.2			
WS previous day	WSX	2.6			

the rain, this parameter was transformed to a binary form, stating the presence or absence of rain. It should be noted that all physical factors related to the 24 hour period prior to emptying the traps at 08.00.

Regression analysis

The pitfall trap catches in winter wheat and sugar beet were investigated in relation to a selection of the following physical factors: the positive net radiation (PNE), the negative net radiation (NNE), sunshine (SUN), relative air humidity (RH), mean windspeed (WS), minimum- and maximum temperature (MIT, MAT), duration of rain (DEP), soil humidity (SH) and the week-factors mentioned above (WK_t).

The data were analysed using a forward stepwise regression technique known as 'maximum R^2 improving technique' (J. Goodnight, MAXR procedure, SAS 1990). At each step, the variable resulting in the highest increase in R^2 is added to the model, followed by a re-examination (and, if necessary, exchange for a better variable) of the variables already in the model. The selection of the best model was based on a significant contribution (at the 95 % confidence limit) of the variables in the model. In case the forward selection resulted in a 'best' model which included a quadratic but not the linear component of a variable, the latter was included in the final model. Correlations between variables and variance inflation of each variable were examined (McGriffen *et al.* 1988).

For regression analysis the logarithm of the daily sum of all arthropods caught in 30 traps was used as the dependent variable. The counts being reasonably high, rarely lower than 10 and never 0, not too many differences were expected between (quasi-) Poisson modelling and lognormal modelling of the counts. The regression model was based on the assumption that the physical factors were related to the catches in a multiplicative way, i.e. a change in one factor affecting the level at which other factors can operate. To ameliorate estimates of nonlinear relationships, mean values were subtracted from the physical factors, used in the regression as independent variables.

Possible changes in population density, which were not related to physical factors, were allowed for by including a dummy variable for every week; the 'week-factors' WK_t (t=1 to 10), which had the value 1 during the days of a specific week and zero for all other days. In this way, the physical factors were analysed within weekly periods, during which population growth was assumed to be negligible.

Possible long lasting effects of physical factors on arthropod activity were analysed for all variables by adding to the multiple regression the values of the previous day $((x_{-1})_j)$.

A choice was made to fit flattening or optimum relationships of physical factors and pitfall trap catches with a second order polynomial, for which the squares of all variables were added to the analysis (x^2_j and $(x_{-1})^2_j$). This resulted in the following model:

$$(2.1)\ Y \quad = \mu + a_j x_j + b_j x^2_j + c_j (x_{-1})_j + d_j (x_{-1})^2_j + e_t WK_t + \epsilon.$$

where:

μ the mean number of spiders trapped during the period not indicated with week variables (Table 2.1), a_j, b_j, c_j, d_j regression coefficients (j=1 to n), x_j and x^2_j the physical factors

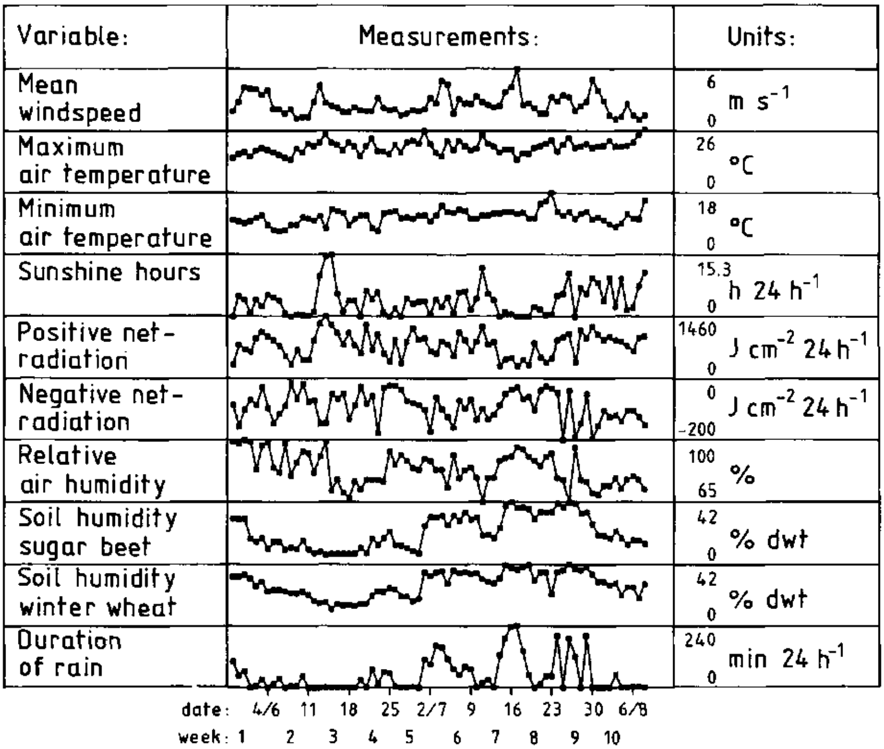

Figure 2.1. Environmental variables measured in relation to arthropod activity.

after substraction of the variable-mean, and their squares, $(x_{-1})_j$ and $(x_{-1})^2_j$ the physical factors of the preceding day after substraction of the variable-mean, and their squares, *WK,* the week-factors for week 1 to t $(t=10)$, ϵ a random contribution from a normal distribution with mean 0 and variance σ^2).

It should be noted that, in the case of corrections for population density in certain weeks as indicated by the inclusion of week-factors, the intercept value represents the mean of the catches in the other weeks.

Although more sophisticated methods (such as differencing) exist to calculate the relationships studied, our choice was based on simplicity of application of method used and interpretation of results. For the use of the density dependent 'week-factor' a certain stability in time was assumed, of the activity of the investigated arthropod groups in relation to crop circumstances and/or effects of physical factors.

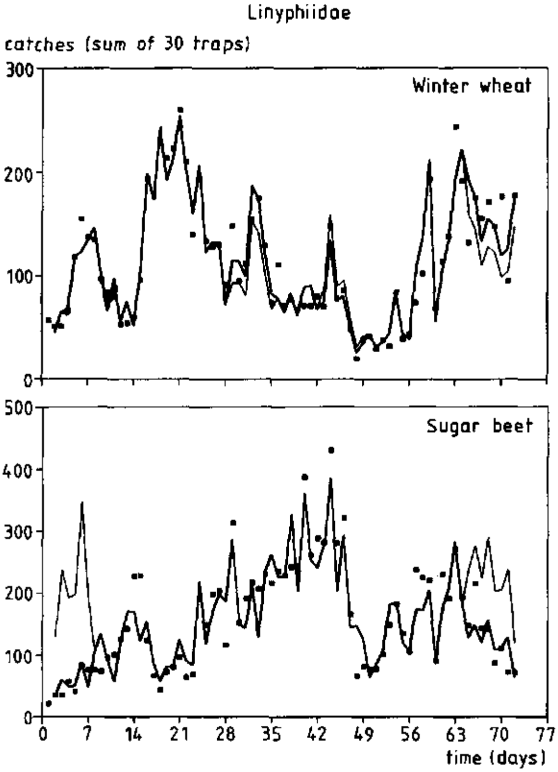

Figure 2.2. Fitted curves for the activity of Linyphiidae in a winter wheat and a sugar beet crop in relation to environmental variables. Solid squares represent the sum of the observed catches in 30 traps per day. The results of the complete regression model (equation (2.1)) are drawn with a thick line. A thin line connects the estimated values after substraction of the effect of the week-factors.

Results

Fig. 2.1 shows that values of most physical factors varied considerably from day to day. Soil moisture showed little variation on a daily basis, lowest values being recorded during the first 5 weeks. A lot of rain was recorded in weeks 6 to 9. As the regression calculations were based on these values after subtraction of the mean, the mean values are presented in Table 2.1.

The results of the regression calculations are shown in Table 2.2. A visual representation is given in Fig 2.2. Differences in crop microclimate between winter wheat and sugar beet resulted in specific activity relationships for each crop.

In winter wheat the mean number of linyphiid spiders caught during the 72 day sampling period was 3.8 trap^{-1}. Daily mean values varied from 0.7 trap^{-1} (15 July, after heavy rain) to 8.7 trap^{-1} (18 June).

Table 2.2. Results of multiple regression calculations aiming at identication of relationships between physical pactors and pitfall trap catches of linypiid spiders in winter wheat and sugar beet. Units are indicated for the basal variable only.

	abbreviation	unit	regression coefficient	standard error	F-value	Prob >F
Winter wheat (DF=70, R^2 = 0.92, mean square of residual = 0.033)						
intercept			4.647	0.008	57.19	0.0001
positive net radiation	PNE	Jcm-2	0.954 E-3	0.128 E-3	7.45	0.0001
PNE on previous day	PNEX		0.891 E-3	0.166 E-3	5.37	0.0001
square of PNEX	PNEX2		-1.06 E-6	0.331 E-6	-3.21	0.0023
negative net radition	NNE	Jcm-2	1.67 E-3	0.728 E-3	2.30	0.0255
square of NNE	NNE2		30.6 E-6	10.7 E-6	2.86	0.0062
NNE on previous day	NNEX		0.374 E-3	0.726 E-3	0.52	0.6083
square of NNEX	NNEX2		26.82 E-6	11.7 E-6	2.28	0.0268
soil humidity	SH	%dwt	-13.36 E-3	3.69 E-3	-3.61	0.0007
air humidity	RH%		-12.28 E-3	3.76 E-3	-3.27	0.0019
RH on previous day	RHX		- 4.08 E-3	4.09 E-3	-0.99	0.3236
square of RHX	RHX2		0.71 E-3	0.35 E-3	2.05	0.0458
incidence of rain	DEP		-0.123	0.058	-2.13	0.0376
DEP on preceding day	DEPX		-0.161	0.066	-2.42	0.0189
sunshine on preceding day	SUNX	h	-29.1 E-3	13.4 E-3	-2.17	0.0344
maximum air temperature	MAT	°C	-29.7 E-3	12.8 E-3	-2.32	0.0243
corrections for density	WK5		0.207	0.085	2.45	0.0179
	WK7		-0.171	0.087	-1.96	0.0552
	WK10		0.190	0.098	1.94	0.0579
Sugar beet (DF=70, R^2 = 0.80, mean square of residual = 0.087)						
intercept			5.599			
soil humidity (mean of begin & end 24-h period)	SH	%dwt	19.40 E-3	4.71 E-3	4.12	0.0001
square of soil humidity	SH2		- 2.75 E-3	0.45 E-3	- 6.09	0.0001
positive net radiation	PNE	Jcm-2	0.573 E-3	0.190 E-3	3.00	0.0039
PNE on previous day	PNEX		48.1 E-6	0.147 E-3	- 0.33	0.7452
square of PNEX	PNEX2		- 2.09 E-6	0.44 E-6	- 4.76	0.0001
negative net radiation	NNE		2.60 E-3	1.04 E-3	2.50	0.0152
maximum air temperature	MAT	0C	86.7 E-3	22.7 E-3	3.82	0.0003
square of MAT	MAT2		-12.1 E-3	3.8 E-3	-3.19	0.0023
minimum air temperature	MIT		21.1 E-3	23.2 E-3	-0.93	0.358
square of MIT	MIT2		-11.9 E-3	4.2 E-3	- 2.82	0.0067
incidence of rain on preceding day	DEPX		0.219	0.099	2.20	0.0319
corrections for density	WK1		- 1.37	0.14	- 9.61	0.0001
	WK10		- 0.62	0.14	- 4.58	0.0

The following selection of physical variables showed the best correlation with the activity of linyphiid spiders in winter wheat:

$$
\begin{aligned}
(2.2)\ Y = {} & 4.647 \\
& + 0.954\ \mathbf{PNE}10^{-3} + 0.891\ \mathbf{PNEX}10^{-3} - 1.062\ \mathbf{PNEX2}10^{-3} \\
& + 1.673\ \mathbf{NNE}10^{-3} + 30.64\ \mathbf{NNE2}10^{-6} + 0.374\ \mathbf{NNEX}10^{-3} + 26.83\ \mathbf{NNEX2}10^{-6} \\
& - 0.0123\ \mathbf{RH} - 4.080\ \mathbf{RHX}10^{-3} + 0.711\ \mathbf{RHX2}10^{-3} \\
& - 0.123\ \mathbf{DEP} - 0.161\ \mathbf{DEPX} - 0.013\ \mathbf{SHX} \\
& - 0.030\ \mathbf{MAT} - 0.029\ \mathbf{ZX} \\
& + 0.207\ \mathbf{WK5} - 0.171\ \mathbf{WK7} + 0.190\ \mathbf{WK10}
\end{aligned}
$$

(For an explanation of abbreviations see Materials and Methods and Table 2.2.) Significance of variables used in equations (2.2) and (2.3) is shown in Table 2.2. It should be noted that the WK_t-factors, apply typically to the specified (by subscript t) weeks.

Variance inflation and collinearity index indicated no problems with any of the variables in the model.

Equation 2.2 implied the following correlations. Trapping success of linyphiid spiders in winter wheat was positively correlated with high positive net radiation (PNE) and high positive radiation during the previous day (PNEX, PNEX2), for the latter an optimum could be calculated at 1203 Jcm^{-2} which together with a negative effect on the catches of sunshine on the previous day (SUNX) indicated low spider activity following a day with high activity. The correlation between trapping success and radiation loss during the night, with an optimum at -95 Jcm^{-2} (NNE, NNE2), indicated the importance of nocturnally active species. The effect of radiation loss of the preceding night (NNEX, NNEX2) was only significant for the quadratic component. Negative correlations with trapping success were found for deposition (DEP), air humidity (RH) and air humidity during the preceding day, the latter showing a calculated optimum at 86% RH (RHX, RHX2). A reducing effect of soil humidity at the beginning of the sampling period (SHX) on the catches indicated that adverse effects of soil humidity were most pronounced during daytime. A high maximum air temperature (MAT) reduced the trapping succes. Equation (2.2) includes corrections for trapping success unrelated to physical factors for week 5, 7 and 10.

In sugar beet, the daily mean number of spiders caught varied from 1.37 trap^{-1} (2 June) to 14.33 trap^{-1} (11 July) with a mean of 5.23 trap^{-1}.

The variance inflation and collinearity index indicated no problems with any of the variables in the model.

In preliminary runs, significant contributions to the model were shown for soil humidity determined at the beginning and at the end of a 24 hour period, and the respective squares.

As the separate use of these variables added considerably to the variance inflation, a choice was made to use the means in the regression, which prevented this problem.

The following correlations were found between trappping success and physical variables:

$$\begin{array}{lllll} (2.3)\ Y & = 5.60 & & & \\ & +\ 0.573\ \mathbf{PNE10}^{-3} & -\ 0.048\ \mathbf{PNEX10}^{-3} & -\ 2.094\ \mathbf{PNEX210}^{-6} & +\ 2.60\ \mathbf{NNE} \\ & -\ 21.48\ \mathbf{MIT10}^{-3} & -11.95\ \mathbf{MIT210}^{-3} & +86.73\ \mathbf{MAT10}^{-3} & -12.13\ \mathbf{MAT21} \\ & +\ 19.4\ \mathbf{SH10}^{-3} & -\ 2.75\ \mathbf{SH210}^{-3} & +\ 0.219\ \mathbf{DEPX} & \\ & -\ 1.37\ \mathbf{WK1} & -\ 0.624\ \mathbf{WK10} & & \end{array}$$

The model indicated that radiation (PNE) had a positive effect on trapping success. However, high radiation on one day, which during that day was associated with high trapping success, was correlated with low catches the next day (PNEX2). Radiation loss at night (NNE) was positively correlated with high catches. At the uncovered soil surface of the sugar beet crop, trapping success showed an optimum relationship with soil humidity (SH, SH2) with an optimum at 30%dwt. The square of minimum air temperature at 1.5 m (MIT) was selected in the sub-final model without linear component, indicating a reduction of trapping success at nocturnal temperatures below and above 11 °C. After adding the linear component in the final model (equation (2.3)) the square remained significant only. Maximum air temperature at 1.5 m (MAT, MAT2) was positively correlated with trapping success, with an optimum calculated at 23 °C. Rain on the previous day was correlated with high catches (DEPX).

The relative contribution of the most abundant species to species composition in the winter wheat and sugar beet plots is shown in Figure 2.3. Most of the trapped spiders were males. The exception was female *O. apicatus* which were abundant in the winter wheat. In winter wheat the species composition changed during the 72 sampling days. The highest percentage of the species *Bathyphantes gracilis* was found during the first and last weeks, female *O. apicatus* peaked in the middle weeks. The males of *Erigone atra*, *E. dentipalpis* and *O. apicatus* showed little temporal variation in relative trapping success. In sugar beet the species composition showed little variation. In order of decreasing abundance, *E. atra*, *E. dentipalpis*, *Meioneta rurestris* and *Oedothorax fuscus* were present in the catches at a constant percentage.

Discussion

The present results show significant correlations between physical factors and trapping success, most of which can be given a biologically meaningfull interpretation. It should be noted that each variable in the regression equations signifies the effect as corrected for

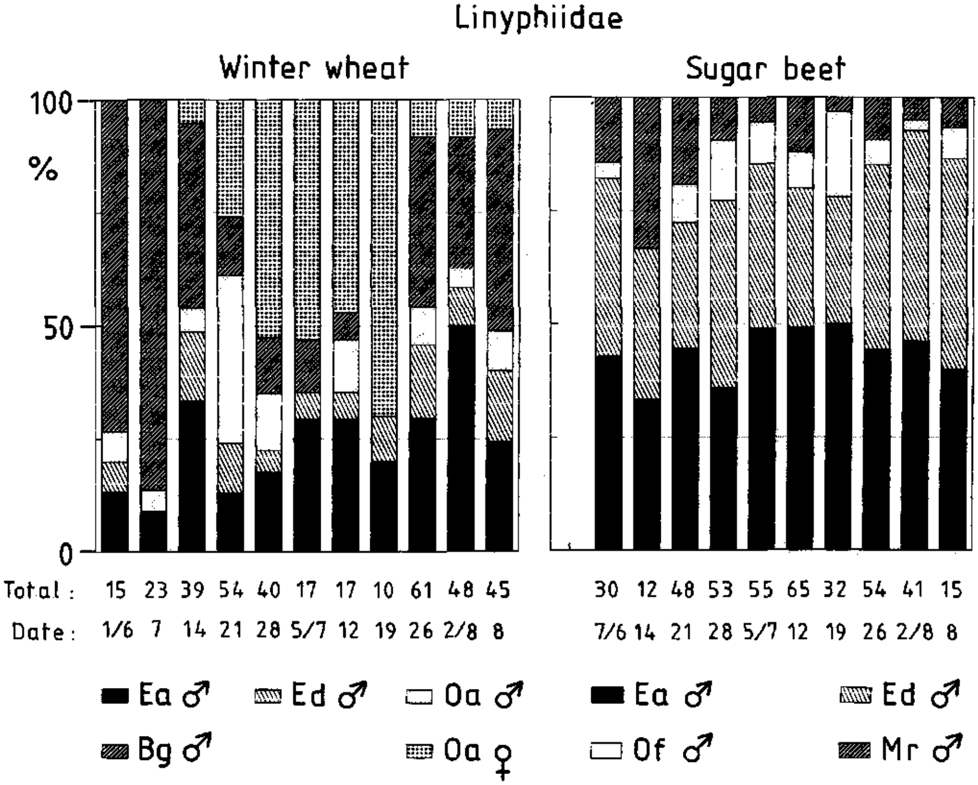

Figure 2.3. Species composition of the linyphiid spider fauna in winter wheat and sugar beet. The relative contribution to the catches of the most abundant species was examined on a weekly basis. The actual number caught and the day for which the species composition was determined are indicated at the bottom of the picture. **Ea** = *Erigone atra*, **Ed** = *Erigone dentipalpis*, **Oa** = *Oedothorax apicatus*, **Bg** = *Bathyphantes gracilis*, **Of** = *Oedothorax fuscus* and **Mr** = *Meioneta rurestris*.

influences of all other factors. The correlations between trapping success in winter wheat and sugar beet and a combination of physical factors selected by the multifactorial analysis, suggest that predictions of changes in spider activity in winter wheat can be based on physical parameters.

Very high radiation values on the previous day were correlated with low catches the next day. Likewise, rain on the preceding day had a positive effect on trapping success. Both situations suggest that spiders compensate the following day for very high or low activity on the day before with regard to some sort of physiological daily activity optimum.

Correlations were not calculated at species level, as this would not have enabled the prediction of the general activity of linyphiid spiders in the field. Indeed, effects of species composition on the regression outcome are not likely in sugar beet, as the species composition of this crop remained practically unchanged during the 72-day sampling period. This does not hold for winter wheat, in which *Bathyphantes gracilis* males and *Oedothorax apicatus* females changed dominance twice during the sampling period. However, despite small differences in the shape and size of these spiders, the walking activity of different species may react similarly with respect to the most important physical factors and may therefore not have had much effect on the regression outcome.

B. gracilis males and *O. apicatus* females were shown to have a preference for nocturnal activity in winter wheat (pers. obs.). This preference for nocturnal activity, in combination with the absence of *B. gracilis* males and *O. apicatus* females in sugar beet, may explain why radiation loss, which was the sole night-related factor in the regression, played such an important role in winter wheat.

The correlations which were found between physical factors and the walking activity of spiders were not meant to imply direct causal relationships. They could, however, serve very well as a starting point for laboratory studies of the factors which are causally related to walking activity. The relevance of such laboratory studies for predicting walking activity in the field situation, however, will still need to be tested under field conditions.

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CHAPTER 3

Factors governing pecies dependent diel activity rhythms in adult linyphiid spiders in winter wheat in relation to ambient physical factors

Summary

Diel activity periodicities in different linyphiid spider species in winter wheat were studied in relation to field, month and physical factors. For this purpose, spider activity was monitored on a 1.5 hourly basis over 96-hour experiments, using 18 pitfall traps.

The results indicated that linyphiid spider species possess an endogenous activity rhythm, of which the expression, but not the temporal pattern is affected by physical factors.

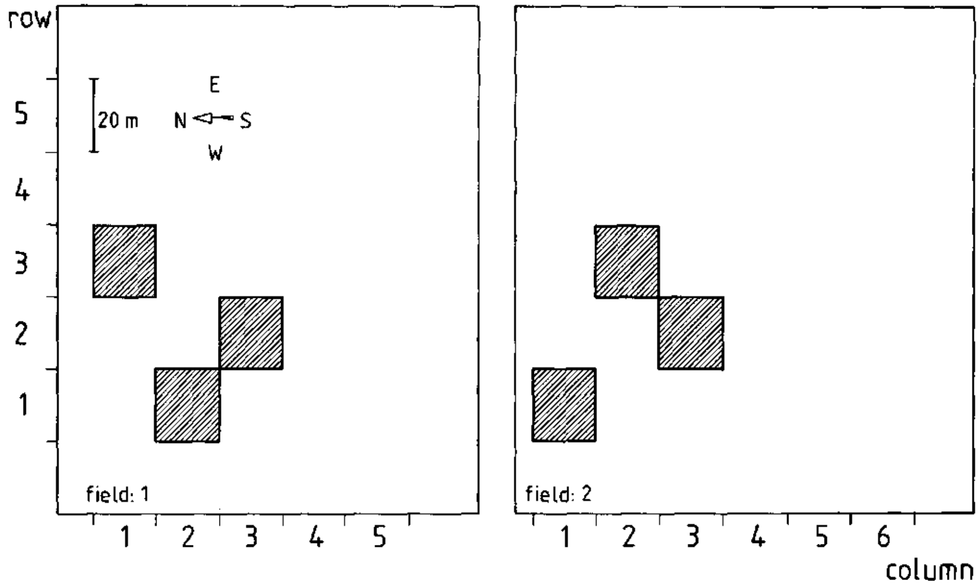
Introduction

Most arthropods show diel (over a period of 24 hours) periodicities in their walking activity. These periodicities may synchronize sympatrically living predators and pray species, but also allow for temporal separation in niches (Park 1941, Corbet 1966). In addition, diel activity rhythms may be of importance with respect to the effects of pesticides with a short halflife of bioavailability. Activity rhythms determine the time after spraying, and therewith the bioavailability of the pesticide at the moment an animal contacts a substrate contaminated by pesticide residues.

The present study deals with the diel activity rhythm in linyphiid spiders. These spiders live at the soil surface and in cracks in the soil, and are highly abundant throughout the year in many different ecosystems. Their diel activity depends on species and sexe (Dondale *et al.* 1972, De Keer and Maelfait 1987, De Keer *et al.* 1989). Species dependent effects of crop microclimate were indicated by the finding that in grazed pasture, male *Oedothorax fuscus* and both sexes of *O. retusus* showed relatively higher catches in high vegetation during the night than during the day, this effect being absent in *Erigone atra* (De Keer *et al.* 1989). These, and other publications on walking activity of linyphiid spiders, have, however, provided little information about the nature of diel activity or the extent to which it may be influenced by ambient physical factors.

According to activity relationships in other spiders, a diel activity rhythm in linyphiidae may be determined by endogenous or exogenous cues. In a review on this subject, Cloudsley-Thompson (1987) states that most spiders will display endogenous periodicities of movement and rest when maintained under constant conditions in the laboratory. The existence of an endogenous activity rhythm, the expression of which was controlled by daylight, has been clearly shown for *Cupiennius salei* (*Ctenidae*) by Seyfarth (1980). The synchronisation of activity in these spiders at LD 12:12 completely disappeared in constant light, and showed an endogenous rhythm with a periodicity of 24.9 h in constant dark. A relationship between diel activity pattern and exogenious stimuli was shown for *Lycosa ceratiola*, which emerges at night from sheltered retreats in response to ambient temperature, being arhythmic without thermal cues (Carrel 1980).

The aim of the present paper was to investigate the following aspects of diel activity in linyphiid spiders in cereals: the effect of field, month and ambient conditions on the species and sex dependent diel activity pattern, the type of rhythm, i.e. exogenous or endogenous, the presence of physical cues with respect to the onset and end of activity, and effects of physical factors on activity.



3.1. Diel activity experiment. The figure shows the position of the plots within fields 1 and 2, the latter being situated 200 m south-east of field 1.

Materials and methods

During the summer of 1990 spiders were caught in winter wheat using pitfall traps. The position of the plots within the fields is given in Figure 3.1. The position of the pitfall traps within the plots is given in Jagers op Akkerhuis and van der Voet (1992). A description of the pitfall traps is given by Everts *et al.* (1989).

With the aim of creating a range of physical conditions, the experiment incorporated sampling on a field with a dense crop (field 1) and with an open crop (field 2), over two different months. Spiders were caught from fields 1 and 2 from 28 May to 1 June, and from field 2 from 2 to 6 July. During these periods the traps were emptied 16 times a day (every 1.5 hours). As depletion of spiders from the area in proximity to a trap may affect initially the trapping success, the pitfall traps were set in function two days before the onset of an experiment, spiders caught during these days not being part of this study.

In each field, spiders were trapped in three different plots of 18*20 m, using 6 pitfall traps per plot situated in the center of the plot as specified in Jagers op Akkerhuis and van der Voet (1992). Spiders were put in 70% alcohol and stored for later identification according to Lockett and Millidge (1951, 1953, 1974).

Temperature (mercury thermometers, ± 0.1 °C) and air humidity (hair hygrometer, ± 3%) were measured in each plot in the crop at 5 cm above the soil each time the traps were emptied. Thermometers and hygrometers were freely exposed to air and shielded from direct radiation. As spiders are poikilotherm and may show different activity patterns in reaction to photoperiod, their activity was expected to depend on radiation and rain. Therefore measurements of net radiation (J/cm^2), being the difference between incoming and outgoing radiation for a given surface, light intensity, measured as global radiation (J/cm^2), and rain (mm hour^{-1}) were obtained from the university weather station. This was situated at 300 m distance from both fields.

Soil samples of the top 2 cm of the soil were taken every morning at 8.00 hours. Soil moisture content was determined gravimetrically as percentage dryweight after oven drying at 105 °C overnight. The soil consisted of a heavy illitic river clay 'medium high Nude silty clay loam', 59% lutum content, 5.3% organic matter.

The cereal was subject to integrated pest management. It was not sprayed during the experimental periods. Crop stages according to Zadoks (1974) were 10 and 8 on fields 1 and 2 during the first, and 11 on field 2 during the second sampling occasion. The crop on field 1 was dense with a moss covered soil, the crop on field 2 was more open without moss cover.

Regression analysis

To study the effect on trapping success of physical factors and a possible endogenous activity rhythm in linyphiid spiders, multiple regression analysis was used, based on the following equation:

$$(3.1) \qquad \ln(Y+1) = \mu + V_2 + V_3 + \alpha\cos(t') + \beta\sin(t') + \alpha_2\cos(t') + \beta_2\sin(t') + aT + bRH + cNR + dDEP + \epsilon$$

in which: Y is the sum of the catches in 18 traps during 1.5 hour, μ the mean value of the logarithmically transformed catches on field 1, V_2 & V_3 are dummy variables in relation to the relative trapping success on the different fields to account for possible differences in population density, α & β the regression coefficients of the first harmonic component of time, α_2 & β_2 the regression coefficients of the second harmonic component of time,
t' the transformed time, i.e. time expressed as $t' = t - t_0$ with t and t_0 in radials, and t_0 corresponding with 360^0, *a to d* the regression coefficients of the physical factors,
T the temperature (°C) in the crop at 5 cm above the soil, ***RH*** the relative air humidity (%) in the crop, ***NR*** the summarised net radiation (Jcm^{-2}1.5h^{-1}), ***DEP*** a variable indicating the incidence of rain (the variable is 1 when it rained in the 1.5 hourly period and 0 otherwise), ϵ_{tv} disturbances of the time and field dependent measurements, which are assumed to have a zero mean and standard deviation σ_ϵ.

Table 3.1. Abundance and species composition of linyphiid spiders in winter wheat fields in May and July. Catches are summarised per field (96 hours and 18 pitfall traps).

sexe	male			female		
month	May		July	May		July
field	1	2	2	1	2	2
species:						
O. apicatus	0	21	101	3	7	225
O. fuscus	0	6	103	9	36	80
O. retusus	2	1	5	1	1	6
E. atra	54	148	69	3	9	8
E. dentipalpis	17	54	44	3	5	7
L. tenuis	34	32	21	0	3	1
M. rurestris	19	3	10	0	1	2
B. gracilis	86	138	39	3	4	5
D. concolor	2	9	72	2	2	21

The moment *(t)* of the maximum (or maxima for α_2 and β_2) and the amplitude *(A)* at this point may be calculated from dissolving the following two equations with two variables:

$$(3.2) \qquad \alpha = A\cos(t') \text{ and } \qquad \beta = A\sin(t')$$

The common significance of the harmonic regression coefficients α and β is given by the F^2_{N-1} statistic which may be obtained after combination of the separate t-values according to :

$$(3.3) \qquad F = [t^2_1 + t_2^2 - 2t_1t_2r]/[2(1-r^2)]$$

A detailed explanation of the use of harmonic components in regression analysis may be found in Anderson (1971).

Results

Table 3.1 shows the total catches in the most abundant species. This species were: *Oedothorax apicatus*, *Oedothorax fuscus*, *Oedothorax retusus*, *Erigone atra*, *Erigone dentipalpis*, *Leptiphantes tenuis*, *Meioneta rurestris*, *Bathyphantes gracilis* and *Diplostyla concolor*. The restgroup consisted of uncommon species and juveniles.

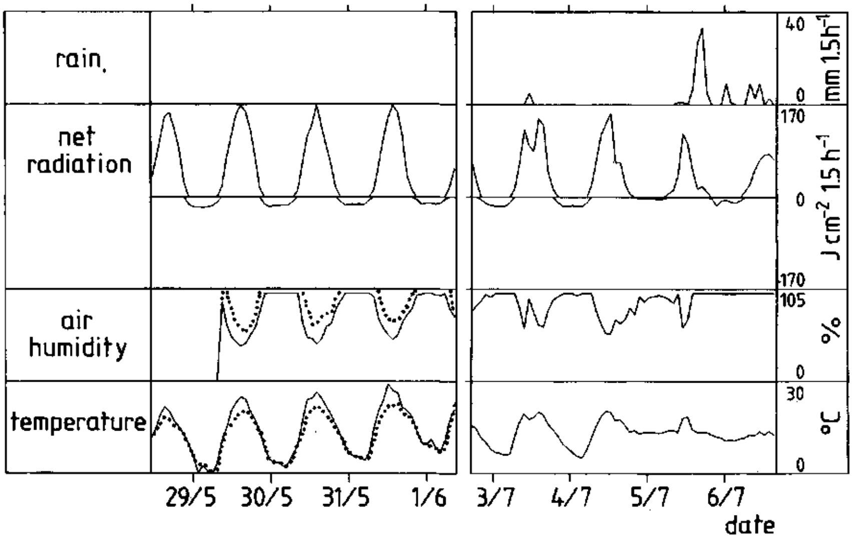


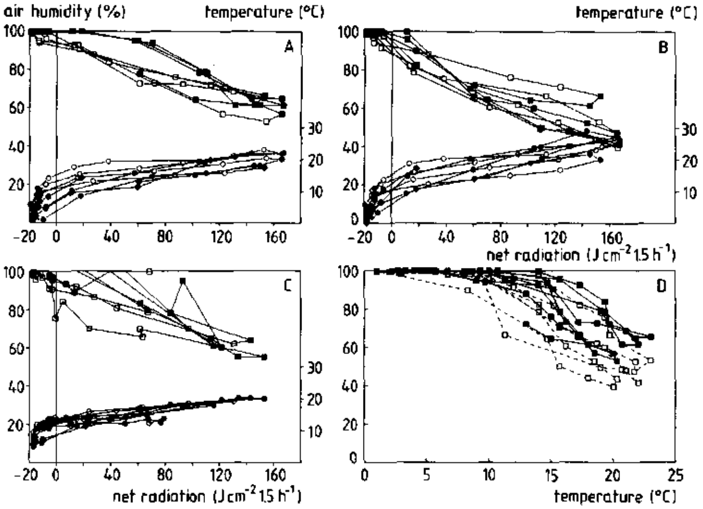
Figure 3.2. Hourly measurement of physical factors in the periods from 28 May to 1 June on field 1 and 2, and from 2 to 6 July on field 2. Measurements relating to field 1 are indicated with a broken line.

Table 3.2. Soil moisture of the top 2 cm of soil in the experimental plots at 8.00 hours (% dryweight).

Date:	Field 1	Field 2
29-5	20.6	12.4
30-5	20.0	10.5
31-5	17.2	9.2
1-6	16.8	7.8
3-7		34.6
4-7		27.1
5-7		30.1
6-7		29.4

Daily variation in physical factors is shown in Fig 3.2. During the first sampling period (28 May to 1 June) diurnal fluctuations in physical variables were very similar between days (Fig 3.2). No rain was recorded in May. In July a change in weather on the 5th (48 hours after the beginning of the experiment) stabilized the diurnal temperature variation in the range of 12 to 14 °C, and air humidity in the crop at values above 80%. Initial soil moisture on field 2 was high due to heavy rainfall on 20 and 26 June (Table 3.2).

Field dependent relationships between physical factors are shown in Figure 3.3. A nonlinear relationship in relation to net radiation was found in the daily course of air humidity and temperature. On all fields, temperature showed a quick rise (white markers)



3.3. Physical factors in relation to crop microclimate measured every 1.5 hour. Fig 3a to 3c show the variation in relative air humidity (left y-axis, square markers) and temperature (right y-axis, round markers) for: 28 May to 1 June on field 1 (Fig 3a), 28 May to 1 June on field 2 (Fig 3b), and 2 to 6 July on field 2 (Fig 3c). In Figs 3a to 3c periods with increasing radiation values are indicated by closed markers, periods with decreasing radiation values with open markers. Fig 3d shows the relationship between temperature and air humidity in the crop on field 1 (closed markers) and field 2 (open markers) in the period of 28 May to 1 June.

with increasing, and a slow return (black markers) with decreasing net radiation. In May, temperatures reached highest and air humidity lowest values in the open crop of field 2. On both fields, air humidity increased in a similar, almost linear way with decreasing net radiation. It should be noted that air humidity at temperatures below 10 °C was mostly 100% in both fields (Fig 3.3d). In July, patterns were much disturbed by a weather change 48 hours after the beginning of the experiment, which brought cloudy weather, rain and low net radiation values (Fig 3.3c).

A comparison of the catches in field 1 and 2 is given in Figure 3.4 for male *B. gracilis*, *E. atra* and *L. tenuis*. *B. gracilis* males show highest activity round 5.00 and 14.00 hours. The 95% confidence limits around the 96-hour means in relative hourly trapping success indicate no field dependent differences for the respective hours, however, there is a trend for high catches during the day, and low catches during the night in field 2, the open field, relative to field 1. The activity of *E. atra* shows a peak during noon hours. Although the 95% confidence limits indicate a significant difference at 11.00 hours, the activity patterns show great similarity on both fields. *L. tenuis* shows a peak in activity towards the evening and slightly elevated activity around 02.00 hours. The catches were similar for the different fields.

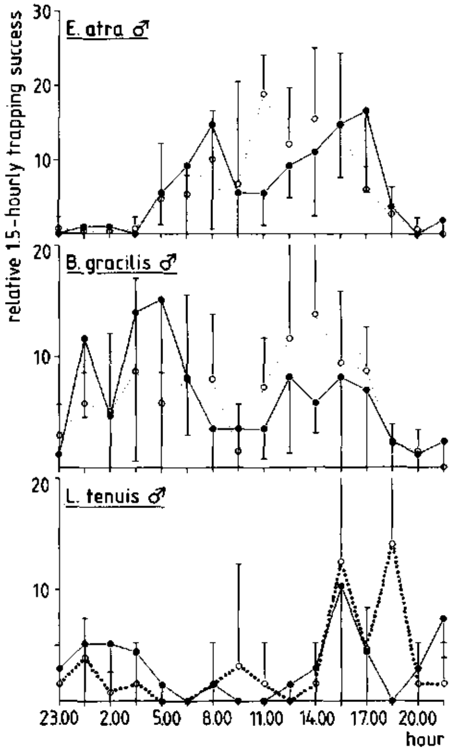


Figure 3.4. Field dependent diel activity distribution for linyphiid spider species in fields 1 (closed cirles) and 2 (open circles). Bars represent the 95% confidence limits of the daily mean 1.5 hourly trapping success.

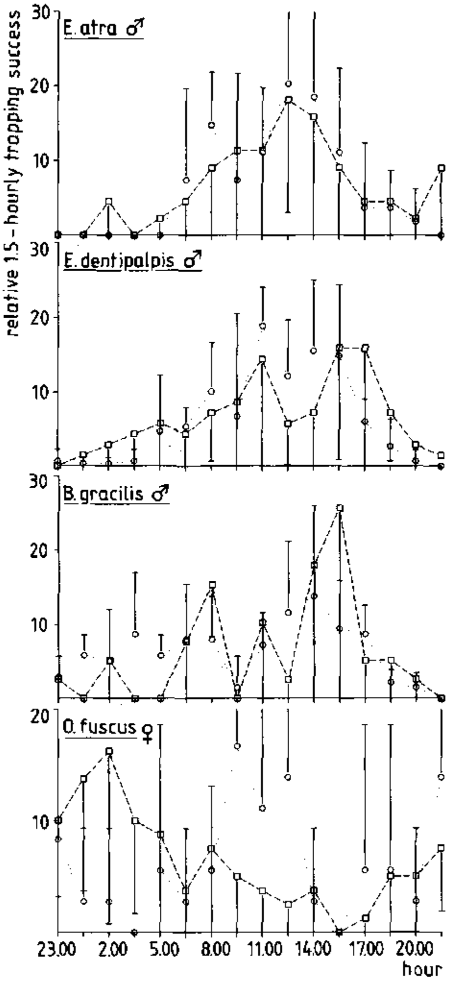


Figure 3.5. Comparison of the diel activity distribution for linyphiid spider species in field 2 in May (open circles) and July (open squares). Bars represent the 95% confidence limits of the daily mean 1.5 hourly trapping success.

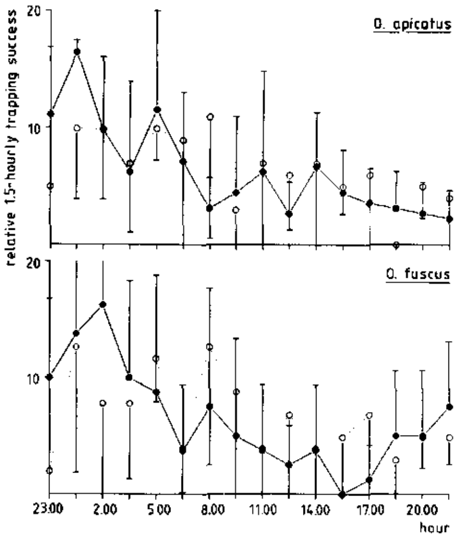


Figure 3.6. Sex dependent diel activity distribution for linyphiid spiders in field 2 in July. open circles = males, closed circles = females.

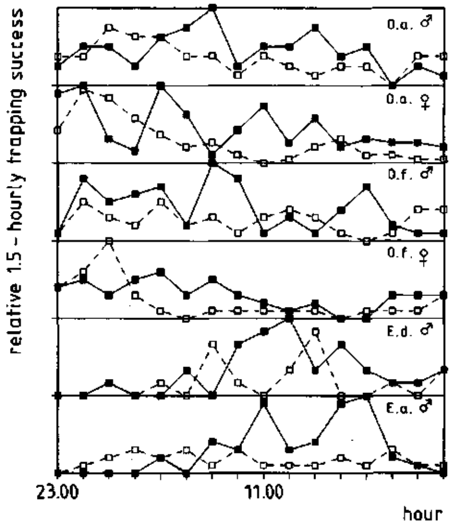


Figure 3.7. The effect of rain on the diel activity periodicity of linyphiid spiders in field 2 in July. solid squares = no rain, open squares = cloudy weather and rain.

The effect of sampling in May or July on the diel activity pattern was studied in male *E. atra*, *E. dentipalpis*, *B. gracilis* and female *O. fuscus*. Figure 3.5 shows that sampling in different months had little effect on the diel activity distribution for male *E. atra*, *E. dentipalpis* and *B. gracilis*, however, considerable differences were found for female *O. fuscus*, which showed an almost inverse activity pattern in July in comparison to May. It should be noted that relatively few *O. fuscus* were trapped in May, which may have affected the latter observation.

The effect of sexe on the diel activity distribution was studied in *O. fuscus* and *O. apicatus*, which were the only species of which high numbers of both sexes were caught. Figure 3.6 shows that apart from slightly higher activity at night in females than in males, the general activity pattern of both species and sexes shows great similarity.

The incidence of rain and cloudy weather during the last 48 hours of the experiment in July allowed for the study of the effects of bad weather on the diel activity pattern. To investigate these effects, plots were made of the hourly catches before and after the weather change, as is shown in Figure 3.7. Numbers are expressed relative to the total number of spiders caught in 96 hours. The catches for the last 48 hours were lower than those of the first 48 hours in all species, indicating a negative effect of rainy weather on spider activity, The pattern of diel activity, however,

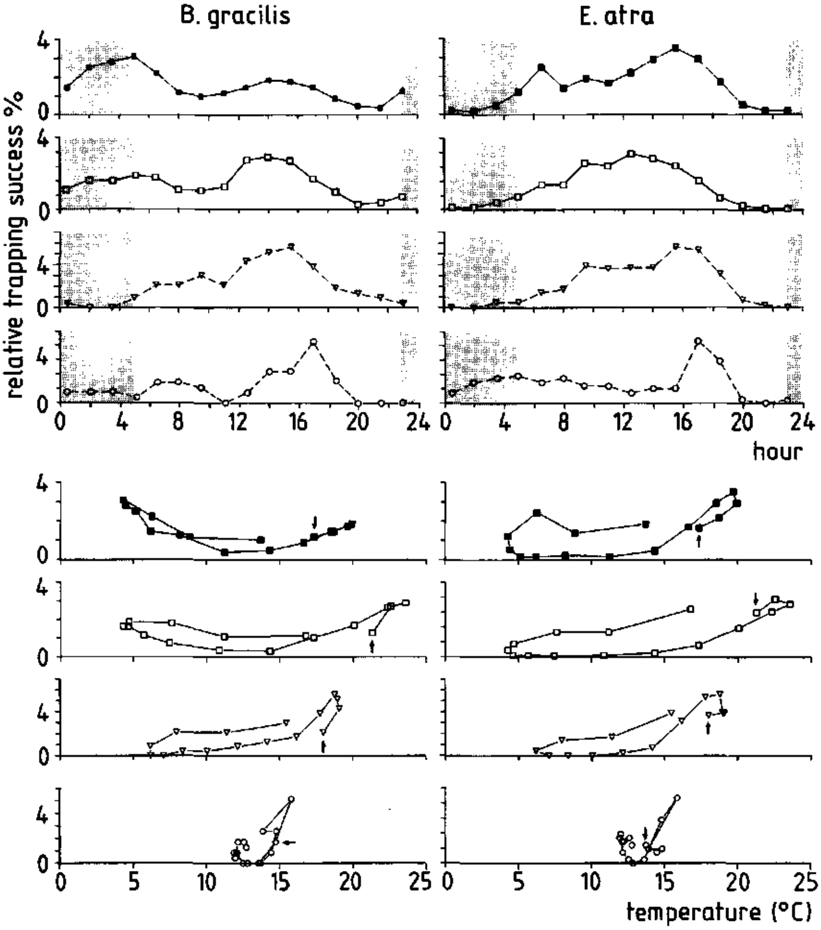


Figure 3.8. Relationship between physical cues and the 1.5 hourly trapping success in *B. gracilis* and *E. atra*. The smoothed relative trapping success (explanation in Results section), is plotted against time of the day in Figs 8a and 8b, and against the smoothed mean values of temperature at 5 cm above the soil in Figs 8c and 8d. From top to bottem each of the figures 8a to 8d show the values in May on field 1 and 2, and in July during the first and the last 48 hours of the experiment on field 2, respectively. Starting at 11.00 hours, indicated by an arrow, lines connect subsequent mean values for each 1.5 hourly period, ending with the relative trapping success at 09.30 hours.

seemed little affected by rain.

The results show that linyphiid spiders exhibit species dependent diel activity which, for most species, depends little on field, month, sexe or rain. It remains to be studied whether this pattern is related to endogenous or exogenous cues and to what extent its expression is modified by physical factors. The possible role of physical factors as a cue for walking activity was studied in male *B. gracilis* and *E. atra*. Figure 3.8 shows the mean hourly

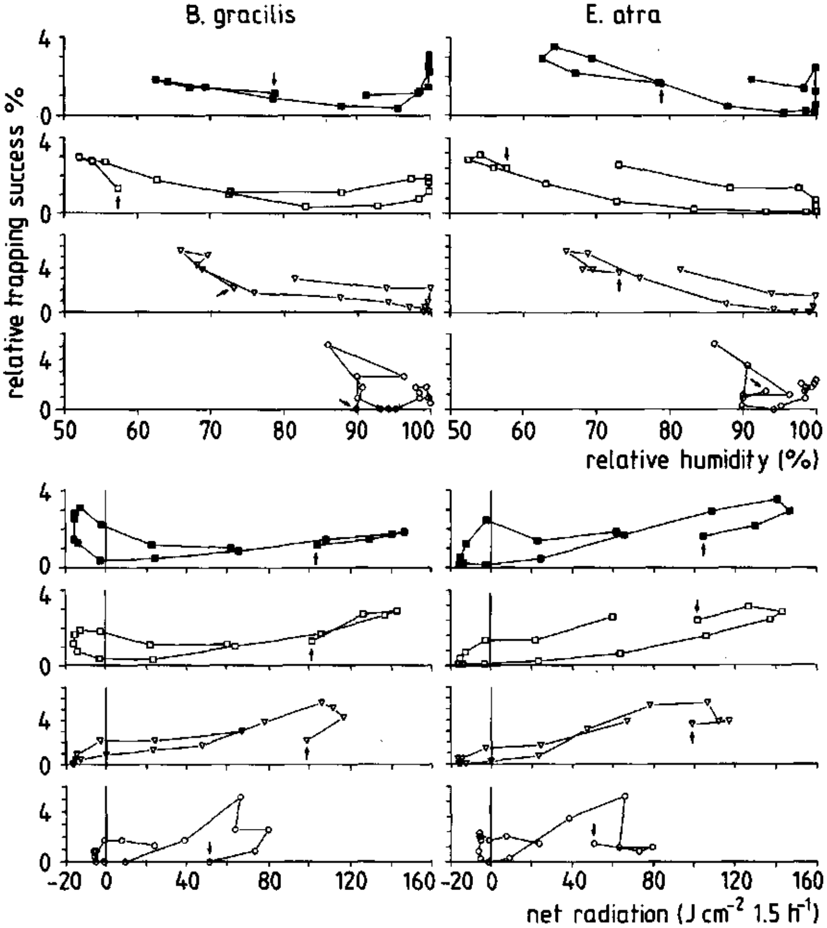


Figure 3.9. Relationship between physical cues and the 1.5 hourly trapping success in *B. gracilis* and *E. atra*. The smoothed relative trapping success (explanation in Results section), is plotted against the smoothed relative air humidity in Figs 8a and 8b, and against the smoothed net radiation values in Fig 8c and 8d. From top to bottem figs 8a to 8d show the values in May on field 1 and 2, and in July during the first and the last 48 hours of the experiment on field 2, respectively. Starting at 11.00 hours, indicated by an arrow, lines connect subsequent mean values for each 1.5 hourly period, ending with the relative trapping success at 09.30 hours.

activity of the spiders in relation to hour of the day, temperature, relative humidity and net radiation. The catches were devided into four subsets: catches in May on field 1, in May on field 2, first 48 hours in field 2 and the last 48 hours in field 2 in July. For these subsets the physical factors for any given hour were relatively stable between days. Therefore catches could be expressed as mean hourly trapping success in relation to time of the day or in relation to

Table 3.3. Parameter estimates and significance of the parameters in equation (3.1). * and ** indicate significance at the 0.05 and 0.01% level.

variable	regression coefficient	standard error	T for H_0: parameter=0	significance
B. gracilis (189 observations, R^2 = 0.37, F value= 10.53)				
intercept	1.224	0.662	1.85	
field 2	0.310	0.099	3.11	**
field 3	-0.246	0.105	-2.33	*
first harmonic component of time				
cost	-0.070	0.155	-0.45	
sint	0.151	0.101	1.50	
second harmonic component of time				
cos2t	-0.050	0.057	-0.87	common significance: *
sin2t	0.163	0.076	2.14	*
physical factors				
T	-0.031	0.015	-2.09	*
RH	-0.004	0.006	-0.67	
NET	-0.004	0.003	1.44	
DEP	-0.040	0.154	-0.26	
E. atra (189 observations, R^2 = 0.53, F value = 19.87)				
intercept	0.568	0.631	0.90	
field 2	0.297	0.095	3.13	**
field 3	0.045	0.100	0.45	
first harmonic component of time				
cost	-0.308	0.148	-2.08	* common significance: *
sint	0.248	0.096	2.58	**
second harmonic component of time				
cos2t	-0.085	0.055	-1.53	
sin2t	0.088	0.072	1.21	
physical factors				
T	0.038	0.014	2.66	**
RH	-0.006	0.006	-1.07	
NET	-0.055 E-3	0.003	-0.02	
DEP	-0.290	0.147	-1.97	*

the corresponding values of physical factors. To account for differences in population density between the fields, the trapped numbers per 1.5 hour were, for each sampling period, converted to percentages relative to the field total. To reduce variation caused by the Poisson distribution of the pitfall trap data, the curves of the hourly mean trapping success were based on smoothed values. The individual percentages for hour x were converted into 4.5 hourly mean values for hours x-1.5, x and x+1.5. The physical factors were smoothed accordingly. The results are shown in Figures 3.8 and 3.9.

The mean, smoothed, 1.5-hourly trapping results for *B. gracilis* and *E. atra* as a function of time of the day and of temperature are shown in Figures 3.8a to d, and as a function of relative air humidity and net radiation in Figures 3.9a to d. Night, defined as the period from the first until the last hour with zero incoming radiation, is indicated by the shaded area in Figures 3.8a and b. It should be noted that the first and the last hour during 'daytime', the incoming radiation values were very low.

B. gracilis showed an activity peak at 04.30 hours and at 14.00 hours, the night peak in July having shifted slightly towards the day peak. The peak catches in May, at night, correlated closely with the coldest hours and, at daytime, with the highest temperature, lowest air humidity and highest net radiation. It should be noted that maximum catches were always observed at extreme values of the physical factors, but showed no relationship with the absolute values of these maxima, nor with the sign of change in the physical variables. *E. atra* showed very little activity during the evening and early night, but had an activity peak during the day. With the exception of the open crop in May, highest activity during the day was observed 1.5 hours after the extremes of temperature, air humidity and net radiation had been reached. At night, however, after 03.30 hours, and during the period with rain (Fig 3.9d) earlier, activity showed an increase, which, except during the rainy period, showed a correlation only with increasing net radiation values, being observed well before dawn and the corresponding increase in temperature or decrease in air humidity (Figures 3.8d and 3.9b).

The possibility of linyphiid spiders showing an endogenous diel activity pattern with the expression influenced by physical factors was studied in *B. gracilis* and *E. atra* using multiple regression analysis as described in the materials and methods section. Unsmoothed data were used for these calculations. Temperature and air humdidity, measured in the crop, net radiation and the incidence of rain, on a 1.5 hourly basis, and the time of the day served as independent variables in the model. No significantly better fit was found for models in which the effect of the physical factors was allowed to change sign during the day according to a sinusoidal function. The results of the regression calculations are shown in Table 3.3.

In *B. gracilis*, the second harmonic component of the time of the day (two sinusoidal waves per 24 hours) was significant only. This indicated a biorhythm with two optima during the day, at 5.30 and 17.30 hours. In addition to this biorhythm, a significant negative contribution for temperature was found, which indicated a negative temperature activity relationship for *B. gracilis*.

In *E. atra* one optimum was found in the 24 hour biorhythm, the optimum being estimated at 9.00 hours. In addition to the biorhythm, significant contributions to the trapping success were found for temperature, showing a positive effect, and rain, which showed a reducing effect for trapping success.

Discussion

The present results show distinct, species dependent, diel activity patterns in linyphiid spiders. Observations of diel activity at a similar level of detail have been recorded by De Keer and Maelfait (1987) for *Oedothorax fuscus* in grazed pasture. Despite many differences with the present experiments, activity patterns show great similarity for the sexes. It should be noted that a reduction in activity at 6.30 hours and 15.30 hours was observed in both studies, which suggests that these events are not determined by chance or random error. Apparently, activity in these spiders responds in both situations to the same impetus, which is, most likely, of an endogenous nature, as it was not markedly influenced by crop, field, country, year or differences in physical conditions.

The presence of an endogenous activity rhythm is the most likely basis for walking activity in male *B. gracilis* and *E. atra* for several reasons. One, in different fields and months, trapping success showed daily optima at the same time of the day. The moment at which these optima occurred was independent of the absolute value of physical factors. Two, the sign of the rate of change of the physical variables did not show a consistent trend with trapping success. This was observed in catches of *B. gracilis*. In *E. atra* increases and decreases in trapping success were correlated with increases and decreases in net radiation. Different activities were found, however, for the same net radiation value depending on whether net radiation was increasing or decreasing. Three, the inclusion of a diel activity rhythm significantly increased the fit of the regression model for *E. atra* and *B. gracilis*. This does not exclude the possibility that the addition of other physical factors will ameliorate the model. The low correlation between the models and the observations must be ascribed to the intrinsically large variation in the poisson distributed 1.5 hourly pitfall trap catches.

Although the above results point to an endogenous activity rhythm, elucidation of the nature of this rhythm and possible synchronisation of it by physical cues, requires laboratory experiments analogous to those presented by Seyfarth (1980).

Assuming that walking activity depends largely on muscle function, the positive relationship for male *E. atra* between trapping success and temperature is in accordance with the general positive relationship in arthropods between muscle performance and temperature (review by Josephson, 1981).

The negative effect of temperature on trapping success for male B. gracilis requires a more complex explanation. The ability of this species to be active at temperatures below 5 °C corresponds with its highest activity in relation to reproduction in the field in February (Tretzel, 1954). Winter activity indicates that the physiology of *B. gracilis* is well adapted to cold. The species may, therefore, have a low temperature optimum for activity, higher temperatures causing a reduction in activity.

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CHAPTER 4

Walking behaviour and population density of adult linyphiid spiders in relation to minimizing the plot size in short term pesticide studies with pyrethroid insecticides

Summary

Temporal changes in spatial distribution, and population density of linyphiid spiders were studied in relation to physical factors and deltamethrin spraying in the field. The results of the study have been used to set the conditions necessary when plot size is reduced in field trials.

The results showed independence of observations at 7 m from an unsprayed reservoir, with respect to migration, in the week following pesticide application. Soil moisture had a positive effect on trapping success, without having apparent effects on the migration rate. No difference in effect was found for deltamethrin spraying on wet and dry soil. Shifts in the spatial distribution of spider activity showed a close correlation with crop microclimatic conditions. The effect of trapping on population density was shown to be limited. The results suggest that small plots surrounded by a border of 7 m are appropriate for assessing short term pesticide effects on adult linyphiid spiders in winter wheat.

Introduction

Jepson and Thacker (1990), stated that pesticide side-effects on terrestrial arthropods may be analysed on different temporal and spatial scales. The use of mathematical models within and between these scales was proposed as a tool for improving interpretation of results at the different levels (Jepson *et al.* 1990); however, modelling studies require an evaluation of the hypothesis used and of the predictive value of the outcome in the field situation. This demands field observations of the model parameters, preferably with replications, on a spatial scale which is in accordance with the temporal scale of the observed effect. As a consequence, short term pesticide effects may be analysed on small plots, the size of which must garantee their independence from treatments in adjacent plots and/or influences from the surrounding field such as migration. The aim of the present study was to find an appropriate, small plot size for short term experiments with linyphiid spiders. This should account for effects of spider migration in relation to physical factors and deltamethrin application.

The choice for using linyphiid spiders is based on the work of Everts (*et al.* 1989, 1990, *et al.* 1991a, *et al.* 1991b), who showed that linyphiid spiders represent an abundant and vulnerable part of the epigeal arthropod fauna. These spiders are highly sensitive to deltamethrin, used in the present study as model pesticide.

Although the above has not yet lead to the definition of an appropriate small plotsize for short term field experiments, different authors have studied linyphiid spiders in relation to factors which determine this plotsize. Thomas *et al.* (1990) calculated 'median estimated recovery times' of 1.1 and 3.7 weeks, respectively, for migration of the linyphiids *Oedothorax apicatus* and *Erigone atra*, in winter wheat at a distance of 15 m from an unsprayed reservoir. Jepson and Thacker (1990), studying the migration of terrestrial arthropods into dimethoate treated winter wheat, showed that the migration velocities could be ranked in increasing order from *Carabidae* via *Linyphiidae* to *Staphylinidae*. Everts *et al.* (1990), in studying the spatial distribution of linyphiidae in oilseed rape, showed that *E. atra* (male) in oilseed rape had an unchanged distribution over the field after an 80% reduction following deltamethrin application. The relationship between soil humidity and residual toxicity of deltamethrin for female *Oedothorax apicatus* (*Erigonidae*) was studied by Everts (1991a) who showed that under controlled laboratory conditions, effects were more severe on wet or very dry soil than on moist soil. The density of spiders, normally mostly *linyphiidae*, in agricultural ecosystems, has been estimated (D-vac, handsorting or enclosures) between 0 and 100 spiders m^{-2} (Schaefer 1976, Sunderland *et al.* 1987, de Keer and Maelfait 1987).

In previous experiments the side effects of deltamethrin on linyphiid spiders have been examined using a sampling frequency of one week (Inglesfield 1989, Everts 1990, Thomas

1990). As, in these papers, the effect of deltamethrin on linyphiid spiders was found to be most severe during the first week after spraying, an *a priori* sampling frequency of once a day was chosen for the present experiment.

The information in the papers quoted above was not conclusive as to the extent to which the temporal and spatial scale of the experiments in our situation could be reduced. The main objectives of the present study therefore were:

1. To investigate the temporal and spatial dynamics of the distribution of linyphiid spider activity in the field, in relation to crop microclimate and deltamethrin application.
2. To determine an appropriate sampling frequency with respect to the observed short-term effect of deltamethrin.
3. To quantify the population density of linyphiid spiders in winter wheat with respect to possible effects of trapping on population density.

Materials and methods

Work addressed to the first and second objective forms the first part of this study. The experimental layout in the field is given in Fig. 4.1. The field was situated on the terrain of the Institute for Plant Protection Research (IPO, Wageningen). It was divided into four equal plots with the following treatments: 1. a 'wet' and 2. a 'dry' control plot, 3. a 'wet' and 4. a 'dry' plot treated with deltamethrin. Fields were sown with winter wheat and treated according to standard integrated pest management practice. Except for experimental impact, crops were not sprayed during the experimental period.

Spiders were caught in round pitfall traps (Everts *et al.* 1990). 81 Traps were placed in a square grid pattern of 9 times 9, 7 m apart. These were emptied daily between 08.00 and 10.30 from 9 to 26 May 1989 and 10 days later on 5 June. Linyphiid spiders were counted in the field and stored in 70% alcohol.

Deltamethrin (Decis^{R}-flow)(Hoechst Holland BV, Amsterdam, The Netherlands) was sprayed on the morning of 20 May using a tractor-mounted hydraulic sprayer with split-nozzles (Douven, nr 11-110-06 (Douven Machinefabriek, Horst))(5 g a.i. in 400 l water/ha, windpeed on 20 May at 9.00 hours: 1.4 m s^{-1}, direction: east). Deposition at soil level was determined using a fluorescent tracer (Tinopal)(68 g/400 l water/ha). For this pupose strips of window glass (50 by 3 by 0.3 cm) were put on the soil in a regular grid (Fig. 4.1). The strips were collected immediately after spraying and placed in a light proof box. The stips were rinsed with distilled water and the tinopal was measured, quantitatively, using a fluorimeter (emission 450 nm, absorbtion 380 nm).

The first 2 cm of soil, a heavy illitic river clay; 'medium high Nude silty clay loam', 59 % lutum content, 5.3 % organic matter, was sampled daily at 9.00 hours, and at 19.00 hours on 20 May, at three different locations per plot to determine soil humidity (% dry weight, oven dried overnight at 105 °C, Fig. 4.1). The 'wet' plots were sprinkled with water using a 15 m wide sprinkling device with split nozzles on the evening of 18 May.

The temperature of the air 5 cm above the soil and of the top soil layer (-0.5 cm) was measured at the center of each plot (Fig 4.1) using thermistors mounted in thin stainless steel tubes freely exposed to the air and shielded from direct radiation. Hourly means were stored for temperature readings taken every 5 minutes (Depex datataker; DT100I). The accuracy of the thermistors was within 0.3 °C (checked before and after placement in the field).

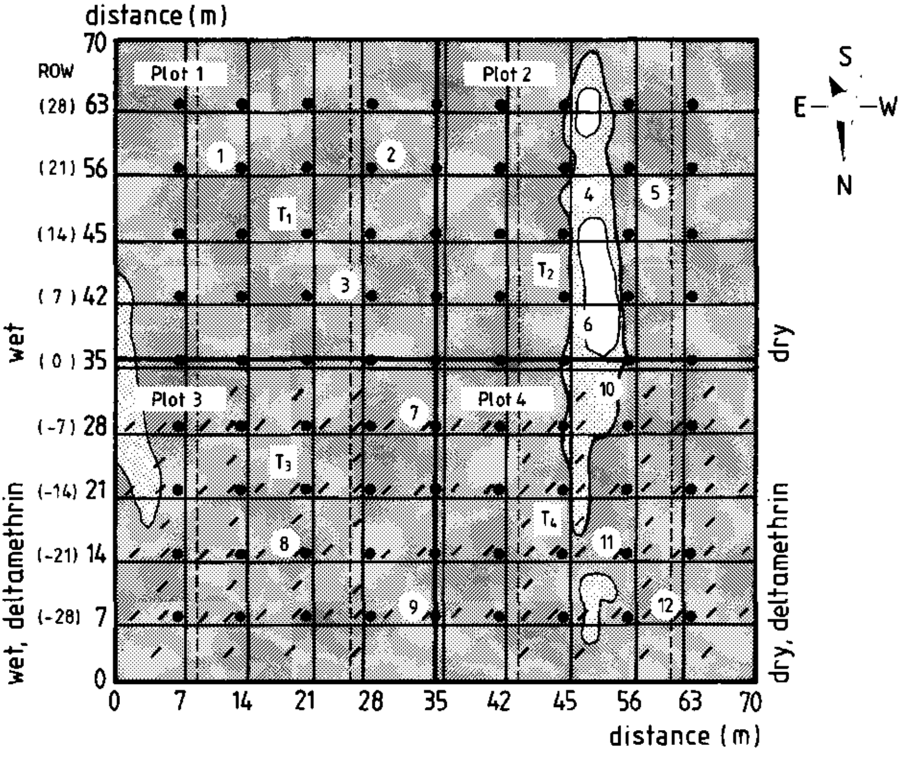

Figure 4.1: Experimental design of the migration experiment. Plot 1 = wet control, plot 2 = dry control, plot 3 = wet deltamethrin, and plot 4 = dry deltamethrin (water spraying: 19 may, deltamethrin application: 20 may). Crop density is indicated by different shades of grey (white = less than 10 plants m^{-2}, grey = 10 to 25 plants m^{-2}, dark = 25 to 60 plants m^{-2}). Dotted lines represent tractor wheelings. Solid lines show the paths used to collect the spiders. Soil moisture and temperature measurement points are indicated by the numbers 1 to 12 and T1 to T4. Bars in plot 3 and 4 give the positions of glass strips used in measurement of deposition.

The graphs of the spatial activity distribution of linyphiid spiders were made using Surfer software (Public Domain, method: kriging). To reduce effects of individual, isolated, extreme catches, the individual values for the 81 x-y coordinates were calculated as the mean of the logarithms of the daily catches of 9 to 14, 15 to 20 and 21 to 26 May, and of the logarithm of the mean value (sum/10) obtained from 27 May to 5 June.

The effects of deltamethrin were analysed using short time series analyses (BACI-analysis, Before and After impact on a Control and Impact plot (Stewart-Oaton *et al.* 1986), Van der Voet (1987)). An example of how this technique can be used in the assessment of pesticide effects in field trials is given in Jagers op Akkerhuis and van der Voet (1992). As in this experiment counts ($plot^{-1}$) were reasonably high and never 0, little difference was expected between (quasi-) Poisson modelling and lognormal modelling of the counts and for computational simplicity the latter approach was chosen. It was assumed that catches might differ for each plot and day, but that, without treatment, day-effects will have the same relative influence on the catches of each plot. Water was assumed to exert a constant influence on all catches from sprinkled plots from the day of spraying onwards. The effect of deltamethrin was modelled assuming a common, instantaneous effect for deltamethrin sprayed plots, followed by exponential recovery. The possability of an interaction between the effect of deltamethrin and that of water was also investigated. These assumptions resulted in the following general model:

$$(4.1)\quad Y_{it} = \mu + \alpha_i + \beta_t + \delta_{IAdm} * n_{dm} * e(h_{dm} * t - t_0) + \delta_{IAw} * n_w + \delta_{IAdm} * \delta_{IAw} * n_{int} + \epsilon_{it}$$

where Y_{it} is $ln(n_{it})$, i.e. the log-transformed counts for plot i and day t, μ the systematic part of the model, α_i and β_t respectively the plot and day effects, δ_{IAdm} an impactvector for which the value is 0 before and 1 after (A) impact (I) of deltamethrin, n_{dm} the instantaneous effect and h_{dm} the rate at which the catches recover from deltamethrin impact, δ_{IAw} and n_w the impactvector (starting one day earlier than deltamethrin impact) and the effect of water, n_{int} the interaction effect and ϵ_{it} a random contribution from a normal distribution with mean 0 and variance s_2.

As the above model consists of simple units arranged in a hierachical way, the significance of using a more complex model was tested using the likelihood ratio test statistic:

$$(4.2)\quad vr = \{(SS_{res,0} - SS_{res,1}) / (df_{res,0} - df_{res,1})\} / MS_{res,1}$$

Table 4.1. Models used for BACI analysis of the effects (for explanation of symbols see text).

model	equation
1	$Y_{it} = \mu + \alpha_i + \beta_t + \epsilon_{it}$ (no-treatment-effect model)
2	$Y_{it} = \mu + \alpha_i + \beta_t + \delta_{IAw} * n_w + \epsilon_{it}$ (model with constant effect of water)
3	$Y_{it} = \mu + \alpha_i + \beta_t + \delta_{IAdm} * n_{dm} * e(h_{dm} * t - t_0) + \epsilon_{it}$ (exponential decaying effect of deltamethrin)
4	$Y_{it} = \mu + \alpha_i + \beta_t + \delta_{IAdm} * n_{dm} * e(h_{dm} * t - t_0) + \delta_{IAw} * n_w + \epsilon_{it}$ (const. eff. of water, exp. decay of effect deltamethrin)
5	$Y_{it} = \mu + \alpha_i + \beta_t + \delta_{IAdm} * n_{dm} * e(h_{dm} * t - t_0) + \delta_{IAw} * n_w + \delta_{IAdm*\delta IAw} * n_{int} + \epsilon_{it}$ (const. eff. of water, exp. decay of effect deltamethrin, with interaction)

where indices 0 and 1 refer to the simple and more complex model (Table 4.1). The variance ratio is identical to the F statistic known from analysis of variance, and under the null hypothesis that the simple model is the true model it has an F distribution with $df_{res,0} - df_{res,1}$ and $df_{res,1}$ degrees of freedom.

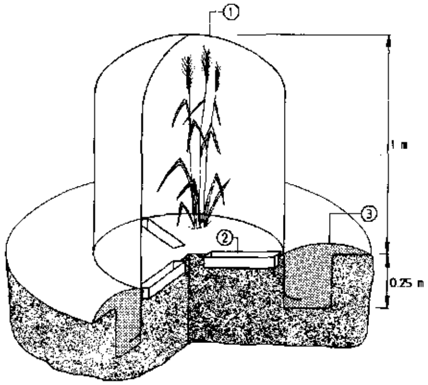

Fig. 4.2. Enclosure trap (1 m²): 1, net (mesh diameter <0.3 mm); 2, pitfall trap; 3, quartz river sand.

The second part of the experiment was aimed at assessing the population density of linyphiid spiders. A replicate experiment was carried out on two plots in an untreated field bordering the field described above. Two stretches of winter wheat (series 1; 5 to 10 July, series 2; 15 to 26 July) measuring 100 by 18 m were devided in 5 plots of 18 by 20 m. Four times 2 enclosures were placed on each border between two plots, 5 m from the center of the field. Enclosures (Fig 4.2) made of fine mesh net (mesh diameter < 0.3 mm) were placed around 1 m^2 circular sections of the crop and the net was dug 25 cm into the soil. As the installation of the enclosures was rather labourous, these were installed on successive days in groups of 5 and 3 (series 1), or 4 and 4 (series 2). Three rectangular (30 by 7.5 cm) pitfall traps, filled with (4%) aqueous formalin solution and a little detergent (Teepol) were placed in each enclosure. 6 Reference samples, for determining natural variation in spider activity, were caught using 5 groups of 6 round pitfall traps in the center of the field (2 m from the center of the plot, 3 m apart). All traps were emptied daily between 8.00 and 10.30 hours. Temperature was measured in the crop at 5 cm above the soil surface and the hourly mean taken from measurements made every 5 min (mean of 2 thermistors, coupled to a data-taker (DT100I), accuracy within 0.3 °C limits, checked before and after the experiment).

Results

Abiotic variables

The results of measurements of abiotic factors for the first part of the experiment are shown in Fig 4.3 to 4.5, and of the second part in Fig 4.6. The mean deposition of deltamethrin on the soil amounted to 0.26 g/ha (std. 0.019, n=53) on the wet plot and 0.26 g/ha (std. 0.018, n=58) on the dry plot and the pesticide was fairly evenly deposited over the sprayed area (Fig 4.3).

The mean soil moisture of the 'wet' plots was raised, using a 30 m wide spraying boom with nozzles every 60 cm, from 35.7 % to 44.8 % (std respectively 3.2 and 3.1, n=6). Soil moisture changes for separate plots are presented in Table 4.2. Fig 4.4 shows that in parts of the field with an open crop canopy the soil was drier and dried out quicker than the soil of more covered parts. Rainfall during the 24 hours preceding sampling on the mornings of 12, 13, 14, and 21 May amounted to 1.0, 9.7, 2.7 and 0.8 mm, respectively and 0 mm on all other days.

Table 4.2: Effect of sprinkling on the soil moisture content of plots.

	soil moisture: 9.00 hours (%dwt, standard dev.)			
	control		deltamethrin	
day	dry	wet	dry	wet
19 May	23.8 (6.2)	32.4 (5.5)	23.1 (4.8)	41.9 (2.1)
20 May	22.1 (4.8)	41.3 (5.6)	28.0 (6.1)	48.4 (1.9)

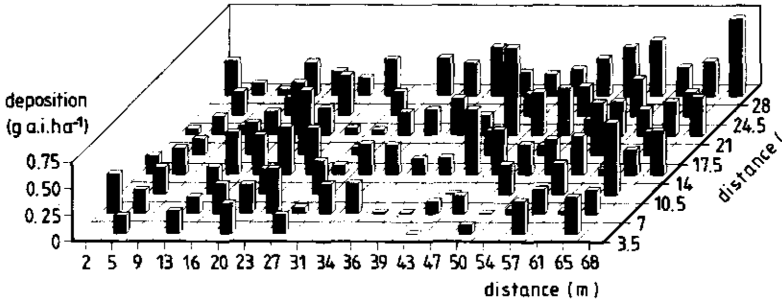

Figure 4.3: Calculated deposition of deltamethrin at the soil surface (g ai ha^{-1}).

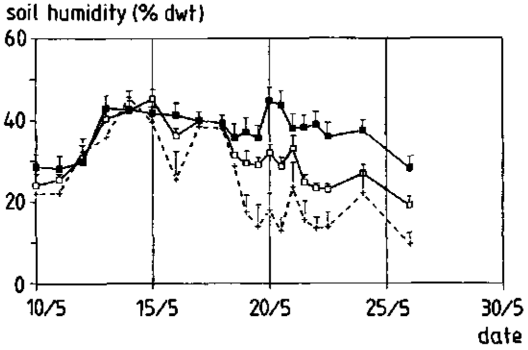

Figure 4.4: Soil humidity in relation to crop density and sprinkling. Mean soil humidity (% dryweight, and std) of: the wet plots (-■-)(n=6), the part of the dry field with a dense crop (-□-)(n=3), and the part of the dry field with an open crop (-+-)(n=3).

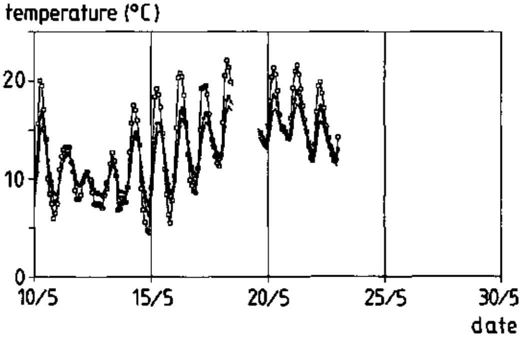

Figure 4.5: Diurnal temperature fluctuations in the crop. Temperatures at 5 cm above the soil (-□-) and 1 cm below the soil surface (-■-) in dry plot (T4), and at 1 cm below the surface in wet plot (-+-)(T3) (5 cm above the soil in the wet plot failed due to a defective thermistor).

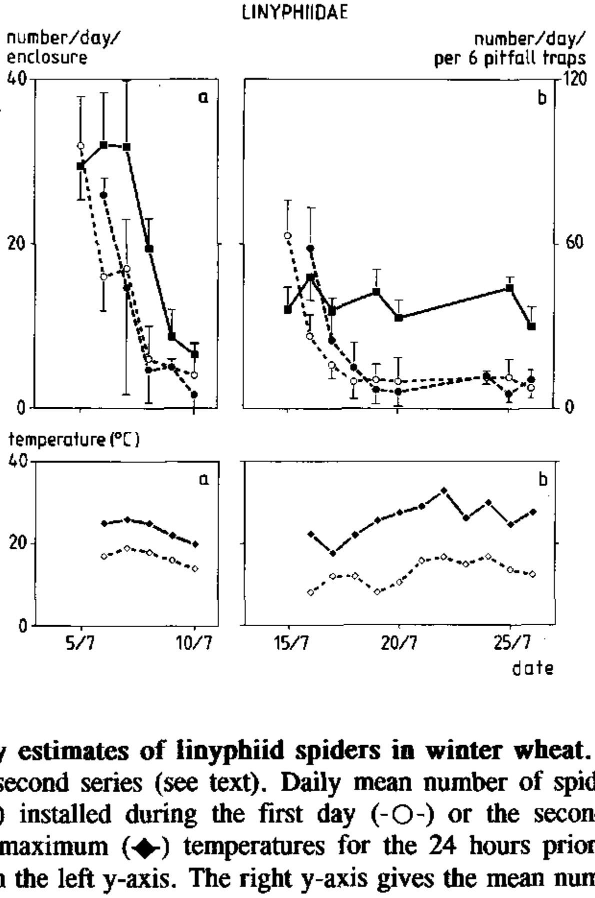

Figure 4.6. Density estimates of linyphiid spiders in winter wheat. Fig 4.6a = first series, Fig 4.6b = second series (see text). Daily mean number of spiders caught in the enclosures (with std) installed during the first day (-○-) or the second day (-●-), and minimum (-◇-) and maximum (-◆-) temperatures for the 24 hours prior to emptying the traps are indicated on the left y-axis. The right y-axis gives the mean number (with std) of spiders trapped in the reference traps in the field (-■-)(sum of 6 traps).

Fig. 4.5 shows the sinusoidal, diurnal fluctuations of the temperature in the crop with a maximum amplitude of 16 °C. The air temperature showed a higher maximum and lower minimum than the soil temperature. The latter effect was seen most clearly for the dry plots.

Spider catches.

Spatial changes in spider activity in relation to effects of deltamethrin and/or physical factors are shown in Figure 4.7 and 4.8. Figure 4.7 shows the trapping success on a daily basis, resulting in high temporal resolution. The Poisson ditribution of trapping data, however, causes low spatial resolution in this figure. In Figure 4.8 spatial resolution was increased by summarising the catches for periods of 6 days. In the latter case 'iso-linyphiid' lines are drawn around areas with the same mean number of trapped spiders.

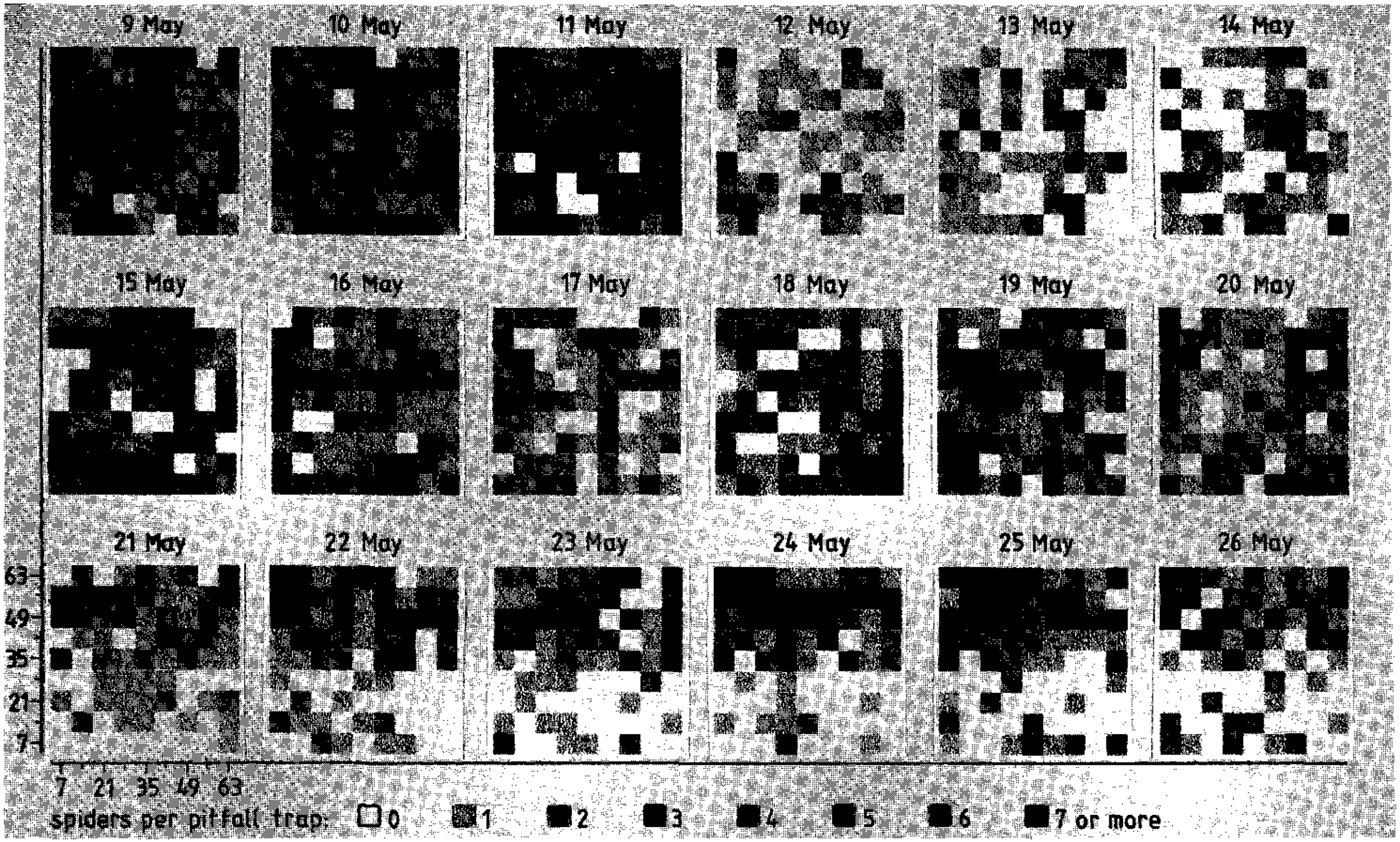

Figure 4.7: Temporal changes in trapping success. For 9 to 26 May, the observed trapping success of 81 individual traps is plotted on a daily basis using different shadings of gray for catches of 0 to 6, and 7 or more. Water sprinkled on 19 May. Deltamethrin applied on 20 May.

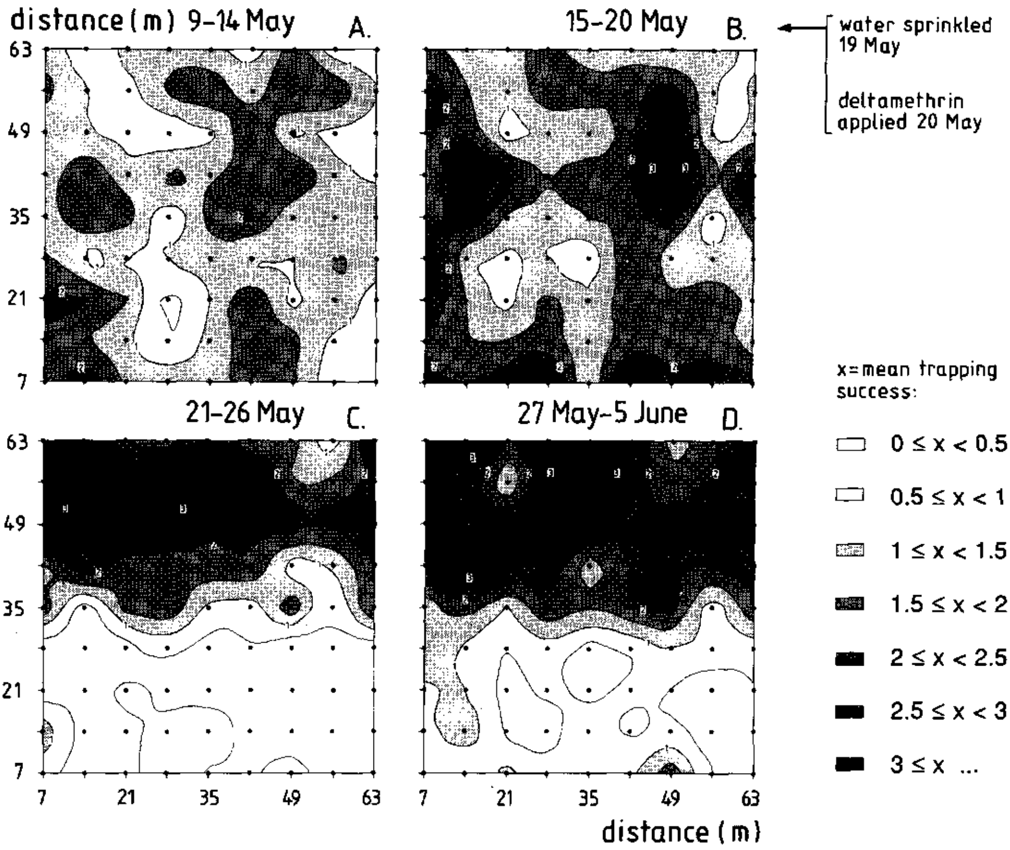

Figure 4.8: Spatial distribution of linyphiid spider activity. 'Iso-linyphiid lines' are drawn between regions with similar trapping success (indicative of spider activity and/or density) during the following periods: 9 to 14 May (A), 15 to 20 May (B), 21 to 26 May (C) and 27 May to 5 June (D). For calculation of the mean catches see text. Water sprinkled on 19 May. Deltamethrin applied on 20 May.

During 9 to 14 May the catches showed an even spatial distribution (Fig 4.8a) including during the period of 12 to 14 May (Fig 4.7) when cathes were very low. During 15 to 20 May high catches were observed in the proximity of the area with an open crop canopy (Fig 4.7 and 4.8b). On the evening of 19 May plots 1 and 3 were sprinkled with water, and plot 3 and 4 were sprayed with deltamethrin on the morning of 20 May. During the next 6 days, the area of highest catches shifted more than 21 m, from the dry control plot to the wet control plot, where it remained until the end of the experiment. Treatment with deltamethrin strongly reduced catches, an effect which showed equal spatial distribution on both dry and wet deltamethrin plots (Fig 4.7 and 4.8c). The frontline between high catches on the control plots and very low catches on the deltamethrin-treated plots stayed close to the border between these plots (Fig 4.7 and 4.8d). The slightly higher catches in all traps during the last period are an artifact of the way in which these particular mean values were calculated (see above).

Figure 4.9 shows the immediate, detrimental effect of deltamethrin on the catches, and an increase in trapping success for the water sprinkled plots after a lag phase of two days. The effects were evaluated using time series analysis. The models investigated are summarised in Table 4.1 and the lack of fit statistics for these models are given in Table 4.3. Results of the F-statistics, indicative for the significance, are presented in Table 4.4.

The results of the time series analysis (Table 4.4) show that model 3 fits better than model 1, indicating the effect of deltamethrin. The effect of sprinkling is not significant in model 2, but, after including the effect of deltamethrin in the model the better fit of model 4 over model 3 shows that sprinkling significantly raised the catches. Model 5 was not significantly better than model 4, indicating no significant interaction between the effect of deltamethrin and water. The residuals revealed no problems with the assumptions of the model. The effect parameters of model 4 were calculated as: $^{n}_{dm}$=-1.27 (s.e. 0.53), h_{dm}=0.03 (s.e. 0.04) and $^{n}_{w}$=0.40 (s.e. 0.13). Deltamethrin thus caused a 72 % reduction of the catches and no significant recovery was found within 6 days. Sprinkling caused a 36 % calculated mean increase of the catches in both plots.

Table 4.3. Lack of fit for the models of Table 4.2.

model	df_{res}	SS_{res}	MS_{res}
1	51	15.40	0.30
2	50	14.75	0.29
3	49	3.95	0.08
4	48	3.30	0.069
5	47	3.18	0.068

Table 4.4. Significance of larger complexity, using likelihood ratio test for hierarchical models. (ns = not significant)

models	extra par.'s	var. ratio	P (approx.)
2 over 1	1	2.24	ns
3 over 1	2	71.50	60.00
4 over 3	1	9.42	0.00
5 over 4	1	0.76	ns

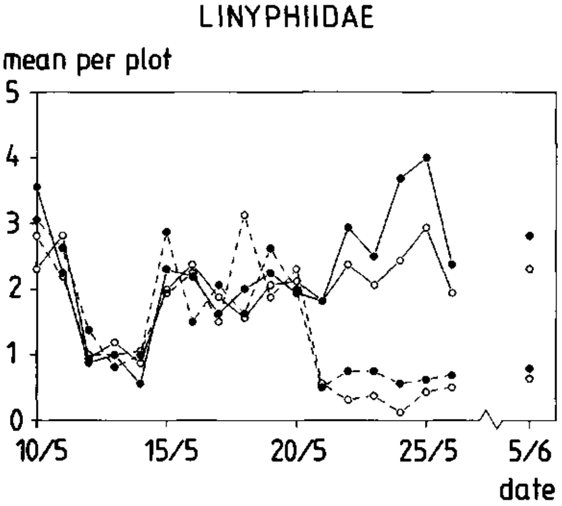

Figure 4.9: Effects of water and deltamethrin on spider activity. ——— = catches of the control plots, - - - = Catches of the deltamethrin treated plots. Closed (●) and open (○) rounds represent catches on water sprinkled and dry plots. Mean values were calculated for the 16 traps in the center of a plot.

As a special case of changing spatial distribution of spider activity, spider migration was investigated by summarising the catches of the traps in rows at different distances from the edge between the sprayed and the unsprayed plots. Figure 4.10 shows that before spraying deltamethrin, catches were equally distributed over the field, with slightly higher catches in the rows at -28 and 7 m. Spraying of deltamethrin caused a strong reduction of all catches in the sprayed plots. The catches of the first day after spraying were relatively low in the control plot, and rather high in some rows of the deltamethrin sprayed plot. 17 days after impact, a slight increase of the catches at -7 m was observed. Comparison of the relative distribution of the summarised pre-spray and post-spray cathes over the rows shows that, after spraying, either the catches at 0 and 7 m showed a relative decrease or those at 14 to 28 a relative increase. Fig. 4.7 and 4.8 show that this effect was mainly due to a shift of the area of high catches to the center of plot 1. The apparent absence at -7 m of recovery due to immigration, gave little reason to model the diffusion component of these data according to the method published by Thomas *et al.* (1990).

Figure 4.6 shows that the numbers of linyphiid spiders caught each day in the enclosures decreased rapidly with time, to reach a constant low level after 4 days. This decrease was not related to environmental factors because it preceded the decrease in catches from the field (caused by rain on 7 and 8 July) during the first series (Fig. 4.6a), and clearly showed no correlation with the field catches during the second series (Fig. 4.6b). This implies that the decrease was caused by a progressive reduction in the number

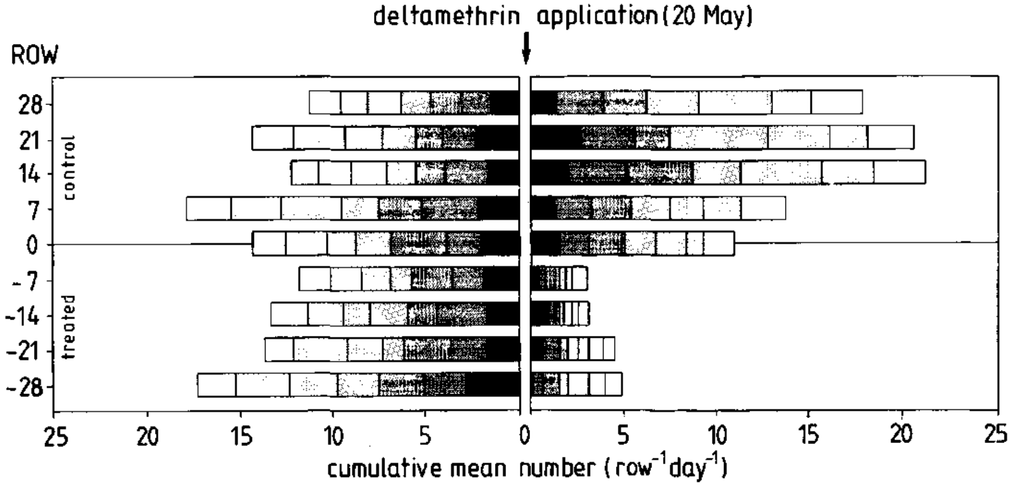

Figure 4.10: Spatial aspects of linyphiid spider dispersal. Means of 9 traps in rows at constant distances from the edge between the deltamethrin treated and untreated half of the field (see Fig 4.1). Left side = before, right side = after deltamethrin application. Means were calculated for the catches of the periods 9 to 14 May (lumped), 15 to 26 May (daily), and 27 May to 5 June (lumped). Dark shading indicate days close to deltamethrin application.

of spiders in the enclosures. A conservative estimate of the population density of linyphiid spiders in each investigated enclosure was obtained by substracting the constant level (mean value of 3 for both series) from the catches, followed by numerical integration of the resultant first 4 days catches to give a mean per m^2. The densities obtained were 59 or 39 (std 14 and 19) for the groups of 5 and 3 enclosures in the first series, and 26 or 23 (std 8 and 10) for the two groups of 4 enclosures in the second series.

Discussion

The present results strongly suggest slow migration of spiders in winter wheat. This is demonstrated by the steep gradient in trapping success crossing the plot edges between the sprayed and unsprayed plots. A steep gradient may be caused by several processes. One, it may result from slow migration. Two, a repellent effect of residually available deltamethrin may prevent spiders from migrating into the sprayed plots. Three, if the sprayed plots remain lethally toxic for a long while, and thus function as a sink with repect to spider migration, even rapid migration will result in a steep gradient. The option of pesticide toxicity remaining at a high level is unlikely, as the trapping success in the deltamethrin sprayed plots was never reduced to zero. With respect to repellence of deltamethrin sprayed substrate Boehncke *et al.* (1990), using radio-labelled deltamethrin, showed that evaporative losses from wheat leaf and soil surfaces respectively amount to 69% and 24% after 24 hours, with 52% loss from soil after 75 hours (wheat was not tested after 75 hours). Moreover, the bioavailability of deltametrin from substrate was shown to decline rapidly, with a halflife time of less than 3 hours on soil under controlled laboratory conditions (pers. obs. G. Jagers op Akkerhuis). Therefore, the second option is only realistic if deltamethrin shows a long lasting deterrent effect at extremely low bioavailability. This requires further investigation. Consequently, the most likely cause for the observed gradient is slow spider migration. It should be noted that, in combination with random movement, this does not exclude the possibility that spiders show high walking activity.

The absence, of recovery at -7 m in the sprayed plots for more than a week after spraying, is in accordance with the results of Thomas *et al.* (1990). Thomas *et al.* (1990) found no significant recovery (Tukey HSD multiple range test) for 15 m distance from an unsprayed reservoir within 3 weeks after deltamethrin spraying in winter wheat (10 g ai ha^{-1}), for Oedothorax apicatus or 4 weeks for *Erigone atra*. It should be noted that the ERT$_{50}$ times given in the paper of Thomas *et al.* (1990) are based on a fixed recovery level, ie half of the pre-spray relative density, instead of recovery relative to the observed reduction in trapping success immediately after spraying. The ERT$_{50}$ times therefore suggest quicker migration than actually observed (demonstrated by the multiple range test). This shows that with regard to migration, experimental plots for short term experiments using deltamethrin can be relatively small, as long as they are surrounded by a border of 7 m to separate treatments.

Shifts in the spatial distribution of trapping success may be related to factors such as crop density, soil humidity and temperature. In Fig 4.7 and 4.8a rain and low temperatures corresponded with low overall catches. After rain on 11, 12 and 13 May, the soil stayed moist for a while (Fig 4.4) which together with locally higher temperatures and more radiation could explain why catches peaked near the area with low crop density (Fig 4.8b). Thereafter temperatures increased further, the soil became dryer, and a shift in catches was observed to the sprinkled and most densely grown part of the field (Fig 4.8c and d) where the soil remained moist and the crop filtered away most sunshine. The quick and massive response to changing physical conditions of locations 21 m apart, as was the case for the shift in trapping success from plot 2 to plot 1, between 3 and 5 days after sprinkling on plot 1 (Fig. 4.7 and 4.8), implies that the observed changes in trapping success are most likely due to local changes in mobility and not to redistriution of the spiders. As this may interfere strongly with migration experiments, this topic requires further investigations. In investigating such rapid changes in spatial ditribution of linyphiid spider acitivity, attention should be payed to the fact that, as a result of the Poisson distribution of pitfall trap data, increasing the temporal resolution of the data results in a lower spatial resolution and *vise versa* (figure 4.7 and 4.8).

In contradiction to the laboratory results of Everts *et al.* (1991a), who found that deltamethrin, sprayed on soil, was more toxic to *O. apicatus* on a wet soil (30 % wet weight) than on a soil with a moderate moisture content (10 or 20 %), the effect of deltamethrin in the present field-experiment was not increased by high soil humidity. Following a lag-phase of two days, which may correspond with a reduction in bioavailability of the deltamethrin deposit byond the detection limit for spiders, sprinkling on 19 May increased the trapping success of both wet plots (1 and 3).

The results showed that deltamethrin affects catches within 24 hours. A sampling frequency of once in 24 hours or more frequently is therefore necessary to study the instantaneous effect and/or short term recovery after application of deltamethrin.

The few spiders which were continuously caught in the enclosures suggested that some immigration occurred. As it was absolutely impossible for spiders to enter the enclosures via the net, immigrant spiders must have entered the enclosure from below, probably via a few deep cracks (deeper than 25 cm and extending for more than 50 cm, this being the area covered with fine sand after closing the tents) present in the heavy clay soil.

The effects of trapping on the population density can be derived from density estimates and trapping frequency. The lowest (conservative) estimate of population density was 23 spiders m^{-2}. The average daily number of spiders trapped in a pitfall trap in the field during series 2 (Fig 4.10) was 7. A trap may therefore catch an average of 49 spiders in a week which is roughly equivalent to the population found in 2 m^{-2} or 0.6 % of the population (8280 spiders) of a plot of (18 times 20=) 360 m^{-2}. This suggests that, for the given activity of the spiders and a moderate density of pitfall traps, the effects of trapping on population density will be modest. The impact of trapping on population density should also be compared to natural mortality and/or regeneration.

Migration by ballooning was not investigated in this paper, and the occurrence of ballooning may have influenced the results, however the role of ballooning in the short time scale may be limited. Quantitative aspects of ballooning as a source of recovery could therefore form an interesting topic for further study.

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CHAPTER 5

A dose-effect relationship for the effect of deltamethrin on a linyphiid spider population in a winter wheat field

Summary

The relationship between application rate and effect of deltamethrin (Decis^R) on a linyphiid spider population was studied in relation to ambient physical factors. A field experiment using 5 application rates and a control was conducted in winter wheat plots. The aim was to determine application rates that allowed for determination of negative as well as positive effects of physical factors on arthropod toxicity using pitfall traps. Several physical variables were measured in the crop canopy and deltamethrin deposition was determined at ground level. The use of Before-After-Control-Impact design (BACI) time series analysis for characterisation of instantaneous pesticide effects and velocity of recovery, relative to the application rate sprayed was evaluated.

An initial reduction in spider catches ranging from 58% to 85% percent for sprayed concentrations of 0.25 to 4 g active ingredient per hectare (ai ha^{-1}) was observed, followed by exponential recovery with a half-life time of 2.8 days for all treatments. The effects observed in this study were relatively mild compared to other field-experiments. It was concluded that an application rate near 0.25 g ai ha^{-1} deltamethrin (i.e. one twentieth of the recommended rate) would be sufficient to study the relationship between physical factors and the effects of deltamethrin. BACI time series analysis was shown to be a useful technique for characterising short-term pesticide effects.

Introduction

Field experiments with insecticides sprayed at the same application rate sometimes show unexplained differences in effect. These differences are generally attributed to the influence of physical factors on bioavailability of the toxic compound, on arthropod vulnerability (Everts *et al.* 1991b) and/or on activity mediated exposure (Chapter 2).

The effects of recommended or higher application rates for different pyrethroid insecticides on (epigeal) arthropods have been described by several authors (Basedow *et al.* 1985, Shires 1985, Fisher and Chambon 1987, Vickerman *et al.* 1987) and have been reviewed by Inglesfield (1989); however, most of the experiments lack information on physical factors and/or the effects of physical factors may have been masked by the use of high application rates which in all circumstances induce a high mortality. To study the relationships between physical factors and toxic effects, application rates which allow for physical factor dependent differences in impact are a necessary prerequisite. Little information on this topic exists in the literature and the resent study was, therefore, designed to determine appropriate application rates.

Basing our decision on the work of Everts (1989, 1990, 1991a, 1991b), epigeal money-spiders (linyphiidae), representing a relevant and vulnerable part of the terrestrial, non-target arthropod fauna, and the insecticide deltamethrin were chosen as model taxon and model pesticide, for the investigation of mechanisms which alter toxic effects of pesticides on epigeal arthropods.

Pesticide effects on arthropods can be characterised generally by an initial, instantaneous, reduction in catches immediately following a pesticide application, and subsequent recovery, with a specific recovery rate. To obtain precise estimates of these variables in the present experiment a **BACI** (**B**efore and **A**fter treatment, on **C**ontrol plots as well as on **I**mpact plots) experimental setup was chosen. BACI requires that measurements of a parameter, here the number of spiders trapped daily in pitfall traps, are available before and after treatment, on control plots as well as on impact plots. This method was described by Stewart-Oaten *et al.* (1986), it allows for detailed evaluation of the effects of a treatment in relation to a control and to the measurements taken before impact. It should be realised that the results are only applicable to the given situation, unless tested under a variety of conditions. Extrapolation to other situations can only be justified when all the influences in another situation are known or identical (external representativeness). A detailed description of an applied BACI analysis is given in this paper.

To allow for comparison with other experiments, soil humidity and pesticide deposition at the soil level have been measured.

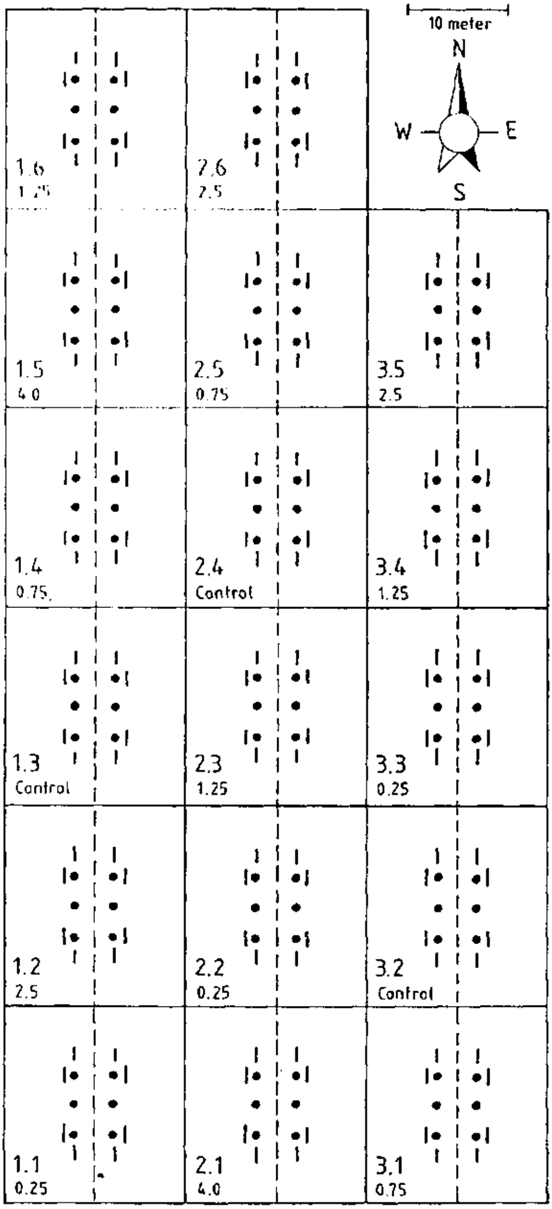

Figure 5.1. Overview of the field situation giving the position of the pitfall traps (dots) the glass strips (stripes) and tractor trails (broken lines). Field number (large) and nominal application rate (small) are indicated at the lower left corner.

Materials and Methods

The experiment was carried out at the Institute for Plant Protection Research, Wageningen. Individual plots measured 360 m^2. A randomized block design was used for 3 blocks, each row with 6 treatments representing a block (Figure 5.1)(plot 3.6 was discarded as the winter wheat lodged).

Linyphiid spiders were caught in pitfall traps in a winter wheat crop. Six round pitfall traps were placed in a rectangular grid in the center of each plot. The traps had a diameter of 10 cm, were filled with a 4% formaline solution, and were shielded from rain by a transparant cover (Everts 1989). These were emptied each morning between 8.00 and 9.30 hours local time (6.00 and 7.30 hours Universal Time Constant). Catches were lumped for each plot and stored in 70% alcohol. The distance between the pitfalls and the edge of the plot was always at least 7 m (Figure 5.1). It was found in earlier experiments (Jagers op Akkerhuis 1993) that this plot size and trapping lay-out was appropriate for assuring that the density of the spiders was not severely affected by trapping, and that spiders would not migrate into adjacent plots within a two-week assessment period. Sampling continued from 10 to 26 July 1989.

Both impact and control plots were sprayed on the morning of 19 July, using a tractor-mounted hydraulic sprayer (Slit nozzles (Doeven, 11-110-06), pressure 2.3 atm, 60 cm above the crop, wind; NNW, 0.8 m s^{-1}). The deltamethrin range used was 0.25, 0.75, 1.25, 2.5 and 4 g ai 400l^{-1} water ha^{-1}, control plots were sprayed with water (400l ha^{-1}). To avoid possible contamination of the lower concentrations, the deltamethrin was sprayed in order of increasing concentration. Fields with the same concentration were sprayed within a time span of 15 minutes. The concentrations were sprayed between 8.00 and 10.30 hours.

A fluorescent tracer (Tinopal^R) was used to measure groundlevel deposition of the pesticide. Five grams of Tinopal was diluted in 70 l of deltamethrin solution and deposition was measured on strips of window glass (50*3 cm) placed according to the pattern shown in Figure 5.1. The glass strips were removed at 11.30 and placed in a light proof box. The slides were rinsed with destilled water at the laboratory and deposition was determined fluorimetrically (filters: emission, 450 nm and absorbtion, 380 nm).

The first two cm of soil (a heavy illitic river clay; 'medium high Nude silty clay loam', 59% lutum content, 5.3% organic matter) were sampled on the day of deltamethrin application in each plot at 8.00 and 17.00 hours to determine soil humidity (% dry-weight, oven-drying overnight at 105 °C).

On the day of spraying soil temperature (-1 cm) and air temperature (+5 cm) were measured at 8 randomly chosen locations in the crop by means of thermometers. The thermometers were shielded from direct radiation.

Before-After-Control-Impact time series analyses

There are many possible methods for analyzing trapping data from BACI experiments (Van der Voet, 1987). In this experiment the numbers of trapped spiders were reasonably high, rarely less than 10 per field, and never 0, therefore little difference was expected between (quasi-) Poisson modelling and lognormal modelling of counts. As this facilitates computation, the latter modelling approach was chosen. This gives the following basic model for estimating the number of spiders per field:

$$(5.1) \qquad Y_{it} = \mu_{it} + \epsilon_{it} \qquad \text{(plot } i\text{, day } t\text{)}$$

Where $Y_{it} = ln(N_{it})$, i.e. the log-transformed number for plot i and day t. μ_{it} repesents the systematic part of the model, specified below, and ϵ_{it} represents a random contribution from a normal distribution with mean 0 and variance σ^2. Note that this additive model for Y_{it} corresponds to a multiplicative model for the counts N_{it}.

It is now assumed that, in the absence of treatments, individual plots, i, may yield different, location dependent, catches, while any specific plot may, on different days, t, yield different, time dependent, catches. It is also assumed that relative differences between plots remain constant through time. This gives a linear model with two main effects and no interaction:

$$(5.2) \qquad Y_{it} = \mu + \alpha_i + \beta_t + \epsilon_{it}$$

Treatments can only have effect after deltamethrin application (A) at the impact plots (I). Let δ_{AI} be 1 for treated plots and 0 for nontreated. τ_{kt} is a treatment term indicating that effects may depend on application rate (k=1..5) and on time (t=11..17), but not on the plot. The model can now be extended using a term associated with treatment and application rate:

$$(5.3) \qquad Y_{it} = \mu + \alpha_i + \beta_t - \tau_{kt}\delta_{AI} + \epsilon_{it} \quad \text{(application rate } k\text{)}$$

One of the objectives of the experiment was to draw clear conclusions about recovery, after deltamethrin application. The above model does not specify how treatment effects are related to application rate and time, therefore further refinement of the model is appropriate.

With respect to time dependence for a fixed application rate k, the most restricted model that is realistic has 2 parameters. It specifies that application of deltamethrin has an instantaneous effect g_k which then decays exponentially with rate h_k, leading to a dose dependent recovery of the catch:

an appropriate application rate

$$(5.4) \qquad \tau_{kt} = g_k \exp[h_k(t-t_0)] = g_k r_k^{t-t0} \quad \text{(with } r_k = \exp(h_k)\text{)}$$

where t_0 is the time of application. Note that the half-life time $t_{1/2}$ is given by:

$$(5.5) \qquad t_{1/2} = -\ln(2)/h_k = -\ln(2)/\ln(r_k)$$

A more complicated model may, for biological reasons, be used to describe recovery after treatment. For example a lag phase before recovery begins, or a residual effect independent of time (non-total recovery) may be required. In this case, the fit of the model given by equations 1, 2 and 4 (Table 5.3) gave little reason to investigate such models using these data.

Finally, two hypotheses leading to the simplification of the model can be made. One, the recovery rate h_k may not be dependent on the application rate used, having the same value for all application rates used. Thus, equation (5.4) is replaced by:

$$(5.6) \qquad \tau_{kt} = g_k r^{t-t0}$$

It should be noted that equal recovery rates can still result in different, total, recovery times, depending on initial effect g_k. If total recovery time is defined as the time between application and the moment that the effect diminishes below a specified treshold ($|\tau_{kt}| < \tau_0$) then total recovery time t_R is:

$$(5.7) \qquad t_R = \ln(\tau_0/|g_k|)/\ln(r)$$

Two, a dose-effect relationship can be assumed, rather than modelling each application rate with its own specific effect. Several relationships between the initial effect g_k and application rate d_k can now be postulated. The following three were investigated in this paper:

$$(5.8) \qquad g_k = bd_k$$

$$(5.9) \qquad \ln(g_k) = a + b\ln(d_k)$$

$$(5.10) \qquad \ln(e^{g_k}-1) = a + b\ln(d_k)$$

All three equations assume that application rate 0 has an effect 0. Equation (5.8) and (5.9) specify a linear relationship between dose and effect, either with or without a logarithmic transformation. Equation (5.10) is based on a linear relationship between the logarithm of the dose, and the logit transformation of mortality. Percentage instantaneous mortality, i.e. mortality at $t=t_0$ can be defined as:

(5.11) $m = 1 - exp(n_{it0})/exp(n_{it0}, control)$

(5.12) $= 1 - exp(\mu + \alpha_i + \beta_t - \tau_k)/exp(\mu + \alpha_i + \beta_t)$

(5.13) $= 1 - exp(-\tau_k)$

and this gives:

(5.14) $logit(m) = ln(m/(1-m))$

(5.15) $= ln((1 - exp(-\tau_k))/exp(-\tau_k))$

(5.16) $= ln((exp(\tau_k)-1)$

Together with (5.6) the relations (5.8), (5.9) and (5.10) lead to the following treatment effect relationships (to be substituted in equation (5.3)):

(5.17) $\tau_{kt} = bd_k r^{t-t0}$

(5.18) $\tau_{kt} = pq^{ln(dk)} r^{t-t0}$ (with $p=e^a$, and $q=e^b$)

(5.19) $\tau_{kt} = ln(1 + pq^{ln(dk)}) r^{t-t0}$ (with $p=e^a$, and $q=e^b$)

All models were fitted using the statistical computer program Genstat 5 (release 1.3). The directive NLIN of SAS 5 (release 3.1) was used for model D (Table 5.3) because the Genstat directive FITNONLINEAR did not allow a fit of model D.

The models investigated are summarised in Table 5.3 in order of increasing complexity as measured by the numbers of parameters. With the exception of the model pairs B-C, B-D and C-D, each simpler model can be seen as a special case of the subsequent more complex model. The significance of using a more complex model can be tested by using the likelihood ratio test statistic:

(5.20) $vr = [(SS_{res,0}-SS_{res,1})/(df_{res,0}-df_{res,1})]/MS_{res,1}$

where indices 0 refer to the simple and 1 to the more complex model. This test statistic - the Variance Ratio- is the familiar F statistic known from analysis of variance. Under the null hypothesis that the simple model is the true model, it has an F distribution with $df_{res,0}-df_{res,1}$, and $df_{res,1}$ degrees of freedom.

Table 5.1: Mean and standard error of deposition of tinopal and, calculated, deposition quantities of deltamethrin, and soil moisture values on the morning of deltamethrin application.

[C]	plot	measured quantity tinopal (g ai ha^{-1})	calculated deposition deltamethrin (g/ha) (8 replications)	(19-7-89) s.e.	soil humidity (% dwt)	s.e.
0.00	1.3	0.7			19.5	
	2.4	1.2			17.8	
	3.2	0.5			18.1	
Mean:					**18.5**	**0.9**
0.25	1.1	10.3	0.05	0.03	16.3	
	2.2	6.5	0.03	0.01	18.1	
	3.3	7.8	0.04	0.02	17.9	
Mean:		**0.04**		**0.01**	**17.5**	**1.0**
0.75	1.4	14.3	0.19	0.06	17.9	
	2.5	9.9	0.13	0.08	14.3	
	5.1	9.6	0.13	0.06	19.3	
Mean:		**0.15**		**0.04**	**17.2**	**2.6**
1.25	1.6	9.2	0.15	0.05	16.9	
	2.3	15.9	0.26	0.07	19.4	
	3.4	15.1	0.25	0.09	16.1	
Mean:			**0.22**	**0.06**	**17.5**	**1.7**
2.5	1.2	17.2	0.46	0.15	18.8	
	2.6	12.0	0.33	0.14	16.2	
	3.5	17.0	0.50	0.19	16.8	
Mean:			**0.42**	**0.09**	**17.2**	**1.3**
4	1.5	16.7	0.60	0.33	20.7	
	2.1	27.8	1.00	0.34	20.1	
Mean:			**0.79**	**0.28**	**20.4**	**0.4**

Results

Physical Variables.

Mean soil moisture values for the day deltamethrin was sprayed are given in Table 5.1 and Fig. 5.2. Little difference existed between fields. Soil moisture during the day decreased from 17.9 (std 0.4) to 15.8 (std 0.6) percent dwt.

Mean soil- and air-temperatures are given in Fig. 5.2. Highest mean soil and air temperature were 21 (std 1.9) °C and 25 (std 5.4) °C.

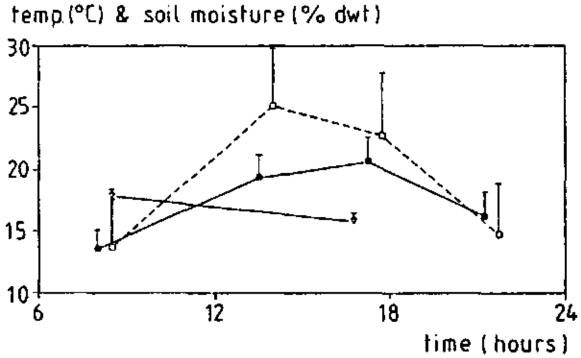

Figure 5.2. Physical factors measured on the day deltamethrin was sprayed. Temperatures in crop 1 cm below the soil surface (-•-) and at 5 cm (-□-) above; plus soil moisture at surface (%dwt) on day of pesticide application (-▽-)(error bars indicate 95% confidence limits).

Analysis of Pitfall-Trap Catches.

The numbers of spiders caught daily in the different plots are summarised in Table 5.2. The lack-of-fit statistics for the 7 models investigated (Table 5.3) are given in Table 5.4. The results of some of the variance-ratio tests are given in table 5.5.

Measurements of the deposition of tinopal, added to all sprayed solutions at the same concentration of 71.4 mg l^{-1}, showed an increase with sprayed concentration. This could only be explained by photodegradation of the tinopal on the glasstrips during the time they lay in the field. An exponential decay was therefore fitted to all tinopal measurements and moments. With the resulting formula; [tinopal(g ha^{-1})] = 31.2exp(-0.00626t(min))(se: 5.4 and 0.0011), where 31.2 represents the calculated deposition on t=0 , correction factors were determined for each spray time, i.e. the calculated concentration on t=0 divided by the calculated value on $t=t_{(min)}$), to calculate the initial deposition on t=0 (Table 5.1). The nominal applied dose for tinopal was used in the models instead of measured deposition values, because of the apparent photodegradation in the field.

The results allow simple interpretation. One, model G does not fit significantly better than model F (Table 5.3 and 5.5). The effect of a specific deltamethrin application rate therefore seems to be adequately described by a simple two-parameter model, that specifies an instantaneous application rate dependent treatment effect, followed by an exponential decay until the pre-treatment situation is restored.

Two, no significantly different recovery rates were found (F over E gives no significant improvement). Half-life times for recovery at the 5 treatment levels were calculated from model F as 2.2, 1.6, 2.9, 2.8 and 2.5 days (in order of increasing application rate). None of the pair comparisons turned out to be significant at the 5% level. In model E a

Table 5.2. Total number of linyphiid spiders caught per day, per plot. The horizontal line separates the days before treatment from those after treatment.

	application rate																
	control			0.25			0.75			1.25			2.5			4	
	plot																
	13	24	32	11	22	33	14	25	31	16	23	34	12	26	35	15	21
day																	
1	22	21	17	22	30	23	19	29	12	12	32	31	21	19	19	16	19
2	10	34	33	29	29	31	23	28	22	6	37	35	22	26	21	7	25
3	24	19	31	29	26	48	23	31	23	14	31	35	20	43	35	30	26
4	20	45	44	46	41	75	27	50	47	18	54	42	44	49	54	31	48
5	18	35	40	27	43	43	27	46	35	20	34	57	30	36	45	27	22
6	22	13	48	25	24	38	24	32	35	27	34	45	23	35	42	39	22
7	32	47	46	40	28	54	23	56	31	30	47	67	35	38	37	28	29
8	13	40	40	27	40	40	34	24	41	11	42	49	27	33	42	22	32
9	18	32	32	24	22	28	25	31	18	13	18	31	20	23	34	27	21
10	31	33	38	33	43	48	44	55	45	21	48	66	28	44	32	32	37
11	26	27	25	15	7	12	6	10	3	9	7	13	3	6	3	7	1
12	27	25	45	27	15	17	13	24	18	11	15	23	10	10	11	7	11
13	34	44	35	19	32	29	20	22	17	16	15	19	12	26	20	20	9
14	18	54	41	36	24	47	28	41	23	17	26	17	12	15	18	11	10
15	23	38	48	38	28	39	25	24	29	17	22	35	11	13	27	13	17
16	35	50	53	24	36	44	29	45	16	30	28	46	27	27	39	26	25
17	23	34	47	33	27	31	47	48	30	28	33	33	13	24	32	23	15

combined half-life time of 2.7 days was found. As indicated by Table 5.5 this did not improve on the data fit of model F.

There were differences in instantaneous effects between the 5 deltamethrin application rates. The values that were found using model E are presented in Table 5.6. There was a general tendency for the higher application rates to have a larger effect, although application rates 0.75 and 1.25 were out of order. It therefore seems justified to postulate a dose-effect relationship. Of the three models with a dose-effect relationship (B, C and D), the versions with the linear log-dose log-effect relationship (C) or the linear log-dose logit-effect relationship showed an almost equally better fit, compared to model A (Table 5.5).

Models C and D are the simplest models in the set capable of adequately describing the experimental results (Table 5.5). The latter, due to its log-dose logit-effect curve is more

Table 5.3. Investigated models. Explanation of symbols in text.

A $Y_{it} = \mu + \alpha_i + \beta_t + \epsilon_{it}$
(**no** treatment effects)

B $Y_{it} = \mu + \alpha_i + \beta_t - b^*d_k r^{t-t0}\delta_{AI} + \epsilon_{it}$
(**linear dose-effect** relation, exponentially decaying effects, equal decay rates)

C $Y_{it} = \mu + \alpha_i + \beta_t - p^*q^{ln(dk)}r^{t-t0}\delta_{AI} + \epsilon_{it}$
(**linear log-dose - log-effect** relation, exponentially decaying effects, equal decay rates)

D $Y_{it} = \mu + \alpha_i + \beta_t - ln(1+p^*q^{ln(dk)})r^{t-t0}\delta_{AI} + \epsilon_{it}$
(**linear log-dose - logit-effect** relation, exponentially decaying effects, equal decay rates)

E $Y_{it} = \mu + \alpha_i + \beta_t - g_k{}^*r^{t-t0}\delta_{AI} + \epsilon_{it}$
(**no** assumed **dose-effect** relation, exponentially decaying effects, equal decay rates)

F $Y_{it} = \mu + \alpha_i + \beta_t - g_k{}^*r_k{}^{t-t0}\delta_{AI} + \epsilon_{it}$
(**no** assumed **dose-effect** relation, exponentially decaying effects, **unequal** decay rates

G $Y_{it} = \mu + \alpha_i + \beta_t - \tau_{kt}\delta_{AI} + \epsilon_{it}$
(**no** assumed **dose-effect** relation **or time-effect** relation).

Table 5.4. Lack of fit for the models from Table 5.3.

model	**df$_{res}$**	**SS$_{res}$**	**MSres**
A	256	31.03	0.121
B	254	23.10	0.091
C	253	20.42	0.081
D	253	20.49	0.081
E	250	19.92	0.080
F	246	19.50	0.079
G	221	17.23	0.078

in accordance with general theory of dose-effect relationships. However, model C was preferred to model D because of its simplicity and for computational reasons, i.e. its applicability both in Genstat and SAS. It has only 3 parameters more than the no-treatment-effect model A, which, not unexpectedly, produced the poorest fit of all the models, indicating definate treatment effects.

Table 5.5. Significance of models with larger complexity using the likelihood ratio test for hierarchical models.

model	number of extra parameters	Variance ratio	P(approximately)
B over A	2	43.57	<0.0001
C over A	3	43.66	<0.0001
D over A	3	43.37	<0.0001
E over B	4	9.94	<0.0001
E over C	3	2.08	0.10
E over D	3	2.37	0.10
F over E	4	1.33	0.26
G over F	25	1.16	0.29

Table 5.6. Significance of application rate dependent reduction factors (Model E). Application rates with effects that were not significantly different from each other are indicated by the same letter in the last column.

application rate	g_k	reduction factor for catches (e^{g_k})	significance
0.25	0.95	2.6	a
0.75	1.27	3.5	bc
1.25	1.13	3.1	ab
2.50	1.86	6.4	cd
4.00	1.94	7.0	d

The fitted values from model C for the separate fields, are shown in Figure 5.3 together with the actual catches. The (unstandardized) residuals are displayed in Figure 5.4 (graph against time), and reveal no problems with the model, although at the time of deltamethrin application (day 20) the fit of the model seems slightly less than at other times, indicated by larger residuals.

The three treatment effect parameters of model C (see equation (5.18)) are estimated as follows: $p=1.29$ (s.e. 0.16), $q=1.34$ (s.e. 0.08), and $r=0.78$ (s.e. 0.04). The estimated time curves in the absence of plot and time effects are shown in Figure 5.5. The instantaneous reduction factors for application rates of 0.25, 0.75, 1.25, 2.5 and 4 g ai ha^{-1} are estimated as 2.4, 3.3, 3.9, 5.4, and 6.9, respectively. The effect half life was estimated as $t_{1/2}=2.8$ days.

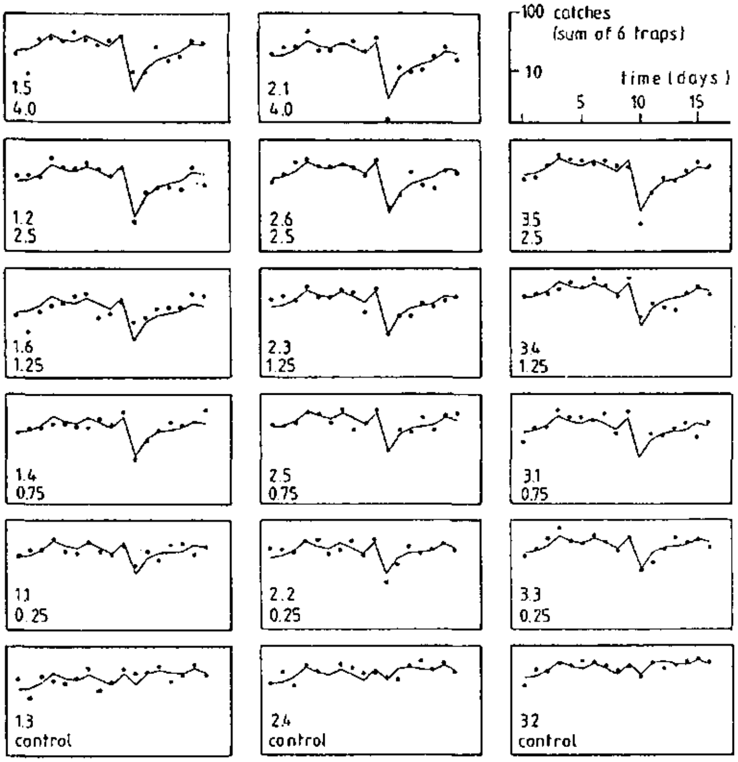

Figure 5.3. Data (points) and fitted curves from model C for all 17 plots. Counts (log scale) against time (days). The nominal application rate of deltamethrin is indicated for each graph. The plots are depicted in order of concentration.

Discussion

The trapping success recovered to within 18% of the control populations within one week after treatment for all application rates. For the following reasons this quick recovery of the activity of the very sensitive spiders was probably caused by a reversible effect of deltamethrin on spider physiology. Generation time, and therefore recovery by population development, of linyphiid spiders is at least several weeks (De Keer and Maelfait 1987, 1988, Lissens 1986). Aerial migration, of linyphiid spiders, is a very large-scale event (Thomas *et al.* 1990), resulting in similar number of spiders entering each plot. This leads to higher recovery rates for fields with low initial effect. As no significant differences in recovery rate were found, immigration due to ballooning can be precluded. Jagers op Akkerhuis (1993) found that epigeal migration of linyphiid spiders in winter wheat was of little importance over a distance of 7 m and a timespan of two weeks. This migration

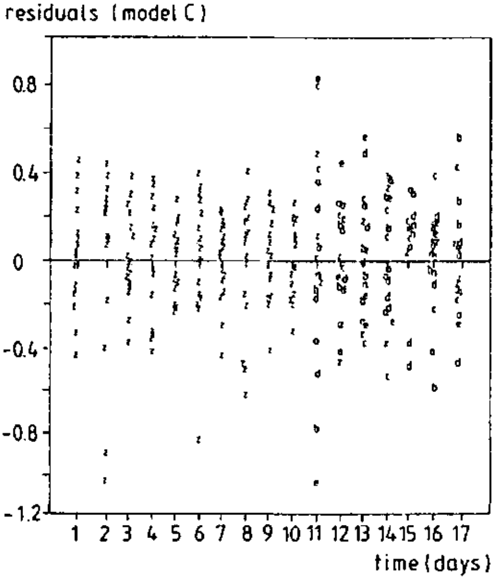

Figure 5.4. Display of the (unstandardised) residuals of model C against time (days).

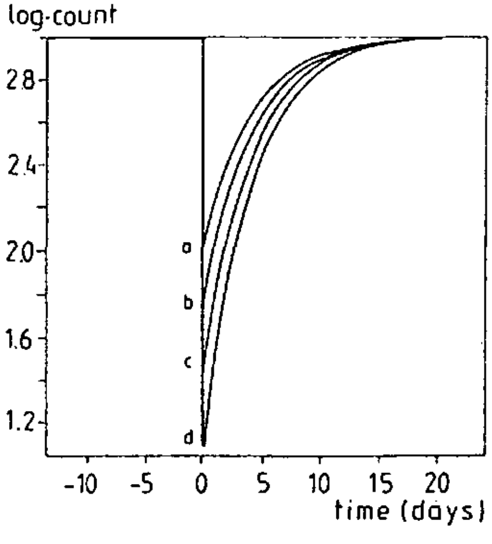

Figure 5.5. Calculated treatment effect curves (model C, without effects of field or time) for application rates 0.5 (a), 1 (b), 2 (c) and 4 (d) g ai ha^{-1} deltamethrin applied on day 20. The choice of the initial number of spiders is arbitrary.

would lead to a sigmoid instead of an exponential recovery curve due to the early arrival of fast-moving spiders. Everts (1991b) found that the walking activity of linyphiid spiders is slow to return (days) after (topical) applications of sublethal doses of deltamethrin. Postuma (unpublished results of an undergraduate study at our laboratory, 1990) has shown that linyphiid spiders were not capable of selectively avoiding sprayed surfaces, which implies that a reduction in the number of trapped spiders shortly after spraying cannot be explained by reduced presence of spiders at the (contaminated) soil surface.

Reversible physiological effects were expected for the lower application rates. We assumed that at least partial mortality of the population would occur for the application rate of 4 g ai ha^{-1}. This assumption was based on an the non recovery of the reduced catch in an earlier experiment using the same plot and an application rate of 5 g ai ha^{-1}. (Jagers op Akkerhuis 1993).

Why the present application rate of 4 g ai ha^{-1} did not show considerable mortality raises the question of why deltamethrin applications at similar application rates may yield considerable differences in effect. Factors which may have contributed to the observed difference and which were measured in both experiments are: 1. Deposition of deltamethrin at the soil stratum, 2. Moisture content of the soil surface, and 3. Temperature of the air at the ground level. The following values for these variables were measured during the experiments: 0.26 g ai ha^{-1}, 35% dwt and 17-20 °C for the previous experiment (Jagers op Akkerhuis, 1993), and 0.79 g ai ha^{-}1, 15-17.8% dwt and 25 °C for the present experiment. This shows that the mild effects found in the present experiment were obtained despite higher deposition. Possible explanations of this phenomenon are, a reduced exposure of the spiders to contaminated soil shortly after spraying due to low walking activity caused by the adverse effects of hot weather and a very dry soil, or the low availability and rapid volatilisation (Boehncke *et al.* 1990) of deltamethrin on a very dry soil in the present experiment.

The moderate effects of all application rates used in this experiment in comparison to other experiments in winter wheat imply that in most situations an application rate of 0.25 g ai ha^{-1} deltamethrin, resulting in an instantaneous reduction of the catches of 58 percent, can be considered appropriate for studying relationships between physical factors and the effects of deltamethrin.

The results of the experiment demonstrate that adequate analysis of pesticide effects is possible using BACI models. An advantage of this technique is that it enables a comparison of different application rates of pesticides on different species by comparing, precisely estimated effect parameters such as initial effect and median recovery time, while correcting for the day-to-day effects of changing environmental conditions.

BACI models can also be used when the treatment model has another functional form e.g. when it includes a lag-phase before recovery, non-total recovery or long-term sigmoid recovery of a population after pesticide application. Only the treatment effect term in the model formula needs to be adapted.

BACI analysis uses control plots as an internal standard. Apart from the obvious advantage of a reduction in temporal variablility of the results, this makes the results dependent on the typical situation of the standard during the experiment. This means that extrapolation of BACI results to other conditions may only be justified using 'external representativeness assumptions'. Strictly, this implies that the results of different experiments are only comparable when all factors, except impact, are the same, or when the effects of other factors are known. In practice extrapolation requires a BACI analysis to be accompanied by detailed description of relevant factors such as soil humidity and soil type, pesticide deposition quantities for the target stratum, pesticide formulation, important environmental variables, and arthropod activity in the hours immediately following pesticide application.

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CHAPTER 6

Effects of walking activity and physical factors on the short term toxicity of deltamethrin spraying in adult epigeal money spiders (Linyphiidae)

Summary

The effect of deltamethrin application on terrestrial spiders (*Linyphiidae*) was studied in the field situation in relation to walking behaviour and/or physical factors. Deltamethrin was sprayed in winter wheat at a dose of 0.5 g ai ha^{-1} in 8 experiments with replicated treatments. Spider walking activity was monitored either on a daily basis or every 1.5 hours for 10 hours after spraying. The deposition of deltamethrin was determined at the soil stratum using a fluorescent tracer. Physical variables were measured daily in the crop, or were obtained from a nearby weather station. Deltamethrin effects on spider activity were analysed using short time series analysis. The species composition of the spider community was determined in May and July.

A strong positive correlation was found between spider activity on the day of spraying and the reducing effect of the pesticide on trapping success. This suggests mobility has an important role in the poisoning process of terrestrial arthropods. Similar trapping success and effect in three experiments conducted with different physical variables but identical walking activity, imply a relative importance of walking in comparison to physical factors. Circumstancial evidence was found for high bioavailability of deltamethrin on moss covered soil.

Introduction

A lot of information has been published on the toxic effects of pyrethroid and other insecticides under field conditions (see for instance Croft and Brown 1975, Basedow *et al.* 1981, Inglesfield 1989), however, there are very few studies which take into account that the response in the field situation is influenced by ambient conditions, such as temperature, soil type, soil moisture, soil cover etc. Therefore, most of the data published cannot be used for the prediction of an effect in a given field situation. Such a prediction can only be made after identification and quantification of the factors which influence the response of populations in the field.

One of these factors is the walking behaviour of the arthropods, which determines the uptake of sprayed pesticide from substrate, i.e. the residual uptake. Salt and Ford (1984) used stochastic modelling to simulate the uptake of insecticide from plant surfaces by larvae of the lepidopteran *Spodoptera littoralis*. Sensitivity analysis indicated that the effect depended mainly on walking velocity, the contact area between insect and leaf surface and the proportion of insecticide transferred per encounter. The relative importance, for terrestrial spiders, of residual uptake in comparison to direct or oral uptake was shown by Mullie and Everts (1991) who sprayed with radiolabelled deltamethrin female *Oedothorax apicatus* (*Linyphiidae*), contained together with their prey in tubs with a clay substrate. Investigations of the residual uptake of deltamethrin by female *Oedothorax apicatus* showed saturation of pesticide uptake with distance walked (Jagers op Akkerhuis and Hamers 1992).

The present study aims at validation, in the field situation, of the assumptions of the importance of walking activity in the poisoning process of terrestrial arthropods. The great difficulty in this is that it can only be assessed properly in experiments which take into account the effect of ambient conditions on pesticide availability and arthropod vulnerability. In a project, using linyphiid spiders, of which this publication is a part, uptake and effects of deltamethrin have been studied extensively, both in the laboratory and in the field; thus offering a good opportunity to study the effect of walking behaviour while correcting for contributions of ambient physical factors.

Everts (1990), showed that linyphiid spiders are a highly sensitive and abundant part of the epigeal arthropod fauna. These spiders are particularly sensitive to deltamethrin and were therefore chosen as pesticide-effect model in the present study.

Linyphiid spiders live hidden in crevices, under leaves (*Oedothorax* spp.) or, sit in webs, which are built in cracks and small shallow pits in the soil (*Erigone* spp.), or between plants near the soil surface (*Leptiphantes*, *Bathyphantes* spp.)(Thornhill, 1983). Hiding in cracks, they are barely exposed to direct spray. Especially in a clay soil, in which deep cracks may be present, this offers shelter opportunities for a large number of spiders.

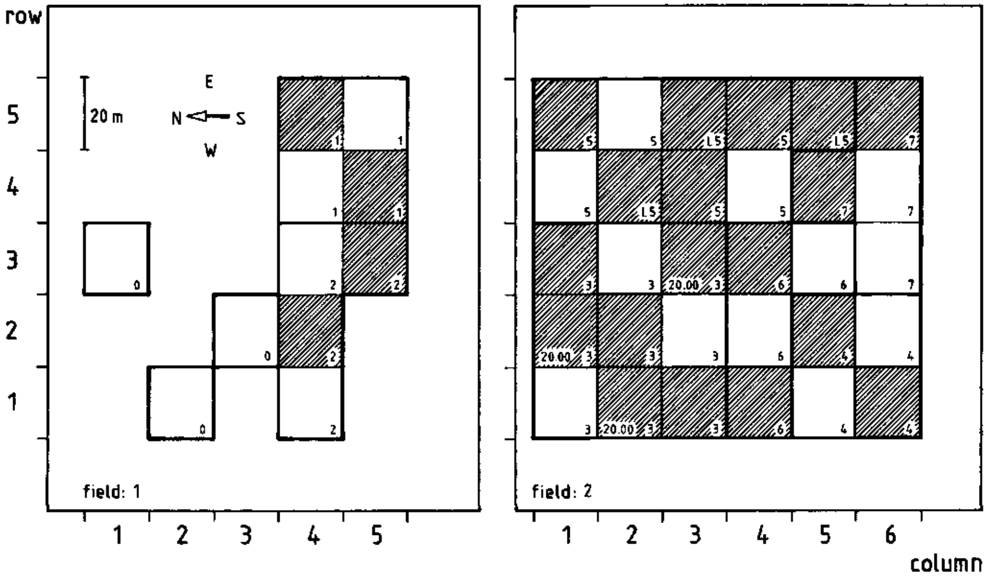

Figure 6.1. Map of experimental fields 1 and 2 showing position and treatment of plots used in the experiments. The experimental number is indicated in the lower right corner. 'L' and '20.00' indicate plots sprayed with low volume droplets or plots sprayed at 20.00 hours (not in the present paper).

Spiders walk when hunting for a prey, searching for a female to mate or a place to lay eggs, or in reaction to a diurnal rhythm (Vickerman and Sunderland 1975, de Keer and Maelfait 1987) or dispersal behaviour. Walking velocity mainly depends on substrate and environmental conditions (pers obs). In part walking behaviour will be performed at the soil surface where spiders are exposed, residually, to sprayed substrate such as soil, moss, plant cuticula, plant debris or spider webbs. Residual uptake has been shown to be an important route of uptake for arthropods (Salt and Ford 1984, Mullié and Everts 1991). The nature of the substrate may cause changes in bioavailability of the compound (Jagers op Akkerhuis and Hamers 1992).

After deltamethrin poisoning, spiders exhibit changes in walking behaviour. Activity shows a quick rise followed by a longlasting reduction. Walking speed is reduced immediately to decline continuously and it is possible that knock-down will occur (Jagers op Akkerhuis 1991). During prostration or quiescense the arthropod are vulnerable to desiccation and predation (Everts *et al.* 1991b).

Based on the scenario described above, the present study aimed at assessing the importance of spider walking activity as a factor which may influence pesticide side-effects. Walking activity and pesticide toxicity were studied in relation to ambient environmental variables.

Table 6.1: Fields, plots, duration and application dates for experiments 1 to 8 (column and row number giving the plot position as indicated in Fig 6.1).

# Experiment	Duration	Appliccation date	Field	Position of plots	
				control	treated
0	28/5 - 1/6	-	1	13,21,32	
1	16/6 - 25/6	20/6	1	44,55	45,54
2	23/6 - 31/7	26/6	1	41,43	42,53
3	1/7 - 9/7	4/7	2	11,23,32	13,22,31
4	7/7 - 18/7	13/7	2	51,62	52,61
5	12/7 - 21/7	16/7	2	14,25,44	15,34,45
6	21/7 - 31/7	26/7	2	42,53	41,43
7	26/7 - 3/8	30/7	2	63,64	54,65
8	26/7 - 1/8	27/7	2	21,22	11,12,31,32

Materials and Methods

A series of 8 experiments was conducted during the summer of 1990 in two winter wheat fields on the terrain of the Institute for Plant Protection Research (IPO, Wageningen). A map of the fields and plots is given in Fig 6.1. The duration of each experiment, application date, the number and position of plots are given in Table 6.1. Fields 1 and 2 differed in that field 1 had a higher and denser crop and a higher soil humidity.

Each experiment typically consisted of 4 or 6 plots of 18*20 m (360 m^2). Field design and trapping lay-out assured that the density of spiders was not severely affected by trapping, and that spider migration from or into adjacent plots within a one week assessment period was unlikely (Jagers op Akkerhuis 1993). Half of the plots served as unsprayed controls, the other plots were sprayed (08.00 hours) with 0.5 g active ingredient (ai) deltamethrin (Decis flow^R) in 400 l water ha^{-1} using a tractor mounted hydrolic sprayer (Slit nozzles (Doeven, 11-110-06), 2.3 atm, 60 cm above the crop). The deltamethrin concentration used was chosen to give a range of effects, from moderate to severe, depending on physical conditions (Jagers op Akkerhuis and van der Voet, 1991).

In every plot 6 round pitfall traps were placed in a regular pattern at a distance of 7 m from the edge of the plot as described in Jagers op Akkerhuis and van der Voet (1991). A description of the pitfall traps (10 cm diameter, partly filled with an aqueous formaline solution) is given by Everts (1990). The traps were emptied daily between 08.00 and 09.30 hours, and during the first 12 hours after spraying every 1.5 hours. Spiders were counted in the field and stored separately for each trap in 70% alcohol.

Table 6.2: Equations used in the time series analysis of the pitfall trap data. In which: Y_{it} is $\ln(N_{it})$, i.e. the log-transformed number for plot i and day t, μ the niveau, α_i the effect term for the different plots i, β_t the effect term for the different days t, g the (field dependent (g_i)) effect term for the initial effect, h the (field dependent (h_i)) effect term for recovery, δ_{AI} the impact-vector indicating deltamethrin application and ϵ_{it} a random contribution from a normal distribution with mean 0 and variance s^2.

A $Y_{it} = \mu + \alpha_i + \beta_t + \epsilon_{it}$
(no treatment effect)

B $Y_{it} = \mu + \alpha_i + \beta_t + g\,exp[h(t-t_0)]\delta_{AI} + \epsilon_{it}$
(treatment effect, common initial effect and recovery)

C $Y_{it} = \mu + \alpha_i + \beta_t + g\,exp[h_i(t-t_0)]\delta_{AI} + \epsilon_{it}$
(treatment effect, common initial effect, separate recovery)

D $Y_{it} = \mu + \alpha_i + \beta_t + g_i exp[h(t-t_0)]\delta_{AI} + \epsilon_{it}$
(treatment effect, separate initial effect, common recovery)

D $Y_{it} = \mu + \alpha_i + \beta_t + g_i exp[h_i(t-t_0)]\delta_{AI} + \epsilon_{it}$
(treatment effect, separate initial effect and recovery)

A fluorescent tracer (Tinopal^R) was used to measure groundlevel deposition of the pesticide. Each time a known quantity of tinopal was dissolved in the sprayed deltamethrin solution, and the deposition measured quantitatively on 8 strips of window glass (50*3 cm) per field, placed flat on the soil, at an angle of 45° to the direction of the crop rows, as described in Jagers op Akkerhuis and van der Voet (1992). To minimize photodegradation of the tinopal, glass strips were removed from the field immediately after spraying and placed in a light proof box. The strips were rinsed in the laboratory with a known quantity of demineralized water and the tinopal concentration was determined fluorimetrically (emission 450 nm, absorbtion 380 nm).

Soil humidity, air humidity and air temperature were measured every morning (08.00 hours). On the day of deltamethrin application soil humidity was also determined 8 hours after spraying, while air humidity and air temperature were measured in the crop every 1.5 hours for 10 hours after spraying. Measurements were taken in each plot of every experiment, except for a few scattered days when less measurements were done or during experiment 3 and 5 when only plots 11, 23, 31 and 15, 34, 55 were taken into account. Soil humidity (% dryweight, oven-drying overnight at 105 °C) was determined for the first two cm of soil (a heavy illitic river clay; 'medium high Nude silty clay loam', 59% lutum content, 5.3% organic matter). Minimum and maximum temperatures and relative air humidity were measured in the crop canopy at 5 cm above the soil using min-max thermometers (± 0.2 °C) and hair-hygrometers, freely exposed to the air and shielded from direct radiation.

The meteorological station of the Agricultural University Wageningen, situated 250 m south-west from field 1 and 300 m west from field 2 provided hourly measurements of the following meteorological variables. These records were used to calculate sum or mean values corresponding with the 24 hour trapping periods: temperature, relative humidity and windspeed at 1.50 m, sunshine hours, net radiation, time and quantity of rain.

Species were identified using Lockett and Millidge (1951, 1953, 1957) for the cathes between 28 May and 1 June on field 1 and 2, while spiders of field 2 where also identified between 1 and 9 July.

Instantaneous effect (reduction in pitfall trap catches) and exponential recovery (Jagers op Akkerhuis and van der Voet 1992) were calculated from the daily pitfall trap catches using BACI Time series analysis (Before and After application on Control and Impact field, Stewart-Oaten *et al.* 1986, van der Voet 1987). The equations used are given in Table 6.2. The significance of using a more complex model was tested using the variance ratio:

$$(6.1)\quad vr = [(SS_{res,0} - SS_{res,1})/(df_{res,0} - df_{res,1})]/MS_{res,1}$$

where indices 0 and 1 refer to the simple and more complex model. Under the null hypothesis that the simple model is the true model, it has an F distribution with $df_{res,0} - df_{res,1}$ and $df_{res,1}$ degrees of freedom. The initial effect, calculated as percentage reduction relative to the control, and halflife time of recovery can be calculated from g and h (Table 6.2) as 100(1-exp(g)) and -ln(2)/h.

As the hidden lifestyle of the spiders hindered population density assessments in the present experiments, a tentative relative measure for spider density was used. This was based on the assumtion that comparable crop conditions during the experiments, i.e. a green crop with a moist soil, implied similar relationships between spider activity and environmental variables on different fields. These relationships were investigated for experiments 1 to 5 using a multipele regression technique (Max-R stepwise regression technique (SAS, 1985). A dummy variable F_l was introduced to account for differences in population density between the fields, resulting in the following general formula:

$$(6.2)\quad Y = \mu + a_j x_j + b_j x_j + c_j(x_{-1})_j + d_j(x_{-1})_j + e_k(F_k) + \epsilon$$

in which: Y is the natural logarithm of the mean number of spiders per treatment (6 traps per plot), μ the intercept, a_j to e_k the regression coefficients, x_j the j environmental variables, x_j^2 the quadrats of the environmental variables, $(x_{-1})_j$ the environmental variables of the preceding day, $(x_{-1})_j^2$ the quadrats of the environmental variables of the previous day F_k a dummy varible for field 1 ($k=1$) which is one for this specific field and zero in all other cases, ϵ a random contribution from a normal distribution with expectation 0 and variance sigma.

The environmental variables used were: the daily means of the air-temperature (°C) and relative humidity (%) at 150 m, and the daily sums of sunshine (h), net radiation devided in radiation gain during the day and radiation loss at night (J cm^{-2}), duration (min) and quantity (mm) of rain. Because soil humidity measurements were not available before 21 June and soil humidity stayed relatively stable during the first 5 experiments, soil humidity data were left out of the regression.

Results

Figure 6.2 shows that the experiments could be arranged into two groups, based on the presence or absence of effect.

A reduction in trapping success following deltamethrin spraying was found for experiments 1 to 5. The significance of the effects was demonstrated using the model with

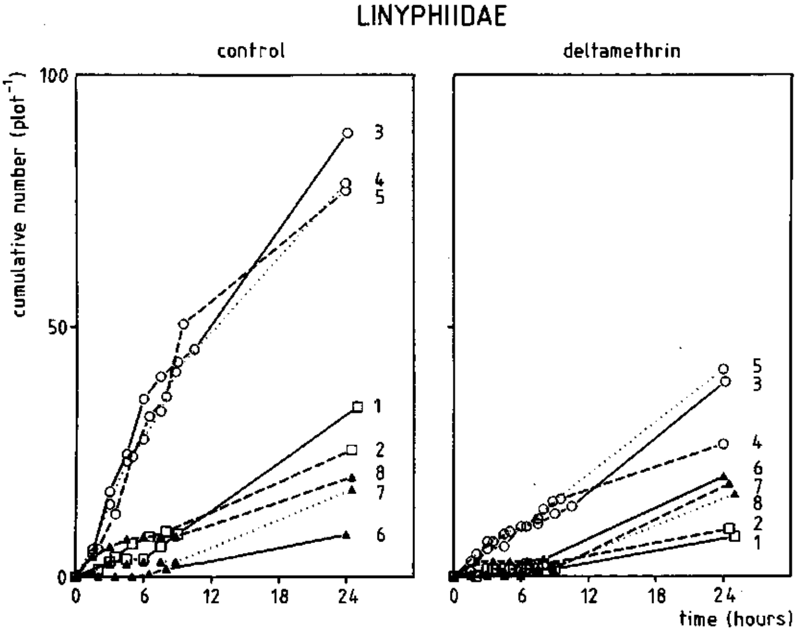

Figure 6.2: Cumulative pitfall trap catches (mean of plot totals) during 24 hours after deltamethrin spraying on control and impact plots. The experimental number is indicated in the figures. Experiments on field 1 are indicated with a □, the first three experiments on field 2 with a ○ , and the last three experiments on field 2 with a □.

Table 6.3: BACI analysis of the pitfall trap catches. Indicated are: mean square (MS), sum of squares (SS) and degrees of freedom (DF) of the residuals (R) for the models with no pesticide effect (equation A, Table 6.2) or with a common initial effect and recovery (equation B, Table 6.2) respectively R_0 and R_1, the F statistic (see text) and significance of model B over model A ('ns' = not significant at 0.05% level, '***' = significant at 0.001% level) and the calculated initial effect and recovery rate with standard error of mean.

Exp #	MSR_0	SSR_0	DFR_0	MSR_1	SSR_1	DFR_1	F	P	Initial effect	Recovery Rate
1	0.106	2.87	27	0.023	0.56	25	51.2	***	-1.3 (±0.1)	-0.2 (±0.1)
2	0.076	1.82	24	0.040	0.89	22	11.6	***	-1.0 (±0.2)	-0.7 (±0.7)
3	0.052	2.07	40	0.037	1.39	38	37.7	***	-0.7 (±0.2)	-0.6 (±0.3)
4	0.049	1.61	33	0.020	0.62	31	25.0	***	-0.9 (±0.1)	-0.4 (±0.1)
5	0.059	4.24	72	0.033	2.31	70	29.2	***	-1.0 (±0.1)	-0.6 (±0.2)
6	0.109	3.26	30	0.106	2.97	28	1.4	ns	-	-
7	0.066	1.60	24	0.056	1.24	22	3.2	ns	-	-
8	0.026	0.47	18	0.021	0.33	16	3.3	ns	-	-

a common initial effect and recovery rate (Table 6.2 model B, Table 6.3). The other models did not improve the fit and are therefore discarded from further presentation. The calculated initial effects (Table 6.3) ranged from a 51% reduction in experiment 3 to a 74% reduction in experiment 1, whilst the recovery halflifes ranged from 1.0 day in experiments 2 and 5, to 2.9 days in experiment 1 (Table 6.4). No clear relationship existed between the initial effect and the recovery velocity.

In the other group consisting of experiments 6, 7 and 8, the effects of deltamethrin were not significant.

In Fig 6.3 cumulative spider catches of the control and impact plots are shown as a measure of arthropod activity during 24 hours after application of deltamethrin. A strong reduction in trapping success shortly after deltamethrin spraying was observed in experiments 1 to 5. For experiments 6, 7 and 8 catches were extremely low during the day with slightly higher numbers at night and no apparent effects of deltamethrin on the catches during the first 24 hours after spraying.

The deposition of deltamethrin at the soil stratum, is shown in Table 6.4. Relatively little deltamethrin reached the soil on field 1.

Based on environmental variables, the experiments could be classified into three groups; the experiments on field one had a high and dense crop (80 cm) and a moss

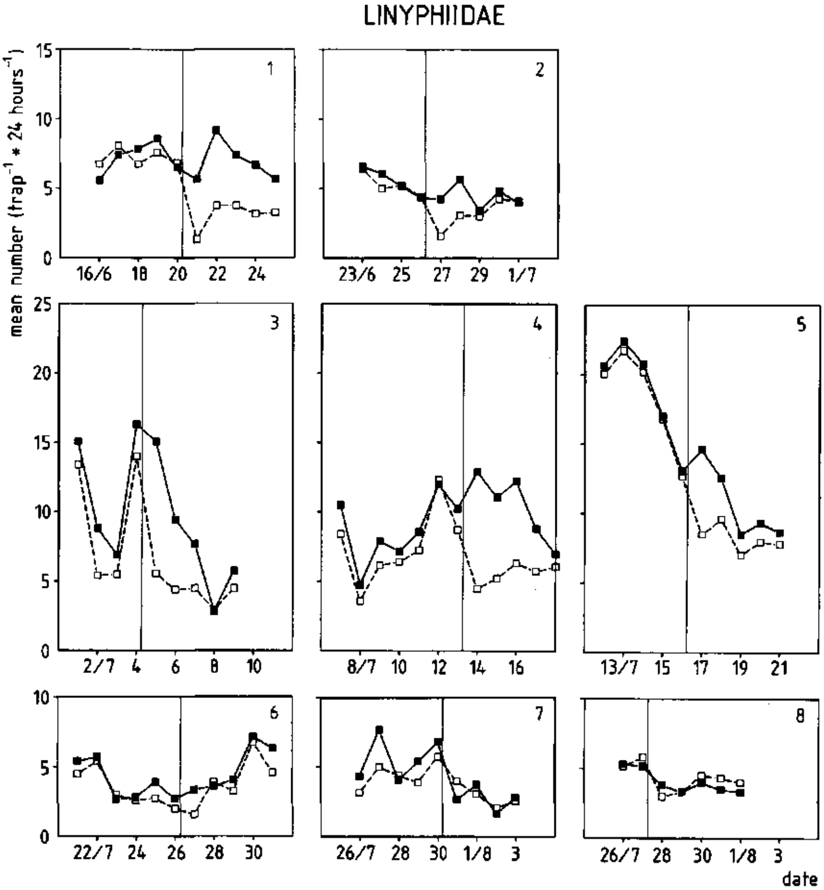

Figure 6.3: The effect of deltamethrin spraying (0.5 g ai ha^{-1}) on a field population of linyphiid spiders in relation to application date dependent walking activity and physical factors. The experiment number is indicated in the top left corner. A vertical line shows the moment of spraying. Values represent means of 2 or 3 fields (see Table 6.1) ■ = control, □ = impact.

covered soil (80% cover, visually estimated), experiment 3 to 5 shared a lower (60 cm), less dense crop and a moist soil without a moss cover, and experiment 6 to 8 were characterised by a shrivelled, dry crop and a bare dessicated soil which was exposed to direct weather influences.

Table 6.4 shows measurements of physical factors assessed during the 24 hours following deltamethrin application. The following data are of special interst in relation to the observed catches. Thunderstorms during the nights following deltamethrin application caused heavy rain during experiment 1 (at 19.00 hours) and 2 (from 23.00 until 01.00 hours). Air humidity in field 1 was relatively high compared to field 2. Soil humidity

Table 6.4: Biotic and abiotic variables measured during the first 24 hours following deltamethrin application. Standard deviations from the deposition measurements were calculated using the plot means (- = missing observation, ns = no significant effect found).

Experimental #	Application date	Initial effect (%)	Recovery halflife (d)	Cumulative catches				Deposition of deltamethrin (g ai/ha)	Air humidity (%RH)			Soil humidity (% dwt)		Temperature (+5 cm, °C)	Sunshine (h)	Sum of netto radiation (j/cm^2)		Deposition	
				control 00.00-08.00	control 09.00-24.00	deltamethrin 00.00-08.00	deltamethrin 09.00-24.00		08.00	13.00	18.00	08.00	14.00			positive	negative	duration (min)	quantity (mm)
1	20/6	74	2.9	9	26	2	7	0.015(±0.001)	90	80	100	37	17	19	1.7	565	-165	54	16.8
2	26/6	64	1.0	9	17	3	7	0.017(±0.000)	100	70	100	43	20	28	3.9	1158	-47	103	30.1
3	4/7	51	1.2	46	43	14	25	0.035(±0.003)	100	50	70	31	14	20	4.1	916	-33	0	0
4	13/7	59	1.7	51	27	16	11	0.040(±0.004)	90	40	90	18	20	30	14.9	1522	-217	0	0
5	16/7	64	1.2	41	47	15	27	0.065(±0.001)	50	-	70	9	18	30	5.0	998	-76	0	0
6	26/7	ns	ns	2	8	4	17	0.038(±0.003)	80	30	60	7	17	32	10.8	1286	-179	0	0
7	30/7	ns	ns	8	12	2	17	0.073(±0.002)	60	30	80	6	18	33	13	1313	-97	0	0
8	27/7	ns	ns	3	21	2	16	0.055(±0.005)	80	30	70	5	20	38	8.4	1153	-70	0	0

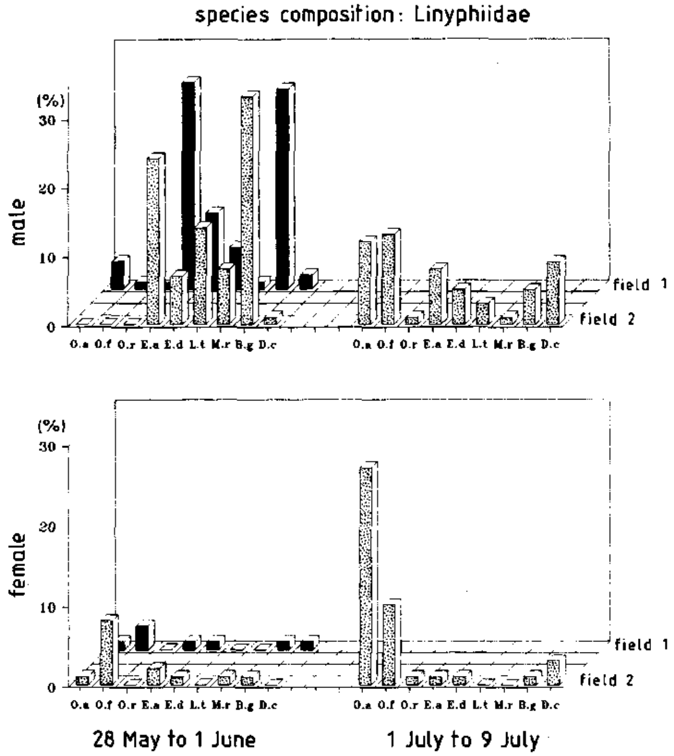

Figure 6.4: Species composition of linyphiid spiders in field 1 and 2. Bars represent the percentage of the specified groups of the total number (N) of spiders cought per field and per period. 28 May to 1 July; Field 1: N=488 (408 male, 71 female), Field 2: N=248 (225 male, 23 female); 1 July to 9 July; Field 2: N=819 (464 male, 355 female). O.a = *Oedothorax apicatus*, O.f = *Oedothorax fuscus*, O.r = *Oedothorax retusus*, E.a = *Erigone atra*, E.d = *Erigone dentipalpis*, L.t = *Leptyphantes tenuis*, M.r = *Meioneta rurestris*, B.g = *Bathyphantes gracilis*, D.c = *Diplostyla concolor*.

decreased slowly during experiments 4 to 8. Air temperature in the crop on the day of deltamethrin spraying fluctuated from 20 °C in the morning to 30 °C at noon, except on 20 June and 4 July when temperatures did not exeed 20 °C.

As shown in Fig 6.4, the species composition of spiders caught on field 1 and 2 from 28 May to 1 July showed considerable similarity. Most trapped spiders were male *Erigone atra* and *Bathyphantes gracilis*, followed in abundancy by *E. dentipalpis*, *Lepthyphantes tenuis* and *Meioneta rurestris*. Few female spiders where caught, most of which were *Oedothorax fuscus*. Identification of spiders caught between 1 and 7 July on field 2 showed a lower percentage of all above mentioned male spiders in favour of *Oedothorax apicatus*, *O. fuscus* and *Diplostila concolor*. Catches of females, consisting mainly of *O. apicatus* and *O. fuscus*, showed an increase.

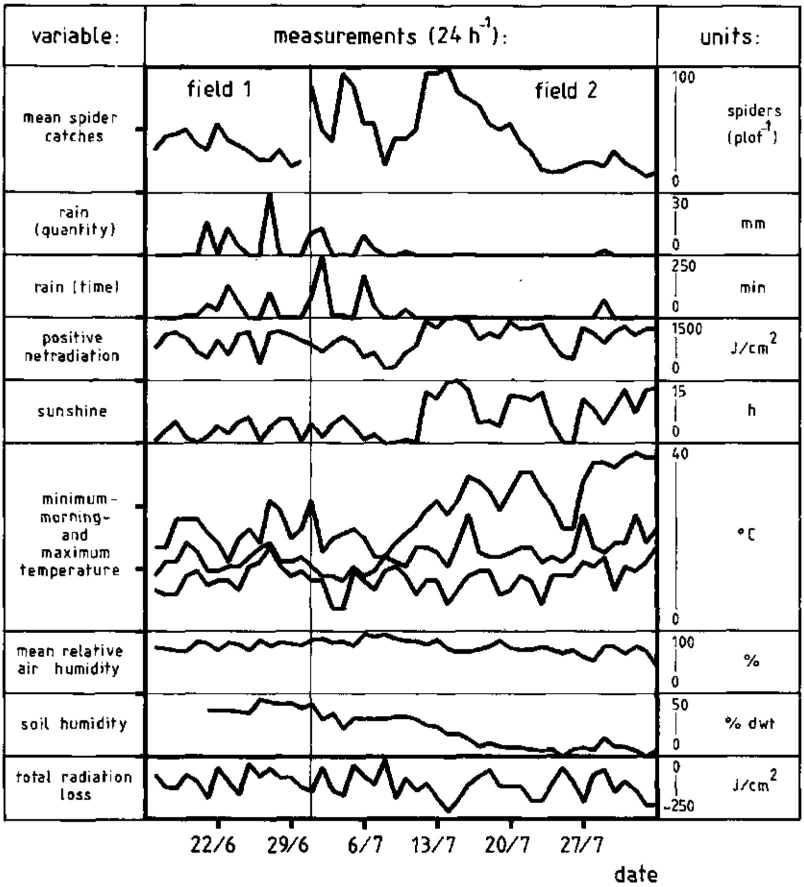

Figure 6.5: Physical factors in relation to mean daily pitfall trap catches.

Figure 6.5 shows the daily measurements of physical factors which were used to deduce changes in spider density. The multiple regression of the catches of the first 5 experiments resulted in a selection of the following factors:

(6.3) $Y = 3.34 + 0.0006Pnetr - 0.002Nnetr - 0.49F1$ (N=49 R^2=0.59)

in which: *Y* is the natural logarithm of the sum of the daily catches, *Pnetr* the net radiation gain during the day (J/cm^2), *Nnetr* the net radiation loss at night (a negative value, J/cm^2), and *F1* the field factor for field 1 (see text).

Statistical information about these results is shown in Table 6.5. The catches showed a close positive correlation with net radiation during the day and eradiation at night. The negative value (-0.49) of the field factor of field 1, indicates a 26% (=$e/e^{-0.49}$) lower population density in this field.

Table 6.5: Results of the multiple regression analysis of the relationship between trapping success and physical factors (for explanation of abbreviations see equation (6.3)) ($N= 49$, $R^2=0.59$)

Variable	R.Coeff.	STD	F	Prob.
interc.	3.34			
F1	-0.49	0.09	27	0.0001
Pnetr	0.0006	0.0001	16	0.002
Nnetr	-0.002	0.001	4	0.05

Discussion

The strong positive correlation between spider activity and reduction in trapping success clearly demonstrated the importance of walking activity in determining pesticide effects based on residual uptake. The present field observations therefore offer evidence for the assumption of Jepson (1989) that "the level of activity of a species will affect residual uptake considerably'. An important conclusion can be drawn from the identification of walking behaviour as a toxicity mediating factor in the field, namely that ambient physical factors are also of great toxicological importance, because of their effect on the walking behaviour.

The dependence of a pesticide's bioavailability on physical factors implies that each combination of physical factors is related to a specific bioavalability. In the case of identical walking activity and physical conditions this should result in similar effects. However, almost identical control activity of spiders, as measured by pitfall trapping, and pesticide effect, as measured by the initial reduction in trapping success, were observed in experiments 3, 4 and 5, despite considerable differences in physical factor conditions. There are two possible explanations for this phenomenon. One, spider behaviour compensated for differences in bioavailability of deltamethrin. As indicated by the above scenario, deltamethrin causes an increase in spider activity. The residual uptake depending on the distance walked (Jagers op Akkerhuis and Hamers, 1992), this will increase uptake. Different bioavailabilities, if not too low, may therefore stimulate spider walking behaviour until a dosis is taken up which induces immobilisation. Two, the simultaneous effect of all physical factors accidently caused a similar bioavailability and walking activity. Verification of these options using a quantitative model which describes the bioavailability and effect of the compound in relation to physical factors will be published later.

The present results provided circumstancial evidence for high bioavailability of deltamethrin from moss which was demonstrated by Jagers op Akkerhuis and Hamers (1992) in controlled laboratory studies using *O. apicatus* and deltamethrin. This follows from the observation that despite low deposition at the soil stratum in experiment 1 and 2, which were the only experiments conducted on soil with a heavy moss cover, these experiments showed considerable effect, suggesting a relatively high availability of the sprayed deltamethrin.

Comparison of spider activities between the experiments implied knowledge of the population density. As the absolute density of spiders in cereal on a clay soil is very difficult to determine, the assumption of a close correlation between density, spider activity and physical factors was used to obtain a rough estimate of differences in spider density in the first 5 experiments. Close and ecologically relevant correlations have been demonstrated earlier using this method (pers. obs.). It is, however, realised that this method needs refinement. The outcome of the regression therefore is of indicatory value. For the experiments on field 2 a stable population was assumed, fluctuations in trapping success being indicative of spider activity. The immediate rise in spider catches following the return of favourable conditions after rain on 29 August, in combination with the selective nocturnal activity of the spiders during these experiments (Figure 6.2) supports the assumption that low spider catches during experiment 6, 7 and 8 were caused by an adverse effect on walking activity of the dry soil and high temperatures, and not by changes in population density.

The results of the present study show the importance of arthropod walking behaviour with respect to residue based short term pesticide toxicity. As, on species level, there is hardly any quantitative information on arthropod behaviour in the field, this requires further investigations. Cicumstancial evidence was found for a high bioavailability of deltamethrin from moss. There is little quantitative knowledge of substrate dependent residual bioavailability of pesticides. With respect to modelling studies, this shows the need for investigations of the bioavailability of compounds from substrate in relation to substrate type, arthropod anatomy and behavioural aspects of arthropod exposure to different substrates in the field. Additionally, the interpretation of pesticide effects in the field would profit greatly from more quantitative information on substrate dependent temporal changes in bioavailability of a compound in relation to ambient physical factors.

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Part II. Laboratory studies

CHAPTER 7

Substrate dependent bioavailability of deltamethrin for the epigeal spider *Oedothorax apicatus* (Blackwall)(Aranaea, Erigonidae)

Summary

Terrestrial arthropods are mainly exposed to pesticides by uptake from the substrate e.g. 'residual' uptake, which depends on the bioavailability of the pesticide. The residual uptake of deltamethrin by female *Oedothorax apicatus*, a linyphiid spider, was studied in dependence of soil cover, soil moisture, walking behaviour, time after spraying and deposition rate. [^{14}C]labelled deltamethrin was used to quantify the uptake processes. Data were analysed using multiple regression analysis.

Uptake was markedly higher for soil covered with fungy or moss than for uncovered soil. For uncovered soils with moisture contents varying of 7 to 49% dry weight, low uptake was measured, while at 63% moisture, the uptake was high. The bioavailability halflife was 157 min. The rate of uptake decreased with distance walked. The possible consequences of these results for field experiments are discussed.

Introduction

Bioavailability, which determines the uptake and hence the dose of toxicant to which an organism is exposed, is an important variable for prediting the effects of pesticides on arthropods. Bioavailability depends on a combination of species-dependent factors and physico-chemical factors. Mullié and Everts (1991), using the linyphiid spider *Oedothorax apicatus* showed that uptake from substrate (residual uptake) is an important route for exposure to pesticides in these spiders. The effect of substrate on bioavailability in the field is, however, largely unknown. Soil type, soil moisture, and the covering of a soil by moss, algae, fungi or plant debris may influence bioavailability directly.

Residual uptake depends on the contact surface per distance walked by an arthropod. Species and substrate dependent differences have been recorded for the uptake of fine cristalline compounds (Lewis and Hughes 1957, Gratwick 1957, Beesley and Chadwick 1982). For permethrin (ULV formulation) Salt and Ford (1984) have demonstrated a high proportion (near 100%) for pick-up of contacted drops by *Spodoptera littoralis* larvae.

The availability of pesticides may change with time. An exponential decrease of availability was found for pesticides in the soil (Cheng 1984). For deltamethrin on a clay-soil Mullié and Everts (1991) reported a bioavailability half-life for *O. apicatus* of 42 hours when the animals were exposed to the substrate for periods up to 4 hours.

Other factors may determine a compounds availability. Availability from a soil, for example, tends to be negatively correlated with the octanol/water partitioning coefficient of a compound (Briggs 1981, Gestel van & Ma 1990), with the organic matter content of the soil (Harris 1967, Briggs 1981), and positively correlated with soil humidity (Harris 1967), temperature (Bailey & White 1964) (except in cases of very strong adsorbtion) and, in dry soil, air humidity (Call 1957, Gerolt 1961, Gerolt 1963). On leaf surfaces, bioavailability is negatively correlated with the octanol/water partition coefficient of a compound and the thickness of the culicular wax-layer (Ford & Salt 1987). Insecticide transfer from a leaf to an insect is influenced by the viscosity of the formulation (Crease & Ford 1987), droplet size (Omar & Matthews 1987, Adams *et al.* 1987, Hall 1987), concentration (Adams *et al.* 1987) and the architecture of the wax layer (Adams *et al.* 1987, Magelhaes *et al.* 1989). A review of these subjects has been made by Ford and Salt (1987). The partitioning of pesticides between the cuticular wax of a plant and an insect depends mainly on the dilution of a compound in the available wax. For both taxa the cuticular waxes have approximately the same constitution (Hadley 1981). Equilibrium between concentrations of the pesticide in body tissues may be reached within several hours (Greenwood *et al.* 1990).

The aim of this paper is to quantify the uptake of deltamethrin by linyphiid spiders in relation to soil moisture and soil cover while taking into account the distance an animal

Table 7.1: Experimental design of the uptake measurements, showing the times after spraying (a to d) when spiders were introduced to the treated tubs. Experiments with control spiders (ctr) were not related to a specific time.

	day 1, 2 and 4 covered soil					day 3 uncovered soil					day 5 and 6 uncovered soil					day 7 moss cover			
	soil humidity					**soil humidity**					**soil humidity**					**soil humidity**			
	7	21	35	49	63	7	21	35	49	63	7	21	35	49	63	35	35	35	35
time																			
a	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
b	16	60	120	60	60	16	60	120	60	60	16	60	60	60	120	60	60	120	120
c		120	240	120	120		120	240	120	120	120	120	120	240	120	120	240	240	
d		180	360	180	180		180	360	180	180	180	180	180	360	180	180	360	360	
-	ctr	ctr	ctr	ctr	ctr	ctr	ctr	ctr	ctr	ctr	ctr	ctr	ctr	ctr	ctr	ctr	ctr	ctr	ctr

has walked on the soil, the deposition rate and the time after spraying. Everts *et al.* (1989) showed that the linyphiid spider *O. apicatus* is a relevant and vulnerable indicator species for side effects of pyrethroids in epigeal arthropods, and is especially sensitive for deltamethrin. Therefore this species and this compound were used in the present study. [C^{14}]deltamethrin incorporated into a commercial formulation was used to quantify the pesticide uptake.

Materials and Methods

The experimental design is shown in Table 7.1. Three types of soil cover were investigated, uncovered soil, soil covered with fungi and soil covered with moss. Each day the residual uptake of deltamethrin by spiders from one, randomly chosen, type of soil cover was determined for 5 soil moisture values, 7, 21, 35, 49 and 63 percent dry-weight (dwt) in random sequence. The uptake from each soil moisture treatment was measured at four successive times over 15 min. There were two exceptions to this. One, the moss-covered soil, which was collected in the field, and had a soil moisture of 35% dwt. Two, the soil of 7% dwt, where the first two times in the time series were investigated for 9 minutes only. This was done because preliminary experiments showed that the bioavailability of deltamethrin on this soil decreased within 30 minutes to control level.
In all experiments adult, F_1 generation, female *O. apicatus* were used; these were reared at 20 °C in the laboratory from females trapped in the field (modified from Aukema *et al.* 1990).

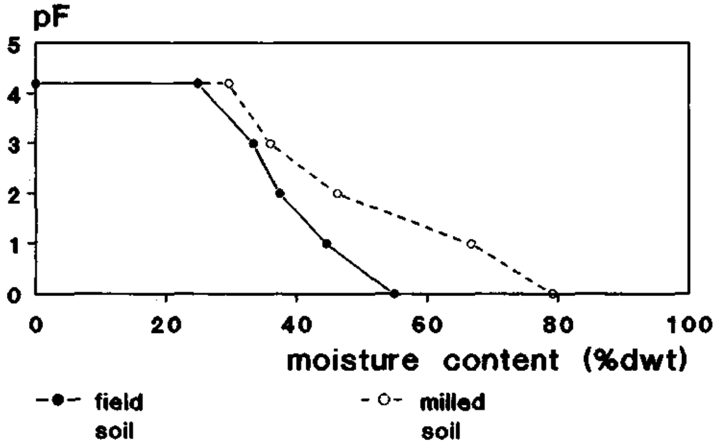

Figure 7.1: pF-curves for the clay soil used in the experiments. Curves are shown for the natural soil from the field, and for the milled soil used in the tubs.

Adult spiders were stored at 5 °C and provided with fruitflies until use. Three days before use the spiders were transferred to 21 °C, given fruitflies *ad libitum* for two days, and then starved for 24 hours.

To enable future extrapolation of laboratory results to the field we used a heavy, river clay, soil from our field plots (lutum content: 59%, organic matter content: 5.3 %, pH (Potassium Chloride): 6.2, from the top 25 cm of the soil of plot 'West 2' of the Institute for Plant Protection Research (IPO, Wageningen, The Netherlands). The soil was dried at 30 °C and milled (1 mm maximum crum size). pF curves of the natural soil and of the ground soil are given in Fig. 7.1. The bulk density of the dried and milled soil was 1.20 kg l^{-1}, and 1.36 kg l^{-1} for the natural soil.

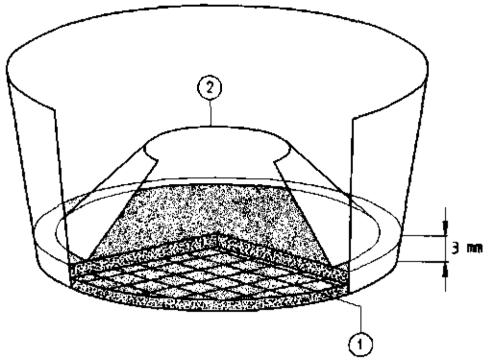

Fig. 7.2. Cross-section of a tub. 1: A thin layer of clay soil, showing the embedded plastic gauze. 2: A conus installed on the soil to prevent spiders from climbing on the walls of the tub.

[^{14}C Benzyl]deltamethrin, suspended in toluene, specific activity 2.18 GBq mmol^{-1}, M: 507.21, was provided by Roussel Uclaf. The toluene was evaporated under nitrogen at 70 °C and replaced by a 'blank' formulation (S276BF) of 'Decis-flow' emulsifiable concentrate (also provided by Roussel Uclaf) to give a stock formulation of 22.54 g a.i. l^{-1}

(the commercially available concentrate is 25 g a.i. l^{-1}), this was kept at -20 °C. A fresh dispersion was made each day by pipetting 28.4 μl of the stock formulation into 8 ml tap-water, in a small, clean glass jar to give a working-emulsion of 80 mg a.i. l^{-1}.

Experiments were conducted in transparant round plastic tubs (5 cm heigh, 9.5 cm diam.)(Fig. 7.2) which were prepared in a strictly standardized way. Into each tub 12 ml of water was pipetted and a round piece of plastic gauze (3 mm mesh width) was placed on top of the water. Then 19 g of dried soil was sieved into each tub. The tubs were repeatedly banged by hand onto a table top, until a smooth and evenly wet surface was obtained. This was allowed to dry at 40 °C to obtain soil moistures of 7, 21, 35, 49 and 63 percent dryweight. The gauze prevented the soil from forming wide cracks. The tubs were then closed and stored at 20 °C in 100% air humidity. The soil moisture content of the tubs was checked by weighing at the start and at the end of each measuring series. Soil moisture values differed by less than 1% dwt from the nominal values.

Tubs with soil referred to as 'uncovered' were always prepared less than 24 hours before use. Tubs with 'covered' soil were stored for three days to permit a thin fungal cover to develop. A 'moss cover' was created by transplanting thin patches of moss and algae from the field to prepared tubs at 35% soil moisture. Photographs of selected soils are shown in Figs. 7.3a to 7.3d.

The soil surface was sprayed at t=0 with 1 ml of the working emulsion (see above) via a Potter Precision Laboratory Spray Tower (Potter 1952)(Burkhard) at a pressure of 0.3 kg cm^{-2}. The deposition pattern was evaluated using a solution of methylene-blue. This revealed a fine and equal pattern, on paper, of small droplets less than 0.4 mm diameter. The deposition of deltamethrin was measured with the aid of two halves of a glass cover slip (18*18 mm) placed randomly on the soil.

A plastic cone with an open top, (8 cm diam. and an inward angle of 30^0) was placed on the soil. If necessary fine purified quartz sand (Merck) was used outside the cone to plug wide cracks (Fig. 7.2). During observations tubs were kept at 21 ± 0.1 °C.

After the introduction of a spider to a tub, the tubs were closed, and the walking pattern and time of activity were recorded for 15 min, divided into periods of three minutes to evaluate the effects of deltamethrin on walking speed, activity and resting frequency. After 15 mins, the spider was anaesthetised using carbon dioxide, and transferred into 2 ml 'Soluene-350' (0.5N quarternary ammonium hydroxide in toluene) in a 6 ml plastic scintillation tube. Any remaining CO_2 was blown out of the tub to prevent effects on the activity of a next spider. Spider tissue was dissolved in 2 ml Soluene^R, using a shaking water bath (55 °C, 4 h). The tubes were filled with scintillation liquid ('Ultima Gold^{TM}') and left for 10 minutes (chemoluminescence decay) prior to liquid scintillation counting (Packard TRI-CARBR 300C, 10 min, C^{14}-spectrum). Recovery was 100%, no quenching (Mullié and Everts 1991). The glass slips used for deposition measurements were counted in pure scintillation liquid.

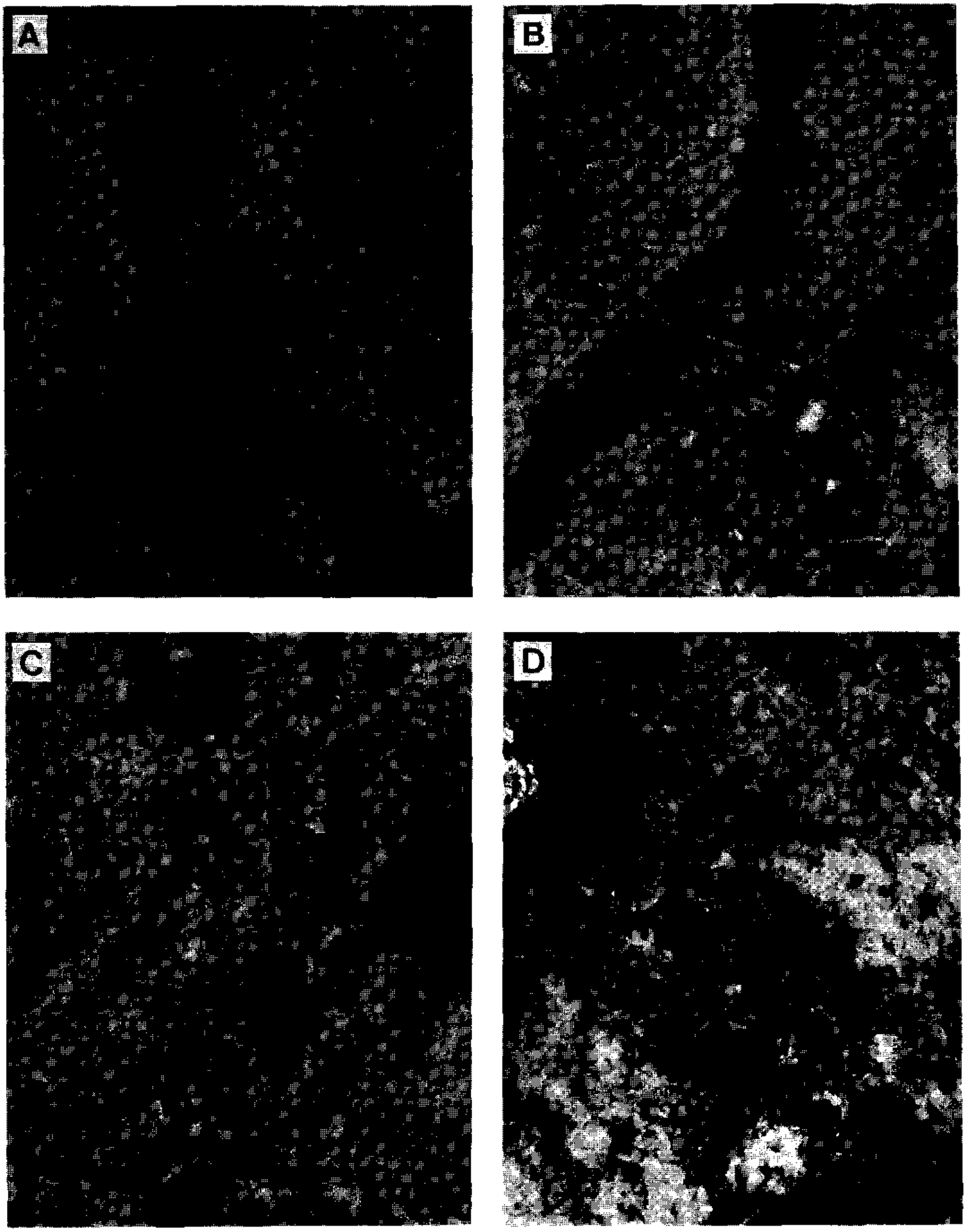

Fig. 7.3. Selected soil treatments showing soil moisture and fungal development. A: 'Uncovered' soil of 35% dwt. B: 'Covered' soil of 35% dwt. with clear fungal development. C: 'Covered' soil of 63% dwt showing little development of fungal hyphae. D: Soil covered with moss.

Spider walking patterns were analysed using a line intersection method originally developed for analysing root lengths (Tennant 1963, Newmann 1966). The accuracy of this method was within 5% limits in terms of the variation coefficient.

Data analysis

For each day the mean number of scintillation counts from the control spiders was subtracted from the measurements of the treated spiders, then the control spiders were excluded from the regression. The subtraction resulted in a few zero or small negative numbers (13 out of 124) which were set to 1 for reasons of calculation (i.e. the logarithmic transformation of the uptake values). Using the specific activity and molecular weight of the deltamethrin, the uptake in dpm was multiplied by a factor $3.877*10^{-3}$ to obtain the uptake in ng spider-1. Subsequently a linear regression analysis was applied to the logarithm of the corrected deltamethrin uptake as the dependent variable. In this way the model reflected the assumption of a multiplicative relationship i.e. a change in one factor influences the level at which all other factors operate. Based on preliminary experiments (R. Kraay, unpublished results, and Mullié and Everts 1991), an exponential decay was modelled for uptake as a function of time, an exponential increase was modelled for uptake as a function of deposition and a power curve was modelled for uptake as a function of distance. Soil moisture and soil cover were modelled as class-variables for the following reasons. Preliminary results of regression calculations showed that the functional relationship between uptake and soil moisture was not well approximated by a polynomial with a low degree. No quantitative information was available on the soil cover. The use of cover classes implies the calculation of class means for each class which is represented in the regression by a vector with the value 1 within the class, and 0 otherwise. This resulted in the following linear regression model:

$$(7.1)\ Y = \beta_0 + \beta_1 ln(D) + \beta_2 DEP + \beta_3 T + \beta_{4..7} SH_j + \beta_{8..13} C_k + \epsilon$$

where Y represents the natural logarithm of the corrected uptake (ng deltamethrin), β_0 the intercept, β_1 to β_{13} regression coefficients, D distance walked (cm), DEP the measured deposition of deltamethrin (g ha^{-1}), T the time after spraying a spider was placed on the substrate (min), SH_j (j=7 to 49) soil humidity classes with 63 as reference (% dwt), C_k cover classes (no cover (k=0), fungal cover on soil of 7 to 63 (k= 7, 21, 35, 49, and 63) with moss cover as reference (k=m)) and ϵ an error term with expectation zero, a constant variance and a normal distribution. The regression allows for comparison of uptake on uncovered and covered soil for each soil moisture treatment. For soil of 35% dwt it also allows for comparison of the uptake on fungal cover with that on moss cover.

Results were calculated using the GLM (General Linear Models) option of the SAS statistical package (SAS 1991).

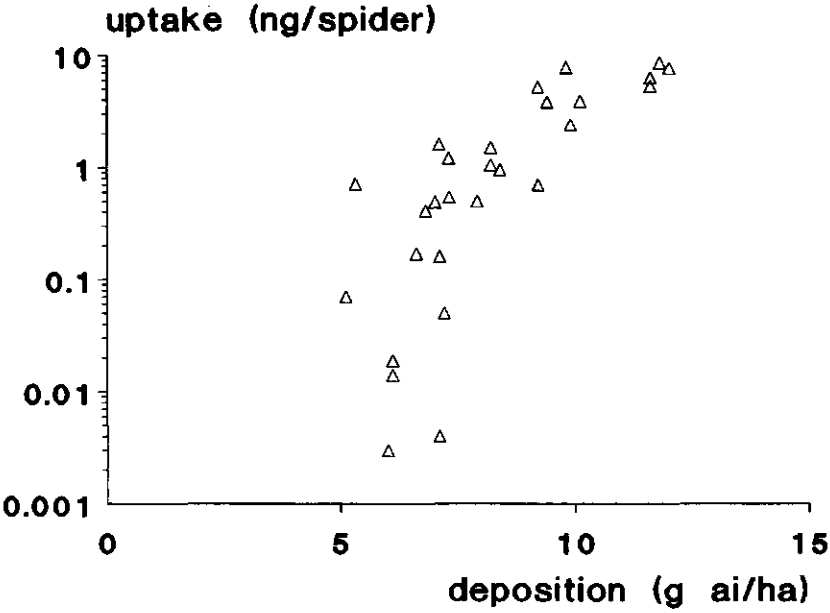

Fig. 7.4. Deltamethrin uptake in relation to application rate, uncorrecten for other factors, showing observations at 10 min after spraying.

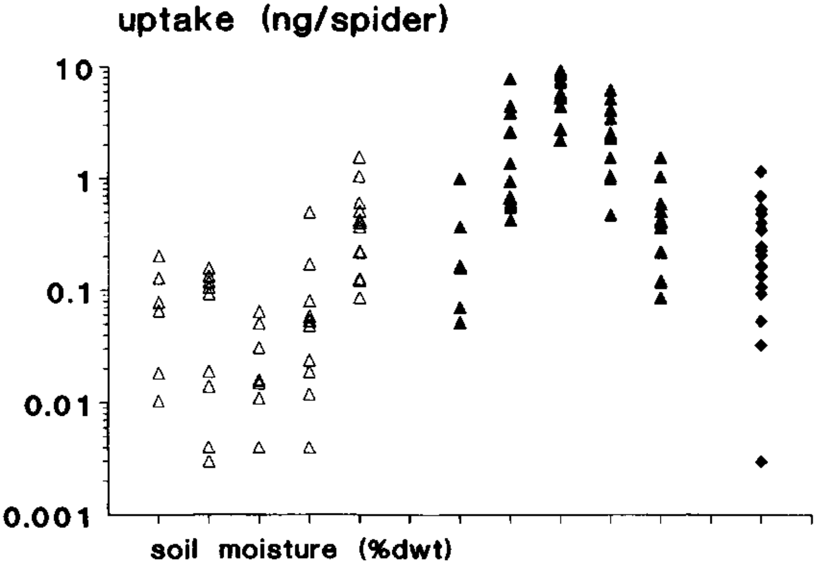

Fig. 7.5. Deltamethrin uptake in relation to soil cover and soil moisture, uncorrected for other factors. (△) uncovered soil, (▲) fungi covered soil, (◆) moss covered soil.

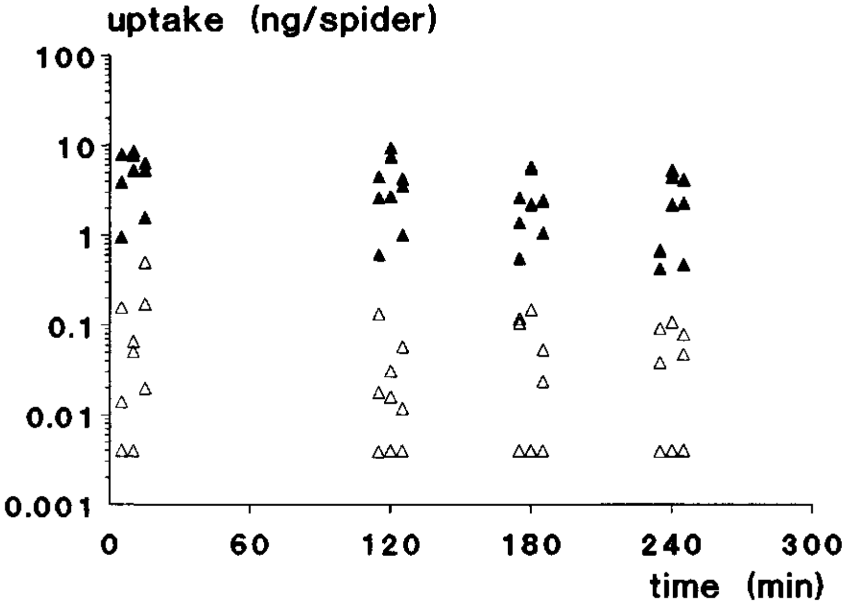

Fig. 7.6. Deltamethrin uptake in relation to time after spraying, uncorrected for other factors. (△) uncovered soil, (▲) covered soil. At each time, individual observations are shown for 21% dwt (first column), 35% dwt (middle), and 49% dwt (last colums).

Results

Fig 7.4-7.6 show the measured deltamethrin uptake in relation to soil humidity and soil cover, time after spraying, and deposition rate, without correcting for variation due to other factors. The latter prevented clear presentation of the uptake in relation to distance walked.

Multiple regression was used for the analysis of the 5-dimensional dataset. Based on the former assumptions about the functional form of the separate variables, this resulted in the following model in which the uptake on bare soil of 63% dwt served as reference for the contributions of soil humidity, and the uptake on moss for the contribution of soil cover:

$$
\begin{array}{llllll}
(7.2) & Y = & & -2.26 & +0.33\ \ln(D) & +\ 0.34\ DEP - 0.0044\ T \\
& & -2.45\ SH_{7} & -2.40\ SH_{21} & -2.91\ SH_{35} & -\ 2.50\ SH_{49} \\
& & -2.55\ C_{0} & -2.23\ C_{7} & -0.33\ C_{21} & +\ 1.67\ C_{35} \\
& & +1.06\ C_{49} & -2.45\ C_{63} & &
\end{array}
$$

(For an explanation of the symbols, see eqn (7.1)).

Table 7.2: Statistical information for the results of the regression model (for explanation of the symbols see text). $N = 124$, significance level of the total model: $p < 0.0001$, $R^2 = 0.82$, standard deviation = 1.02, SH_{63} and C_{moss} served as reference classes. The following table gives for each regression coefficient the values of the estimate, the chance that the value equals 0 (no significant contribution to the model), and the standard deviation of the estimate.

Parameter	Estimate	P>|T|	SD of estimate
Intercept	-2.26	0.12	1.44
Dep	0.34	0.0008	0.098
T	-0.0044	0.0001	0.00083
ln(D)	0.33	0.064	0.18
SH7	-2.45	0.0001	0.53
SH21	-2.40	0.0001	0.44
SH35	-2.91	0.0001	0.44
SH49	-2.50	0.0001	0.43
SH63 (reference)	0		
C0	-2.55	0.0001	0.39
C7	-2.23	0.0035	0.75
C21	0.33	0.62	0.66
C35	1.67	0.01	0.64
C49	1.07	0.08	0.61
C63	-2.45	0.0001	0.61
Cmos (reference)	0		

The model had a good fit (R-square of 0.82) and reasonable accuracy (std of 1.02). The residuals revealed no problems with the model (Fig. 7.7). Relative to the reference treatments, significant contributions were found for most of the soil moisture and soil cover treatments (significances in Table 7.2).

The results of the analysis allow a quite simple interpretation. Using the calculated regression coefficient for each separate variable at fixed values of other variables, curves were calculated in which uptake is plotted for covered and uncovered soil as a function of soil

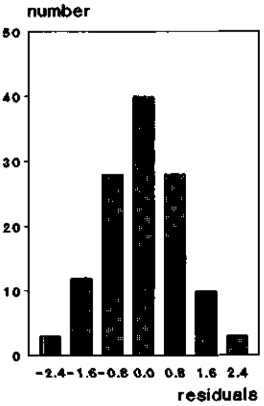

Figure 7.7: Histogram of the (unstandardised) residuals from the regression model supporting the normal distribution of the residuals.

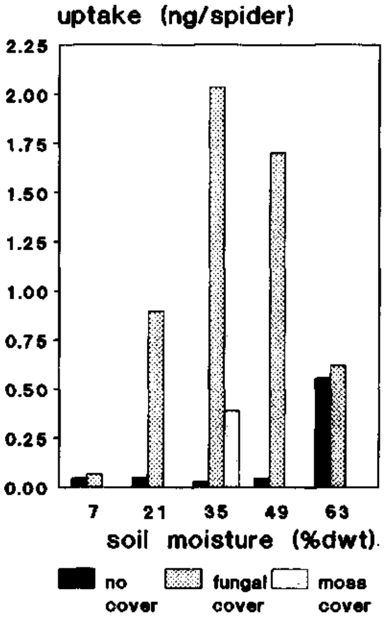

Figure 7.8: Calculated uptake of C^{14}-labelled deltamethrin (model (2)) by female O. apicatus from soil in relation to soil cover and soil moisture. (distance walked = 200 cm, deposition = 7.5 g ha^{-1}, time after spraying = 30 min).

moisture, deposition quantity, time after spraying or walked distance (Figs 7.8-7.11).

Uptake on uncovered soil was low for all classes of soil moisture except for 63% dwt (Fig. 7.8). Uptake of deltamethrin on fungal covered soil of 21, 35 and 49% dwt (not on 7 or 63% dwt), or on soil with a moss cover was higher than on uncovered soil. Significant differences between uptake for the classes of soil cover and soil moisture are given in Table 7.3.

Table 7.3: Significant differences at p=0.01 between the regression estimates for soil humidity and soil cover classes.

soil humidity		soil cover	
		no cover	a
		moss cover	b
7	a	fungal cover on 7%	a
21	a	" " 21%	b
35	a	" " 35%	b
49	a	" " 49%	b
63	b	" " 63%	a

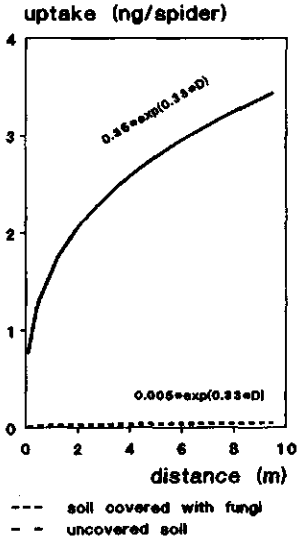

Figure 7.9: Calculated uptake of C^{14}-labelled deltamethrin (model (2)) by female *O. apicatus* from soil in relation to the distance walked on the substrate. The thick line represents the uptake from fungal covered soil (35% dwt) and the broken line the uptake from bare soil (35% dwt)(deposition = 7.5 g ha^{-1}, time after spraying = 30 min, soil moisture = 35% dwt).

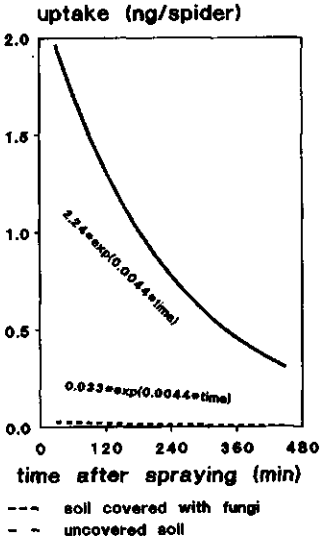

Figure 7.10: Calculated uptake of C^{14}-labelled deltamethrin (model (2)) by female *O. apicatus* from soil in relation to the time after spraying on which a spider is placed on the substrate. The thick line represents the uptake from fungal covered soil (35% dwt) and the broken line the uptake from bare soil (35% dwt)(distance walked = 200 cm, deposition = 7.5 g ha^{-1}, soil moisture = 35% dwt).

A rapid decrease in the rate of uptake was found with an increase in distance (Fig. 7.9). Bioavailability decreased exponentially with time (Fig. 7.10). The bioavailability half-life (measured as uptake per distance (ng cm^{-1})) was calculated as 157 min (SD 133-198 min).

An exponential increase was found for uptake against deposition (Fig 7.11). The 'doubling concentration' of uptake as a function of deposition amounted to 2 gr a.i. ha^{-1} (SD 1.6-3 g).

The mean walking speed and percentage activity of the control spiders and the spiders on sprayed soil are given in Table 7.4. It was concluded that control spiders on a covered soil generally walk more slowly (ANOVA, $F_{(6,145)}=21.5, P<0.0001$) and are more active ($F_{(6,145)}=19.2, P<0.0001$) than spiders on an uncovered soil. In general treatment of the soil with deltamethrin caused spiders to walk more slowly ($F(1,145)=23.1, P<0.0001$) but raised the percentage of time that they were active ($F(1,145)=41.38, P<=0.0001$). A notable exception to this was found for the soil at 7% dwt where the effects of deltamethrin on the behaviour of the spiders were faint or absent.

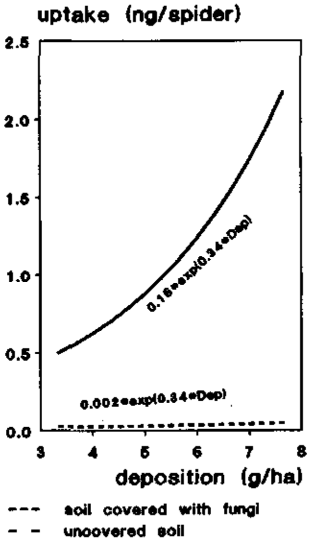

Figure 7.11: Calculated uptake of [^{14}]Clabelled deltamethrin (model (2)) by female *O. apicatus* from soil in relation to deposition. The thick line represents the uptake from fungal covered soil (35% dwt) and the broken line the uptake from bare soil (35% dwt)(distance walked = 200 cm, time after spraying = 30 min, soil moisture = 35% dwt).

Discussion

The results showed a marked difference between pesticide uptake from covered and uncovered soil. High uptake was related to a rich fungal cover as qualitative observations showed (Fig 7.3). Poor development of the fungal cover on soils of 7% and 63% dwt coincided with uptake values comparable to the corresponding uncovered soils. It is likely that both the wet and dry soil formed an unsuitable substrate for rapid fungal development and that fungal hyphae could not protrude from the water film on the wet soil. A combination of the following two mechanisms may explain the positive correlation between soil cover and high bioavailability of deltamethrin. The lack of a waxy cuticula limits diffusion or adsorbtion of deltamethrin on fungal hyphae or moss. Furthermore, the thin threadlike structure of fungal hyphae may prevent the superficial spread of pesticide drops and cause the deposit to remain concentrated over a small area in relation to the impact site.

On bare soil an immediate decrease in bioavailability after spraying was observed. This may be explained by instantaneous absorbtion of droplets of the pesticide solution by the clay, leaving only a minor fraction of the deltamethrin available at the soil surface. Low uptake values for all except the highest soil moisture classes of the bare soil indicate no clear relationship to soil moisture of the assumed absorption process. This is in agreement with the moderate effect of soil moisture on the (low) toxicity to crickets of several pesticides mixed with a muck soil, reported by Harris (1967). However, using mineral soils, Harris found a gradual increase in toxicity with increasing moisture content of the soil. In our observations no gradual increase was found. A relatively high uptake was found selectively on the very wet uncovered soil, which implied that absorption or adsorption processes on this water-logged soil were strongly delayed or blocked.

Table 7.4: Walking activity and walking speed of female *O. apicatus* on untreated (n=3) and on deltamethrin treated (n=12) soils with different moisture contents and soil covers (with 95% confidence limit).

	mean walking speed (cm s^{-1})				mean walking activity (% of time)			
	control		treated		control		treated	
humidity and cover	mean	95%cl	mean	95%cl	mean	95%cl	mean	95%cl
bare soil								
7	1.95	0.33	1.79	0.26	21	6	16	9
21	2.45	0.56	1.90	0.34	19	5	25	6
35	2.47	0.57	1.67	0.20	17	13	34	8
49	2.30	0.57	1.87	0.26	10	10	32	8
63	2.55	0.65	1.43	0.25	16	16	29	5
mean	2.34	0.27	1.73	0.25	17	6	28	7
covered soil								
7	1.88	0.40	2.55	0.80	27	17	17	8
21	1.84	0.36	1.31	0.18	22	19	51	7
35	1.53	0.34	1.12	0.19	22	18	41	8
49	1.30	0.23	1.10	0.22	17	14	43	4
63	1.63	0.48	1.21	0.18	20	8	44	6
35 moss	1.89	0.90	1.48	0.37	6	3	30	7
mean	1.78	0.14	1.37	0.15	10	4	39	3

With respect to the distance walked, the functional form of the uptake-curve suggests that the distal parts of the tarsi of the spiders may act as small reservoirs. Due to saturation of these parts with deltamethrin, uptake decreases with distance walked. The observed relationship between uptake and distance walked implies that for residual uptake the term bioavailability is best defined over short distances, as species dependent uptake per distance walked.

The bioavailability halflife of 157 min is in the same order of magnitude as the value Salt and Ford (1984) deduced from their model for the uptake of cypermethrin from leaves by *Spodoptera littoralis* (1 h). Cilgy *et al.* (1988), using *Bembidion lampros*, observed a reduction in time for the contact toxicity of deltamethrin sprayed on wheat leaves, with a half-life of approximately 4 days, when the animals were exposed to the substrate over 24 hours. Differences between the latter results and the outcome of our experiment may be explained by the fact that in our observations, the bioavailability was corrected for the walked distance. This implies that measurements were not affected by the influences of poisoning or substrate condition on the activity of the animals during the measuring period.

The exponential increase in uptake found with increasing deposition may be explained in the following way; as the concentration of our spraying solution was always near 80 mg ai l^{-1}, and assuming that the droplet size of the spray remained constant at the constant spraying pressure used, high deposition implies a high droplet density. The nonlinear increase of bioavailability may therefore, be caused by a higher availability of deltamethrin from surfaces which were pesticide-saturated as a result of overlapping droplets.

Effects of deltamethrin on the behaviour of spiders in relation to a soil cover may be explained as follows; a low walking speed and high activity on covered soil indicates that covered soil is a complex structural environment for spiders to encounter. When exposed to deltamethrin, the spider is active for a larger percentage of time, but the walking speed is reduced immediately after contact with the compound. This suggests that spiders experience an unpleasent sensation at the extremities immediately after contact with deltamethrin, which urges avoidance, but in the meantime disables walking ability. As the deltamethrin will stick to the tarsi, the repellent effect of deltamethrin enhances uptake.

Strictly, the experimental design relates the effect of soil cover to its fellow soil moisture. However, not too many difficulties are expected in extrapolation to different cover classes for a given soil moisture because uptake on bare soil was very low and showed little relation with soil moisture. Therefore, if a rich fungal growth would be present on very dry soil or very wet soil, the uptake would be estimated with reasonable accuracy by the respective regression coefficients for dry or wet soil and a rich cover.

The residual uptake of a pesticide by arthropods is a process which involves many different parameters. It may therefore be economic to model uptake of an easy-to-handle and easy-to-rear arthropod species under known circumstances and calibrate this model with crucial species dependent factors to predict uptake in other arthropods on other substrates.

The results presented in this paper may have important implications for the field situation. Soil cover (fungi, moss) was shown to raise bioavailability by a factor of 100. A quantitative estimate of the natural soil cover in the field thus seems essential for the prediction of uptake. Preliminary observation gave the impression that most of the soil surface in a field of winter wheat is covered, during the summer, with algae, fungi, moss or plant debris which therefore suggests a high bioavailability.

Another factor which may be important in the field when pesticides are used with a short bioavailability half-life (not to be misstaken for decay), is the activity of the animals. Even for compounds with a high intrinsic toxicity to arthropods such as most pyrethroids, circumstances may occur in which momentarily low arthropod activity due to environmental factors (diurnal activity, too hot or too wet for activity) in combination with a short half-life time for a compound may lead to no observed effects in the field. Theoretically this phenomenon can be used to enhance the selectivity of a pesticide.

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CHAPTER 8

Water balance, *respiration* and *immobilisation* in relation to deltamethrin poisoning and physical conditions in the epigeal spider *Oedothorax apicatus* (Blackwall) (Linyphiidae).

Summary

The effect of a pyrethroid insecticide, deltamethrin, on water loss, metabolic rate and immobilisation was studied, using the epigeal spider *Oedothorax apicatus* as a test species. Water loss was measured gravimetrically at different temperatures, air humidities, and doses of deltamethrin (Decis^R). Metabolic rate and immobilisation were measured at different temperatures and doses. Immobilisation was measured at different relative humidities and temperatures. Water loss due to evaporation increased linearly with vapour pressure deficit, the slope of which showed a positive relationship with temperature, indicating an increase in cuticle permeability with temperature. In addition to water loss by evaporation, deltamethrin treatment induced water excretion, which was positively related to dose and temperature, but independent of air humidity. Metabolic rate increased with temperature and dose. Immobilisation was highest at low temperatures in combination with low air humidity.

The consequences of pesticide induced water excretion are discussed with respect to arthropod mortality in the field and LD_{50} or EC_{50} values for pyrethroids presented in the literature. The present results seem to offer an explanation for hitherto unexplained minima in temperature-response curves in pyrethroid toxicity in some arthropod species. It is concluded that simultaneous measurements of metabolic rate, water loss rate and pesticide effects are a prerequisite for an understanding of the effects of pesticides on arthropods.

Introduction

The treatment of arthropods with pyrethroids may cause neurotoxic effects in different parts of the nervous system, including the central nervous system and sensory, motory, or neurosecretory neurons (Miller and Salgado 1985, Ruigt 1985). The effects may differ in magnitude and duration, as a result of differences in intrinsic sensitivity of the target cells, metabolism of the coumpound and the velocity and mode of penetration to the target site. The exact route of uptake is the subject of controversy see Ford *et al.* (1981), Gerolt (1983), Welling and Paterson (1985).

It has been shown that pyrethroids induce increased water loss in arthropods (e.g. Ingram 1955, Hewlett and Gostick 1955, Sun and Johnson 1972, Gerolt 1976, Sundukov *et al.* 1989). Pesticide induced water loss involves active water excretion as has been demonstrated by strong reductions in cuticular water loss in *Musca domestica* after exposure to pyrethrum in an oxygen free, metabolism blocking, atmosphere (Gerolt, 1975). Active water excretion is controlled by the release of a diuretic hormone (Casida and Maddrell 1971, Gerolt 1983). Greenwood *et al.* (1985) demonstrated the relationship between pesticide induced water excretion and diuretic hormone production, by showing that, after radiocautery of the *corpora cardiaca*, which are in control of the diuretic hormone production, no significant increase in water loss was found in permethrin-treated desert locusts (*Schistocerca gregaria*). Pyrethroids may also cause rapid immobilisation, or 'knockdown'. This has led to the suggestion that water loss in immobilised arthropods which are exposed to heat and drought on the soil surface may increase mortality (e.g. Casida and Maddrell 1971, Everts 1991a).

Water loss in arthropods also comprises passive, evaporative water loss which depends on ambient temperature and air humidity. Evaporative water loss is influenced by vapour pressure deficit and the permeability of the cuticle. Under the assumption that the surface temperature of an arthropod is similar to its surrounding air, and the arthropod's body fluids exists of near 100% water, water loss may be expressed as a function of vapour pressure deficit and cuticular conductivity (equation (8.1)). The vapour pressure deficit can be expressed as a function of the saturation partial pressure of water vapour, and relative air humidity as shown in equation (8.2). Finally the saturation partial vapour pressure is function of temperature (Nobel, 1970, equation (8.3)).

$$J = C\,P \tag{8.1}$$

$$P = (1 - RH/100)\,P^{*} \tag{8.2}$$

$$P^{*} = 6.107 \exp((17.4\,T)/(239+T)) \tag{8.3}$$

where: J is the water loss rate (g m^{-2} h^{-1}), C is the conductivity of the cuticle and surrounding air layer. C (g N^{-1} h^{-1}) may be expressed as $1/R$ with R being the temperature dependent resistance for water vapour of the cuticle, P is the deficit of vapour pressure (N m^{-2}), P^* is the saturation partial vapour pressure (N m^{-2} 10^{-2}), RH is the relative air humidity (%) and T is the temperature (°C).

It should be noted, that water permeability of the cuticle increases exponentially with temperature, as has been shown for the black widow *Latrodectus hesperus* (Hadley and Quinlan, 1989) and other spiders (Vollmer and MacMahon 1974, Hadley *et al.* 1981).

As no quantitative information is available in the literature which would enable a prediction of combined water losses for an organism in the field, the aim of the present study was to quantify processes of water loss and immobilisation in relation to physical factors and pesticide poisoning. The following aspects were studied. Passive water loss was studied in relation to different combinations of air humidity and temperature. Pesticide induced water excretion was studied in relation to temperature and air humidity. Immobilisation was studied in relation to temperature and relative humidity, for its possible contribution to dissication by preventing drinking. The active nature of the pesticide-induced water loss implies a relationship with metabolic rate; measurements of the latter, in relation to temperature and pesticide poisoning were therefore included in this study.

Everts (1990) and Everts *et al.* (1991a, 1991b) showed that linyphiid spiders are sensitive indicators for side-effects of pyrethroids on epigeal arthropods in the field, and that the species *Oedothorax apicatus* could be handled and reared easily under laboratory conditions, *O. apicatus*, therefore was used in the present study. The pyrethroid insecticide deltamethrin was used as a model compound.

Materials and Methods

The experiments were conducted using laboratory-reared female *O. apicatus*. The spiders were reared as described by Jagers op Akkerhuis and Hamers (1993, in press). Spiders were acclimatized at 20 °C for 72 hours and provided with fruit flies *ad libitum* until 24 hours before the experiment. During a short period of anaesthesia (CO_2, 3 min) individual spiders were treated, using a Burkhard Electric Microsyringe Applicator, on the dorsal abdomen with either a solution of the synthetic pyrethroid insecticide Deltamethrin in a commercial formulation (Decis^R) or tap water as a control treatment. Previous experiments had showed a similar effect for a solution of the blanc formulation of Decis^R to that of tap water (A. Boersma, 1987. Unpublished results at our laboratory).

Water loss

The experiments were conducted in closed 12 dm^3 containers using glycerine-water solutions (Schoorl 1946), to create the required air humidity. A hygrometer was used to check the relative air humidity (EWHS 31 Eliwell capacitive hygrometer). Spiders were handled during the water loss experiments in small (± 1 cm^3) tetraeder-shaped cages folded from perforated aluminium foil, the weight of which remained constant under different air humidity conditions. To assure stable air humidity in the containers during the experiments, spiders were handled on sledges of half open, round plastic tube (2 cm diameter) with a closed end, which entered the containers horizontally through round holes, exchange between air in and around the containers was thus minimised.

The rate of water loss over time at different doses was determined as weight loss using 6 groups of 8 spiders, exposed to 0, 0.0025, 0.025, 0.25, 2,5 and 25 ng ai (active ingredient) spider^{-1} (20% relative humidity (RH), 19 °C). Spiders were weighed (over about 2 minutes, Mettler ME30 balance) at t=0, 90, 150, 210 and 270 minutes after application. Transpiration was expressed as the weight loss per hour relative to the initial weight (mg mg$_{i-1}$ h^{-1}). Given the number of spiders used, the treatment with deltamethrin and installation of the spiders in the cages took in total more than an hour, individual spiders were weighed for the first time 90 minutes after pesticide or tapwater application.

The relationship between relative humidity, temperature and dose was investigated for each of the following treatments: 0, 0.25 or 25 ng ai spider^{-1}, at 15 °C and at 25 °C, using 6 spiders per treatment. The vapour pressure deficits used are given in Table 8.1. The transpiration rate (mg mg$_i^{-1}$ h^{-1}) was determined as the slope of the weight loss curve (regression through measurements at t=90, 150, 210 and 270 minutes after application).

Table 8.1. Experimental design of the study of water loss rate in relation to vapour pressure deficit (P), temperature and dose of deltamethrin (n=3 treatment^{-1} day^{-1}).

Temperature	15°C		25°C	
Day	1	3	2	4
vapour pressure deficit				
	0	0	0	0
	8.8	10.1	14.3	16.2
	13.8	14.7	26.3	25.7
dose (ng ai spider)				
	0	0	0	
	0.25	0.25	0.25	0.25
	25	25	25	25

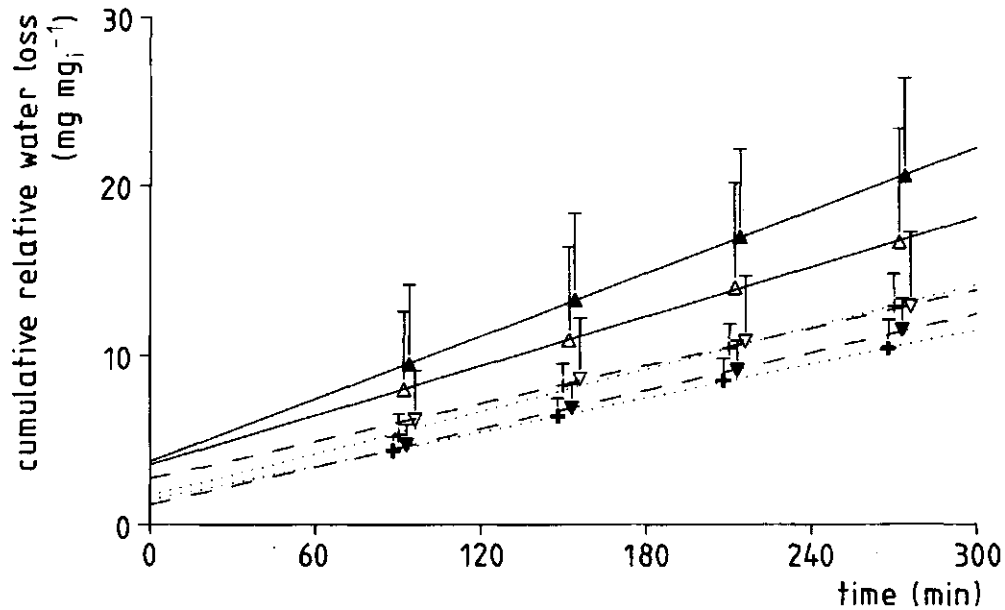


Figure 8.1: Cumulative water loss of *O. apicatus* in respons to deltamethrin poisoning. Water loss and standard error. for .+ . = 0, .✚. = 0.0025, -▽- = 0.025, -▼- = 0.25, -△- = 2.5 and -▲- = 25 ng ai deltamethrin spider^{-1} (19 °C, RH: 20%, n=48).

Table 8.2. Water loss rate (WLR, mg mg$_i^{-1}$ hour^{-1}) and intercept (Intc) (with standard errors, SE) for spiders exposed to doses between 0 and 25 ng ai spider^{-1} deltamethrin. Significant differences are indicated with different characters.

Dose	#	WLR	SE		Intc	SE	
0	8	2.5	0.17	b	1.7	0.43	a
0.0025	7	2.1	0.14	a	1.2	0.28	a
0.025	8	2.3	0.19	a	2.8	0.84	a
0.25	8	2.1	0.11	a	1.5	0.36	a
2.5	8	2.9	0.29	b	3.6	1.38	a
25	8	3.7	0.32	c	3.9	1.66	a

Metabolic rate

The oxygen consumption of spiders was measured at 15, 20, 25 and 30 °C at doses of 0, 0.25 or 25 ng ai spider^{-1} (9 spiders per treatment). Respiration of individual spiders was measured using the Cartesian diver technique (Keklowski 1971, Janssen and Bergema 1991). During the measurements the divers are submersed in a 0.1 M NaOH solution in open contact with the chamber of the diver. CO_2 produced by the spiders is absorbed by the NaOH solution and the volume loss is a measure for O_2 consumption. Volume is adjusted to a constant value, allowing for buoyancy, by lowering the pressure on the system using a high precision manometer. The low molality of the solution caused the air humidity in the divers to be near to 100%. O_2 consumption was expressed as metabolic rate (MR, ul O_2 h^{-1} g^{-1}), ie the slope of the oxygen loss curve (measurements in a linear part of the curve between 90 and 300 min after introduction of a spider).

Immobilisation

Original data from an experiment by Everts (results published in Everts *et al.* (1991b)) were used. In this experiment, spiders were exposed to deltamethrin in 30 liter containers at 10, 20 and 30 °C at different RH (12, 33, 70, 85 and 100%). 20 spiders per treatment were treated topically with 2.5 ng ai $spider^{-1}$. Free drinking water was available to the spiders in 4 cm long plastic tubes of 4 mm diameter which were refilled daily. Spiders were classified as immobile when they were unable to walk after repeated gentle stimulation with a fine brush. This definition included the possibility that immobile spiders moved limbs, so long as this did not result in walking behaviour. The percentage of immobile spiders was scored. For the present study, only the observations at 2, 3, 4 and 5 hours after treatment were used. This was done to minimise the possible metabolism of the dose of deltamethrin applied. Dose-dependent relative immobilisation rates were calculated from the observed immobile fractions, assuming that spiders had a constant chance to become immobilised. This resulted in the equation:

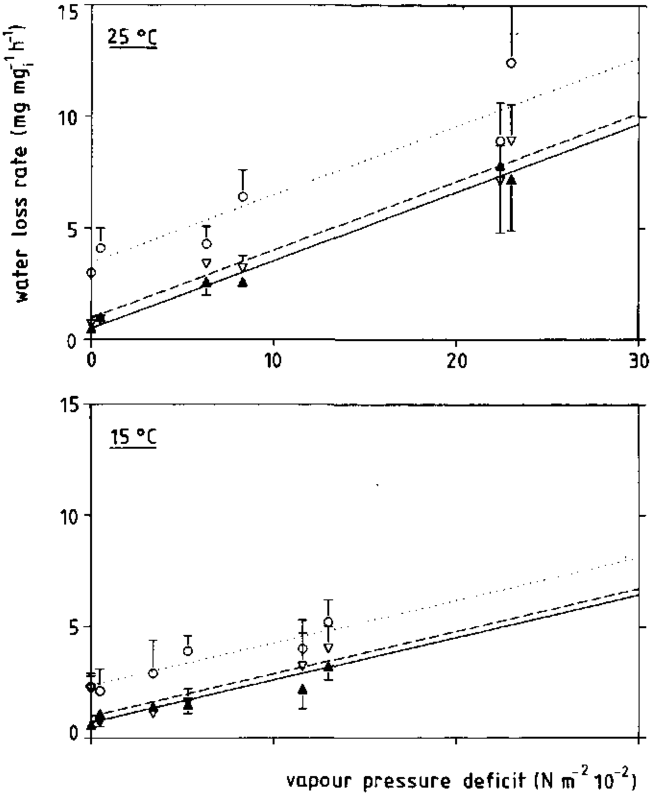
$$(8.4) \qquad N_t \quad = N_0 exp(-ct)$$

in which N_0 and N_t are the numbers of mobile spiders at t=0 and t=t respectively, and c is the relative immobilisation rate (h^{-1}).

Results

Fig 8.1 shows that for all doses, cumulative water loss increased linearly with time between 90 and 270 minutes after application. The water loss rate during this period was, therefore, taken as the standard measure of water loss in this and the following tests. Linear regression resulted for all treatments in a positive intercept ($P > 0.05$) which was not significantly different between treatments. The dose-effect relationship at 20% RH showed no effect on water loss rate (WLR) at the doses between 0.0025 and 0.25 ng ai $spider^{-1}$ and an increase in water loss at 25 ng (Table 8.2).

The observed relationships between water loss rate, temperature, dose and vapour pressure deficit are shown in Figure 8.2. Spider water loss showed a linear increase with vapour pressure deficit, the slope of which increased with temperature. In addition,



Figures 8.2 A and B: Regression curves of the water loss rate (with standard deviation) of *O. apicatus* in relation to vapour pressure deficit. Fig's 8.2a and b show the water loss of spiders given doses of 0 (- ▲ -), 0.25 (- ▽ -) and 25 (.○.) ng ai spider^{-1} at 15 °C and 25 °C, respectively.

Table 8.3: Water loss rate in relation to vapour pressure deficit, temperature and dose deltamethrin. For an explanation of symbols see equation (5). The result reflect the outcome according to equation (5) (mean square of residual: 0.00015)(ns = not significant at the 0.05 level).

parameter	estimate	standard error	significance
MWLR	0.0059	0.0026	
$Q1_{10}$	0.72	0.55	ns
C	0.0024	0.0002	
$Q2_{10}$	1.62	0.31	
$WER_{0.25}$	0.0036	0.0028	ns
WER_{25}	0.022	0.003	
$Q3_{10}$	1.76	0.47	

irrespective of the vapour pressure deficit, deltamethrin caused additional dose-dependent rates of water loss. For analysis of the results it was assumed that total water loss rate was a function of metabolic, passive and pesticide induced water loss. Passive water loss was related to cuticular conductivity and vapour pressure deficit. Active water excretion was modelled in dependence of dose. For simplicity reasons all measurements were taken relative to the initial weight and initial surface. The observations were analysed using a regression based on the following equation in which independent Q_{10}-values were allowed for each type of water loss:

$$(8.5) \quad TWLR = MWLR\ Q1_{10}^{((T-20)/10)} + C_p\ P\ Q2_{10}^{((T-20)/10)} + (I_{0.25}\ WER_{0.25} + I_{25}\ WER_{25})\ Q3_{10}^{((t-20)/10)}$$

In which: *TWLR* is the sum of evaporative, passive water loss and water excretion (g g_i^{-1} h^{-1}), *MWLR* is a low level of (metabolic) water loss contributing to all measurements, $Q1_{10}$ is the factor by which *MWLR* at 20 °C changes when the temperature changes 10 °C, C_p is the cuticular conductivity (g g_i^{-1} m_i^{-2} N^{-1} h^{-1}), *P* is the vapour pressure deficit, $Q2_{10}$ is as *Q1* but now for the increase of cuticular conductivity with temperature, *T* is temperature (°C), I_α is an impactvector which is 1 when the applied dose is α ng ai spider^{-1}, and 0 otherwise, WER_β is the active water excretion rate (g g_i^{-1} h^{-1}) for dose β, $Q3_{10}$ as *Q1* but now for the increase of active water excretion with temperature. The results are given in Table 8.3. All variables showed a significant contribution to the model except *Q1*, indicating a non-significant decrease of the basal metabolic water loss with temperature, and $WER_{0.25}$, indicating no significant effect on water excretion at a dose of 0.25 ng ai spider^{-1}.

The metabolic rate of spiders increased exponentially with temperature (Fig 8.3). The

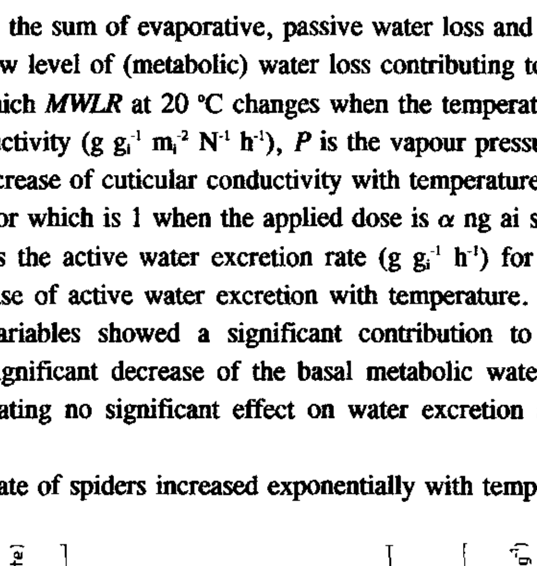


Figure 8.3: Linear regression of logarithmically transformed metabolic rate against temperature for different doses of topically applied deltamethrin:-□- = control, -△ - = 0.25 ng ai spider^{-1}, -○- = 25 ng ai spider^{-1} (bars represent standard errors).

Table 8.4. The effect of dose and temperature on the metabolic rate. Analysis of variance and regression calculations for the logarithmically transformed metabolic rate at dose (D) 0, 0.25 and 25 and temperatures (T) 15, 20, 25 and 30 °C (number of observations = 156, standard error in brackets). The Q_{10} value is calculated from the regression results.

Dose	Intercept	regr.coefficient	R^2	Q_{10}
0	5.27(±0.18)	0.051(±0.0001)	0.42	1.67
0.252	5.13(±0.12)	0.067(±0.005)	0.76	1.95
25.2	5.38(±0.16)	0.065(±0.007)	0.65	1.92

ANOVA

Source	DF	F VALUE	PR > F
D	2	25.03	0.0001
T	3	79.50	0.0001
D*T	6	2.15	0.0511

relationship between *MR* and temperature was analysed using linear regression and ANOVA (Table 8.4) on the logarithmically transformed data. Significant effects were found for dose, temperature and the interaction between these parameters ($T \cdot D$), the latter indicating a steeper slope of the temperature dependent increase in metabolism in deltamethrin treated animals.

Figure 8.4 shows the results of the immobilisation experiment. At high temperatures the immobilisation rates were low. But at low temperatures spiders were immobilised relatively rapidly. It should be noted that immobilisation showed a strong increase with decreasing air humidity.

Discussion

The linear relationship between water loss rate and vapour pressure deficit in *O. apicatus* in the present study accords with Fick's law for evaporation of water. This stresses the relative importance of the cuticular route for water loss, as is indicated also by results of Nemenz (1954), for different spider species, and Löw (1983), for the bird-spider *Eurypelma californicum*, showing that cuticular water loss determines 88 to 99% of the water loss in spiders.

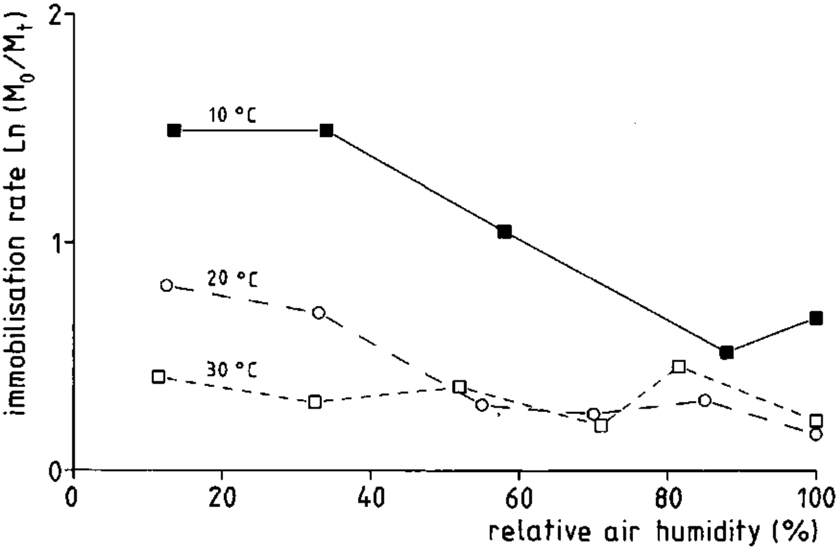


Figure 8.4: Immobilisation of spiders after application of 2.5 ng ai deltamethrin spider^{-1} in relation to relative air humidity. Experiments were conducted at the following temperatures: -■- = 10 °C, -○- = 20 °C, □ = 30 °C.

The positive relationship with temperature of the slope of the passive water loss rate with vapour pressure deficit (*Q2* of 1.62±0.31 between 15 and 25 °C) indicates an increase in cuticle conductivity. Exponential increase in cuticular permeability has been shown by Hadley and Quinlan (1989) who studied this phenomenon in specimens of the black widow spider *Latrodectus hesperus* which had their mouths, anuses, book lung slits, gonopores and spiracles sealed with beeswax (Q_{10} of living individuals between 30 and 40 °C was 2.79).

A low basal level of weight-loss was observed in control as well as in deltamethrin treated spiders which amounted to 0.7% h^{-1} at 15 and 0.5% h^{-1} at 25 °C, the temperature effect, however, was not significant. This weight-loss may be caused by basal metabolism, having been raised by stress due to the experimental conditions. Other effects which may have added to basal water loss are: an increase in water loss rate following anaesthesation with CO_2, or treatment-independent water loss in relation to exposing the spiders for a few minutes every hour to dry air during weighing. The latter also explains why evaporation of water could be measured in the 100% air humidity treatment. Elucidation of the nature of basal water loss requires more detailed observations.

In addition to evaporative water loss, water loss in *O. apicatus* was induced by deltamethrin treatment. Deltamethrin-induced water loss was not related to air humidity as was clearly shown by considerable water loss at 100% *RH*. This confirmed the involvement of active water excretion. The independence of pesticide induced water loss from air humidity was also shown by Hewlett and Gostick (1955) when measuring weight loss in flour beetles (*Triboleum castaneum*) treated with pyrethrum, at relative humidities between 40 and 90%.

Active, deltamethrin-induced, water excretion implies a relationship with metabolic activity. Therefore, one may expect a similar temperature dependence in these processess, metabolic rate generally showing an exponential increase with temperature (Wigglesworth 1965). Based on the observations at 15 and 25 °C at 25 ng ai spider^{-1}, a Q_{10} of 1.76 ± 0.47 was calculated for active water excretion (Table 8.3). A Q_{10} value of 1.92 was calculated for metabolic rate at the same dose over the range of 15 to 30 °C (Table 8.4). The difference between these Q_{10} values was not significant, which supports the assumption of the involvement of metabolic activity in active water excretion. The somewhat lower value of the presently observed Q_{10} values in comparison with generally observed Q_{10} values for biological processes of 2 to 3 requires further investigations.

Active water excretion due to pesticide poisoning may have important ecotoxicological consequences. Clearly, poisoned arthropods are unable to avoid excretion of water, even when they succeed in reaching humid locations, as may be found in cracks in the soil or other hiding places.

Immobilisation in *O. apicatus* showed a strong negative temperature-effect and humidity-effect relationship. A negative temperature-effect relationship is observed in many animal species, and is explained by better binding at low temperatures, of pyrethroids at sodium channels in neurons, and the subsequent high intensity of repetitive discharges (van de Bercken and Vijverberg 1986). The effect of temperature on immobilisation ('knockdown velocity') has been clearly shown *in vivo* for the cabbage looper, *Trichoplusia ni* (Hübner), for different pyrethroids, at different temperatures and doses (Toth and Sparks, 1990).

The marked negative humidity-effect relationship for immobilisation suggests the involvement of integumental humidity receptors in the immobilisation process. This may very well be explained by an amplification of the signals of humidity receptors by deltamethrin. In *Periplaneta* these receptors were shown to behave as hair hygrometers, producing few signals at 100% air humidity while signalling at a high frequency in dry air (Yokohari and Tateda, 1976). Rapid immobilisation at low air humidity may be induced by the inability of the CNS to coordinate arthropod mobility during intensive signalling of the humidity receptors. Immobilisation may occur rapidly due to the direct contact of the peripheral humidity receptors with deltamethrin, and need not necessarily be accompanied by deltamethrin effects on the CNS.

In *O. apicatus* signals from the humidity sensors are an important trigger for knockdown, however, different sources of sensory information may serve the same purpose in other arthropods.

The importance of peripheral information in the immobilisation process has been suggested by Page et al. (1949), for the musquito *Aedes aegypti*, and by Clements and May, working with locusts (1977), and is supported by observations by other authors. Scott and Matsumura (1983) observed a higher rate of knockdown in *Blatella germanica* when allethrin or cypermethrin was applied to the tarsi in comparison to the abdomen. This may be explained by the rich sensory innervation of the tarsi, found in most arthropod species, in comparison to little sensory innervation of the abdomen. Finally, Miller and Adams (1977) showed that knockdown was correlated with the ability of a compound to cause repetitive firing in sensory nerves.

It should be noted that a negative temperature-effect relationship was found for knockdown, whilst a positive temperature-effect relationship was found for water excretion. This may indicate differences in the mode of action of deltamethrin on sensory neurons and neurosecretory cells, or be related to increased activity of the diuretic hormone at high temperature. A negative temperature effect relationship for knockdown in combination with a positive temperature-effect relationship for water excretion offers an explanation for temperature dependent minima for pyrethroid effects. Such curves have been found by different outhors, when no discrimination was made between the effects immobilisation or death. Toth and Sparks (1990) observed minimum-effect curves after treatment of third instars of the cabbage looper (*Trichoplusia ni*) with cis-permethrin or esfenvalerate (minimum LD_{50} at 27 °C, and mortality defined as 'no movements within 30 seconds after prodding'). Everts *et al.* (1991b) observed a minimum effect curve after treatment of the terrestial money spider *O. apicatus* with deltamethrin (minimum effect at 20 °C, effect referring to immobilisation as well as death). More generally the combination of positive and negative temperature relationships may explain the small effect of temperature on the effectiveness of deltamethrin sprayed in the field.

The present study shows that an integrated approach is necessary to investigate effects of pyrethroids on arthropods. With respect to the effect of temperature on the relationships between pesticide, metabolism and water loss, there is much work still to be done. The independence of immobilisation and water excretion, and the influence this has on measured effects such as knockdown and mortality, should be accounted for in interpretations of existing observations of LD_{50} or ET_{50} values.

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CHAPTER 9

Mortality in females of the spider *O. apicatus* after treatment with deltamethrin caused by water loss; a simulation study

Summary

Natural and deltamethrin induced water loss was studied as a cause of mortality in the epigeal linyphiid spider *Oedothorax apicatus*. A quantitative deterministic model was constructed for the simulation of water loss in a laboratory population for 144 hours after topical application of deltamethrin. Weight loss of spiders was modelled as the sum of water loss by evaporation, which is a function of vapour pressure deficit and cuticular permeability, and of water excretion in reaction to deltamethrin poisoning, which is a function of temperature and dose. The model distinquished between mobile, immobile, recovered and dead spiders. Irreplaceable water loss occurred selectively in immobile spiders, other spiders being able to drink.

The model predicts the onset of mortality and the number of dead spiders satisfactorily at different temperatures and air humidities. This strongly indicates that water loss is the main cause of death for spiders poisoned by deltamethrin. The results of the model support the existence of two independent toxic effects of pyrethroid insecticides; the well known rapid immobilisation or knockdown effect, and pesticide induced water excretion.

Introduction

Pyrethroid pesticides may cause a range of neurotoxic effects which are considered the primary lesion. Death, however, is likely to result from secondary lesions, as arthropods recover from extended periods of anoxia in which nervous system function is blocked (Gerolt 1983).

Secondary effects of neurotoxicity may be reflected in behaviour and physiology. Disruption of behaviour may increase the chance of predation, hinder selection of favourable living conditions and reduce uptake of food or water. Physiological regulation of a range of processes, including water balance, is disturbed by pyrethroids via effects on neurosecretion (Casida and Maddrell 1971, Orchard and Osborne 1979). It has been suggested that the combination of pesticide induced immobilisation and water loss renders the animal vulnerable to lethal water loss (Casida and Maddrell 1971, Everts *et al.* 1991a). This may be of great ecotoxicological importance in the field. No quantitative information is, however, available in literature to test this hypothesis. Therefore the possible contribution of water loss to mortality of arthropods in the field was investigated in the present study.

Water loss in arthropods includes passive water loss and pesticide induced water excretion. Passive water loss depends on temperature dependent permeability of the cuticle and the vapour pressure deficit of the air (Edney 1977, Appel *et al.* 1986, Hadley and Quinlan 1989). Water excretion depends on pesticide dose and temperature (Hewlett and Gostick 1955, Jagers op Akkerhuis *et al.* submitted) and is controlled by a diuretic hormone (Casida and Maddrell 1971, Greenwood *et al.* 1985).

The poisoning of arthropods by insecticides involves a sequence of events including the uptake of the pesticide from the substrate, penetration and distribution of the pesticide in the body tissues, target specific sensitivity, and metabolism and excretion of the compound. Quantitative models have been proposed or developed to integrate these factors in order to explain mortality after treatment for different pesticides and arthropods (Hollingworth 1971, Burt 1972, McFarlane *at al.* 1977, Welling 1977, Ford *et al.* 1981a, Salt and Ford 1984, Greenwood *et al.* 1990). So far, however, none of the models has incorporated the possibility of water loss as a cause of mortality.

The aim of the present study was to investigate the significance of water loss for mortality in arthropods treated with deltamethrin. For this purpose a deterministic simulation model was developed which predicts mortality in a laboratory population of spiders on basis of measurements from a series of experiments concerning dose dependent effects of deltamethrin on hygrothermal physiology of spiders and spider behaviour. In the model, the different behavioural responses after treatment have been considered as various stages of the same neurotoxicological process.

Their high abundance in the field situation and high sensitivity to deltamethrin having been demonstrated by Everts *et al.* (1989), females of the linyphiid spider *O. apicatus* were used in the present study as a model species. The synthetic pyrethroid deltamethrin, in the commercially available emulsifiable concentrate formulation Decis^R, was used as model compound.

Experiments

The simulation model required quantitative information about water loss in relation to physical factors, and about the behavioural response of spiders in relation to dose, temperature and air humidity. This was obtained from the following three laboratory studies.

Experiment 1: Natural and pesticide induced water loss

After treatment with deltamethrin, spiders were exposed at 15 and 25 °C to air of different vapour pressure deficits. The water loss of the spiders was measured gravimetrically for several hours after treatment. Oxygen consumption of the spiders was measured at different temperatures and doses using a Cartesian diver technique (Janssen and Bergema 1991). A detailed description of the experiment and results is given in Jagers op Akkerhuis *et al.* (submitted).

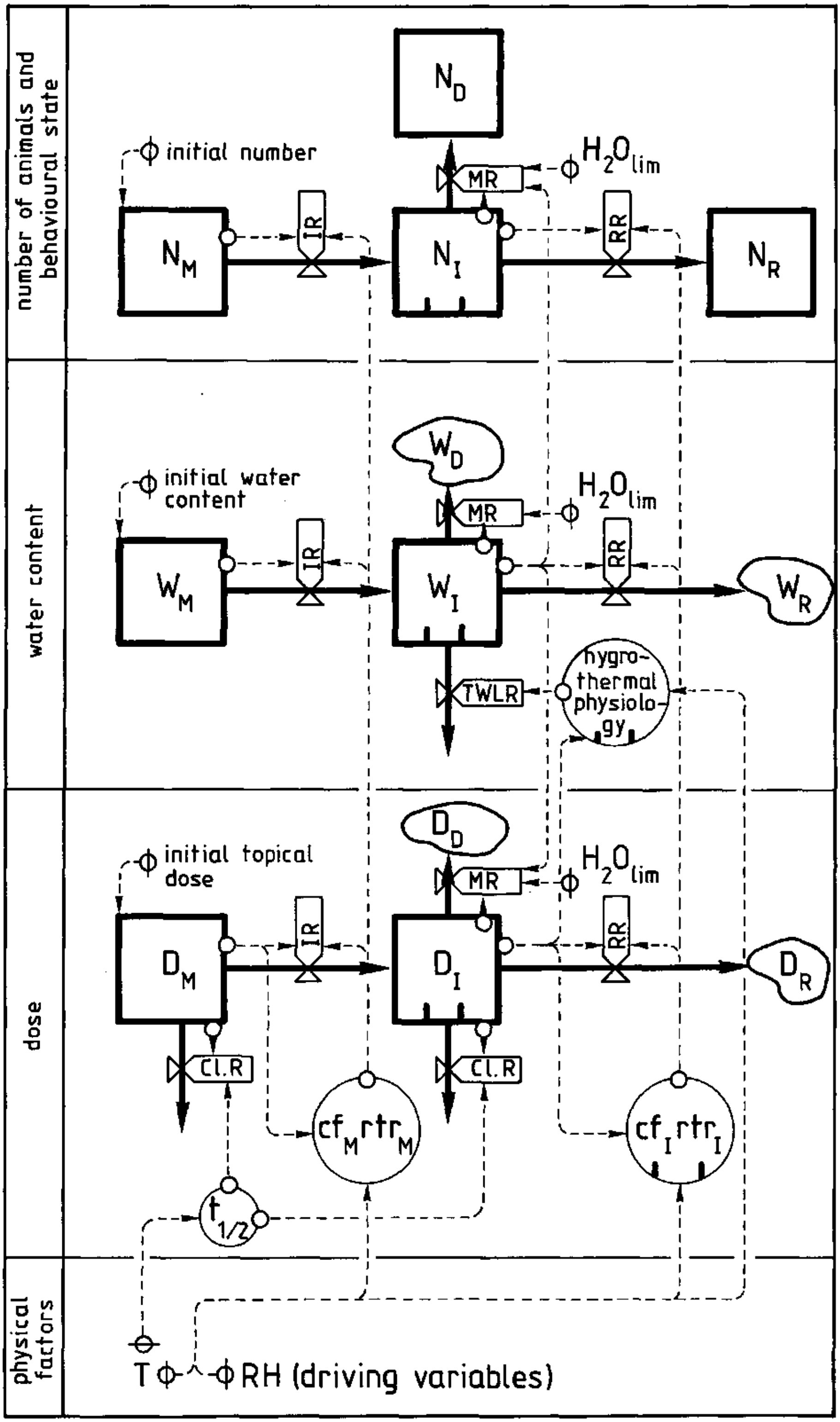
Experiment 2: Spider behaviour in relation to dose

Female *O. apicatus* originating from fields in the Flevopolder (mid-Netherlands) were kept in the laboratory in plastic cups (6.5 cm high, 9 cm diameter) at 15 °C for two months. They were provided *ad libitum* with Drosophila (vestigial type) and collembola. Spiders were acclimatized at 20 °C for three days before the onset of the experiment. During these three days and the 7 days of the experiment, spiders were not fed. The spiders were treated with deltamethrin on the dorsal abdomen during a short period of CO_2 anaesthesia, using a 10 ul Exmire microsyringe mounted in a Burkhard Electric Mycrosyringe Applicator^R. The following doses of the emulsifiable concentrate Decis^R in tap water were used: 0.29, 0.63, 1.3, 2.9, 6.3, 13, 29, 63, 130 ng ai spider^{-1} in a 50 nl droplet. 20 Spiders were used for each treatment. After application, individual spiders were kept in 1 cm high, 5 cm diameter petri dishes with a bottom of moist plaster of Paris. The behaviour of the spiders according to the classification of mobile, immobile or dead, was recorded, the person recording did not know which treatment the spider had received. Observations were made 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72 and 96 hours after application.

Experiment 3: Spider behaviour after deltamethrin treatment, relation with temperature and air humidity

Spiders were reared as F1 generation from female *O. apicatus* trapped in the field. The animals were kept separately in a 5 cm diameter petridish with a substrate of moist plaster of Paris. The first four instars were provided *ad libitum* with a mixture of different *Collembola* species trapped from a lawn. From the fourth instar onwards, *Drosophila* were also fed. Spiders were acclimatized and starved for two days at 20 °C before the start of the experiment. Application of deltamethrin confirmed the description given above, but at 2.5 ng ai spider^{-1}. Experiments were conducted in 12 dm^3 aquaria in climatic chambers at temperatures of 10, 20 and 30 °C. The following air humidity treatments were created using saturated salt solutions according to Winston and Bates (1960): 100, 85, 70, 55, 33 and 12.5% relative air humidity (RH). A control treatment consisting of untreated spiders at 12.5% RH was included at all temperatures. Spiders were kept in 6 cm high, 9 cm diameter plastic cups with a bottom and lid of fine wire gauze to allow for free exchange of air. Drinking water was available to the spiders from the ends of 4 cm plastic tubes of 4 mm diameter. Behavioural response was recorded as above, at 2, 3, 4, 5, 6, 8, 12, 24, 48, 96 and 144 hours after application (methods and results partially published in Everts *et al.*, 1991a).

(>) **Figure 9.1. Mortality of deltamethrin treated spiders (female *O. apicatus*) in relation to temperature and air humidity; Forrester diagram of modelled relationships.** The model uses information about behaviour and water loss of spiders in relation to dose, to predict mortality. State variables are represented by rectangles, rates by valve symbols, axiliary variables by circles, sinks by irregularly shaped rounds. Solid lines represent flow of matter, broken lines represent flow of information. Age cohorts in variables are indicated by two bars at the bottom of the respective symbols. Capitals N, W and D represent the total number of spiders, the total water content and the total dose of N spiders, respectively. Subscripts M, I, R and D indicate the behavioural states mobile, immobile, recovered and dead. IR, RR and MR represent the immobilisation rate, recovery rate and mortality rate, for the respective processes. Temperature and air humidity dependent relative rates of transition from the categories mobile to immobile (rtr_M) and immobile to recovered (rtr_I) are corrected for dose using a correction factor (cf_M and cf_I). Total water loss rate in immobile spiders ($TWLR_I$) is calculated as the sum of transpiration as a function of the vapour pressure deficit and cuticular water conductivity, and pesticide induced water excretion as a function of temperature and dose. Mortality occurs when the water content of immobile spiders (W_I) surpassess the water loss that is assumed lethal (H_2O_{lim}).


number of animals and behavioural state
initial number
N_M
IR
N_I
N_D
MR
H2O lim
RR
N_R
water content
initial water content
W_M
IR
W_I
W_D
MR
H2O lim
RR
W_R
TWLR
hygro-thermal physiology
dose
initial topical dose
D_M
IR
D_I
D_D
MR
H2O lim
RR
D_R
Cl.R
Cl.R
cf_M rtr_M
cf_I rtr_I
t_1/2
physical factors
T
RH (driving variables)

The model

Forrester diagram

The forrester diagram of the model is given in Fig 9.1. In this model, mortality in female *O. apicatus* is explained by the underlaying processes of the effects of deltamethrin on behaviour and water loss rate. For 144 hours after deltamethrin application at a treatment dose of 2.5 ng ai spider^{-1}, the model simulates water loss for spiders at 10, 20 and 30 °C, at each of the following air humidities: 100, 85, 70, 55, 33, 12.5 %RH. Mortality occurs when the water content of immobile spiders drops below a lethal threshold (H_2O_{lim}). The model combined information about water loss and behaviour from experiments 1-3, to predict mortality as observed in experiment 3.

The relationships on proces-level given in equation (9.1) to (9.5) were analysed using regression techniques. Appendix I presents the explicit form in which these relationships were used in the simulation model.

Passive water loss and pesticide induced water excretion

Total water loss at 15 and 25 °C and 0, 0.25 or 25 ng ai deltamethrin spider^{-1} was described as the sum of water loss due to passive water loss and pesticide induced water loss based on results presented in Jagers op Akkerhuis *et al.* (submitted). A dose-effect relationship was estimated for the pesticide induced water loss rate, which had to be based on the two doses used. Following results of Hewlett and Gostick (1955) a logit(pesticide induced water loss rate)-ln(dose) relationship was assumed. Maximum effect on water loss (100% effect) in the present study was assumed to occur at the same dose as maximum transition, 130 ng ai spider^{-1}, doses of 25 and 0.25 ng spider^{-1} respectively causing 80% and 12% effect. Assuming no interaction between active water excretion and evaporative water loss, this resulted in the following equation for the total water loss rate:

$$
\begin{aligned}
(9.1)\quad TWLR &= C_{ref}\, P\, Q1_{10}^{((T-20)/10)} \\
&\quad + Q2_{10}^{((t-20)/10)}\, WER_{max}\, [1+exp(-(C_1+C_2 ln(D)))]^{-1}
\end{aligned}
$$

In which:

TWLR is the total water loss rate (as percentage of initial weight), i.e. the sum of evaporative water loss and water excretion (g g_i^{-1} 100 h^{-1}, with g_i = initial weight), C_{ref} the cuticular conductivity at 20 °C (g g_i^{-1} N^{-1} m_i^2 h^{-1}), *P* the vapour pressure deficit (N m^{-2}), $Q1_{10}$ the factor by which C_{ref} changes with a 10 °C change in temperature, *T* temperature (°C), $Q2_{10}$ as Q1 but now for the increase of WER_{max} with temperature, WER_{max} the maximum observed water excretion rate (g g_i^{-1} h^{-1})in relation to a high dose (130 ng ai spider^{-1}), *C1* a constant indicating the *WER* at a doses of 1 ng ai spider^{-1}, *C2* a constant indicating the increase of logit(*WER*) with ln(*D*), *D* the applied dose of deltamethrin (ng ai spider^{-1}). (For reasons of symplicity, the surface of spiders was taken as a constant and eliminated from the equations by division). The values of the variables in equation (9.1) were determined in a previous study (Jagers op Akkerhuis *et al.*, submitted) and are given in Appendix I.

Immobile spiders could not drink the free water offered in the experiments. Age-cohorts of immobile spiders were distinquished, each cohort consisting of spiders which had become immobile during the short period of time equal to the time step of integration. Mortality was assumed to occur when the water loss in the spider had reached a lethal threshold value, expressed as percentage of the average bodyweight of unexposed spiders. Pulz (1987) reviewed lethal water loss percentages for 9 different spider species. The values ranged from 16 to 31% of the average initial bodyweight. The average of 23% is used in the model.

Behavioural state

To quantify the relationship between behavioural response and the stage of the poisoning process after treatment, spiders were classified in behavioural states. The following states were defined: **Mobile**; walking behaviour varying from not affected to uncoordinated. **Immobile**; unable to stand upright after repeated stimulation. **Recovered**; Mobile animals which had previously been classified as immobile. **Dead**; repeated stimulation induces no movement of any of the extremities (checked by using a binocular microscope); no improvement in behaviour during following observations.

Relative transition rates

For several hours after the application of deltamethrin, a, dose dependent, constant fraction of the spiders, per time, made the transition from mobile to immobile and from immobile to recovered. Accordingly, the logarithmically transformed fraction of spiders in the original state showed a linear reduction with time, in which the slope of the relationship, signifying the 'relative transition rate', depends on dose. In Fig. 9.2 these relationships are presented as a linear increase in the logarithm of the number of spiders in the original state devided by that in the original state on t=t. As the observations originated from the first hours after treatment, metabolic or other inactivation of the pesticide was assumed to be limited. The present study did not include the modelling of relative transition rates in relation to penetration of the pesticide through the cuticle (Ford *et al.* 1981b, Greenwood *et al.* 1990). The transition of spiders between behavioural classes was modelled using relative transition rates (C_r):

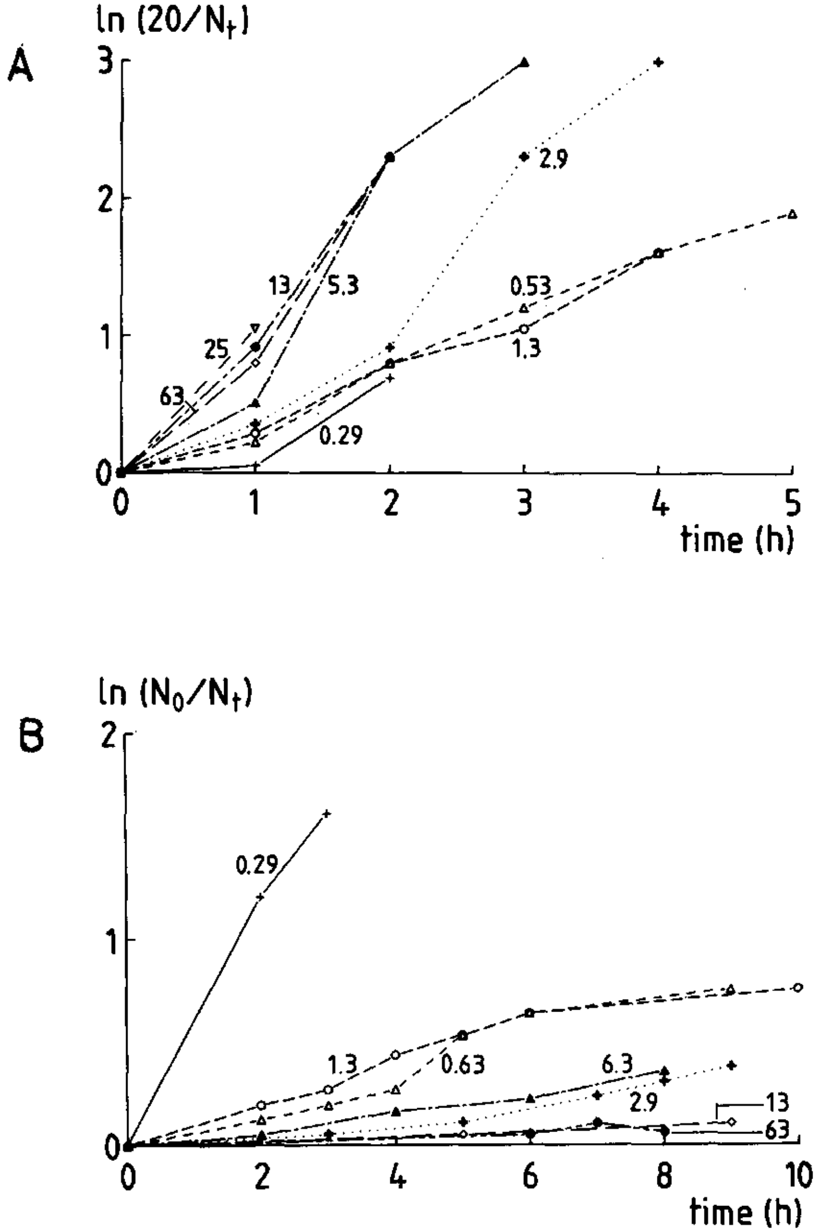


Figure 9.2. **Immobilisation (2a) and recovery (2b) of spiders at different topically applied doses deltamethrin (20 °C, 100%).** The Fig's give a linear representation of the observed exponential decrease of the number of mobile (9.2a) and immobile spiders (9.2b). Doses used (ng ai spider^{-1}) are indicated in the Figure.

$$(9.2)\ N_t \quad = N_0 exp(C_r\, t)$$

in which N_t=the number of spiders in a certain behavioural state at t=t (h), N_0 the number of spiders in that behavioural state at t=0 and C_r the relative transition rate (h^{-1}).

Although not explaining underlaying processess, this model has the advantage of giving a simple, satisfactory description of the observed phenomena.

Relative transition rates: effects of dose

Based on the results shown in Figure 9.2, the relationship between dose and the relative rate of immobilisation or recovery was investigated. The expression of a dose-effect relationship as logit(effect) against log(applied dose), which is commonly used for the description of toxic effects on (sub-) populations (Berkson 1944, 1951) was used for the relative transition rates as follows:

$$(9.3)\ ln[E/(E_{max}-E)] \quad = E_0 + C_D ln(D)$$

in which:

E is the observed effect, i.e. relative rate of immobilisation or recovery (h^{-1}),

E_{max} the maximum possible effect at high dose, E_0 the intercept at 1 ng ai spider^{-1} i.e. at $ln(D)=0$, C_D the increase of logit(effect) with ln(dose), and D the topically applied dose (ng ai spider^{-1}).

Fig 9.3 shows that the relative immobilisation rate was positively, and the relative recovery rate negatively related to dose. Regression coefficients of the relationships are given in Table 9.1. Calculation of the logit(effect) required the inclusion of a maximum possible effect E_{max}. Extrapolating from the data the respective maxima were estimated somewhat above the effects observed at the highest dose used (130 ng spider^{-1}). Recovery at 0.29 ng ai spider^{-1} was left out of the regression in Fig 9.3, as recovery occurred before the first moment of observation.

Table 9.1: Relative transition rates in relation to applied dose. N = number of observations, E_{max} = estimated maximum effect (i.e. relative transition rate), E_0 and C_r see equation (9.2), R^2 = correlation coefficient (regression coefficients with standard errors).

	N	E_{max}	C_0	C_r	R^2
Relative immobilisation rate (h^{-1}):	9	1.3	-0.50(+0.43)	0.62(±0.07)	0.91
Relative recovery rate (h^{-1}):	8	0.6	-1.95(±0.21	-0.47(±0.04)	0.95

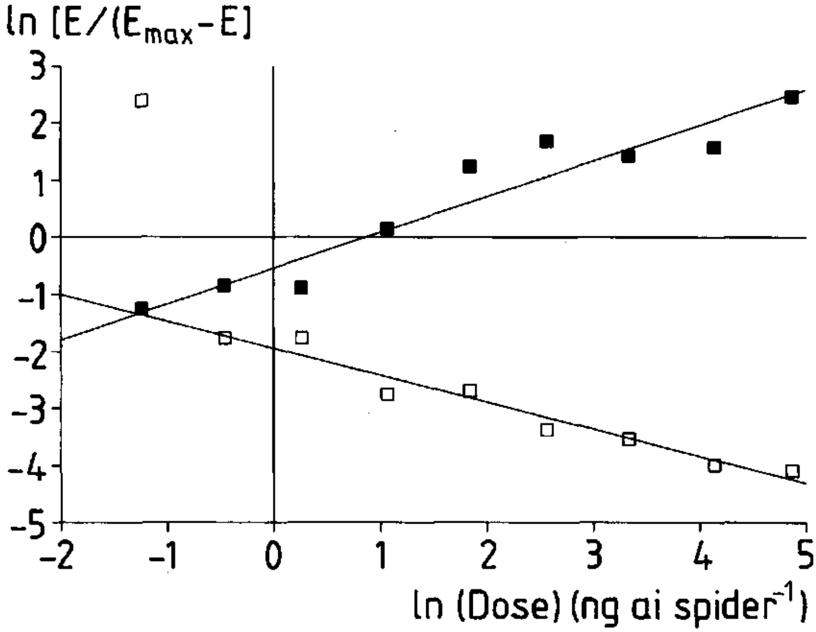


Figure 9.3. Dose-effect relationship for relative immobilisation rates (solid squares) and dito recovery rates (open squares) of topically treated spiders (20 °C, 100% RH). Fitted curves according to equation (9.3).

Table 9.2: Statistics of the relationship between relative immobilisation rate (h^{-1}), temperature (°C) and relative air humidity (%) (number of observations 17, mean square of residual 0.02)(all factors significant at the 95% level). $RTRMI_{ref}$ = reference relative transition rate (at 20 °C, 100% RH and 2.5 ng ai spider^{-1}, C_{RH} = rate of decrease of RTRMI with relative humidity, Q_{10} = change of RTRMI with temperature, relative to 20°C.

Parameter	Estimate	Std. Error
$RTRMI_{ref}$	0.25	0.05
C_{RH}	0.006	0.001
Q_{10}	0.49	0.04

Relative transition rates: effects of temperature and air humidity

Results of experiment 2, in which the effect of physical factors were studied on the rates of immobilisation and recovery in spiders treated with 2,5 ng ai spider^{-1}, showed that the immobilisation rate was negatively related to air humidity and temperature (Fig 9.4a).

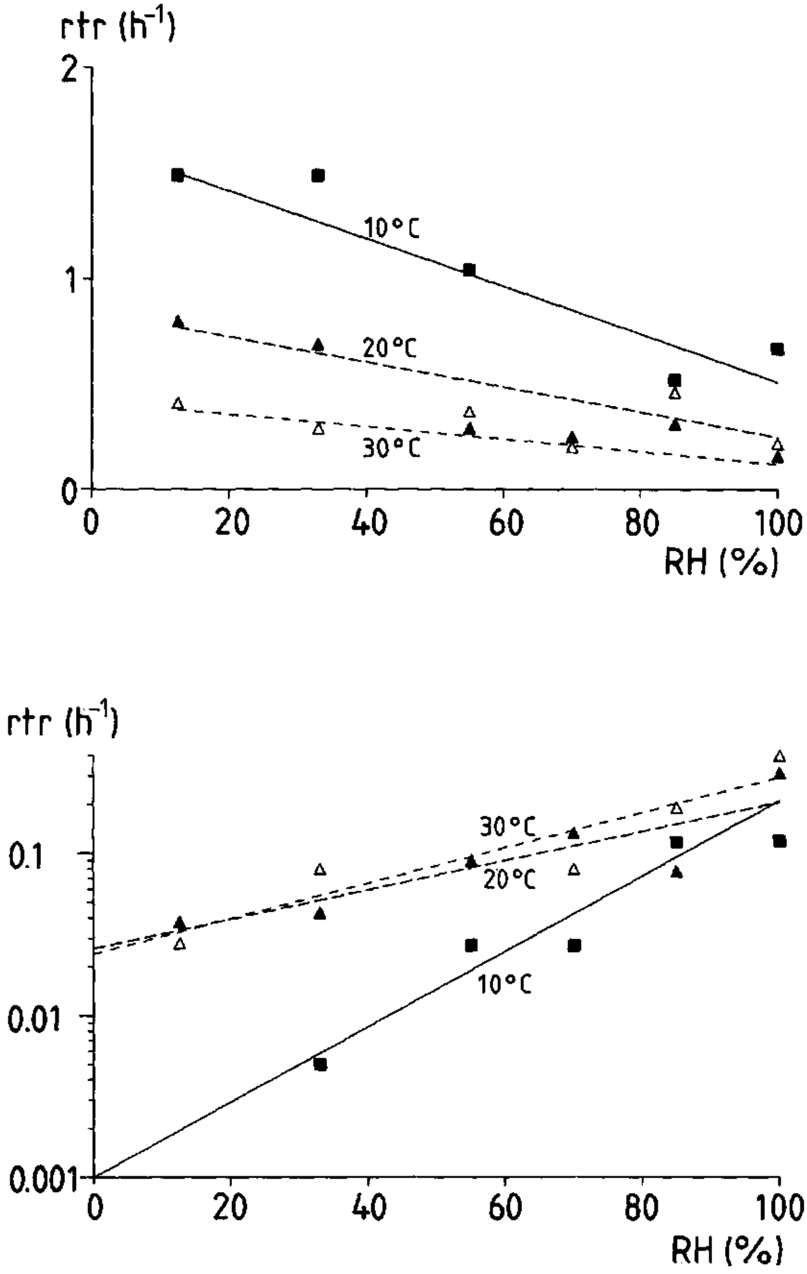


Figure 9.4. Effects of relative air humidity and temperature on relative immobilisation rate (4a) and relative recovery rate (4b). Fitted curves according to equation (9.4). Temperatures shown in the Figure.

Table 9.3: Relationship between relative recovery rate and relative air humidity at different temperatures. N = number of observations, C_0 = intercept, C_1 = slope of ln(relative recovery rate) with relative air humidity, R^2 = correlation coefficient (regression coefficients with standard error).

Temp.	N	C_0	C_1	R^2
10 °C	5	-6.96(+0.34)	0.054(±0.007)	0.96
20 °C	6	-3.66(±0.41)	0.021(±0.006)	0.78
30 °C	5	-3.77(±0.44)	0.025(±0.006)	0.86

Rapid immobilisation was related to dry air. A linear relationship was found to explain variation in the data satisfactorily. A regression was performed, the results of which are given in Table 9.2, based on the following equation:

$$RTRMI = [RTRMI_{ref} + C_{RH}\,(100\text{-}RH)]\;Q_{10}^{((20-T)/10)} \tag{9.4}$$

in which *RTRMI* is the relative transition rate from mobile to immobile at 2.5 ng ai spider^{-1} (h^{-1}), $RTRMI_{ref}$ reference transition rate at 2,5 ng ai spider^{-1}, 100% RH and 20 °C, C_{RH} change of *RTRMI* with air humidity, *RH* the relative air humidity (%), Q_{10} the factor by which the relationship between *RTRMI* and relative humidity changes given a 10 °C change in temperature relative to 20 °C. A Q_{10} value of 0.49 for the relative immobilisation rate (Table 9.2) indicates a temperature dependent reduction of the process with a factor 2.04, which is in the same range as increases of biological reaction rates with temperature, which generally are in the range of 2 to 3.

Fig 9.4b shows that at 10, 20 and 30 °C a linear, positive relationship with air humidity was found for the logarithmically transformed recovery rate. No simple relationship with temperature was found for the slopes of these lines, relative recovery rates being markedly lower at 10 °C, compared to 20 °C and 30 °C (results of regression calculations in Table 9.3). The relationship between recovery and temperature was therefore modelled by linear interpolation between the separate relationships at the three temperatures.

Relative transition rates: combining effects of dose, RH and temperature

The combined effects of dose, temperature and air humidity on transition rates were modelled using the relative transition rates from experiment 2 as a basis with the value at 20 °C and 100% RH and 2.5 ng ai spider^{-1} as reference value. No interaction was assumed between the effect of dose on the one hand and the effects of temperature and air humidity on the other. Relative transition rates at the prevailing temperature and relative air humidity were calculated on basis of the reference dose (2.5 ng ai spider^{-1}) using results of

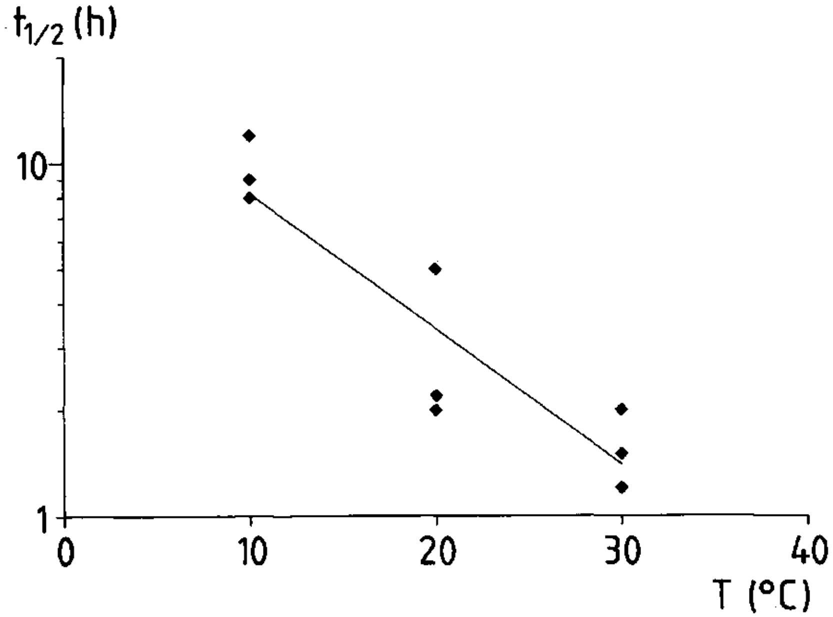


Figure 9.5. Estimated pesticide halflife as a function of temperature. Points represent the halflife values estimated in separate runs of the model, based on prediction of the numbers of immobile spiders. Fitted curve according to equation (5).

experiment 2, and multiplied by a correction factor derived from experiment 1 to account for a different actual dose.

Relative immobilisation rates at 2.5 ng ai spider^{-1} differed considerably between experiments 1 and 2, spiders being approximatily ten times more sensitive to deltamethrin in experiment 1. The difference is attributed to differences in origine of the spiders. The spiders in experiment 1 were collected in the field while in experiment 2 a laboratory reared F1 generation was used. In the simulation model the effect of dose, relative to the effect at 2.5 ng ai spider^{-1}, is used to correct the calculated relative transition rates.

Metabolisation of deltamethrin in relation to temperature

Assuming negative exponential time dependent inactivation of deltamethrin in the spiders, the model included a halflife of pesticide dose applied. The numbers of immobile spiders in experiment 3 were used to calibrate the halflife time of deltamethrin in the model in relation to temperature. The fit of each curve was judged by eye. Due to complete immobilisation or rapid mortality in other air humidities, only the observations in the range of 100 to 70% RH were used. The results of the individual fits are given in Figure 9.5. The fitted curve of the logarithmically transformed half-lifes against temperature was of the following form:

$$t_{1/2} = C/Q_{10}^{((T-20)/10)} \quad (R^2 = 0.83) \tag{9.5}$$

in which $t_{1/2}$ is the estimated halflife of effective pesticide dose (h), C the pesticide halflife at 20 °C, Q_{10} the change induced by a 10 °C change in temperature in the inactivation rate of deltamethrin, T temperature (°C). Values in equation (9.5) were estimated as: $C = 3.38$ and $Q_{10} = 2.44$.

Calculations

Calculations were carried out using a program written in Fortran 77 and executed on a VAX machine or a PC. The results presented have been obtained by the Euler integration method, the time step always smaller than one tenth of the smallest time constant.

Results and discussion

Model predictions of immobilisation and mortality are shown in Fig 9.6 for a dose of 2.5 ng ai deltamethrin spider^{-1} at 100, 77, 55, and 12.5% relative air humidity and 10, 20 and 30 °C. Figure 9.6 also shows observations from a laboratory experiment including control mortality at 12.5% RH. The graphs at 85 and 33 % RH are not shown due to similarity with those at 100 and 12.5 % RH respectively.

The model gives a good prediction of spider mortality at the different temperature and air humidity conditions. At low temperature, late mortality is predicted at low air humidities. At high temperature, the model predicts rapid mortality, the number of dead spiders increasing with decreasing air humidity. Mortality is predicted less accurately at 20 °C than at 10 and 30 °C.

The predicted mortality deviates in certain aspects from the observed numbers of dead spiders. In the experiment spiders died in excess of the control level, after all spiders had recovered from immobilisation. The model typically predicts mortality in relation to lethal water loss in immobile spiders and offers, therefore, no explanation for mortality after recovery. Late mortality may be caused by an overestimation of the capacity to drink free water by recovered spiders, or, may indicate long term damage of deltamethrin to targets not considered in the present study.

The model normally predicts rather abrupt mortality, whilst lethal water loss in the experiments shows a more gradual effect. This is considered to be related to the absence in the simulation of variance between individuals for parameters such as: body weight, intrinsic sensitivity and dose applied.

Simulated mortality at 20 °C consequently starts as much as 24 hours earlier than observed. This may be caused by the overestimation of the air humidity dependent relative immobilisation rate at this temperature by regression according to equation (9.4), in combination with overestimation of the pesticide halflife at 20 °C (Fig 9.5). It seems that some of the relationships with temperature, which were assumed to show linear or log-linear relationships, may show a more complex form. This requires further investigations.

In the present model, the halflife of inactivation of deltamethrin within the body was fitted on basis of observations of immobile spiders. The best fits in the present model were obtained using halflife values ranging from 1.4 to 8.2 hours at temperatures of 30 to 10 °C. Ford *et al.* (1981b) have calculated elimination constants (k_e) for methylbenzyl chrysanthemates topically applied to mustard beetles (*Phaedon cochleariae* Fab.) ranging from 0.001 to 0.1 h^{-1}, which implies pesticide halflifes of 6.9 hours or more (at 20 °C). It seems therefore that the metabolism of deltamethrin in *O. apicatus* is relatively rapid.

The present results indicate that water loss is the most important cause of death in arthropods treated with pyrethroids. In addition, it is shown that temperature and humidity are of major importance with respect to a proper prediction of both immobilisation and mortality. The major effect of physical factors on the effect, indicates that caution should be taken in interpreting published LD_{50} or EC_{50} values for pyrethroids, as these may refer to immobilisation as well as to mortality and may vary considerably depending on the duration of, and physical conditions during an experiment.

The existence of different toxic actions of pyrethroids, i.e. effect on knockdown or water excretion, is supported by literature. Ford *et al.* (1981b) found little correlation between knockdown and mortality in mustard beetles (Phaedron cochleariae Fab.) poisoned with different pyrethroids. Scott and Matsumura (1983) observed the effects of the pyrethroids allethrin, pyrethrin, permethrin, fenvalerate, deltamethrin and cypermethrin on the cockroach *Periplaneta americana* and a susceptible and resistant strain of the German cockroach *Blattella germanica*. Based upon data on resistance, electrophysiological data and observations of the effect of temperature on toxicity, they concluded that there are two distinct types of pyrethroid actions. 'Type I actions were represented by symptoms which exhibit a distinct negative temperature correlation for both knockdown and mortality factors, while Type II action shows no temperature correlation for knockdown and a positive temperature correlation for mortality'. They also found that 'Pyrethroids with predominantly Type I action had greater knockdown activity, while pyrethroids with mainly Type II action were toxic.'Deltamethrin was classified in their work as showing predominantly Type II action. The results of Scott and Matsumura (1983) support the negative temperature relationship for knockdown, and, assuming water excretion to be the main reason for mortality, the positive temperature relationship for water excretion shown in the present study. In spiders deltamethrin also exhibits 'Type I' action as it causes rapid immobilisation.

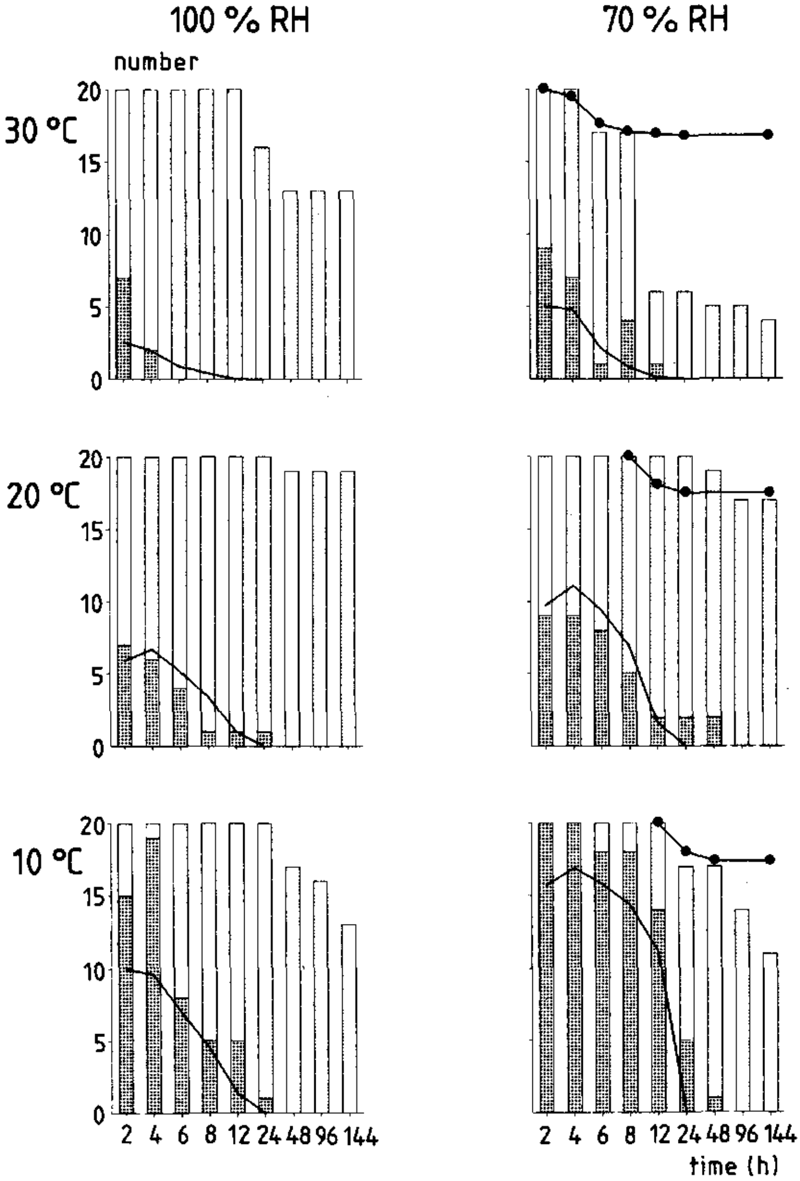
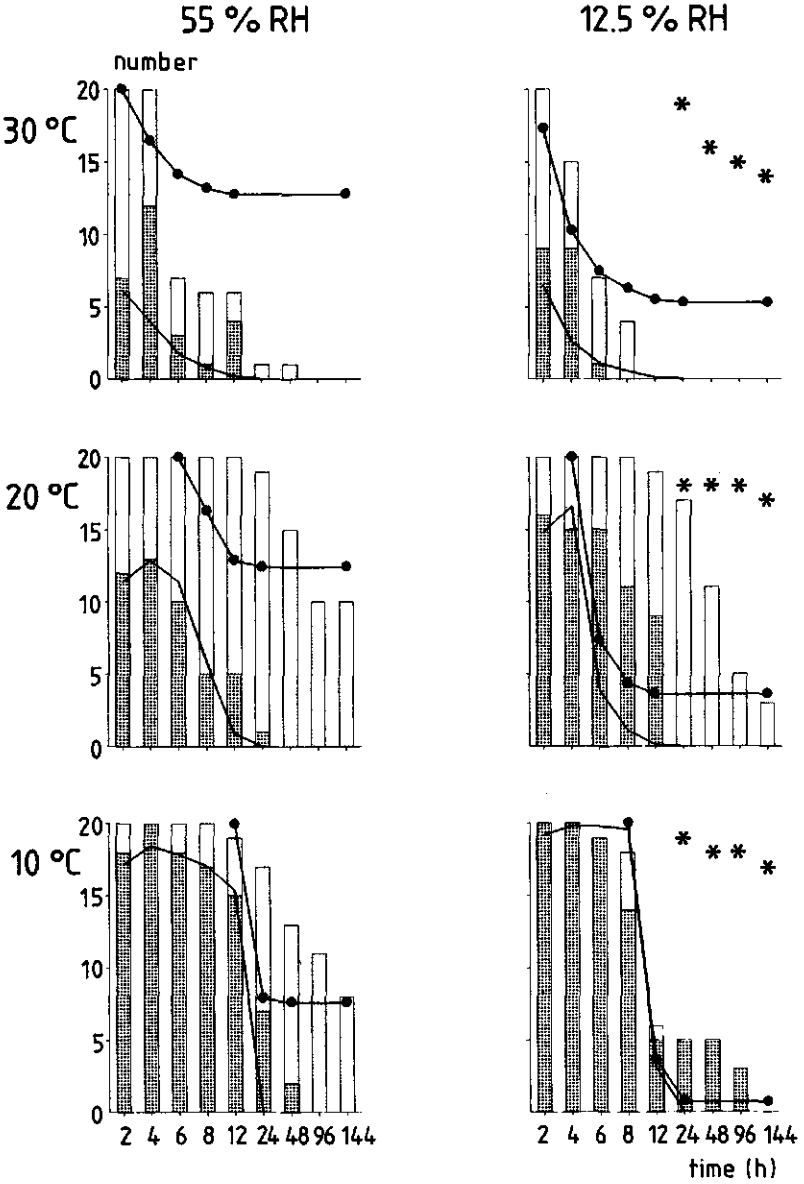


Figure 9.6. Simulation of mortality in the spider *O. apicatus* in relation to behaviour and water loss as a function of temperature and air humidity. Mortality as observed in experiment 3, and simulated numbers of mobile, immobile and dead spiders for a dose of 2.5 ng ai spider^{-1} deltamethrin. Observed numbers are represented as bars; dark shading = immobile spiders, light shading = mobile spiders. Bar lenght indicates the



number of surviving spiders. Lines with solid round markers = surviving spiders after substraction of the simulated number of dead spiders, lines without markers = simulated number of immobile spiders. Controls, receiving the 'blank' formulation (S276BF) of 'Decis-flow' in the same dilution as the other treatments, were exposed to 12,5% RH with free drinking water available. Control survival is indicated with '*'.

The positive temperature relationship of mortality caused by water excretion after treatment with 'Type II' pyrethroids has important ecological consequences. It should be noted that the present study showed that pesticide induced water excretion in arthropods is independent of the vapour pressure deficit of the air, as was also demonstrated by Hewlett and Gostick (1955) for pyrethrum treated flour beetles. The animal can only reduce pesticide induced water excretion by hiding in a cold place, which it cannot search for, if it is immobilised by the simultaneous occurrance of 'Type I' action, i.e. immobilisation. Yet, the relationship between pesticide action, water excretion and temperature has received very little attention in the literature.

The internal concentration of deltamethrin, as a function of time, affects both the duration of knockdown and the intensity of the water loss rate. This indicates the great need for reliable, quantitative, methods, enabling the assessment of the, effective, internal pesticide dose.

The present results, in combination with the literature, suggest the following conceptual model for the effect of deltamethrin on linyphiid spiders. Spider behaviour is disrupted shortly after treatment with deltamethrin. Deltamethrin causes abnormal signalling intensity of humidity sensors in the cuticle, especially at low relative air humidity. The distortion of peripheral information about humidity affects the coordination of movement, resulting in immobilisation and an inability to drink. These topics deserve further investigation as little or nothing is known about the role of humidity receptors in the relationship between knockdown and air humidity, or about the nature of the positive relationship between temperature and water excretion. After penetration into the haemolymph, a dose-dependent stimulation of neurosecretion by deltamethrin occurs, including the production of a diuretic hormone which causes active water excretion. Additionally, water is lost due to basal metabolism and evaporation, the latter depending on the vapour pressure deficit of the air and the permeability of the cuticle. The above hypothesis may serve further development of insight into pyrethroid action.

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Appendix I: Equations used in the water-loss model. Symbols are explained in Appendix 2.

Changes in numbers of spiders in behavioural states:
(6) Mobile : $dN_M/dt = -rtr_M\ cf_M\ N_M$
(7) Immobile : $dN_{I,t}/dt = rtr_{M,t}\ cf_M\ N_{M,t}$ $(t=0)$
: $dN_{I,t}/dt = -rtr_{I,t}\ cf_I\ N_{I,t}$ $(t>0)$
(8) Recovered : $dN_R/dt = \int_0^t dN_{I,t}/dt$ $(t>0)$
(9) Dead : $N_D = \int_0^t N_{des,I,t}$

Dose of spiders i.r.t. behavioural state:
(10) Mobile spiders : $dD_M/dt = (-rtr_M\ cf_M - \beta_M)D_M$
(11) Immobile spiders : $dD_{I,t}/dt = rtr_M\ cf_M\ D_M$ $(t=0)$
: $dD_{I,t}/dt = (-rtr_{I,t}\ cf_{I,t} - \beta_{I,t})D_{I,t}$ $(t>0)$

Water content of spiders i.r.t. behavioural state:
(12) Immobile spiders : $dW_{I,t}/dt = rtr_M\ cf_M\ W_M$ $(t=0)$
: $dW_{I,t}/dt = (-rtr_{I,t}\ cf_{I,t} - TWLR_{I,t})W_{I,t}$ $(t>0)$
(13) Dead spiders : $W_{I,t}$ $<77\%$

Relative transition rates:
(14) Immobilisation : $rtr_M = (0.252 + 0.006\ (100-RH))\ 0.489^{((T-20)/10)}$
(15) Recovery : $rtr_{I,t} = \exp[(-10.3+0.33T) + (0.087-0.0033T)RH]$ $(T \leq 20\ °C)$
and $rtr_{I,t} = \exp[(-3.44+0.01T) + (0.012+0.0005T)RH]$ $(T > 20\ °C)$

Reference relationship: Dose-dependent correction factors:
(16) Immobilisation : $cf_M = [\ \{1+\exp(0.5 - 0.62\ln(mD_M))\} / \{1+\exp(0.5 - 0.62\ln(D_r))\}\ ]$
(17) Recovery : $cf_{I,t} = [\ \{1+\exp(1.95 + 0.47\ln(mD_{I,t}))\} / \{1+\exp(1.95 + 0.47\ln(D_r))\}\ ]$

Metabolism of pesticide:
(18) rel. metab. rate : $\beta_M = D_M \ln(2)/t_{1/2}$
(19) : $\beta_{I,t} = D_{I,t}\ln(2)/t_{1/2}$
(20) fitted halflife : $t_{1/2} = 3.38\ 0.41^{((T-20/10)}$

Water loss rate:
(21) Total Rate : $TWLR_{I,t} = EVAP + WER$, with:
(22) evaporation : $EVAP = 0.00237\ P\ 1.616^{((T-20)/10)}$
(23) water excretion : $WER = [0.027\ 1.76^{((T-20)/10)}] / (1+\exp(0.84-0.72\ln(mD_{I,t}))$
(24) vap. press. def. : $P = (1-RH/100)\ 6.107\exp[17.4\ T/(239+T)]$

Appendix 2: List of symbols used in the equations presented in Appendix 1.

N	Indicates the total number of spiders in a specific state (starting with 20 mobile spiders).	
D	Indicates the total dose of spiders in a specific state (starting at 2.5 ng ai spider^{-1}).	ng ai spider
D_r	Reference-dose for connecting the doses-transition rate experiment and the RH-T-transition rate experiment (2.5 ng ai spider^{-1}).	ng ai spider
W	Indicates the total water content of all spiders in a specific state (starting at 100% spider^{-1}).	%
t	Subscript referring to division of a variable in age cohorts of size of the time step "delt".	
i	Subscript referring to the initial state.	
M, I, R, D	Subscripts referring to the behavioural states: Mobile, Immobile, Recovered or Dead.	
m	Prescript used for mean values.	
$N_{des,I,t}$	Number of immobile spiders with water content of less than 77% ("dessicated", Immobile, time t).	
rtr_M	Relative immobilisation rate of mobile spiders i.r.t T and RH.	s^{-1}
$rtr_{I,t}$	Relative recovery rate of immobile spiders i.r.t. T and RH.	s^{-1}
cf_M, $cf_{I,t}$	Dose dependent correction factor for immobilisation and recovery rates.	
$ß_M$, $ß_{I,t}$	Decline in total dose of Mobile or Immobile spiders.	s^{-1}
$t_{1/2}$	Halflife of total dose.	s
T	Temperature.	^{0}C
RH	Relative air humidity.	%
P	Vapour pressure deficit.	Pa
EVAP	Evaporative water loss rate	% s^{-1}
WER	Pesticide induced water excretion rate.	% s^{-1}
$TWLR_{I,t}$	Total Water Loss Rate (for immobile spiders only).	% s^{-1}

Part III. Summary and concluding remarks

CHAPTER 10

Summary and concluding remarks

Relationships were studied between physical factors, the behaviour and physiology of arthropods and the bioavailability and toxicity of pesticides in the field and under laboratory conditions. Based on their abundance in agro-ecosystems, money-spiders (Linyphiidae), notably female *Oedothorax apicatus*, served as model taxon. The widely used insecticide deltamethrin was used as a model pesticide.

This chapter summarises the results for the relationships studied and discussess some of the practical consequences this study may have for extrapolation between the laboratory and the field, hazard assessment and the development of highly effective pesticides. Finally, possible directions for future study are proposed.

Part I: Field studies

Trapping success under natural conditions

During the fieldwork most of the effort was directed towards aspects of natural walking behaviour which contributes to pesticide uptake by spiders during or after application. Special attention was paid to the influence of physical factors and internal activity rhythms on the walking speed of spiders and the onset of activity during the day. Results presented in Chapter 2 and 3 show that walking activity of spiders at the soil surface is strongly correlated with physical factors and/or nicthemeral activity rhythms. This correlation may be used to predict spider behaviour, which, in combination with information about the bioavailability of a pesticide, may serve as a measure for exposure and expected effect.

Using multifactorial analysis of correlations between daily catches and daily recordings of physical factors over a period of 72 days, it was shown that trapping success, as a measure for spider activity, can be modelled accurately on the basis of easily measurable physical factors, especially irradiation and soil or air humidity. In addition, days with very high or low activity of the spiders in the field were correlated with relatively low or high catches the next day, indicating a not previously reported 'compensation behaviour' for the spiders.

In contrast to 24-hour mean activity, the nicthemeral activity rhythms showed remarkable stability under different physical conditions (Chapters 3). Simple combinations of species dependent variation in internal activity rhythm and species dependent relationships between walking activity and physical factors, such as temperature, soil humidity, air humidity, etc. may explain complex patterns in trapping success for spiders over a 24 hour period.

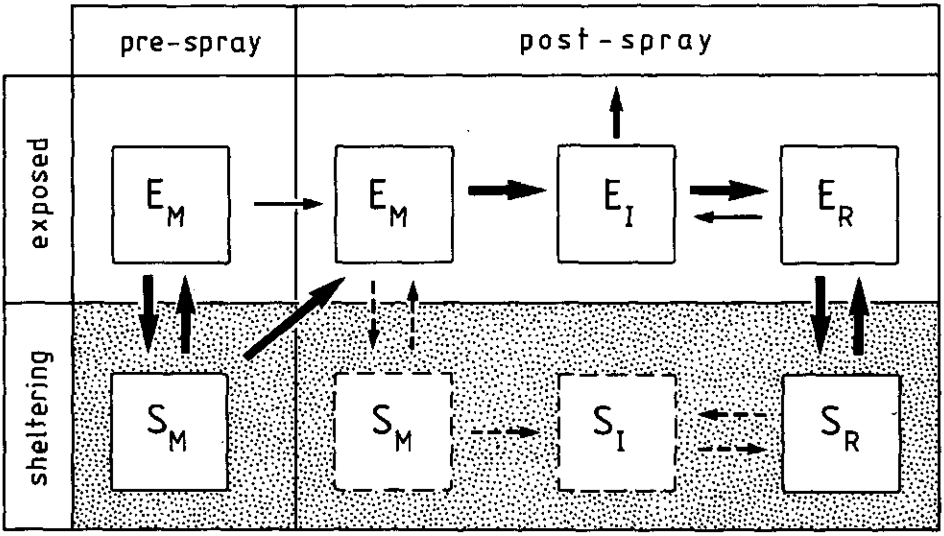


Figure 10.1. The figure shows the most important ways in which linyphiid spiders are exposed to sprayed pesticides in the field. S = sheltering pool of spiders, E = exposed spiders. The subscrips M, I and R indicate spiders that are mobile, immobile or recoverd. Arrow width is indicative for relative imortance of transitions between cathegories.

As immigration of spiders from outside a plot may interfere with the restauration of trapping success after deltamethrin application due to the recovery of poisoned spiders, spider migration was studied in relation to physical factors and deltamethrin application (Chapter 4). The migration of spiders into a sprayed area was shown to be slow. No immigration could be detected at a distance of 7 m into a sprayed plot over a period of one week. In close correlation with spatial changes in physical conditions in the crop, rapid shifts were observed in the spatial distribution of spider activity in the field, despite slow migration.

Furthermore it was shown that the interactions between physical factors and pesticide effects can be studied best when relatively low dosis are used (Chapter 5).

Deltamethrin effects on trapping success in the field

A series of 8 separate experiments in which deltamethrin was sprayed under field conditions demonstrated the importance of spider walking activity at the soil surface (Chapter 6). The information about natural waking activity of spiders obtained in preceding experiments was, in these experiments, used to apply deltamethrin in the field on days for which the respective ambient conditions were associated with great differences in walking activity.

Under field conditions, spiders, hiding most of the time in cracks or under plant debris, will not be exposed to the pesticide before eating contaminated pray or walking over droplet residues (Fig 10.1). As little as 10-15% of the sprayed pesticide reaches the soil (Chapter 4, 5 and 6). The uptake by consumption of contaminated prey seems to be limited by the observation that deltamethrin acts as a deterrent (e.g. Mullié and Everts, 1991). Residual uptake seems to contradict a deterrent effect of a pesticide, however, once deltamethrin has been absorbed by the tarsi, effects of the pesticide will also be experienced on uncontaminated soil. The spiders, therefore, rapidly loose the ability to discriminate between contaminated and uncontaminated soil. In addition, effects of deltamethrin on spider behaviour seem to aggravate exposure.

Immediately following contact with deltamethrin, spiders start preening, show more activity, i.e. movement on the same spot or short walks, and a reduced walking speed (Jagers op Akkerhuis 1990, Jagers op Akkerhuis and Hamers 1992). The walking pattern becomes increasingly more convoluted and the periods of rest, which normally alternate with activity, become longer (unpublished results at our laboratory, A. Boon). The increasing convolution of the walking pattern of deltamethrin treated spiders indicates a decreasing ability for directional movement and loss of orientation in the spiders. This implies that searching for shelter will become more difficult. As a result of higher mobility and reduced orientation, the exposure of spiders to deltamethrin residues at the soil surface increases. Finally the spider becomes immobilised.

Part II: Laboratory studies

Laboratory experiments were used to study the bioavailability of pesticide from substrate, and the disturbance of behaviour and hygrothermal physiology of spiders by deltamethrin in relation to physical factors.

Bioavailability

Using radioactively labelled deltamethrin it was shown that the bioavailability of deltamethrin residues from soil cover such as fungi or moss, is much higher than from clay soil. Moreover bioavailability showed a relative independence from soil moisture content. It should be noted that this indicates that the uptake in the field will largely be determined by the presence of soil cover, and that effects of soil type may be relatively unimportant. In addition it was shown that the rate of uptake showed a reduction with distance walked. This was explained by assuming low chemical conductivity of the tarsi or slow transport of the compound from the tarsi to the arthropod body. These assumptions explain a reduced gradient between residue on soil and cuticle, which limits uptake in subsequent contacts.

The assumption of a relationship between uptake and species dependent anatomy is supported by the work of Salt and Ford (1984). No reduction in pesticide flux from leaf surface to the animal body in subsequent contacts was observed by Salt and Ford (1984) for fourth instar larvae of *Spodoptera littoralis*. These larvae look like caterpillars, from which it may be assumed that the transport of a toxicant from the short leggs of the caterpillar to the haemolymph is faster than is the case for transport via the tarsi and long legs of the spider to the haemolymph.

In experiments, in which bioavailability halflife *sensu stricto* is determined, it may be important to correct for animal behaviour, as is indicated by the following example. Different halflifes of bioavailability from substrate for deltamethrin have been shown of 2.5 hours (Chapter 7) and 42 hours (Mullié and Everts 1991). The difference can be explained by differences in exposure time and the correction for distance walked. In the case of Mullié and Everts (1991) the effect of bioavailability halflife may have been counteracted by a reduction in potential uptake over four hours by rapid immobilisation shortly after deltamethrin spraying, whilst long lasting (over-)activation of behaviour may have caused relatively high uptake long after pesticide spraying.

Although penetration of the pesticide through the cuticle depends on temperature (Ahn *et al.* 1987) and the gradient between external and internal concentration (Greenwood *et al.* 1990), these factors were not studied in the present thesis.

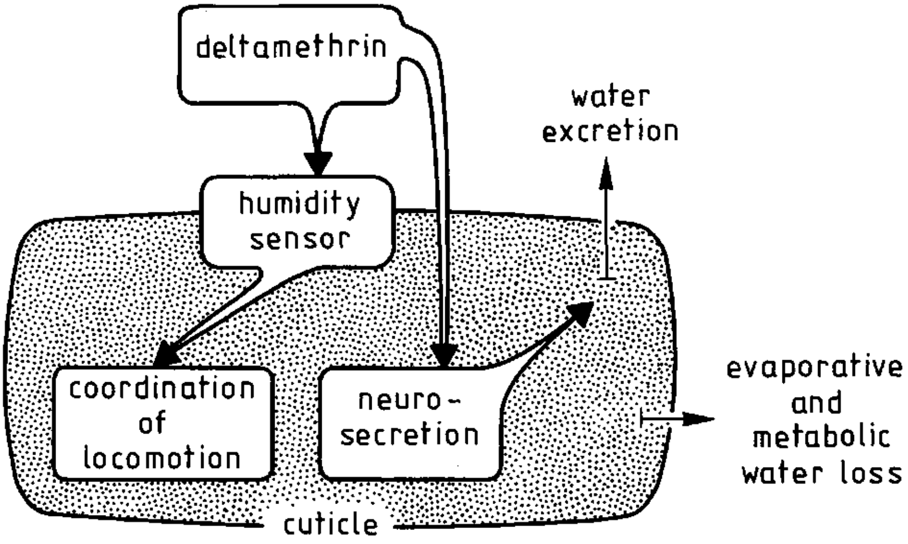


Figure 10.2. Hypothetical model of toxicokinetics and toxicodynamics of deltamethrin in *O. apicatus*.

Behaviour and hygrothermal physiology

The results presented in Chapter 8 and 9 demonstrate in various ways the important role of waterloss as a cause of mortality in spiders poisoned by deltamethrin. Lethal water loss is mainly determined by effects of the insecticide on behaviour and hygrothermal physiology.

Immobilisation in spiders appeared to depend strongly on the relative humidity of the air (Chapter 8). To explain this effect, a hypothesis was proposed which is graphically presented in Figure 10.2. Affected by a high concentration of deltamethrin at the surface of the cuticle, the signalling of humidity sensors in the cuticle, which is most intense in dry air, is increased, which disturbs the peripheral information. This may affect the functioning of general pattern generators (GPG's), which coordinate pattern and speed of motory function. Disturbance of this coordination may result in rapid immobilisation. In addition, the results in Chapter 8 show that deltamethrin causes a dose dependent stimulation of water excretion. This was independent of air humidity and showed an increase with temperature. Figure 10.2 also includes water loss due to evaporation. Evaporation does not depend only on the vapour pressure deficit of the air, it also depends on the water conductivity of the cuticle.

A synthesis of the relationships of the behaviour and hygrothermal physiology of *O. apicatus* in a quantitative, deterministic, model is offered in Chapter 9. Based on behaviour of spiders and water relationships, the model predicts the increase with time in the number of dead spiders in a laboratory population during 144 hours after application of deltamethrin. The model enables predictions of mortality at different combinations of temperature and air humidity.

Practical implications

The results of this study may have practical implications with respect to the following topics: extrapolation from the laboratory to the field, the reducing of pesticide-effects on non-target organisms in the field and the development of highly effective pesticides.

Extrapolation from laboratory to the field

In 'standard toxicity tests' physical factors are often not varied, nor is the relationship between toxic effect and animal behaviour taken into account. In such tests, only the dose is varied, and therefore the results represent only one situation in the effect-space, offering little possibility for extrapolation to other situations. Extrapolation requires knowledge of effect-relationships for at least the biotic and abiotic factors which are important in a given (field-) situation.

Yet, in a restricted number of situations, laboratory based predictions of effects may show some relevance for the field situation: 1. When physical conditions and arthropod behaviour and exposure in the field differ little from the laboratory situation, or, 2. when effects in the field have little dependance on bioavailability, physical factors, or behaviour. The latter may be true for non-reactive and slowly accumulating toxicants, for toxicants where the effect is independent of temperature and/or metabolism, for toxicants with a very long bioavailability halflife and/or the use of high doses. Similarities between results of standard laboratory tests and field observations in other cases must be considered accidental.

Reducing pesticide-effects on non-target organisms

The results of this thesis show that, for non-selective pesticides, applied within the crop at all levels, side-effects may be prevented by using an insecticide with a short bioavailability halflife at a time when few non-target organisms are active. This would create temporal separation of the pesticide and non-target organisms.

Development of effective pesticides

The results of the present study indicate ways of developing highly effective pyrethroid pesticides. Knockdown and water excretion induced by pyrethroid poisoning are unrelated effects. As a result, the combination of two compounds, one with a very high knockdown potency and the other causing a very high water excretion rate, may yield a potent combination-insecticide.

Suggestions for further study

During the present study, it was felt that the considerable discrepancy between information available about certain topics, and their importance for pesticide side-effects may justify exploration of these fields.

One, type I pyrethroids may cause a very rapid, deleterious effect on the ability of arthropods to fly, or walk, the 'knockdown' effect. Although different authors have mentioned the relationship between knockdown and peripheral, sensory information, see also Chapter 9 of this thesis, the explanatory link between peripheral information and behaviour is still lacking. In this perspective, the role of peripheral information in the functioning of a neuronal system called the central pattern generator (CPG) needs to be studied. The CPG forms the basis of the coordination for walking, or flight behaviour in different animals (Wendler 1974, Rossignol *et al.* 1988). The functions of the CPG have been found to rely on proprioreceptive information and/or peripheral sensory information.

Two, the causes of the positive temperature-effect relationship for pesticide induced water excretion are not yet understood.

Three, the long term recovery of spider behaviour following deltamethrin poisoning seems to be relatively slow (Baatrup and Bayley 1992). No information exists about the reasons for this slow recovery, or the extent to which spiders may resume normal behaviour in relation to dose.

HOOFDSTUK 10

Samenvatting en slotopmerkingen

In de voorafgaande hoofdstukken werd verslag gedaan van onderzoek naar de effecten van fysische factoren, de biologische beschikbaarheid van bestrijdingsmiddelen en het gedrag van arthropoden op de opname en het effect van een insecticide onder veld- en laboratorium-omstandigheden. Hierbij werden het pyrethroïde insecticide deltamethrin en de in landbouwecosystemen zeer algemeen voorkomende hangmatspinnen (Linyphiidae) als model gebruikt.

In dit hoofdstuk wordt een samenvatting gegeven van de resultaten van dit onderzoek. Daarnaast worden mogelijke praktische consequenties van de huidige resultaten besproken met betrekking tot extrapolatie van laboratorium-experimenten naar de veldsituatie, de reductie van neveneffecten en de ontwikkeling van efficiëntere insecticiden. Tot slot worden enkele aanbevelingen gedaan voor verder onderzoek.

Deel I: Veldonderzoek

Natuurlijk loopgedrag

Tijdens het veldonderzoek lag de nadruk op aspecten van het natuurlijke loopgedrag, die een bijdrage leveren aan het in contact komen van linyphiide spinnen met een bestrijdingsmiddel tijdens of na een bespuiting. Met name werd de invloed van fysische factoren en interne activiteitsritmes op het moment en de mate van activiteit bestudeerd. Tussen de loopactiviteit van spinnen op het grondoppervlak, fysische factoren en interne activiteits-ritmes, bleek een duidelijke correlatie te bestaan (Hoofdstuk 2 en 3). Deze correlatie maakt het mogelijk om de loopactiviteit van spinnen te voorspellen, hetgeen in combinatie met gegevens over de biologische beschikbaarheid van het pesticide kan dienen als maat voor de blootstelling en effect.

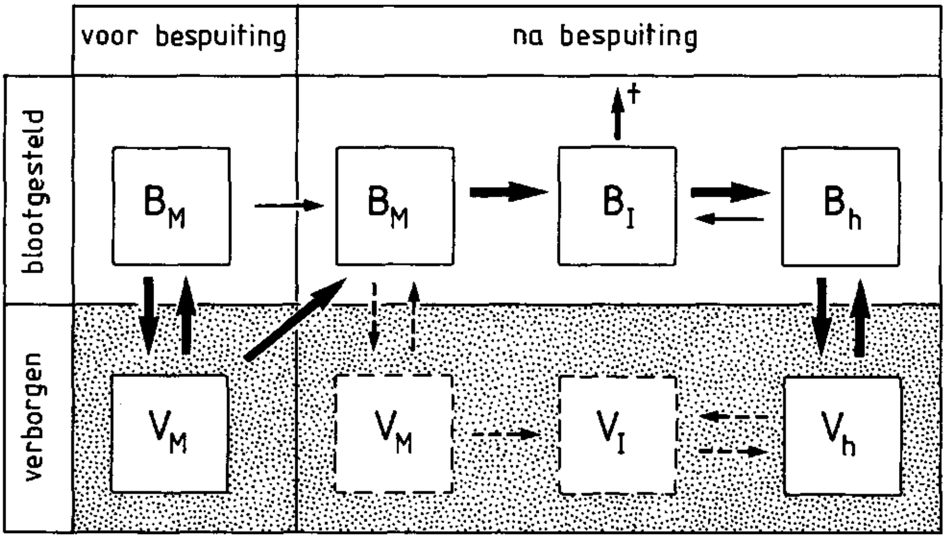
De gemiddelde dagelijkse loopactiviteit van spinnen in het veld kan worden voorspeld op basis van eenvoudig te meten fysische variabelen. Dit bleek uit resultaten van multifactoriële analyse van correlaties tussen vangstgegevens en metingen van fysische factoren van 72 opeenvolgende dagen. De dagelijkse vangsten vertoonden een nauwe relatie met bijvoorbeeld de instraling ($Jm^{-2}h^{-1}$) en bodem- en luchtvochtigheid. Bovendien vertoonden spinnen onder veldomstandigheden een tot nog toe onbekend compensatie gedrag, aangezien hoge of lage activiteit op een bepaalde dag gecorreleerd was met relatief lage of hoge activiteit op de volgende dag.

In tegenstelling tot dagelijkse vangsten, waren de 24-uurs ritmes in de activiteit van spinnen nagenoeg ònafhankelijk van fysische factoren (Hoofdstuk 3). Uit de resultaten bleek dat de gecombineerde effecten van fysische factoren en dag-nacht ritmes op eenvoudige wijze zeer complexe patronen van activiteit in het veld kunnen verklaren.

Om te komen tot een betere statistische onderbouwing van de veldexperimenten en tegelijkertijd de duur van experimenten te korten, werd onderzocht hoe op een verantwoorde wijze de veldgrootte per behandeling en de duur van een experiment konden worden bekort. Een belangrijke factor, die afbreuk kan doen aan de onafhankelijkheid van naast elkaar liggende plots, is de migratie van spinnen. Daarom werd de migratiecapaciteit van spinnen bestudeerd in relatie tot fysische factoren. In een bespoten veld kon 7 meter van de rand gedurende meer dan een week geen migratie worden aangetoond. Wel werden in nauwe samenhang met variaties in het microklimaat in het gewas, snelle verschuivingen waargenomen in de ruimtelijke verdeling van het vangstsucces (Hoofdstuk 4).
Verder bleek dat effecten van fysische factoren op de werking van deltamethrin onder veldomstandigheden het best kunnen worden onderzocht bij lage dosering deltamethrin, varierend van 0.2 tot 0.5 g actieve ingredient ha^{-1} (Hoofdstuk 5). Bij deze concentraties bepalen fysische factoren sterk de mate van effect.

Effecten onder veldomstandigheden

Het belang van loopactiviteit als oorzaak van effecten in het veld werd aangetoond in een serie van acht afzonderlijke bespuitingsexperimenten (Hoofdstuk 6). Door gebruik te maken van de inzichten in effecten van fysische factoren op het natuurlijk loopgedrag van spinnen (Hoofdstuk 2) konden bespuitingen in deze experimenten worden uitgevoerd op dagen met grote verschillen in loopactiviteit. In experimenten waarin tijdens de eerste uren na bespuiting weinig loopactiviteit werd gemeten, konden geen effecten van deltamethrin worden aangetoond.
Omdat de meeste spinnen in het veld beschut zitten, komen ze pas in contact met deltamethrin door opname via voedsel of door over residuen van druppels spuitvloeistof te lopen. Ongeveer 10 tot 15% van de spuitvloeistof komt op de grond terecht (Hoofdstuk 4, 5 en 6)(Figuur 10.1). Opname via voedsel is beperkt, waarschijnlijk omdat deltamethrin een deterrent effect heeft (Mullié en Everts 1991). Het deterrent effect van deltamethrin doet vermoeden dat spinnen bespoten grond mijden. Echter, omdat al na het eerste contact een geringe concentratie deltamethrin achterblijft op de tarsen, zullen spinnen daarna ook op schone grond gevolgen van het middel blijven ondervinden. Ze zijn daardoor slecht in staat zijn om bespoten en onbespoten grond te onderscheiden. Bovendien veroorzaakt deltamethrin gedragsveranderingen die tot meer opname kunnen leiden.



Figuur 10.1. Weergave van de belangrijkste processen die leiden tot blootstelling van linyphiide spinnen aan deltamethrin in het veld. V = verborgen spinnen, B = blootgestelde spinnen, de subscripten M, I en H geven aan dat spinnen mobiel, immobiel of hersteld zijn. De pijldikte geeft het belang aan van de overgangen tussen de klassen.

Gedragswaarnemingen toonden aan dat spinnen zich direct na contact met deltamethrin beginnen te poetsen. Gelijktijding nam de loopactiviteit toe, terwijl de loopsnelheid afnam, het looppatroon steed kronkeliger werd en de periodes rust tussen periodes van activiteit in sterk toenamen (Hoofdstuk 7, A. Boon, ongepubliceerde resultaten, Jagers op Akkerhuis 1990). Een kronkeliger looppatroon duidt op een afname van het vermogen van spinnen om zich te oriënteren na vergiftiging met deltamethrin. Hierdoor zal ook het zoeken naar omstandigheden waaronder de spin minder effect ervaart van deltamethrin worden bemoeilijkt. Een toename van de loop-activiteit en het onvermogen om bespoten grond te mijden zullen ertoe leiden dat de opname van deltamethrin door de spin toeneemt, wat uiteindelijk resulteert in immobiliteit.

Deel II. Laboratorium studies

In laboratorium-experimenten richtte het onderzoek zich op de residuele biologische beschikbaarheid van deltamethrin en op veranderingen in gedrag en hygrothermale fysiologie van spinnen na vergiftiging met deltamethrin.

Biologische beschikbaarheid

Experimenten waarin de residuele opname van radioactief gelabelled deltamethrin werd gemeten, toonden aan dat de bedekking van grond met schimmel of mos altijd tot (veel) hogere opname leidt. Een belangrijke consequentie hiervan is dat opname in het veld sterker zal afhangen van de aanwezigheid van bodembedekking dan van de grondsoort. Daarnaast werd aangetoond dat de opname-snelheid van deltamethrin afneemt met de afstand die spinnen over substraat lopen. Dit kon worden verklaard door aan te nemen dat deltamethrin slecht doordringt in de tarsen of dat het transport van deltamethrin naar het lichaam langzaam is. Beide aannames resulteren in een geringe deltamethrin gradiënt tussen tarsen en grond, waardoor de opname wordt beperkt.

De mogelijkheid van een verband tussen opname en soortgebonden anatomie wordt bevestigd door het werk van Salt en Ford (1984). In experimenten waarin larven van *Spodoptera littoralis* werden gebruikt, die er uit zien als rupsen, werd een lineaire relatie gevonden tussen afgelegde weg en opname van een insecticide. Dit ondersteunt de theoretische relatie tussen anatomie en opname, omdat bij rupsen de korte en brede poten weinig belemmering zullen vormen voor transport van insecticide naar het lichaam.

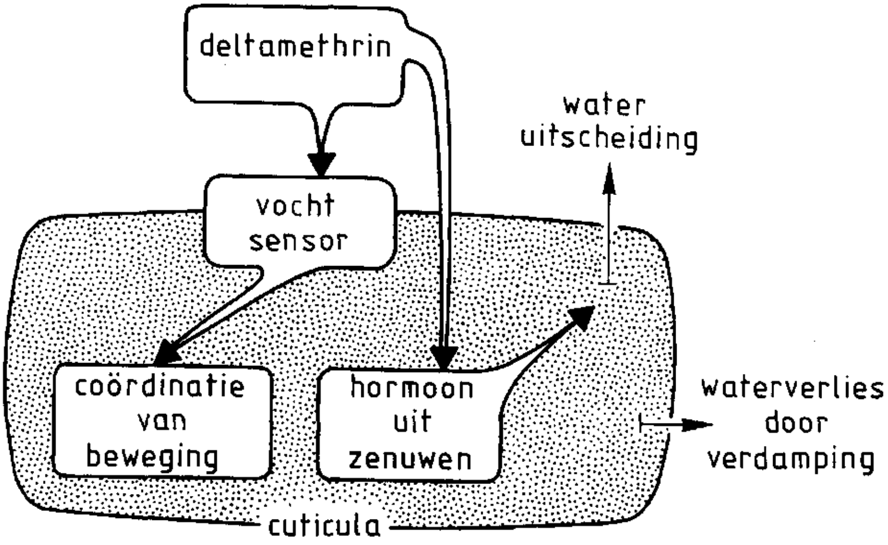
De halfwaardetijd van biobeschikbaarheid, zoals berekend in Hoofdstuk 7 (2.5 uur), verschilt sterk van de waarde die werd bepaald door Mullié en Everts (1992) (42 uur). Dit verschil hangt waarschijnlijk samen met gedragsfactoren in combinatie met verschillen in blootstellingsduur tussen beide experimenten en het al of niet corrigeren van de opname voor de afgelegde weg.

Lethaal waterverlies in relatie tot gedrag en hygrothermale fysiologie

De resultaten in hoofstuk 8 en 9 onderbouwen dat uitdroging de belangrijkste reden is voor sterfte van spinnen als gevolg van deltamethrin-vergiftiging. Lethaal waterverlies wordt veroorzaakt door een combinatie van natuurlijke verdamping en actieve wateruitscheiding onder invloed van deltamethrin. Omdat deltamethrin ook tot immobilisatie leidt, waardoor spinnen niet meer kunnen drinken, veroorzaakt een voldoende hoge dosis sterfte door uitdroging. Op basis van gegevens uit Hoofstuk 8 en 9 werd een kwantitatief deterministisch model opgesteld, wat de toename in de tijd voorspelt van het aantal dode spinnen voor een laboratoriumpopulatie gedurende 144 uur na toediening van deltamethrin. Het model biedt de mogelijkheid voorspellingen te doen over mortaliteit bij verschillende combinaties van temperatuur en luchtvochtigheid.

De waarneming dat de luchtvochtigheid grote invloed had op de immobilisatie-snelheid van spinnen vormde een aanwijzing voor het bestaan van een relatie tussen luchtvochtigheid en de eerste fase van het vergiftigingsproces met deltamethrin. Als mogelijke verklaring voor deze relatie tussen immobilisatie en luchtvochtigheid werd een hypothese opgesteld. Deze is schematisch weergegeven in Figuur 10.2. Vochtreceptoren in de cuticula vertonen bij lage luchtvochtigheid een hoge signaalfrequentie. Versterking van dit signaal onder invloed van deltamethrin verstoort de perifere informatie, wat van invloed kan zijn op het functioneren van Centrale Patroon Generatoren (CPG). Deze laatsten reguleren volgorde en frequentie van beweging in vele diersoorten. Verstoring van de CPG kan tot immobilisatie leiden.

Daarnaast toonden resultaten in Hoofdstuk 8 aan, dat vergiftiging met deltamethrin leidt tot een proces van actieve wateruitscheiding onafhankelijk van de effecten op gedrag. Dit laatste vertoont geen relatie met de luchtvochtigheid maar hangt positief samen met de temperatuur. In Figuur 10.2 is ook het natuurlijk vochtverlies van spinnen door verdamping opgenomen. Verdamping hangt niet alleen af van het dampdrukdeficit maar ook van de doorlaatbaarheid van de cuticula voor water, die exponentieël toeneemt met de temperatuur.



Figuur 10.2. Schematische voorstelling van de toxicokinetiek en toxicodynamiek van deltamethrin in *O. apicatus*.

Toepassingsmogelijkheden van de resultaten

Extrapolatie van laboratorium naar veld

Dit promotieonderzoek heeft laten zien dat voor stoffen met een vergelijkbare werking als deltamethrin, voorspellingen van effecten in het veld op basis van laboratoriumonderzoek sterk zouden kunnen worden verbeterd indien rekening zou worden gehouden met de relaties tussen de belangrijkste biotische en abiotische omstandigheden in het veld en effecten van het middel.

In 'standaard toxiciteits toetsen' wordt alleen de dosis gevarieërd. Dit soort resultaten geeft maar één situatie weer uit de effecten-ruimte. Dit biedt weinig mogelijkheden voor extrapolatie naar andere situaties. Toch kunnen in een aantal situaties laboratorium resultaten wel worden vertaald naar het veld. Dit is mogelijk wanneer de laboratorium en veldsituatie grote overeenkomst vertonen of wanneer effecten in het veld slechts in zeer geringe mate afhangen van biobeschikbaarheid, fysische factoren en het gedrag van dieren. Dit laatse kan gelden voor niet-reactieve stoffen die langzaam accumuleren, voor stoffen waarvan metabolisatie en effect nauwelijks afhangen van temperatuur, voor stoffen met een erg lange halfwaardetijd van biobeschikbaarheid, of bij gebruik van hoge doseringen. In andere gevallen berusten overeenkomsten tussen resultaten van standaard laboratorium toetsen en de veldsituatie op toeval.

Reductie van neveneffecten van insecticiden

Uit dit proefschrift blijkt dat, ook wanneer niet-selectieve middelen worden gespoten, neveneffecten van insecticiden kunnen worden voorkomen door toepassing van een middel met een snelle afname van de biologische beschikbaarheid op een moment dat weinig onschadelijke dieren actief zijn. Dit reduceert ongewenste effecten door een scheiding in de tijd van het insecticide en niet-doelwit organismen.

Ontwikkeling van nieuwe bestrijdingsmiddelen

De resultaten van dit proefschift tonen aan dat pyrethroïde insecticiden zoals deltamethrin twee onafhankelijke werkingsmechanismen bezitten; het gedrag raakt verstoord, en actieve wateruitscheiding wordt geïnduceerd. De effectiviteit van pyrethroïde pesticiden kan waarschijnlijk sterk toenemen door gecombineerd gebruik van twee componenten die ieder zeer effectief één van beide effecten veroorzaken.

Suggesties voor verder onderzoek

Op grond van dit proefschrift kunnen enkele onderwerpen worden aangegeven die dringend om meer onderzoek vragen:

1. Bepaalde pyrethroïden veroorzaken een snelle afname van het vermogen van geleedpotigen om te vliegen of te lopen, het zogenaamde 'knockdown' effect. Hoewel het werk in Hoofdstuk 8 en 9 van dit proefschrift en resultaten van andere onderzoekers hebben aangetoond dat pyrethroïden effect hebben op het perifere zenuwstelsel, ontbreekt de link tussen knockdown en de verstoring van sensorische informatie door pyrethroïden. Deze link zou kunnen worden gelegd wanneer een groep zenuwcellen, die samen de Centrale Patroon Generator (CPG) vormen, in het onderzoek wordt betrokken. Deze CPG zorgt in verschillende diersoorten voor de coördinatie van loop- en vlieg-bewegingen (Wendler 1974, Rossignol *et al.* 1988). Het is bekend dat het functioneren van de CPG kan afhangen van informatie van het perifere zenuwstelsel.
2. Over door deltamethrin geïnduceerde wateruitscheiding (discussie van Hoofdstuk 9), die een positieve temperatuur-effect relatie vertoont, is nog erg weinig bekend. Gezien het belang van waterexcretie voor effecten van pyrethroïden in de veldsituatie, is onderzoek op dit gebied dringend gewenst.
3. Onderzoek naar het herstel van individuele arthropoden na vergiftiging met pyrethroïden is een nog nagenoeg onbetreden terrein. Er zijn aanwijzingen dat het herstel traag is, ook bij lage dosis, wat van grote invloed zou kunnen zijn op het functioneren van predatoire arthropoden in een ecosysteem.

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Curriculum vitae

Gerard Jagers op Akkerhuis werd geboren op 21 maart 1960 te Hengelo. Hij behaalde zijn Gymnasium ß-diploma in 1978 aan de scholengemeenschap De Grundel aldaar. In dat zelfde jaar begon hij met de studie Planteziektenkunde aan de Landbouwuniversiteit Wageningen. Tijdens de doctoraalfase werd onderzoek uitgevoerd aan de vakgroep Dieroecologie in combinatie met Dierfysiologie (Prof. dr. M.W. Sabelis, Prof. dr. W.F. Tjallingi), de vakgroep Entomologie in combinatie met Bodemkunde en Geologie (Prof. dr. J.C. van Lenteren, Prof. dr. L. Brussaard), en Nematologie (Prof. dr. A.F. van der Wal). Een stage werd doorgebracht in Brunoy, Frankrijk, aan het Laboratoire d'Ecologie Generale (Dr. G. Vannier). De studie werd in 1986 met lof afgerond. Na vervangende dienst aan het Instituut voor Bodemvruchtbaarheid in Haren (Gr.)(Prof. dr. L. Brussaard) en drie maanden onderzoek aan de Vrije Universiteit van Amsterdam (Prof. dr. N.M. van Straalen), werd in 1988 in opvolging van dr. J.W. Everts gestart met promotieonderzoek aan de vakgroep Toxicologie onder begeleiding van Prof. dr. J.H. Koeman. Vanaf Augustus 1992 is hij aangesteld door de Programma Commissie Basiskennis Bodem als onderzoeker aan de Vrije Universiteit van Amsterdam in het kader van een topping-up studie van het onderdeel 'toxiciteitstoetsen met bodemorganismen' van het speerpuntprogramma bodem.

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