SENSITIVE INDICATORS OF SIDE-EFFECTS OF PESTICIDES ON THE EPIGEAL FAUNA OF ARABLE LAND

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
in het openbaar te verdedigen
op woensdag 4 april 1990
des namiddags te vier uur in de Aula
van de Landbouwuniversiteit te Wageningen

The studies described in this thesis were partly financed by the Ministry of Housing, Public Planning and the Environment and by the Dutch Programme for the Promotion of Toxicological Research

CONTENTS

To Gabriella

PREFACE

The idea for of present study originates from Jan Koeman, my professor and friend. In 1985 it became clear that next to my work in the tropics, in committees and on long-term infrastructural plans something had to be done, here and now. Therefore, a one season monitoring progamme was started (in view of my experience of large-scale monitoring in Africa) in cooperation with the State Authority for the Lake Yssel Polders, with a rather open concept of the continuation. The work soon drew the attention of financiers, and with the support from the Ministry of Housing, Public Planning and the Environment and the Toxicology Research Promotion Programme and material help from Roussel UCLAF/PROCIDA the continuation was ensured until 1991.

Ecotoxicological field work calls for a great deal of 'specialist's slave labour'. The persons who carried out the vast majority of this work (assistants, graduate students, guest workers and stagiairs) deserve my greatest respect and gratitude. The base of the success was formed by their enthousiasm, creativity, criticism and energy. I thank you, Hans van den Berg, Berend Aukema, Alberto Zuppelli, George Thomas, Eric Hol, Tom Buyse, Kees Beurskens, Harry Bouwhuis, Hans van den Heuvel, Bert Lankester, Peter Nieuwveld, Miranda Diependaal, Renske Postuma, Arien Scholtens, Mark Hoekstein, Niels Goedejohan, Joost Lahr, Anja Boersma, Anja van Gemerden, Alette Rottier, Ruud van Katz, Wim Mullié, Ingrid Willemsen, Marinus Stulp, Luc Simons, Jan Kammenga, Tim Sikking, Anton Gerritsen, Jan Willem Kamerman, Theo Ariëns, Jolanka Eeftink, Marjanne van Rens, Charlotte Schmidt and Manon Vaal!

Because of the integrative character of the study the advice from specialists from several scientific disciplines was of extreme importance. I therefore thank the members of the advisory committee Drs C. Roghair, Th. van Dijk, C. Booij, CAM van Gestel, H. van der Voet and N. van Straalen, as well as the occasional advisors Drs Th. Heyerman, H. van der Valk, B. Brouwer, M. van Montfort, W. Rossing, F. Moriarty and M. Leistra. Of the State Authority for the Lake Yssel Polders the following persons dedicated much of their time to the project: Drs L. Wouters, J. Daling and J. Meems and the farm managers who kindly admitted us to their fields were D.J. van 't Zet and P. van Andel. My good friend Dr Rob Hengeveld was my principal advisor throughout the study.

The manuscript was edited with the invaluable help of Ms Irene Bruggeman and with the assistance of Ms A. Lam and G. Heitkönig. The graphs and maps save been drawn by Mrs C. Rijpma, M. Schimmel and A. Bergwerff. The english was corrected by Ms M.L. Vaalburg-Darbon.

Dr R. Jocqué, Museum for Central Africa, Tervueren, Belgium, kindly provided the drawing used for the cover illustration.

Finally but most of all I thank Gabriella Meerbach for her support and her putting up with me throughout a hard period.

PART 1 : GENERAL

CHAPTER I. EXTRAPOLATION FROM SINGLE SPECIES TEST TO BIOSPHERE: PERSPECTIVES AND LIMITATIONS

Introduction

Ecotoxicology is concerned with the study of undesirable effects of chemicals on organisms in the natural environment. The main objectives are to preserve:

1. the functions of ecosystems, such as primary production and mineral cycling
2. species of organisms which should be protected in connection with the ecosystem functions or which we want to preserve for other reasons (e.g. edibility, aesthetic values).

Both depend on the availability of air, water and soil of adequate quality. It is unfortunate that we have started to pollute our environment at a stage in history when our understanding of the properties of most chemical pollutants and ecosystems is still in its infancy. Through retrospective studies (field studies supported by laboratory experiments) a large number of cases of poisoning of populations and communities have been elucidated. For instance it has been demonstrated unequivocally, that a variety of species of birds and mammals have been affected by pesticides and other environmental pollutants. It should be added, however, that there are also cases where there is suspicion that the decline of populations or changes in the composition of communities are due to chemical pollutants, but where is has not yet been possible to prove that a causal relationship exists, for instance because the hypothetically disturbing variable (the chemical) cannot be separated from other (confounding) variables, which may influence the size and structure of population and communities. Moreover, the vulnerability of species may vary considerably, in time and space.

The methods available for estimating risk of chemical injury to man, however, largely outnumber the methods available for other species and ecosystem functions (Vouk et al. 1985). Furthermore, the heterogeneity of the natural world and the poor knowledge of its fundamentals strongly restrict the possibilities for the development of ecological criteria for environmental quality with more than local applicability. In a small country like the Netherlands, for instance, 23 different water types are identified and for 16 of these quality criteria have been developed (Anonymous 1988). The criteria concern species composition and a few indicator parameters for ecosystem processes.

Ecotoxicology is developing into a predictive science and many attempts have already been made to design laboratory models with predictive value with regard to the real world. Examples are the standard tests with algae, Daphnia spp. and fish adopted under auspices of the OECD (1987) and also adopted in the guidelines of other organizations at national and international level.

Among the scientists in this field, however, there is an increasing awareness that these tests can only be used as a starting point for the establishment of adequate guidelines for environmental protection. The standard laboratory toxicity tests fail to account for the complexity of ecosystems and the strong interactions that may occur between species (Cairns and

Table 1.1 Criteria for the selection of indicators of ecological effects (after Hellawell 1986, Phillips 1980, Kelly 1989).

A. Ecological criteria

- representative of specific functional group
- representative of important taxonomic group
- cosmopolitan distribution

B. Practical criteria

I. Laboratory

- low variability, both genetic and with respect to behaviour
- long-lived
- large
- providing rapid, reliable and specific response
- ease of growing and handling

II. Field

- readily identifiable
- possibility of easy and quantitative sampling
- availability of abundant autecological data
- small activity range
- abundance
- regularity in time and space
- possibility of non-destructive monitoring

C. Regulation criteria

- relevance to ecological endpoint
- possibility to monitor feedback to regulation

Pratt 1987). If indirect effects are likely to occur either by secondary poisoning (e.g. the secondary effect of the rodenticide warfarin on birds and invertebrates (Townsend et al. 1981, 1983)) or by disturbance of interactions (e.g. the increase in prey populations after a toxic effect on predators, mentioned by Crossland (1984) and in Chapter VI) toxicity data from laboratory indicators are of limited value.

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STELLINGEN

Het effekt van deltamethrin in lage doses bij *Oedothorax apicatus* Blackwall berust op een combinatie van een verstoring van de fysiologische homeostasis en een verminderd vermogen tot het vermijden van ongunstige habitatcondities.
Dit proefschrift

Het effekt van bestrijdingsmiddelen op de epigeïsche spinnengemeenschap is niet groter dan het effekt van mechanische verstoring, zoals ploegen en eggen.
Dit proefschrift

De microcosmos methode voorgesteld door Giddings beantwoordt niet aan het gestelde doel: het vaststellen van veilige niveaus van blootstelling aan toxische stoffen van littorale gemeenschappen.
Giddings JM (1986) in: Cairns J (Ed.) Community Toxicity Testing. ASTM STP 920:121-131

Gezien het risico voor de aquatische fauna van het gebruik van carbofuran in de geïrrigeerde rijstbouw, met name in aride gebieden, verdient de ontwikkeling van alternatieve bestrijdingsmethoden grote aandacht.
Mullié WCM et al. (1989) ICBP Study Report No. 36

De resultaten van Girling, gepresenteerd met de bedoeling een nieuwe toxicologische toetssoort voor het marine milieu te introduceren geven niet aan dat deze soort nieuwe informatie oplevert.
Girling AE (1989) Proc 1st Europ Conf Ecotoxicol, Copenhagen. 151-166

Voor de evaluatie van het risico van de emissie van een stof zijn gegevens omtrent het gedrag van gevoelige soorten, in relatie tot de blootstelling, onontbeerlijk.
Gray RH, Dauble DD & Skalski B (1985) Environ Impact Assess Rev 4:84-96

Om theoretische en methodologische redenen verdient het aanbeveling ecotoxicologisch veldonderzoek te richten op gilden van soorten.
Petersen RC (1986) in: Cairns J (Ed.) Community Toxicity Testing. ASTM STP 920:180-198

Lay, Krieger and Korte gaan in hun driejarige studie naar het effekt van PCP in een proefvijver voorbij aan het belang van verschillen in opnameroutes bij predatore insekten, door de wijze van ademhaling buiten beschouwing te laten.
Lay JP, Kriege T & Korte F (1989) Proc 1st Europ Conf Ecotoxicol, Copenhagen. 84-90

Een vergelijking van het verloop van de eischaaldikte met de ringgegevens van enkele roofvogels in Noord-West Europa, van 1935 tot 1980, geeft aan dat de gevolgen van het gebruik van chloorkoolwaterstoffen in de gewasbescherming voor de meeste soorten van ondergeschikte betekenis zijn geweest, in verhouding tot andere faktoren die vermoedelijk verband houden met het klimaat.
Newton I & Haas MB (1984) Br Birds 77:47-70; Hengeveld & Koeman (1986) Computers in Biogeography, Linz

Het verbod op het gebruik van dieldrin bij preventieve woestijnsprinkhaanbestrijding is niet gebaseerd op ecotoxicologische gronden.

De stelling dat de discussie over klimaatsverandering op een hetze berust, is irrelevant voor het natuurbeheer.

Voor een vogelteller is het schatten van een mensenmassa, indien gewenst ingedeeld naar leeftijd, sexe, ras en voedingstoestand, een eenvoudige opgave.

James W. Everts

Sensitive indicators for side-effects of pesticides on the epigeal fauna of arable land.

4 April 1990

The objectives of the present study are:

1. To assess the impact of certain pesticide applications on epigeal arthropod communities in agricultural ecosystems
2. To identify species which may serve as sensitive indicators for possible effects
3. To derive a laboratory model which can be used to predict possible undesirable effects of pesticides on this fauna
4. To help to improve the predictive value of ecotoxicological laboratory tests in general.

The value of indicators

Indicators are either species of organisms or processes, which by virtue of their sensitivity to changing environmental conditions can be used as signals for change. For instance when it's known that a certain species is relatively sensitive to a particular pesticide, such a species can be used to monitor possible effects of that pesticide under field conditions. One of the aims of ecotoxicology is to identify indicators which can be used to protect the environment against the possible undesirable effects of a wide range of chemicals. Preferably the same indicator species should also be suitable model organisms for predictive testing of chemicals under laboratory conditions. Presently standard tests with algae, daphnids and certain fish are used to predict possible negative side-effects on aquatic ecosystems. A main limitation of the use of such predictive indicators is, that this sensitivity generally is restricted to a limited number of chemicals. Slooff et al. (1986) demonstrated for instance that in aquatic invertebrates 'a most sensitive species' to all chemicals does not exist. Likewise universal field indicators do not exist either.

A further complication in the selection of ecotoxicological indicators is, that the sensitivity of a species is not only determined by the intrinsic vulnerability for a certain chemical but also by the likelihood of exposure, which is a function of autecological characteristics (Jepson, 1988) and the environmental fate of the chemical.

Because these factors may differ considerably between species, laboratory toxicity data from one 'sensitive' species (a fish, a bird, an earthworm) representing an ecosystem compartment have little value for risk assessment for that compartment. For soil ecosystems there are at present, however, no standard animal indicators other than earthworms (e.g. *Lumbricus terrestris*, *Eisenia fetida* (Edwards 1983, Ma 1983). The urgent need for more toxicity tests with soil animals has been expressed by OECD (1989) and others and new tests with nematodes, springtails, mites and spiders are under development in a few member countries.

Although the number of (optional) indicators for the terrestrial environment is growing, above-ground arthropods, partially or exclusively living in the vegetation, seem to be overlooked. Virtually all arable land is frequently invaded or visited by non-target invertebrates that are of high ecological value or appreciation, such as butterflies, hymenopterans, beetles (Cuthbertson 1988, Sotherton and Rands 1987) and more often than not field margins and surrounding vegetation such as hedgerows, known for their ecological richness (Bunyan et al. 1981) are polluted by pesticides through drift outside the treated field during application (Elliot and Wilson 1983). In the actual risk assessment procedures, however, the above ground fauna is only represented by vertebrate indicators (mammals, birds). There are no methods available for estimating risk of any pollutant for the vast majority of species and biomass, i.e. the invertebrates, other than methods for the assessment of selectivity of pesticides, using beneficial species.

Existing monitoring methods for beneficial species in arable land

Beneficial arthropods have been used for decades for the assessment of the selectivity of pesticides in agroecosystems (reviewed by Hammons (1981)). In recent years the test methods have been standardized under the auspices of the International Organization for the Biological Control of Noxious Plants and Animals (IOBC, Hassan et al. 1988). The species are selected for their specific role as pollinators of specific crops or as antagonists of certain pest species. The methods involve the observation of effects of pesticides applied to standard substrates in dosages representing the field dosage on survival and functional activity of a limited number of organisms. Toxicity is expressed as percentage effect and at a certain level of toxicity the laboratory tests are followed by semi or full field observations.

Most species used in these tests, however, as well as many species used for testing the efficacy of a pesticide (i.e. the pest species) should also be considered as indicators of complexes of species that should be protected. Insect species of the same family show a remarkable homogeneity in sensitivity to pesticides. An example is given by Croft and Whalon (1982), who reviewed the toxicity of pyrethroids for 25 beneficial species from 7 families. A list of the taxonomic groups represented by some of the species tested under IOBC standards is given in Table 1.2. Data from the tests, for instance, developed for the staphylinid beetle *Aleochara bilineata* (Samsø-Petersen 1987) and the carabid *Pterostichus c-upreus* (Chiverton, pers. comm.) can be used to estimate the toxicity of a compound for as much as 100 associated species of staphylinids and carabids in arable land (e.g. Sunderland et al. 1985 and Chapter II). Data for honeybees can be extrapolated to solitary bees and other Apoidea.

Toxicity data from these organisms are, therefore, at present exclusively applied for pesticides intended for use in integrated pest management programmes. They could be extremely useful for hazard assessment of unintentional release of pesticides and other pollutants. The actual testing methods, however, are of limited value for environmental risk assessment for other compounds and habitats, for a number of reasons. The most important shortcoming of IOBC selectivity testing data for risk assessment is the fact that these tests do not provide median effective dosage levels.

The procedures for selectivity assessment, on the other hand, prescribe field observations if toxicity in the laboratory exceeds a certain level of acceptability (e.g. 20 or 50 % effect) (Hassan et al. 1988). Only in the US field observations are part of the environmental risk assessment procedure for pesticides (Fite et al. 1989). A certain level of damage in the field is accepted, both in selectivity testing (50 %) and in environmental testing (US, 20 %). These levels are based on the assumption that they do not exceed the fluctuations caused by natural disturbing factors, from which populations generally recover. For other chemicals than pesticides no limits of acceptance have been set as yet. Van Straalen (1987) proposes an acceptable loss of 5 % of the species in the soil. This level is based, however, on extrapolation from laboratory data.

Table 1.2 Examples of beneficial and pest species used for testing the selectivity and efficacy of pesticides and the taxonomic groups they may represent in hazard assessment. (Beneficial tests according to IOBC standards.)

Test Species	Taxonomic or Ecological Group
1. Beneficials	
Apis mellifera (1)	Hymenoptera: Apoidea
Encarcia formosa (2)	Hymenoptera: Parasitica (3,4)
Chrysopa spp (1)	Neuroptera (directly exposed larvae)
Coccinella spp (1)	Coleoptera: Coccinellidae (3,5)
Aleochora bilineata (6)	Coleoptera: Staphylinidae
Pterostichus cupreus (7)	Coleoptera: Carabidae (8)
2. Pests	
Aphids & Planthoppers	Homoptera
Caterpillars	Lepidoptera (larvae)
Houseflies, Stableflies, Mosquitoes (adults)	Diptera (adults)
Mosquito larvae	Diptera (aquatic larvae)

1: Hassan et al. 1988; 2: Oomen 1985; 3: Waddill 1978; 4: Whalon et al. 1982; 5: Coasts et al. 1979; 6: Samsø-Petersen 1987; 7: Chiverton, pers. comm.; 8: Hagley et al. 1980

The indicators presented in the present study and the testing methods developed for these species are considered to be a first step to develop an adequate environmental risk assessment model for insecticides in agro-ecosystems.

Extrapolation of laboratory data to the field

The main routes for extrapolation are: (1) extrapolation from laboratory to field populations, (2) from a limited number of species to an association or community and (3) from small to large spatial/temporal scale. The first level is subject of our study and will therefore be discussed in more detail. The latter is one of the most complicated problems in ecology (Hengeveld 1987, Levin 1989, Harwell and Harwell 1989). On the broadest scales the spatial distribution of biota is determined by physical factors but finer scale patchiness is determined by biological processes and interactions. Mathematical models can be designed that include various scales of the ecosystem (Isawa et al. 1987). Specifically in above-ground ecosystems, however, temporal variation due to physical factors, such as weather, may operate at an hour to hour level, limiting the predictability of temporal events at an acceptable level of uncertainty to a few days (e.g. Zadoks 1989). Because there is no correct level of detail the importance is to determine how variability changes across scales. In ecotoxicology this implies the use of mesocosms and (semi-)field tests alongside small scale laboratory studies.

The extrapolation of toxicity data from a single laboratory species to a natural association or community is favoured by the fact that, especially for chemicals with a non-specific activity, the taxonomic relatedness between species is often coupled with a certain similarity in sensitivity (see, for instance, the toxicity data for organic chemicals collected by Verschueren 1983). For other compounds, such as pesticides and heavy metals, however, substantial differences in susceptibility exist between related genera and between sexes of the same species (a few examples are presented in the following Chapters). Kooijman (1987) and Van Straalen (1987) developed techniques to extrapolate from data from a limited number of species to natural associations. Given the toxicity of a certain compound to a few species, the models estimate the sensitivity of an infinite number of species for that chemical. The models, however, assume an equal exposure of all organisms and therefore cannot be applied in situation where exposure is highly variable, such as most above ground ecosystems and when boundaries between ecosystem components (sub-soil - above-ground; sediment - water) are crossed.

At the third level, from laboratory to field populations, extrapolation is restricted by limitations in scale, variability, number of species, genetic composition of laboratory populations and duration of tests in indoor systems. Laboratory conditions are almost always unnaturally stable and unnaturally simple. The classical approach of varying only one characteristic at a time may result in a false indication of toxicity. Examples are given by Solbé (1988) and in Chapter V. Tests may be too short, too 'gross', on insensitive stages in the life cycle or may seek irrelevant and unnaturally responses. Just as lab systems are too simple, the field situation is too complex. Most habitats are damaged at the same time by more than one pollutant and by other, non-chemical factors. Seasonal changes are unpredictable. To measure the right parameter at the right moment with the right sampling effort is often a mere question of chance. (An illustration is given in Chapter VII.) As a consequence, nobody has succeeded yet in predicting quantitatively the toxic effect of one compound on one species in the field situation. Goodman (1982), in an attempt to model the toxicity of azinphosmethyl in orchards on isopods, conducted 1500 toxicity tests but failed to predict quantitatively the population effect because migration and related exposure were not accounted for.

To compensate for the gap in our knowledge safety factors can be applied. The unknowns that a safety factor is supposed to cover are discussed by Cairns (1984) and Van Straalen (1987). The authors list 11 and 6 factors, respectively, that may interfere with effects predicted from single species tests, such as the interactions mentioned above, combined exposure and compensation processes. The value of safety factors is a matter of opinion. Odum (1984) argues that by not using tests with more environmental realism we are not likely to have a scientifically sound determination of the real world response. Kimerle (1986), however, stated that field or multispecies data are not needed if the 'margin of safety concept' is used in hazard assessment and Tebo (1986) considers tests at levels other than that of single indicator species impractical.

The need for intermediate steps and field studies

If the species used in the laboratory is the ecological endpoint in itself the safety factor is small. In this case the margins are primarily given by the acceptability of certain levels of damage. With this example, however, we arrive at the core of the problem. Extrapolation procedures for the protection of species that are actually under development focus on uncertainties concerning sensitivity of members of the community represented by the indicator(s) (Kooijman 1987) i.e. on toxicological data, while the ecological endpoint, the survival of the populations concerned can only be achieved by an assessment based on estimates for extinction. The latter approach involves not only toxicological data but also data on resilience of populations and communities. A simple example is given by Everts (1985) who describes a situation where two species of shrimps disappeared from a river after application of an insecticide against tsetseflies. The most sensitive species of the two recovered three times as quickly as the lesser sensitive one. Brown and Sharpe(1988) propose a model for the computation of the chance of extinction by toxic effects. Extinction, however, cannot be predicted from laboratory single species tests. Therefore, without the incorporation of data from complete communities (mesocosms, test fields) any risk assessment aimed at the protection of species is bound to fail. The same holds for toxicity data with functional parameters. Without validation in the field situation predictions based on these data lack realism despite the sophistication of the method and the preciseness of their application.

Sometimes intermediate steps are possible. These include micro- and mesocosms (Giesy 1978, Kuiper 1981, Gearing 1989) or semi-field tests, such as outdoor ponds (Crossland 1988), enclosures (Shires 1985, Vickerman 1988), cages (Oomen 1985) or the open containers described in Chapter II. Promising results were obtained recently by various authors with qualitative prediction of effects by means of micro- and mesocosms (Crossland and Hillaby 1985, Crossland and Wolf 1985, Larsen et al. 1986, O'Connor et al. 1989). No mesocosm or semi-field test, however, can replace the real field situation. On the contrary, it is the discrepancy between laboratory based prediction and field observations which gives the most interesting insights into ecotoxicological processes (an example is given in Chapter II) and which makes many field studies very rewarding, despite the expense.

Sensitive field methods

Because the relevance of any toxicological event can only be determined in the presence of all other disturbing factors field testing should be an early step in toxicological evaluation procedures. In the actual risk assessments procedures, however, the field situation is either not included or it represents the last step (Bengtsson and Torstensson 1988, Fite et al. 1989), the main reason being the cost and the uncertainty of success.

In the field, the hazard of chemicals has to be distinguished from the constant changes within ecosystems due to natural processes. Because evaluation of these test data in the field lacks internationally adopted protocols it is not surprising that the results are not always consistent (e.g. Basedow 1973, Sunderland 1987) and sometimes even contradictory (e.g. Koeman et al. 1978 and Müller and Nagel 1980).

Field tests are time consuming: from three to six months in situations with short lived effects to several years in chronically polluted situations. In addition to the normal scientific skills involved in laboratory based ecotoxicology (toxicology, histopathology, biochemistry) the field requires ecologists, taxonomists, environmental chemists, application technologists and statisticians. The large amount of data needed to reduce variation in the results has to be generated by specialists during endlessly repeated efforts. Because there are no internationally adopted field protocols often the results are disputed. The discrepancy between the results of some very meticulously conducted field assessments has been referred to above.

For an improvement of the situation not only does indicator selection require further attention but also test and monitoring site selection, the establishment of causality instead of correlation, the sampling techniques, and statistics.

In this study, which concerns the side-effects of above-ground applications of pesticides, the epigeal predatory arthropods, carabid beetles and spiders have been chosen for monitoring, for the following reasons. The species represent a group of predators of herbivorous and saprophagous insects and they are known beneficials in a number of crops (Basedow 1973, Sunderland et al. 1985, Chiverton 1984, Sunderland 1987). This implies a high relevance to the endpoint: acceptable damage to beneficial non-target populations. They can easily be sampled by pitfall traps and the species of most genera are easy to identify. Their numbers are more regular than of virtually all other epigeal arthropods hitherto studied in ecotoxicology. Although the visitors of the vegetation are more exposed to spraying and might therefore react more sensitively to treatment, the epigeals are much more regular in time (Everts, unpublished data; Fischer and Chambon 1987). During our study, a small group of spiders was found to be specifically useful as an indicator. They are presented in Chapter III. The group, a guild of five linyphiid and erigonid spiders, meets most of the requirements listed in Table 1.2.

The establishment of a causal relationship between the immission or presence of a chemical compound and observed changes is the primary goal for ecotoxicological field work. In many cases the possibilities for classical latin-square or randomized field design are restricted, mainly due to the minimum field size imposed by the spatial scale of the events to be measured. The problem of the trade-off between plot size and replication is discussed by Sotherton et al. (1988). Because of this limitation circumstantial evidence for causality between exposure and observed changes is often needed.

In non-replicate tests strong evidence is provided by a dose related response. Examples are given by Crossland (1988) and in Chapter II. Another important observation is the recovery of disturbed populations or processes after disappearance of the compound or its toxic metabolites. In virtually all communities recovery will occur, in one way or another, when the stressor has been removed. A characterization of the various forms of recovery is given by Sheehan et al. (1984) and Kelly and Harwell (1989). If the immission is repeated, in time or space, the multiple subsequent changes observed should be of the same character. An example is given by Everts et al. (1983) who described the effect of a series of sprayings on the aquatic fauna of a river. The reduction observed in a shrimp species could be related to the treatment because the same reduction was observed in three subsequent treatments.

Evidence for an effect is further supported by observations in individuals exposed in *in situ* bioassays and, most overlooked, in the field. Descriptions of acute or subacute effects shown by individual organisms in the field are rare, despite their relevance for an understanding of the events recorded by other methods (Takken et al. 1978, Smies et al. 1980, Everts and Koeman 1987). Both categories of observations however, although important, have to be classified as early warning indications with a high chance of false positives. The same holds for chemical techniques. Both residue analysis and measurement of biochemical response to exposure such as developed by Thompson et al. (1988) are only relevant within an ecological framework.

The scientific basis for the present study

Careful selection of indicators is a waste of time if the ecosystems concerned are poorly known and the monitoring sites are too heterogeneous for between-site comparison. For the study described here the Flevoland Polder Area in the Netherlands was chosen for the following reasons. Arable ecosystems are generally the simplest and best known ecosystems in the temperate zone. Moreover, the history of this polder area since reclamation is fully known. The farmed fields are generally large (40 - 90 ha) allowing large-scale observations. The area of 80.000 ha is managed by one authority (State Authority for the Development of the Lake IJssel Polders) who favors field research. The area is flat and the soil conditions are homogeneous over the major part of the area (Anonymous 1961). The epigeal fauna has been described in various published (Turin et al. 1977) and unpublished records (Nijeveldt 1975). Moreover, at the beginning of the study one farmed area of 250 ha was available that had never been treated by chemicals since reclamation. This area was considered to be a good unpolluted control area. During the survey, however, this assumption proved to be false (Chapter II).

Pre-exposure homogeneity between differently exposed plots is the first prerequisite for a field test. Yet this homogeneity is virtually never statistically proven. The absence of a pre-exposure difference during a single observation is generally accepted as proof of homogeneity. If there is a difference, however, some authors use that difference as a standard for all post-exposure observations (Jepson 1987, Sotherton et al. 1987). In so doing the following fundamental mistake is made. If the purpose of an ecotoxicological field test is to observe effects in time (that is, the onset of an effect, the intensity, the time-lag for recovery and the moment of full recovery (Everts 1983), the baseline data have to be handled as (short) time series. This implies that when the events are expressed in terms of changes from a pre-exposure situation this situation should have the form of a time series as well (for instance: the difference in time between numbers of an organism in a to be treated and a control plot (Stewart-Oaten et al. 1986). It also has to be demonstrated that the characteristics of this time series would remain the same over the period of observation if left undisturbed. If this is not the case, conclusions on the impact of the exposure should be formulated in qualitative terms. Conclusions on total impact and quantitative comparisons between taxa are not allowed. A simple method to overcome this problem is presented in Chapters II and VII.

There are numerous ecological sampling techniques and strategies which can be used for ecotoxicological field work (Southwood 1978). An important choice to be made before starting is whether active or passive methods will be applied. The most important difference for ecotoxicology is the fact that passive methods (trapping) measuring both activity and abundance integrate often unknown events over a certain period of time. Trapping at weekly or even daily intervals masks the very short lived acute effects some chemicals may bring about. An example is the hyperactivity shown by many organisms after exposure to pyrethroids (Ruigt 1984) which interferes with the measurement of a possible reduction in abundance. Active methods, such as hand catching or visual observation may also be biased by changes in behaviour, but these changes can be recorded during the sampling activity. These techniques, however, are too time consuming and depend too much on personal effort to be applied in long-term and large-scale programmes. In the study described in the following chapters pitfall traps were used for sampling epigeal arthropods. The main disadvantage, i.e. the fact that the method does not provide a measurement of absolute densities (Luff 1975) was overcome by the use of control plots, assuming that possible changes in activity induced by the characteristics of the fields were similar in the treated and the control plots despite the different crops. The acceptability of this assumption for the present situation has been demonstrated (Chapter II).

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PART 2 : INITIAL FIELD STUDIES

CHAPTER II

SIDE-EFFECTS OF PESTICIDES ON GROUND-DWELLING PREDATORY ARTHROPODS IN ARABLE ECOSYSTEMS[1]

JW Everts, B Aukema, R Hengeveld[2] and JH Koeman

Abstract

The effects of above-ground applications of pesticides in field crops on the epigeal predator fauna in the Lake Yssel polders in the Netherlands was assessed in a 16-month period by means of pitfall trapping in 6 commercially farmed fields and in one experimental field. The pesticides used in this period were the herbicides 2,4-D-amine and MCPA/MCPP and the insecticides deltamethrin, fenitrothion and bromophos-ethyl. Deposition of the spray on the soil was determined. Bioassays were carried out in the fields in two cases. Of the taxa identified, i.e. staphylinids, carabids and spiders, the erigonid and linyphiid spiders appeared to be sensitive to the three insecticides. No effect was observed from the herbicide applications. The effect of deltamethrin appeared to depend on weather conditions. The effect was stronger at higher temperatures than at lower temperatures; under rainy conditions no effects were observed. A guild of 5 species, i.e. *Erigone atra*, *E. dentipalpis*, *Oedothorax apicatus*, *Meioneta rurestris* and *Bathyphantes gracilis*, is recommended as an indicator for early detection of side-effects of insecticides on the epigeal predator fauna.

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[1] Partly published in Environmental Pollution, 59 (1989) 203-225

[2] Research Institute for Nature Management, Arnhem, The Netherlands

Introduction

The present study describes the first part of a combined field-laboratory programme for the assessment of the risk to sensitive components of the soil ecosystem in arable areas from above-ground field applications of pesticides.

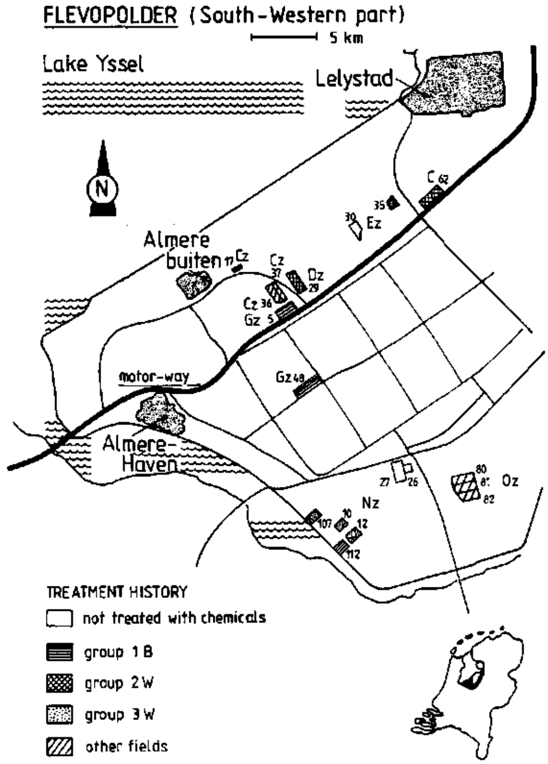

Figure 2.1 Map of the area under study. The sampled fields are indicated by their code. The meaning of the treatmenthistory codes is given in table 3.1.

Of the surface fauna, carabid and staphylinid beetles and spiders are dominant in both biomass and diversity, as well as for their quality as predators of saprophagous and phytophagous organisms (e.g. Sunderland et al. 1980, 1986, Basedow 1973). The toxic effects of pesticides on this group may give rise to an increase in pest populations (e.g. aphids, Edwards et al. 1979).

The objectives of this study are:

a. the assessment of changes in epigeal predator arthropod communities caused by applications of pesticides;
b. the identification of indicators for side-effects of pesticides;
c. the assessment of ambient environmental conditions which may affect the response of organisms to chemical exposure.

Material and methods

The strategy chosen for this study included the following steps:

1. selection of a study area and inventory of epigeal predators;
2. observation of changes in the predator communities that correlate to pesticide treatments, and selection of species that change most markedly in number;
3. establishment of a causal relationship between treatments and the observed changes;

In practice these steps were often concurrent at the same sites.

The study was divided into three sampling programmes, numbered I to III. In each programme the numbers of epigeal arthropods were monitored in commercially farmed fields. In Programmes II to III field experiments were carried out simultaneously. Conclusions concerning effects of pesticides were primarily based on comparisons made between commercially farmed fields and experimental field data. Further support of the conclusions was obtained from observations of acute effects in *in situ* bioassays. For the final step, the results of all programmes were combined.

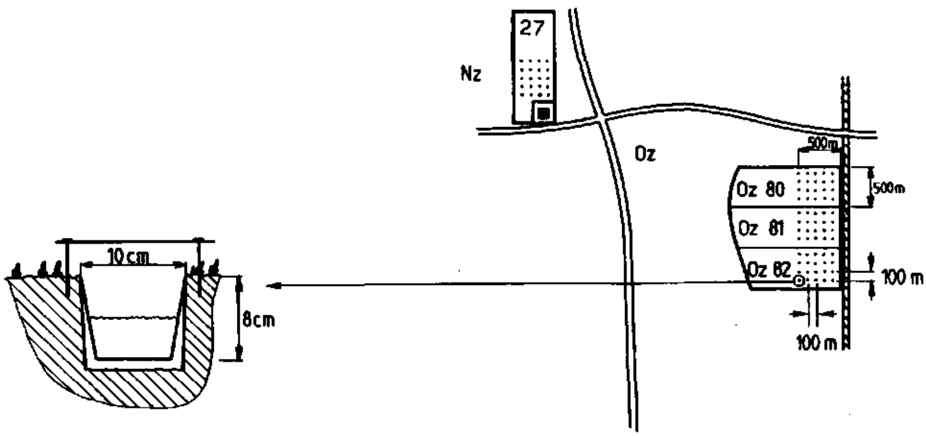

Figure 2.2 Plan of the commercially farmed fields sampled in programme I; the chemical-free field Nz27, and details of a pitfall trap.

Selection of study sites

The study was carried out in the part of the South Flevoland Polder (Figure 2.1) that is managed by the Agricultural Section of the State Authority for the Lake Yssel Polders. With regard to soil type (clay), crops and cultural practices, the area is representative of the northern and south-western polders in the Netherlands The cultural history of this land, reclaimed in 1972, is fully known. The arable area of the polder is divided into parcels of 20 to 90 ha, commonly cultivated in a rotation scheme including oilseed rape, winter wheat and summer barley. Since reclamation a limited number of fields have been cultivated without the use of pesticides.

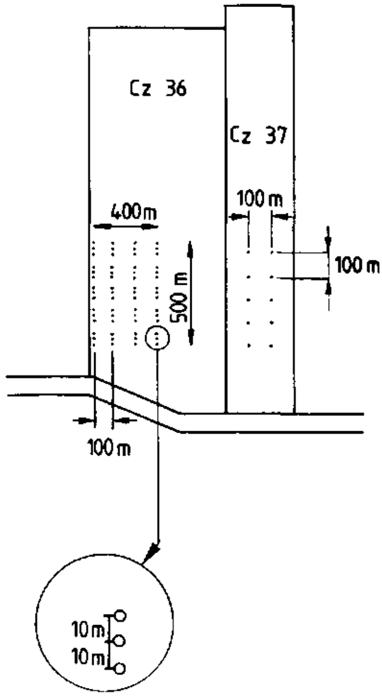

Figure 2.3 Plan of the commercially farmed fields sampled in the programmes II and III. Dots represent pitfall traps.

The crop chosen for this study was primarily oilseed rape. In the Netherlands this is one of the crops which is most frequently treated with insecticides (the group of pesticides which is most toxic to the terrestrial fauna). As untreated oilseed rape fields were not available, untreated fields of winter wheat were used as controls. The correlation of the numbers of the organisms from the different crops was tested for the sampling period before each treatment. Seven fields were selected for observations of the epigeal fauna; six commercially farmed fields and one for field experiments. The fields used in the programmes, and details on sampling and treatments, are given in Table 2.1 and in Figures 2.1 to 2.5.

Sampling techniques

The arthropods were sampled using pitfall traps (plastic cups with a diameter of 10 cm, held in position by a PVC tube and protected by a lid, Figure 2.2) which contained a 4 % formaldehyde solution and a detergent. In periods of frost the liquid consisted of a 50 % glycol solution. The pitfalls were emptied weekly and kept in position throughout the sampling period of each programme. When catches were very low, during the winter, the intervals between pitfall clearance were prolonged to 2 or 3 weeks.

The arthropod groups studied were identified into species level, except Staphylinidae, which were counted as a group. The sex of adult spiders was also recorded. The species were identified according to Locket and Millidge (1951,1953) and Locket et al. (1974). Carabidae were identified according to Lindroth (1974). Species of the carabid genus Amara were not identified. Females of the spiders *Oedothorax apicatus* and *O. retusus* were not separated, they are very similar in appearance. (At a later stage the ratio between the species was estimated and the consequences will be discussed).

In all the fields except the experimental field Cz17, pesticide applications were carried out by means of a tractor in 24m wide swathes. Cz17 was treated with lighter equipment in 6m wide swathes.

Deposition of the pesticide at soil level was deduced from estimates of the vegetation cover. In situations where the vegetation was too high for estimation of the cover, deposition was estimated as follows: at the top of the vegetation and at the soil level MgO-covered glass slides were exposed to the spray. The deposition at soil level was estimated from the difference in droplet number and diameter between slides at the two levels, assuming that the upper level represented the field dose.

Temperature and air humidity were measured at a height of 150 cm in, or near to, the fields, or they were obtained from the Swifterbant meteorological station, which is situated in the polder.

Sampling started as long before the treatments as practically feasible and continued until harvest (Figure 2.5).

Data processing
Of the identified taxa, the total number caught per trap per sampling date was stored in a computer for analysis. Basic community parameters were then calculated by means of a programme developed by Dr C. Booy[3]. Catches per sampling date (n) were transformed to log (n+1). In the commercial farm situation, conclusions concerning possible effects were based on a comparison between the relative numbers, in time, of taxa in fields with different crops. For each taxon it was checked whether these numbers were comparable. The correlation between the numbers from the treated and the control fields was calculated over the pre-treatment periods. For the analysis of the effect, the differences between the values from the treatment and the control area in the pre-treatment period ($\log(n+1)_{treatment}$ - $\log(n+1)_{control}$) were plotted against time. Post-treatment values were then predicted from these pre-treatment data by linear regression. The differences between the observed post-treatment values and the predicted ones were tested for significance by analysis of variance. The acute effect was calculated as the mean difference over three weeks after treatment, expressed as a percentage of the predicted difference, in absolute numbers. This method assures that conclusions on post-treatment differences depend on the variability of the differences between treated and control sites throughout the pre-treatment period. The time-span of the effect is the number of post-treatment observations that is significantly different from the pre-treatment series. Since differences in vegetation structure may interfere with the recovery of populations, the latter was only calculated from the experimental field data. When catches were low, numbers from distinct periods before and after spraying were summed and tested against the control situation by the Students' t-test.

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[3] Dr C. Booy, Institute for Plant Pathology, Wageningen, The Netherlands

The communities were described according to species richness and Mid-Q diversity (Kempton and Taylor 1976). This diversity index was chosen for its sensitivity to changes in the taxa of the middle group between the most abundant species (which are covered by the other methods) and the rare ones (which are of lesser importance in this case).

Variation within a field in time and space was analysed in accordance with Taylor's Power Law, an empirical function describing aggregation (Taylor et al. 1983).

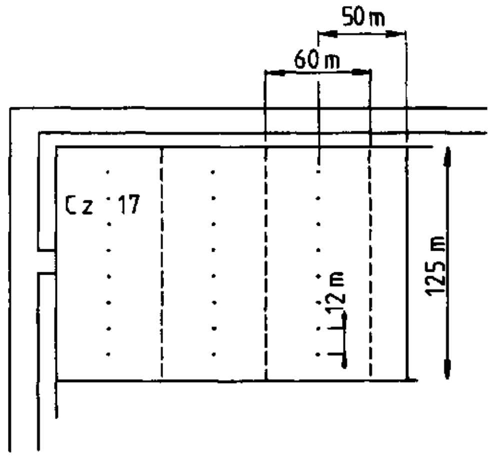


Figure 2.4 Plan of the experimental field used for the programmes II and III. Dots represent pitfall traps.

Programmes I and II

The first Programme concerned an inventory of the epigeal predator fauna (species composition and numbers in time) and changes correlated to pesticide treatments (step 1 and 2) during the spring. It was carried out in three commercially farmed fields (Figures 2.1 and 2.2): two oilseed rape (Oz81, Oz82) and one winter wheat field (Oz80). The fields were surrounded by agricultural land. They were treated once or twice with pesticides (MCPA/MCPP, 2,4-D-amine, bromophos-ethyl and deltamethrin; Table 2.1). Traps were installed at least 100 m from the field edges, in regular grids of 400 x 500 m. Deposition was measured during two deltamethrin treatments.

Alongside this Programme a chemical-free field (Nz27) was sampled by the same methods.

In the second Programme the monitoring in commercially farmed fields had to be carried out at another site, for management reasons. It was a continuation of Programme I, but it was modified in such a way that it permitted an analysis of spatial within-field variation in the treated area. Arthropod numbers were monitored in two rape fields treated with fenitrothion (Cz36 and Cz17) and in an untreated winter wheat field (Cz37). Application rates are given in Table 2.1, numbers and disposition of traps in Figures 2.3 and 2.4.
In the rape field Cz36 20 groups of 3 traps, 3m apart, were placed in a 400 x 500m grid. Cz17 was an experimental field, divided into three equal plots of 0.75 ha. The field was bordered by shrubs on the E and W sides and by ditches at the other sides. Fenitrothion was applied in three dosages (table 2.1).

The combined results of the Programmes I and II, covering 9 months of observation, were used for the selection of indicator species.

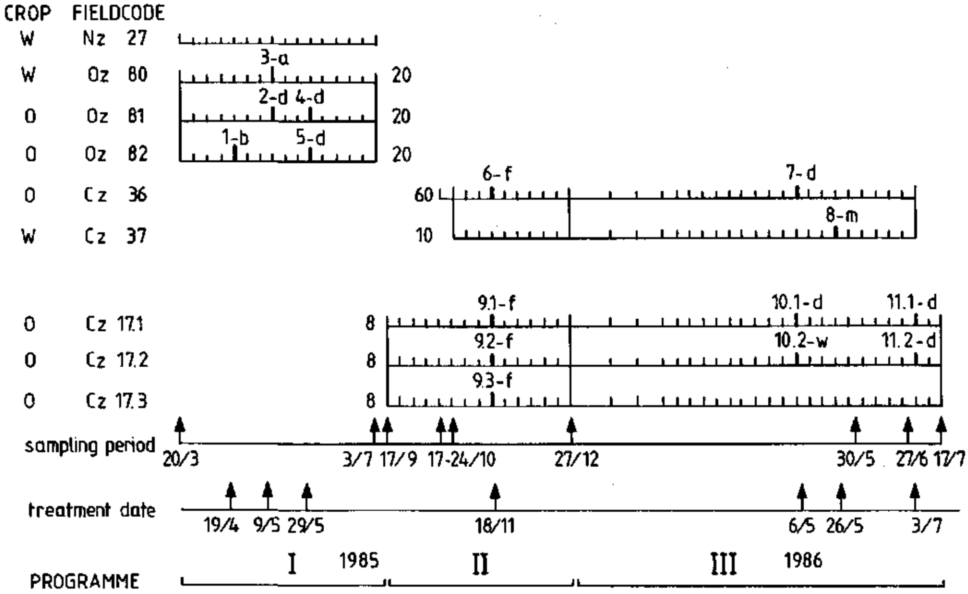


Figure 2.5 Scheme of the sampling programmes. Each field is represented by a horizontal bar, on which sampling dates are marked. Thick marks indicate treatment dates. Fields used in one programme are connected. Codes of the treatments, corresponding to the codes in Table 2.1, are given above the bars. The number of traps in each field is given at the end of the bar for the field. Crop codes: O = oilseed rape, W = winter wheat, C = cereals. Cz17.1-3 are experimental plots. All the other fields are commercially farmed.

Results of Programmes I and II

The total number of specimens identified was 200,653, in 145 taxa. The most abundant ones are shown in Tables 2.2 and 2.3. Linyphiidae and Erigonidae represented 13 % of the total number of staphylinids, carabids and spiders. *Erigone atra* and *Oedothorax apicatus* were the most abundant spiders (76 %) and *Pterostichus cupreus* was the most abundant carabid (66 %).

A significant reduction was found in two species of carabid: *Clivina fossor* after the first deltamethrin spraying at Oz82 and in *Trechus quadristriatus* after fenitrothion spraying in Cz36. In all other situations the numbers of these species were too low or too variable to draw any conclusions. In the Staphylinidae no effects could be demonstrated.
The changes observed in the numbers of the spider species and the period needed for recovery after the treatments, the deposition at soil level and meteorological data at the time of spraying, are given in Table 2.4. The numbers of *Erigone atra* males, *Oedothorax apicatus* females and *Meioneta rurestris* males are presented in Figure 2.6. In Programme I there was a marked reduction compared with the control in *Meioneta rurestris* males, coinciding with the bromophos-ethyl treatment. The catches were virtually reduced to zero after treatment.

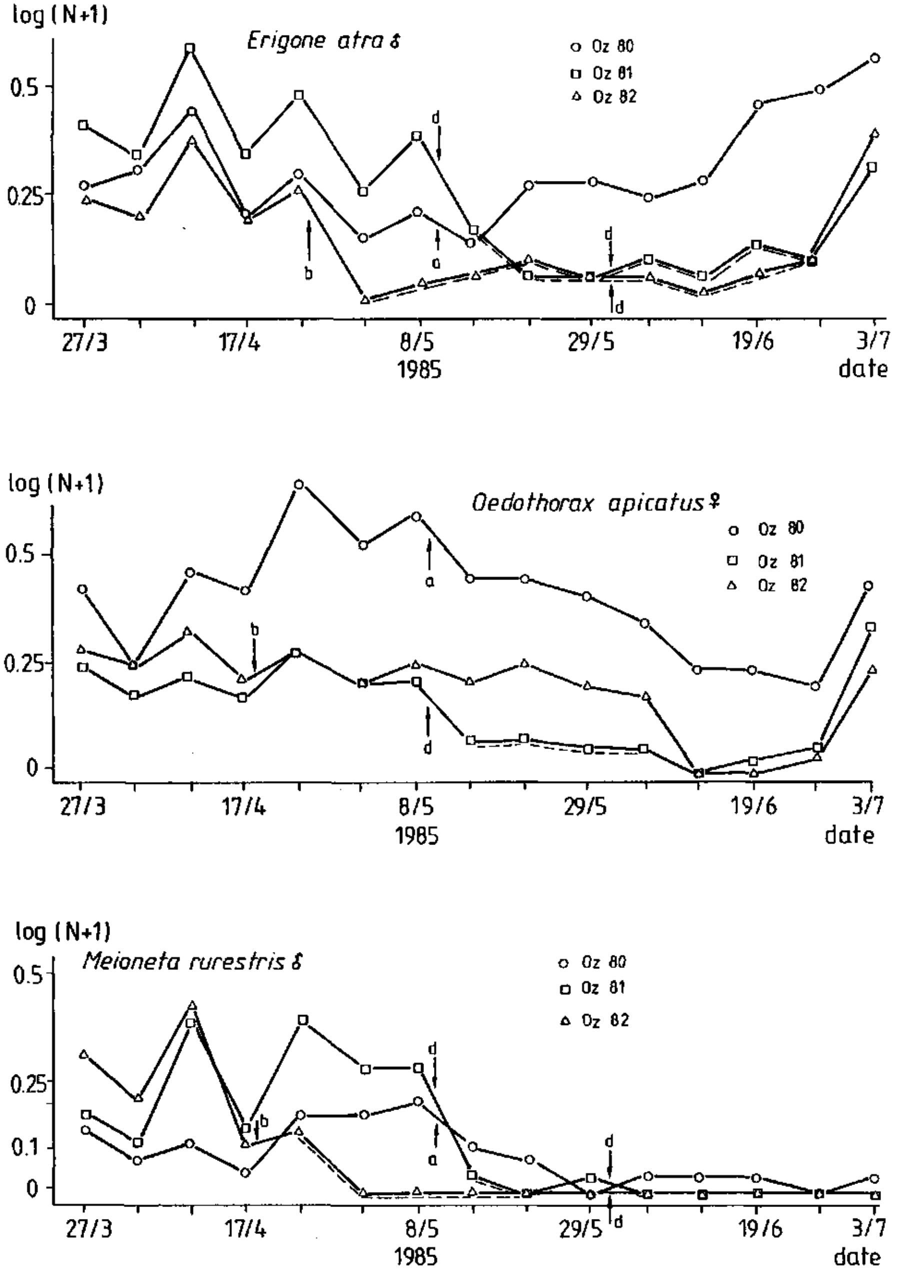


Figure 2.6 Numbers per trap of three spider species sampled in three fields. Pesticide treatments are indicated by arrows. Double lines indicate periods that were different from the control (Oz80). a = herbicide; b = bromophos-ethyl; d = deltamethrin.

Table 2.1 Data of the fields and treatments.

Pro-gramme	Fields				Treatment		
	Code	Size	Type (ha)	Crop (1)	Code (2)	Treatment or Cultivation Method	Dosage (3)
I	Oz80	67	C	winter wheat	3-a	MCPA/MCPP +	1200
						2,4-D-amine	1250
	Oz81	67	C	oilseed rape	2-d	deltamethrin	5
					4-d	deltamethrin	5
	Oz82	61	C	oilseed rape	1-b	bromophos-ethyl	380
					5-d	deltamethrin	5
II	Cz17.1	.75	T	oilseed rape	9.1-f	fenitrothion	1500
	Cz17.2	.75	T	oilseed rape	9.2-f	fenitrothion	1000
	Cz17.3	.75	T	oilseed rape	9.3-f	fenitrothion	500
	Cz36	60	C	oilseed rape	6-f	fenitrothion	500
	Cz37	37	C	winter wheat			
III	Cz17.1	.75	T	oilseed rape	1.1-d	deltamethrin	5
	Cz17.2	.75	T	oilseed rape	10.2-w	water	200[4]
	Cz17.3	.75	T	oilseed rape			
	Cz17.1	.75	T	oilseed rape	11.1-d	deltamethrin	5
	Cz17.2	.75	T	oilseed rape	11.2-d	deltamethrin	10
	Cz17.3	.75	T	oilseed rape			
	Cz36	60	C	oilseed rape	7-d	deltamethrin	5
	Cz37	37	C	winter wheat	8-m	MCPA/MCPP	1200

1) Field type: C = commercially farmed; T = test plot
2) Treatment code refers to Figure 5
3) g a.i. ha^{-1}
4) litre ha^{-1}

Reduced numbers were also found in *Erigone atra* ♂. Both species and *Oedothorax apicatus* ♂ and ♀ showed a reduction after the first deltamethrin treatment when, deposition at soil level was 48 % of the application rate. There was no change coinciding with the herbicide spraying, or after the second deltamethrin treatment, when deposition at soil level was only 10 %.

Table 2.2 Taxa and numbers of Coleoptera identified in Programmes I and II.

Taxon	Programme I	Programme II
A. Taxa that were caught in sufficient numbers for analysis.		
CARABIDAE		
Agonum dorsale	1513	658
Amara spp	4507	-[1])
Bembidion guttula	353	-
B. properans	471	-
B. tetracolum	822	-
Clivina fossor	554	-
Harpalus aeneus	272	-
Loricera pilicornis	225	-
Nebria brevicollis	-	216
Pterostichus cupreus	38629	-
P. melanarius	-	425
P. niger	-	215
P. strenuus	3038	-
Trechus quadristriatus	1542	1286
STAPHYLINIDAE	14772	
B. Other CARABIDAE[2)]		
specimens	5718	1768
species	30	28

1) - = numbers too low for analysis
2) including above-mentioned species when caught in low numbers

Table 2.3 Taxa and numbers of spiders identified in Programmes I to III

Taxon	Programme I	Programme II	Programme III
A. Species that were caught in sufficient numbers for analysis			
LYCOSIDAE			
Pardosa amentata	428	-[1])	
P. prativaga	1498	-	
Trochosa ruricola	150	-	
TETRAGNATHIDAE			
Pachygnatha clercki	-	-	1129
ERIGONIDAE			
Erigone atra	3426	-	5477
E. dentipalpis	444	-	686
Oedothorax apicatus	5237	8241	21401
O. fuscus	385	-	530
LINYPHIIDAE			
Bathyphantes gracilis	-	309	1177
Centromerita bicolor	-	1404	400
Centromerus sylvaticus	-	742	-
Diplostyla concolor	428	-	327
Lepthyphantes tenuis	247	2047	1032
Meioneta rurestris	296	-	-
B. Other spiders[2])			
specimens	4824	2419	3075
species	54	35	46

1) - = numbers too low for analysis
2) including above mentioned species when caught in low numbers

Table 2.4 Mean difference (E) in numbers of spiders collected during three weeks after application o insecticides under various conditions, in percentages of the control (- = difference cannot b calculated, underlined = significantly different from control, between brackets = standar error of the mean (SE) and period before recovery in weeks (p), n = no period could b calculated).

Compound		Bromophos-ethyl	Deltamethrin							
Programme		I	I	I	I	III	III	III	III	III
Treatment code		1-b	2-d	4-d	5-d	7-d -d	10.1 -w	10.2 -d	11.1 -d	11.2
Deposition (g a.i. ha^{-1})		190	2.4	0.5	0.5	4	2.5	0	0.7	1.4
Temperature (°C)		15	12	20	20	18	18	18	28	28
Relative Humidity (%)		45	52	55	55	64	54	54	45	45
Weather[1)]		d	d	d	d	r	d	d	d	d
ERIGONIDAE										
Oedothorax apicatus ♂	E	-62	<u>-63</u>	+43	-	+ 1	<u>-82</u>	-15	<u>-72</u>	<u>-82</u>
	SE	(30)	(8)	(18)		(0)	(9)	(7)	(8)	(9)
	p						7		>2	>2
O. apicatus/ retusus ♀	E	-74	<u>-43</u>	+15	-	+19	<u>-90</u>	+10	<u>-35</u>	<u>-61</u>
	SE	(30)	(5)	(6)		(5)	(11)	(5)	(4)	(5)
	p						4		>2	>2
Meioneta rurestris	E	<u>-94</u>	<u>-94</u>	-	-	-	-	-	-	-
	SE	(13)	(10)							
Erigone atra ♂	E	<u>-34</u>	<u>-85</u>	-67	-70	+14	-19	-16	<u>-97</u>	<u>-95</u>
	SE	(4)	(3)	(23)	(19)	(5)	(6)	(11)	(17)	(23)
	p								>2	>2
E. atra ♀	E	+36	-20	-	-	-	-	-	<u>-72</u>	<u>-100</u>
	SE	(18)	(10)						(6)	(18)
	p								>2	>2
E. dentipal- pis	E	-	-	-	-	-	-	-	<u>-98</u>	<u>-100</u>
	SE								(14)	(17)
	p								>2	>2
LINYPHIIDAE										
Bathyphantes gracilis	E	-	-	-	-	-	-	-	<u>-82</u>	<u>-82</u>
	SE								(9)	(12)
	p								>2	>2
TETRAGNATHIDAE										
Pachygnatha clercki	E	-	-	-	-	-	<u>-94</u>	-50	-70	<u>-100</u>
	SE						(21)	(21)	(33)	(16)
	p						5		>2	>2

Table 2.4 (continued)

Compound			Fenitrothion	
Programme		II	II	II
Treatment code		6-f	9.1-f	9.2-f
Deposition (g a.i. ha^{-1})		450	1350	900
Temperature (°C)		10	8	8
Relative Humidity (%)		60	55	55
Weather[1]		d	d	d
Species				
ERIGONIDAE				
Oedothorax	E	-68	-61[2]	-71
apicatus ♂	SE	(35)	(60)	(101)
O. apicatus/	E	-48	-	-
retusus ♀	SE	(52)		
LINYPHIIDAE				
Lepthyphan-	E	-10	-81	-40
tes tenuis	SE	(8)	(6)	(8)
	p		n	n
Bathyphantes	E	-95	-100	-100
gracilis	SE	(13)	(9)	(21)
	p		16	16

1) d: dry; r: rainy. 2) differences from numbers in plot 9.3 treated at field dose (450 g a.i. ha^{-1}).

In Programme II, the species *Bathyphantes gracilis* virtually disappeared from the fields treated with fenitrothion. In the field experiment, a decrease in numbers corresponding to the spraying of fenitrothion was observed in *L. tenuis* (Figure 2.7).

Recovery occurred generally within 7 weeks, with one exception. *Bathyphantes gracilis* did not recover until 16 weeks after depletion by fenitrothion.

The correlation coefficients from the commercial fields of the pre-treatment numbers of the species mentioned above, are given in Table 2.5. The correlations of most species were high, which implies that the cereal fields were good controls for the rape fields. Exceptions were *C. fossor* and *L. tenuis*.

The Mid-Q diversity and the species richness of the fields are presented in Figure 2.8. The values are given for the periods before, between and after the treatments. The oilseed rape fields Oz81 and Oz82 treated by bromophos-ethyl and deltamethrin, show a lower species richness than the field Oz80. Species richness decreased, compared with the untreated fields, after all the insecticide treatments except the deltamethrin application in Cz36. Mid-Q diversity was not consistently affected by the treatments.

No groups of spiders other than the species mentioned above, and none of the Coleoptera taxa, were as sensitive to these pesticides. Further studies therefore concentrated on Linyphiidae and Erigonidae.

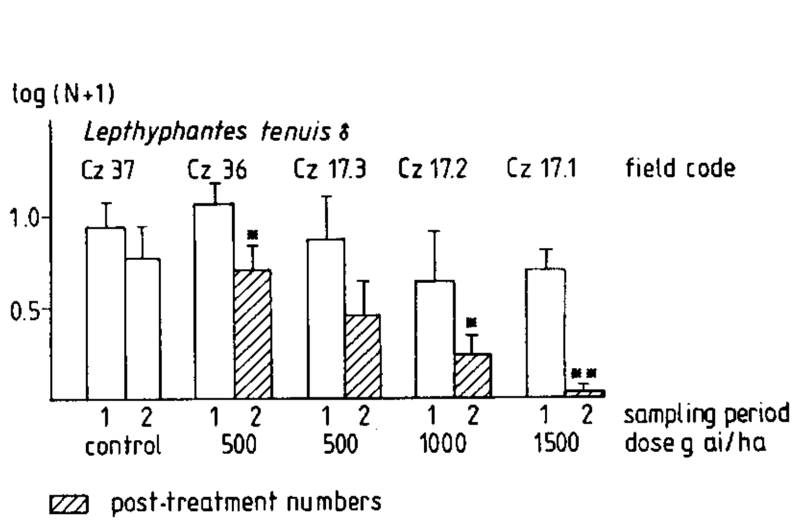


Figure 2.7 Numbers of *Lepthyphantes tenuis* per trap summed over the pre-spray and post-spray sampling periods of programme II. Significance of difference between treated and control numbers: * = $p < 0.05$; ** = $p < 0.005$. Cz17.3 is the control for Cz17.1 and Cz17.2.

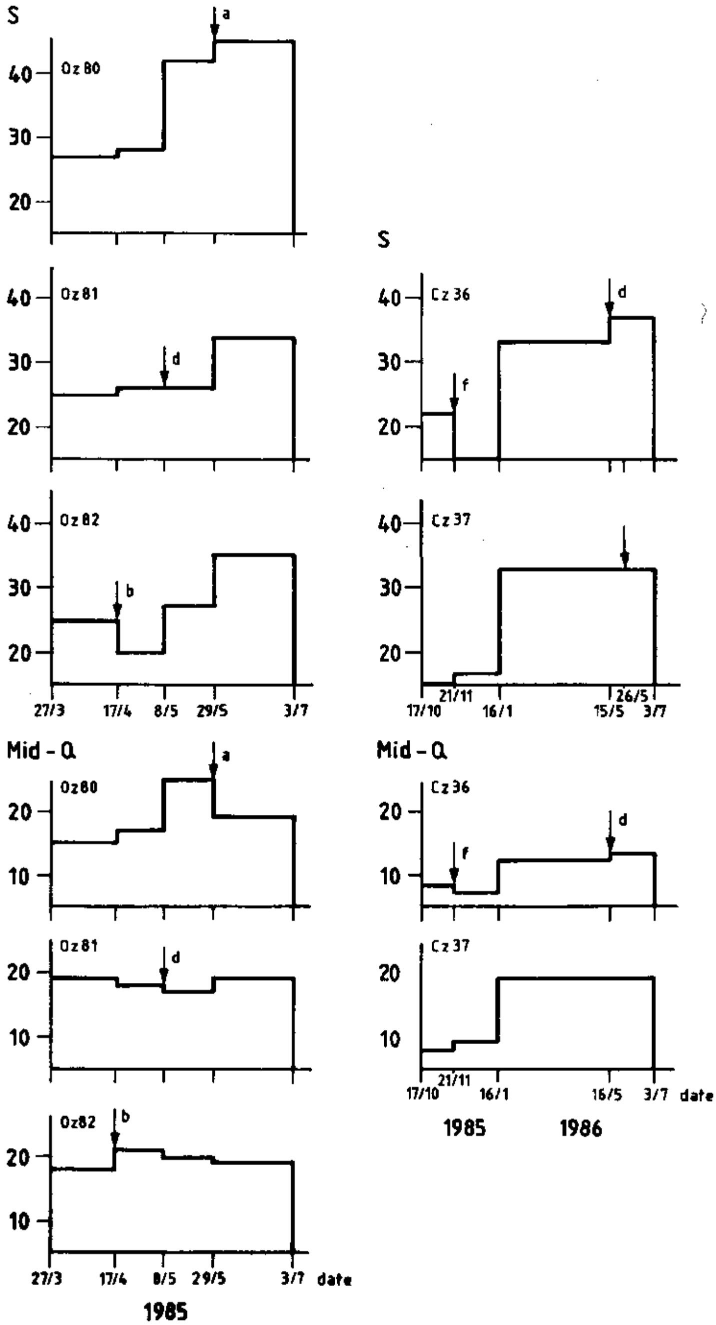


Figure 2.8 Species Richness (S) and Mid-Q Diversity of the linyphiid and erigonid spiders in the fields included in the Programmes I and II. The dates mark the periods between treatments which are indicated by arrows. a = herbicide; b = bromophos-ethyl; d = deltamethrin; f = fenitrothion.

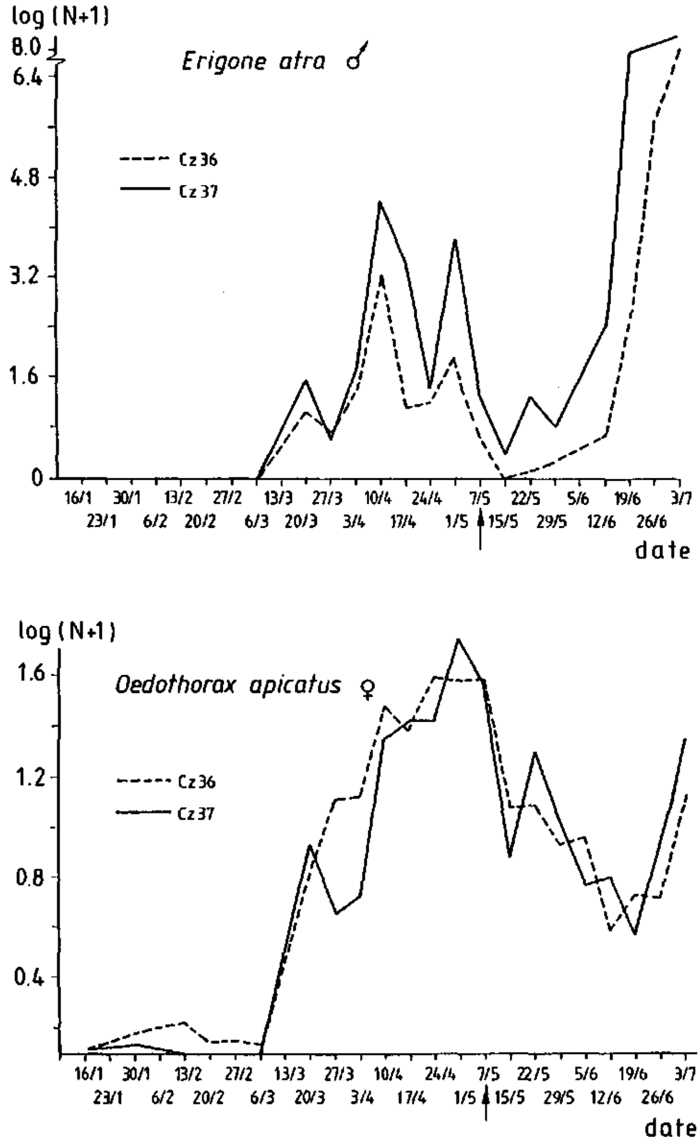


Figure 2.9 Numbers per trap of *Erigone atra* males and *Oedothorax apicatus* females in the fields sampled in programme III. Treatment with deltamethrin is indicated by an arrow.

In spite of the apparent correlation between the population declines and pesticide treatments in Programme I, the observed changes need not be an effect of the insecticides applied, bearing in mind the possible differences induced by the different crops. In order to establish a causal relationship to the pesticide treatments, a comparison was made with the results from Programme III.

Material and methods of Programme III

The third Programme studied the effects in time and space of deltamethrin treatment on the linyphiid spider species, which were selected as indicators in Programmes I and II. Field Cz36 was sprayed commercially with deltamethrin in May 1986 (table 2.1, Figure 2.5). Unlike all the other treatments the spraying was carried out under rainy weather conditions.

In field Cz17 two experiments were carried out. The first experiment (on 6 May 1986) concerned the effect of deltamethrin at field dose in one plot (Cz17.1) with two control plots: one treated with water (Cz17.2) and one untreated (Cz17.3). The second experiment was carried out on 27 June 1986 on the same plots. (At that time the population of *O. apicatus*, affected by the preceding treatment, had fully recovered.) This experiment concerned the effect of deltamethrin at different dosages (i.e. 5 and 10 g a.i. ha^{-1}) with an untreated control (table 2.1, Figure 2.5).
Bioassays were carried out in both tests with captive spiders. The animals were exposed in open containers (dimensions: l x h x w = 30 x 26 x 13cm), with soil and litter on the bottom

Table 2.5 Correlation coefficients of the spiders from cereal and oilseed rape fields, during periods preceding insecticide treatments in rape fields. In programme III the correlation is given over the whole sampling period (underlined: significant at $p < 0.05$; broken line: $p < 0.10$)

	Programme			
	I		II	III
Field codes				
oilseed rape	Oz81	Oz82	Cz36	Cz36
winter wheat	Oz80	Oz80	Cz37	Cz37
Number of sampling dates	7	4	3	22
Trechus quadristriatus	-	-	95.3	-
Clivina fossor	75.2	-	-	-
Oedothorax apicatus m.	95.7	63.3	51.3	99.0
O. apicatus/retusus f.	81.2	72.5	28.0	89.8
Meioneta rurestris	81.0	98.0	-	-
Erigone atra m.	92.4	97.1	-	75.2
E. atra f.	61.0	83.3	-	-
Lepthyphantes tenuis	-	-	43.2	-

Table 2.6 Mortality (%) of captured spiders in fields treated with deltamethrin and at untreated control sites.

Field Code	Cz17.3	Cz17.1	Cz17.2	Cz36	
Treatment code		11.1-d	11.2-d	7-d	
Dosage (g a.i. ha^{-1})	0	5	10	0	5
Oedothorax apicatus					
number exposed	32	33	26	31	28
mortality	19	67	54	0	100
Erigone atra					
number exposed	5	8	8		
mortality	40	87	100		

and caterpillar glue (Tanglefoot®) on the inner side of the walls to prevent the spiders from escaping. At the times of application, the containers were put in both the treated and untreated control sites. Mortality was recorded 96 hours after exposure.

Results of Programme III

E. atra and *O. apicatus* were common in field Cz36 but they showed no significant reduction compared with the untreated field Cz37 (Figure 2.9) despite the high concentration of pesticide at ground level (> 80 % of field dose). The numbers of other species were too low to draw any conclusions.
In the field experiments, however, a dose-related response to deltamethrin treatment was shown by *O. apicatus, E. dentipalpis* and *E. atra*, (table 2.4, Figure 2.10), while the water treatment did not induce a reduction in numbers (Figure 2.11). Although not dose-related, *B. gracilis* and the tetragnathid *Pachygnatha clercki* were also affected by the deltamethrin treatments.

The results of the bioassays are presented in Table 2.6. In the first test, which was carried out during deltamethrin spraying on Cz36 (treatment code 7-d, rable 2.1), mortality was 100 %. In the second and third tests, in which deltamethrin was sprayed in single and double dosage, respectively (treatment codes 11.1-d and 11.2-d) there was an increased mortality in the treated containers. This mortality, however, was lower than in the first test and it was not dose-related.

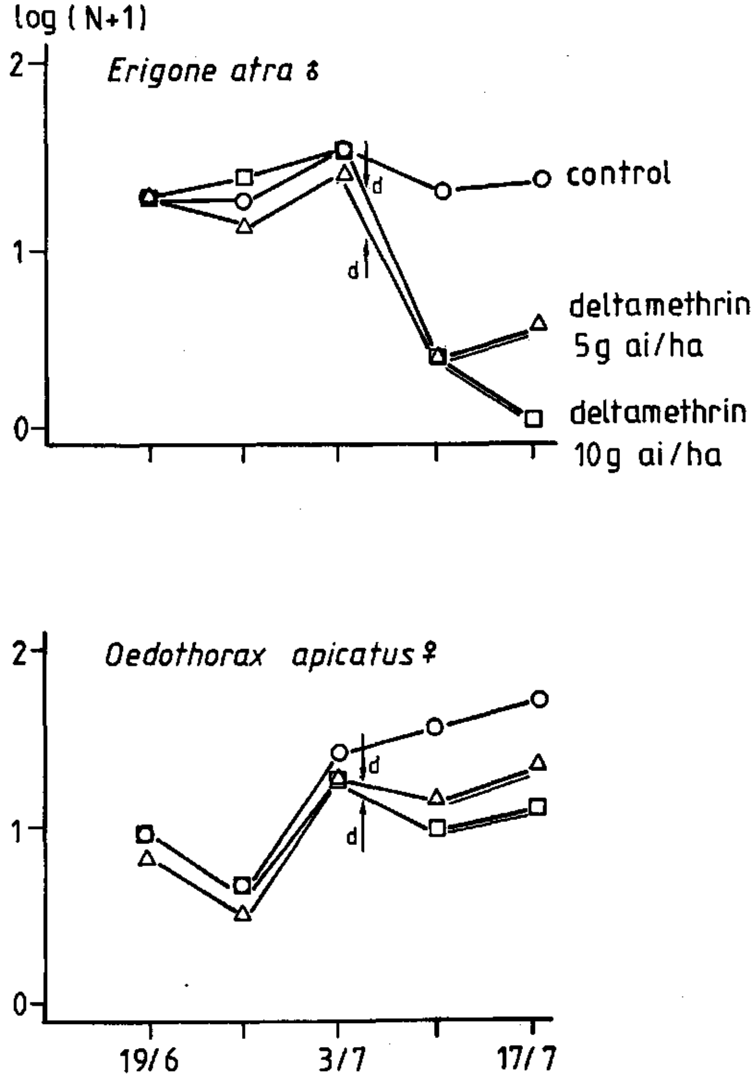
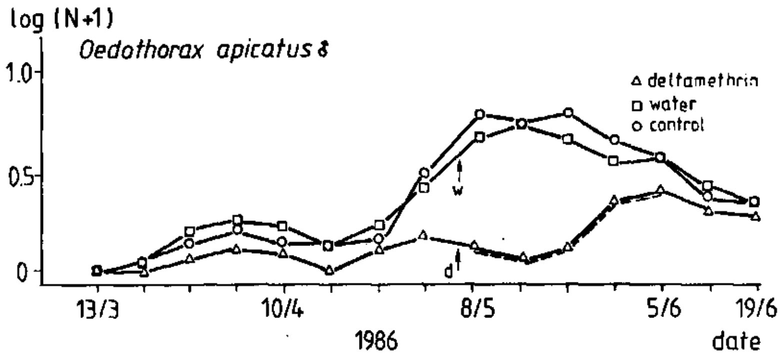


Figure 2.10 Numbers per trap of *Erigone atra* ♂ and *Oedothorax apicatus* ♀ in test plots treated with different dosages of deltamethrin and in an untreated control plot (field: Cz17).



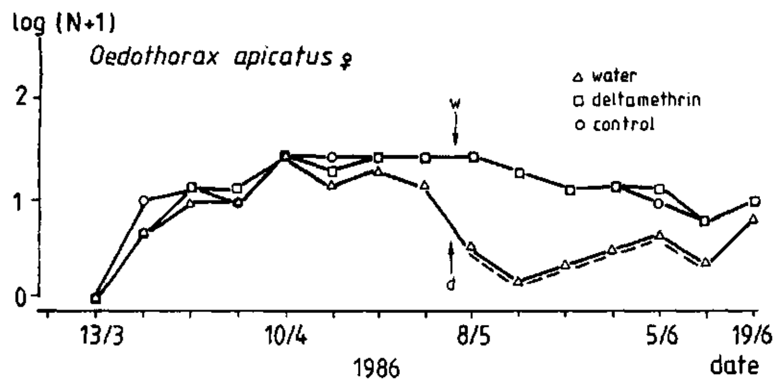


Figure 2.11 Numbers per trap of *Oedothorax apicatus* in test plots treated with deltamethrin and with water and in an untreated control plot (field: Cz17).

Variation within the field was analysed using data from the fields Oz81, Oz82 and Cz36. In the latter field, species richness and abundance differed markedly between sites near the edge and further to the centre of the field, especially during the winter (respectively 38 and 21 spp). In Figure 2.12 the phenology of *L. tenuis* at various distances from the edge is given as an example. The results, however, justify the presumption that the community sampled in Cz17 had mid-field characteristics. (A detailed study of the within-field variation of Cz17 has been carried out by G. Thomas. His results will be published elsewhere).

When Taylor's Power Law was applied, no difference was found in aggregation of the most abundant species in time (Oz81) and in space (Cz36) after treatments by pesticides (Figures 2.13 and 2.14).

Correlation coefficients of the numbers of *O. apicatus* and *E. atra* from Cz36 and Cz37 have been calculated over the whole sampling period (table 2.5). The correlations are high, indicating that the cereal field was a good control for the rape field throughout the season.

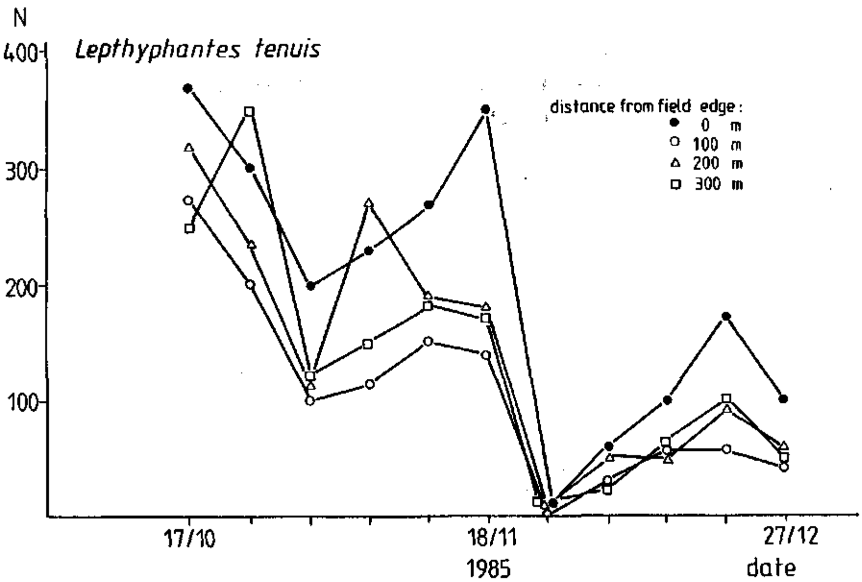


Figure 2.12 Numbers of *Lepthyphantes tenuis* at various distances from the edge in field Cz36.

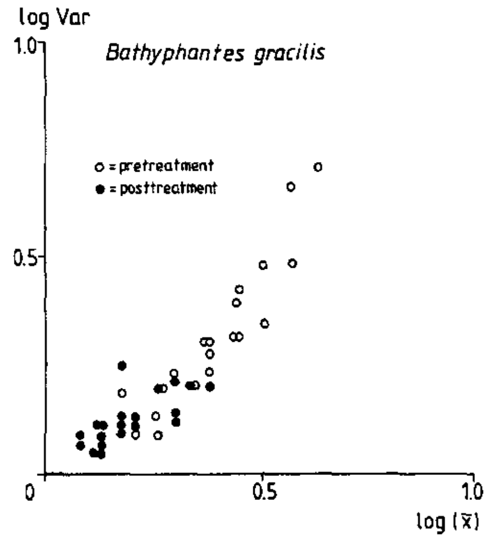


Figure 2.13 Spatial distribution of *Bathyphantes gracilis* in a field before and after a treatment with insecticides, expressed by Taylor's Power Law.

Combined results

Table 2.4 gives a summary of the changes observed in spiders after the different insecticide sprayings. Six species decreased after the deltamethrin treatments, and two after the bromophos-ethyl and the fenitrothion treatments. *Meioneta rurestris* and *Erigone atra* appear

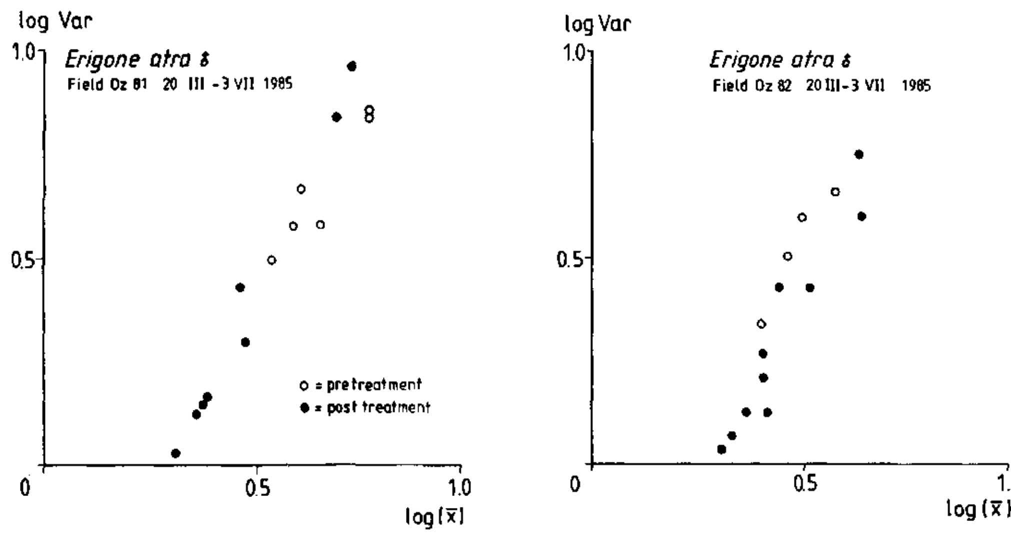


Figure 2.14 Spatial distribution of *Erigone atra* in time over periods before (open dots) and after (closed dots) an insecticide treatment, expressed by Taylor's Power Law

to be more sensitive than *Oedothorax apicatus* to bromophos-ethyl and deltamethrin. There is a marked difference, however, in the effect of deltamethrin, depending on the condition under which it is applied. In the experimental field Cz17 the effect appeared to be dose-related. When divided by the logarithm of the dose, the effect also appears to depend on temperature. In Figure 2.15 the reduction in numbers of *E. atra* and *O. apicatus*, observed on 6 occasions after deltamethrin treatments is given at the different temperatures. When sprayed under rainy conditions, however, deltamethrin did not reduce these species, despite the high deposition on the soil.

Discussion

The results of this study demonstrate the importance of environmental factors on the response of populations of non-target organisms to deltamethrin spraying. There is considerably more impact on the populations under dry and hot, than under cold or rainy conditions. Here we disagree with the literature reviewed by Ruigt (1984) concerning the relation between bioavailability and toxicity of the compound on the one hand, and moisture of substrates and temperature on the other. For most species there is a negative correlation between temperature and toxicity of deltamethrin, whilst residues of pyrethroid are more toxic to a noctuid in wet than in dry soils (Tolman et al. 1981). The explanation of our deviant results could be the following. In most studies with arthropods the temperature-toxicity relationship has been established in short-term laboratory tests, in which mortality was used as the main measure of effect (LD_{50} tests). Pyrethroids, however, are known for their long lasting knock-down effect, followed by either death or recovery. This knock-down effect may last for up to 4 days (observed by the authors). The effect of temperature on knock-down can differ from that on mortality (Ruigt 1984) and in the field other mortality factors, such as predation and drought

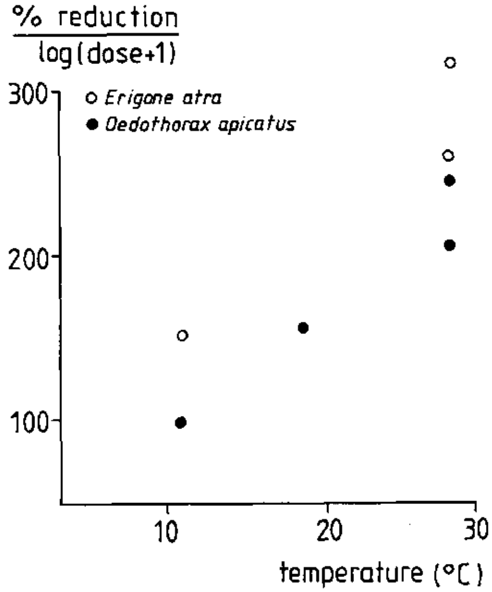


Figure 2.15 Reduction in numbers after correction for the deposition at soil level of two erigonid species after field applications of deltamethrin at varying temperatures. "dose" = deposition at soil surface level.

could interfere with this knock-down (e.g. a paralysed spider cannot escape from predators or drought). It is known that one effect of pyrethroids is an increase of diuresis (Soderlund 1979 and observed by the authors), which may reduce resistance to drought. This could explain the small effects observed after spraying under rainy conditions.

Recovery from the effect of deltamethrin in Cz17 has been described in detail by Thomas (1988). An analysis of the underlying mechanisms, required for an extrapolation to the commercial field situation, will be published elsewhere.

Data on temperature and humidity are rarely given in comparable studies. Despite the fact that these factors may have affected their results, some comparisons with other studies can yet be made. Toxic effects of deltamethrin on spiders have been observed previously in oilseed rape and cereals. The authors found a reduction of > 90 % at applications at 7.5 g a.i. ha^{-1} in Linyphiidae and "small spiders" (Rzehak and Basedow 1982, Basedow et al. 1985) and 60 % in Erigonidae (Fischer and Chambon 1987). In the present study, a marked difference in sensitivity between the species was found in sensitivity under different field conditions. The family-level observations, therefore, may lead to an underestimation of effects. The high toxicity of fenitrothion and bromophos-ethyl to spiders has not been reported before.

The use of guilds of species in ecotoxicological field studies, as proposed by Petersen (1986), appears to be an appropriate approach for the present situation. It concerns a group of taxonomically related species that use resources in the same way. In the present case, the guild would include the following species: *Oedothorax apicatus*, *Erigone atra*, *E. dentipalpis*, *Meioneta rurestris* and *Bathyphantes gracilis*. The response to the treatments observed in members of this group correlated with effects observed in species richness of the spiders. The group represents > 90 % of the Erigonidae and Linyphiidae in our fields, and throughout northern and western Europe (Nyffeler 1982, Thornhill 1983, Sunderland 1987). Spiders appeared to be a sensitive taxon with regard to insecticides (see also Basedow 1985 and Sunderland 1987) and this guild may represent a useful tool for monitoring programmes for side-effects of these compounds.

For two reasons it is very unlikely that the lumping of females of *O. retusus* and *O. apicatus* will give rise to erroneous conclusions. A specialist's check-up of a representative number of samples showed that at most 10 % of the complex belonged to the former species. In a toxicity test (Chapter VI) this species appeared to be less sensitive than *O. apicatus*. Conclusions concerning the toxicity of these pesticides in the field may therefore have been slightly biased, though in a conservative way.

There was no reduction of the taxa studied following the use of the herbicides MCPA/MCPP and 2,4-D. The acute and long-term toxicity of these pesticides to arthropods is known to be low (Edwards 1970, Prasse 1979). The use of herbicides, however, may cause unfavourable changes to the vegetation structure for spiders (Duffey 1974, Raatikanen and Huhta 1968). The effects of an alternative method, mechanical weeding, are discussed in Chapter III.

Similarly, the effect of ploughing on the population of O.apicatus was much as the most toxic effects observed in this study. Edwards (1977) and Blumberg and Crossley (1983) demonstrated a generally harmful effect of cultivation on epigeal arthropods.

The species *Erigone atra* and *Oedothorax apicatus* can be recommended as indicator organisms in ecotoxicological tests for three reasons. Both species can be reared in the laboratory, according to methods developed by the authors (to be published elsewhere) and De Keer and Maelfait (1987); they are generally abundant and easy to collect in various vegetation and soil types, and they are relatively sensitive to many insecticides.

Conclusions

Out of 145 taxa of epigeal predator arthropods identified in this study, seven spider species were shown to be particularly sensitive to field applications of either deltamethrin, fenitrothion, or bromophos-ethyl. The species represented 13 % of the total numbers of predatory arthropods caught.

A guild comprising the most abundant and sensitive species, i.e. *Erigone atra*, *E. dentipalpis*, *Oedothorax apicatus*, *Meioneta rurestris* and *Bathyphantes gracilis*, may be a useful tool for the early detection of side-effects of insecticides on the above-ground terrestrial fauna of arable land.

Erigone atra and *Oedothorax apicatus* are of interest as potential ecotoxicological indicators (test species) for the terrestrial environment.

Under the conditions of the present study, the effect of deltamethrin on populations of spiders was affected by temperature and soil humidity: populations were more reduced under dry and hot, than under rainy and cold conditions.

Acknowledgements

We thank the following persons for their invaluable assistance: JHJ van den Berg, H Bouwhuis, K Beurskens, T Buyse, H van den Heuvel, B Lankester, P Nieuwveld, M Diependaal, R Postuma, A Scholtens, M Hoekstein, N Goedejohan, J Lahr carried out the practical work; Dr H van der Voet developed the test for short time series, Dr Th Heijerman identified many of the carabids; Dr C Roghair was chairman of the advisory comittee; J Wouters, J Daling, Dr J Meems, DJ van 't Zet, P van Andel welcomed us at their farms and provided much technical assistance and Drs F Moriarty, F Florelli, Th van Dijk, H van der Valk and N van Straalen read the manuscript. The study was partly financed by the Netherlands Ministry of Housing, Public Planning and the Environment.

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CHAPTER III

VARIATION IN LINYPHIID AND ERIGONID SPIDERS IN ARABLE LAND RELATED TO FACTORS OTHER THAN INSECTICIDES

JW Everts, B Aukema, R Hengeveld and JH Koeman

Abstract

The present study concerns the long-term impact of cultivation history and the short-term effects of chemical vs. non-chemical weeding in cereals, and of ploughing, on the erigonid and linyphiid spiders in the South Flevoland Polder. The long-term effects were observed in a transversal study using 12 commercially farmed fields divided into 4 groups with a similar cultivation history. The variation in the composition of the spider communities in these fields appeared to be related to the distance between the fields rather than to their history. The spiders in cereal fields that were regularly treated with herbicides showed a higher diversity than those from mechanically weeded fields. The effect of ploughing was tested in an experimental plot. Ploughing had a detrimental effect on the dominant species, *Oedothorax apicatus*. An additional treatment with fenitrothion (500 g ai/ha) did not add much to this effect.

Introduction

In Chapter II linyphiid and erigonid spiders have been introduced as indicators for side-effects of insecticides. It was demonstrated that the abundant species are easy to sample and identify, and that they are regular in numbers and sensitive to a number of insecticides. The usefulness of a biological group as an indicator, however, also depends on the specificity of the response. The present study is therefore concerned with the variation in the community of these spiders related to non-chemical factors. The impact of the following factors was studied:

a. cultivation history since reclamation
b. chemical vs. non-chemical cultivation methods
c. drastic mechanical alteration of the topsoil.

Cultivation history
A transversal study was carried out in 12 commercially farmed fields widely distributed throughout the South Flevoland Polder (Figure 2.1). The fields formed 4 groups with similar cultivation history (table 3.1). The pesticides generally used in oilseed rape are fenitrothion or methiocarb, bromophos-ethyl or -methyl and phosalone or deltamethrin. Pirimicarb and a number of fungicides and herbicides are applied in cereals. Since insecticides are more toxic to arthropods than fungicides and herbicides and in view of the known low toxicity of pirimicarb to spiders (De Clercq and Pietraszko 1985; Cole and Wilkinson 1984; Powell et al. 1985) possible long-term effects on spiders were expected to be related to the preceding cultivation of oilseed rape. This would imply a stronger effect in group 3W than in group 1B (table 3.1). The spiders were sampled over a 4 weeks' period, from 30/5 to 27/6 1986 with 8 pitfall traps per field. Linyphiid and erigonid spiders were identified into species. The composition of each spider community was tested against the Renkonen similarity index (Renkonen 1938) and against the Mid-Q diversity index (Kempton and Taylor 1976). Differences between the groups were tested for significance by analysis of variance (ANOVA).

Chemical vs. non-chemical cultivation
As a part of the study mentioned above the spider communities of cereal fields that were cultivated by non-chemical methods (group 0B + 0W) was compared with that of the regularly treated fields in the South Flevoland Polder (Figure 2.1, Table 3.1). The data were combined with those from Oz80 and Nz27, which were sampled during Programme I (Chapter II).

Soil cultivation
Ploughing is one of the most drastic interventions in the top soil system. In the present study a test was carried out to assess the effect of ploughing on the linyphiids and erigonids. The experiment was conducted in the experimental plot Cz17 (Figure 2.4). In each sub-plot 16 pitfall traps were installed which were emptied weekly. After one week's sampling, one subplot was ploughed; the second was ploughed and sprayed with fenitrothion at 500 g ai/ha, and the third was left untreated as a control. Post-treatment sampling was continued for 5 weeks.

(The fact that there was only one pre-treatment sample was justified by earlier observations which showed a high similarity between the subplots (Chapter II)). The post-treatment numbers of the spiders were compared by discriminant analysis with a programme of SPSSX (1986) using sampling dates as variables.

Results and discussion

The numbers of the dominant erigonid spiders trapped in the 12 cereal fields are presented in Table 3.2. They were analysed by ANOVA. There is no difference between the field groups. The similarity of the species composition of these fields is presented in a dendrogram (Figure 3.1). Fields with similar cultivation history were not closely related but fields that are close to each other show a high similarity. The fields can be divided into two main groups:

Table 3.1 Cultural history of the fields included in Programme IV. B = summer barley; O = oilseed rape; S = summer wheat; W = winter wheat. OB and OW are chemical-free fields, the other fields undergo the normal pesticide treatments. - = field not yet under cultivation.

Field Group	OW	OB		1B			2W			3W		
Code	Nz	Nz 26	Ez 27	Gz 30	Gz 5	Nz 48	Cz 112	Dz 62	Ez 29	Nz 35	Nz 10	Nz 112
Year												
1984	O	-	S	O	O	O	-	-	-	O	O	O
1985	B	W	O	W	W	W	O	O	O	O	O	O
1986	W	B	B	B	B	B	W	W	W	W	W	W

those situated north and those situated south of the highway. The combined results of this study and of Programme I, however, consistently indicate a lower diversity of spiders in fields that are harrowed instead of sprayed against weeds (Figure 3.2; $F = 10.1$; $p < 0.001$).
These observations are not in agreement with Vickerman (1988), who found markedly higher numbers of linyphiids and various other taxa in winter wheat fields in integrated farm systems than in chemical farms. On the other hand, Booij and Noorlander (1988) analysed the carabid fauna in a variety of crops in conventional integrated and organic cropping systems in the North-East Polder, which bears great resemblance to the South Flevoland Polder. They demonstrated that both short-term and long-term effects were obscured by other management factors related to differences in crops. This difference between the British and the Dutch experience is assumed to be partly due to the use of less selective aphidicides (such as demeton-s-methyl and pyrethroids) in Britain than in the Netherlands (mainly pirimicarb). As a

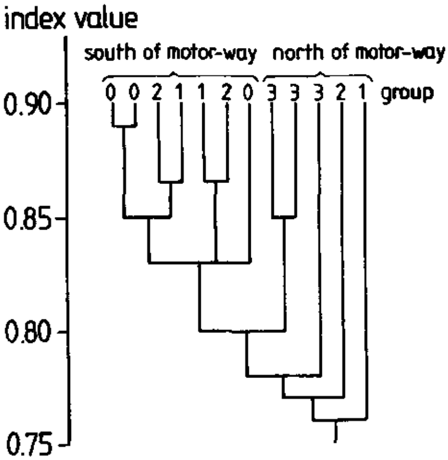

Figure 3.1 Similarity in spider communities in 12 fields grouped into four groups of equal cultural history. 0 = group 0B and 0W, 1 = 1B, 2 = 2W, 3 = 3W. The codes refer to the groups mentioned in table 3.1.

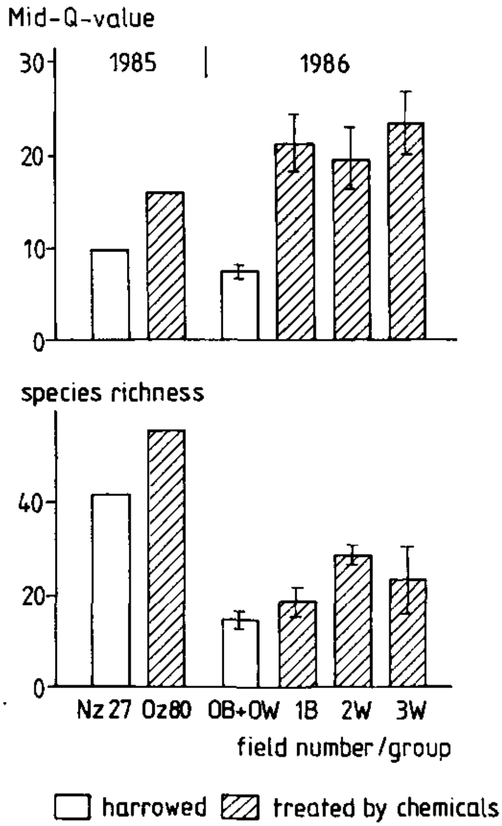

Figure 3.2 Species richness and Mid-Q diversity of Erigonidae and Linyphiidae in cereal fields treated with herbicides or weeded by harrowing. The codes 0B to 3W refer to the groups mentioned in table 3.1.

consequence the effect of other disturbing factors, such as harrowing, are more pronounced in The Netherlands.

The effect of ploughing is shown in Figure 3.3. There was a marked effect on the dominant erigonid species, *O. apicatus* (other species were virtually absent). The additional treatment with fenitrothion did not add much to this effect.

These observations agree with the results of Edwards (1977) and Blumberg and Crossley (1983) who studied the effects of cultivation practices on epigeal arthropods at the order level. They demonstrated a generally harmful effect on this fauna.

Regarding the use of erigonid and linyphiid spiders as indicators for side-effects of insecticides, the results imply that fields selected for monitoring should be situated as near to the control situation as possible. As the spiders are highly sensitive to mechanical alteration of their microhabitat, their use should be limited to observations on short-term effects.

Conclusions

Differences between erigonid and linyphiid spider communities of cereal fields in the South Flevoland Polder are related to distance rather than to cultivation history.

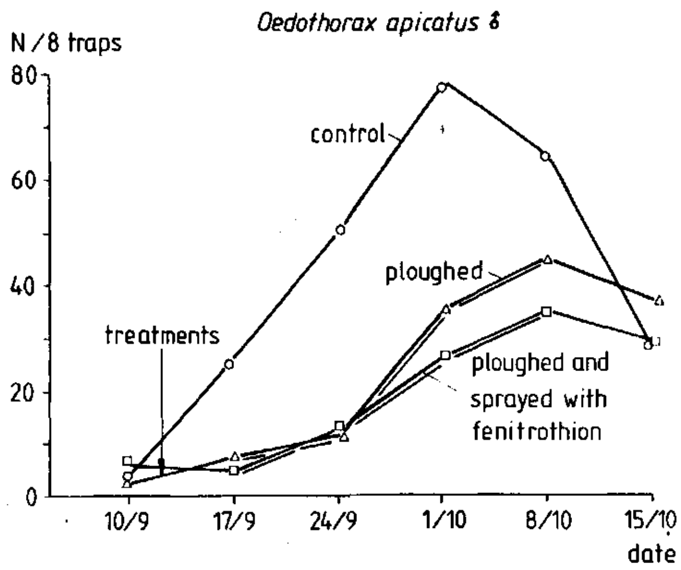

Figure 3.3 Numbers of *Oedothorax apicatus* ♂ from three plots in field Cz17, before and after the indicated treatment. Double lines indicate periods that differ significantly from the untreated control ($p < 0.005$)

The diversity of linyphiid and erigonid spiders is lower in fields that are weeded by harrowing than in fields that are treated by herbicides.

Ploughing causes a strong reduction in erigonid spiders.

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Table 3.2 Mean numbers of three abundant spider species in four groups of cereal fields with different cultural history and significance of differences between species and groups. n.s. = not significant. Group codes refer to table 3.1

Species	Group			
	OB+OW	1B	2W	3W
Erigone atra	127.7	280.3	622.3	331.0
E. dentipalpis	34.0	86.7	115.7	92.0
Oedothorax apicatus	119.3	65.3	83.7	47.3

Source of variation	F	Significance
Species	7.37	$p < 0.005$
Groups	1.43	n.s.
Species x Groups	1.13	n.s.
Explained	2.34	$p < 0.05$

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PART 3: LABORATORY EXPERIMENTS

CHAPTER IV

EXPOSURE OF THE GROUND DWELLING SPIDER *OEDOTHORAX APICATUS* (BLACKWALL) (ERIGONIDAE) TO SPRAY AND RESIDUES OF DELTAMETHRIN

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Abstract

Experiments were carried out on the exposure to deltamethrin spray, the availability of residues and the uptake with food with *Oedothorax apicatus* a member of the indicator group previously selected in the field. Exposure in the field, of spiders and their major prey items collembolans to the spray, was measured by means of a fluorescent tracer. The quantities of spray fluid recovered from these organisms was lower than the quantity measured on soil surfaces exposed to the spray on the soil. The availability of residues on soil was assessed by exposing individuals to sprayed soils of varying moisture content, to a soil showered after spraying and by exposing topically treated spiders to unsprayed soils. The effects of the residues were strongly related to the water content of the soil. On sprayed soils at field capacity, the toxic effects were highest. A shower shortly after spraying reduced the effect of residues considerably. On very dry soils (12.5 % of field capacity) high effects were also observed both on sprayed and unsprayed soils. This effect could be attributed to unfavorable air humidity conditions near the soil surface. The relative contribution of the three routes of uptake, i.e. exposure to spray and to residues and consumption of contaminated prey, was studied with radio-labelled deltamethrin. Spiders were exposed to sprayed soil surfaces (moisture content 75 % of field capacity), topically treated and fed with topically treated fruit flies. The latter route contributed little to the body burden. The amount of deltamethrin absorbed from the soil was twice the amount absorbed after topical treatment. It was concluded that, compared with oral uptake and exposure to spray, exposure to residues on the soil was the most important route of uptake of deltamethrin for *Oedothorax apicatus* (Blackwall).

Introduction

Oedothorax apicatus (Blackwall) was chosen as a good representative of the indicator group identified in the previous field study for laboratory studies with deltamethrin. The species is easy to handle and to grow and very abundant in the field. Exposure, toxicity and the factors interfering with, and modifying the toxic effect of deltamethrin were examined. Deltamethrin ((S)-α-cyano-3-phenoxybenzyl(1R,3R)-cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate) is currently used in oil-seed rape in a number of European countries and has been the subject of other terrestrial ecotoxicological field observations by the same authors under different conditions (Everts et al. 1983, 1985).

The present paper concerns observations on the exposure and routes of uptake. The factors modifying and interfering with toxicity are presented in Chapter V. The exposure of ground-dwelling arthropods to a spray is determined by the deposition of the spray at ground level. The routes of uptake are direct contact with the spray, contact with contaminated surfaces and consumption of contaminated food. In this study we measured deposition on the soil and on individuals of *O. apicatus* and of their prey in the field.

The availability of residues on a soil surface depends on the composition of the soil and its moisture (Harris and Turnbull 1978). In this study, we observed the effect of moisture content of the soil on the residual toxicity of deltamethrin. In the field studies mentioned above it was observed that, when sprayed under rainy weather conditions (i.e. rain shortly after spraying) deltamethrin did not reduce the populations studied. Therefore, the effect of a simulated rain shower on the toxic effect of deltamethrin residues on soil was also studied.

At a given deposition, the oral uptake of the insecticide depends on the amount of contaminated food items consumed and the availability of the compound. In this study, we measured the amount of food eaten and the resulting uptake. The last part of the study concerns the relative contribution of these different routes to the total body burden and the related effects.

Material and Methods

Deposition

Observations on deposition of deltamethrin on soil and epigeal fauna were carried out on 27 May and 29 June 1987 in four plots in field Gz5, a 75ha oil-seed rape in South Flevoland (fig. 2.1). The field was sprayed on both occasions in 24 m wide swathes at a height of 0.6 - 0.7 m above the canopy with swing nozzles (pressure 4 bar). Weather conditions are given in Table 4.1. The vegetation was in flowering and seed setting state. Deltamethrin was applied as Decis Flow 25 [R], in a 0.1 % concentration in 200 l/ha (5 g ai/ha). Droplet deposit on the soil surface was measured by means of water-sensitive paper (Ciba-Geigy) located on the soil and the top of the canopy. Droplet size and density were then determined by means of a Quantimet [R] Surface Pattern Analyzer (Cambridge Instruments). Deposit at soil level was estimated from the difference in deposit at the two levels, assuming that deposit at vegetation level represents the field dose. Deposit on spiders and food items (collembolans) was measured as follows. Uvitex OB [R] (benzoazole-2,2-(2,5-

thiophenediyl)bis(5-(1,1-dimethyl(ethyl))) was added as a fluorescent tracer (1.5 g/l) to the spray fluid. This formulation is highly soluble in organic solvents which assures association with Decis flow ®. After the spraying, the arthropods were caught by hand in the field and stored individually in ethyl acetate. Within 24 hours, the solution was evaporated and the fluorescence was measured in 100 % ethanol in a spectro-fluorimeter at 375 nm excitation and 435 nm fluorescence. Deposition was calculated after gauging with topically treated spiders and collembola. The values were compared with the deposit on the water-sensitive paper, representing the maximal exposure of an object on the ground.

Test animals

Laboratory toxicity tests were carried out with both captive and reared spiders. *O. apicatus* were caught by pewter in the Lake IJssel Polder area. They were kept individually at 20 °C and at an air humidity of 95±3 %. They were fed with collembolans and fruit-flies. Some specimens were used for breeding. Details of the rearing method have been published by Aukema et al.(in press). The method assures over 90 % survival and a full cycle of 30 days.

Effect of soil moisture on availability

The effect of soil moisture on the availability of deltamethrin to *O. apicatus* was studied with females of this species in a series of bio-assays. Three tests were carried out; one with spiders caught in the field and two with spiders reared in the laboratory. Observations were made with soils of varying moisture content. In the first test, plastic cups with a diameter of 9 cm containing 100 g soil were sprayed by means of a spray tower designed by Ten Houten and Kraak (1949). The tower ensures a deposit of 1 mg of spray liquid per cm^2. The soil used was collected from a field in the polder area which has never been treated with chemicals since reclamation (Nz27, Figure 2.1). It is a moderately humic clayey soil with the following characteristics: pH 6.6; carbonates 6.9 %; organic matter 4.3 %; clay 28.3 %; silt 28.2 % and sand 30.0 %. The water retention curve of this soil was determined by methods according to Richards (1965). For this purpose metal rings of 100 cm^3 were packed with moist soil and placed on sand or clay tables or in a pressure membrane apparatus for the low (0 - 0.1 and 0.2 - 0.5 bar) and high (2.5 - 15 bar) suction values, respectively. The 100 bar suction value is determined by air-drying the soil. Soil used for the toxicity tests was dried for 24 hours at 80 °C. Tap water was then added to obtain moisture contents of 5, 10, 20 and 30 % wet weight. The soil was divided among three series of cups. In each series there were 10 cups per moisture class. The soil of the first series was treated in the spray tower. In the second series treated spiders were exposed to untreated soil and in the third series untreated spiders were exposed to untreated soil as a control. The soil of the first series was sprayed at 0.5 g ai/cm^2, an equivalent of a field dose of 5 g ai/ha. Ten spiders per moisture class were exposed individually to the treated soil for 144 hours, at a relative air humidity of 90±5 %, at 20±2 °C. Effects were recorded after 2, 4, 8, 24, 48, 72, 96, 120 and 144 hours of exposure. Spiders used in the second series were exposed topically to 2.5 ng of deltamethrin in 25 nl Decis flow ® solution using a Burkard Electric Microsyringe Applicator. They were incubated under the same environmental conditions as the first series. The effects recorded were: no effect (code 0), incoordination (code 1), paralysis (code 2) and death (code 3). The second test was carried out with reared spiders. Design and methods were the same as in the first test, with two modifications. In this test soil was sprayed with a Potter Precision Laboratory Spray Tower (Potter 1952) and the observations were carried out at 16, 40, 64, 88, 112, 136 and 160 hours after the

Table 4.1 Field data and deposition of deltamethrin sprayed as Decis[R] over oil-seed rape
[1] gram active ingredient per individual

FIELD DATA

Plotnumber	1	2	3	4
Date (1987)	27/5	26/6	29/6	29/6
Dose (g ai/ha)	10	20	20	20
Wind speed (m/s)	4.4	2.6	2.6	2.6
Temperature (°C)	8	12	12	12
Relative Air Humidity (%)	70	78	78	78

DEPOSITION

Soil (g ai/ha)	1.7	2.8	10.0	4.0
Spiders (g ai/ind x 10^{-9} [1])				
Calculated Maximum	0.85	1.4	5.0	2.0
Measured Mean	0.39	0.28	0.48	0.35
Standard Deviation	0.47	0.30	0.56	0.31
Collembola (g ai/ind x 10^{-9})				
Calculated Maximum		0.7	2.5	1.0
Measured Mean		0.11	0.15	0.10
Standard Deviation		0.08	0.11	0.07

beginning of exposure. The third test was also carried out with reared spiders. In this test, the spiders were exposed to treated soil only. The same methods of application and observation were used as in the first test, under the same environmental conditions. In this

Table 4.2 Levels of significance of differences in toxic effect of Decis[R] between moisture levels of soil and periods of observation. The analysis was carried out with data from the whole observation period ([1]) including all moisture levels ([2]) as well as with groups of moisture levels ([3]) and shorter periods ([4]). n.s. = no significant difference.

Groups of observations				Levels of significance			
Origin	Exposure	Period h	Moisture %	Main effects	Time	Moisture	Explained
Captured	Residual	2-144[1]	5+10+20+30[2]	.000	.090 (n.s.)	.000	.000
		2-144	10+20[3]	.652 (n.s.)	.605 (n.s.)	.555 (n.s.)	.555 (n.s.)
		2-144	5+40	.003	.002	.102 (n.s.)	.003
	Topical	2-144	5+10+20+30	.019	.424 (n.s.)	.002	.019
		2-24[4]	5+10+20+30	.000	.000	.058 (n.s.)	.000
		24-144	5+10+20+30	.004	.460 (n.s.)	.001	.004
Reared	Residual	5-144	13.5+20+30	.025	.715 (n.s.)	.002	.028
		16-160	5+10+20+30	.000	.000	.002	.000
		16-160	20+30	.014	.058 (n.s.)	.002	.014
		16-160	5+10	.000	.000	.002	.000
	Topical	2-144	5+13.5+20+30	.004	.002	.258 (n.s.)	.004
		2- 48	5+13.5+20+30	.000	.000	.281 (n.s.)	.000
		48-144	5+13.5+20+30	.081 (n.s.)	.386 (n.s.)	.025	.081 (n.s.)

test, however, soil moisture contents were 13.5, 20 and 30 % wet weight. An extra series was showered after treatment, simulating 4 mm rain.

The qualitatively recorded effects were quantified by summation of the code values. The null-hypothesis of no difference between the series of observations was tested by analysis of variance. For a quantitative comparison of effects in different situations the integrals of the time-series were used.

Routes of uptake
The relative contribution of the three routes of uptake to the body burden was measured by exposing spiders to Decis flow [R] containing 14C-labelled deltamethrin. Fifty four laboratory reared spiders were treated topically at 1.3 ng/spider in 25 nl solution, 48 spiders were exposed to polder soil at 26 % wet weight moisture content, sprayed in a Potter Tower (Potter 1952) at 0.16 μg ai/cm^2. In order to reduce mortality during the test the dosages were lower than the ones used in the other tests. A third group of 24 spiders were fed fruit flies (Drosophila melanogaster) treated with deltamethrin in the Potter Tower at the same dosage as the spiders. Flies were fed to the spiders immediately after being treated. A fourth group was exposed to the spray as well as to the treated surface and they were offered treated flies. The study was carried out at an ambient temperature of 25 °C. Body content of deltamethrin was measured by liquid scintillation counting.

The maximal period of exposure was 144 hours for both the residually and the topically exposed groups and 72 hours for the orally exposed spiders.

Results

Deposition
The results of the deposition measurements in the field are given in Table 4.1 and Figure 4.1. The theoretical exposure to the spray of the organisms was calculated by multiplying the estimated deposition at ground level, in g ai/ha, by their horizontal surface. The exposure in the field of the spiders and the collembolans to Decis was highly variable and far below the maximum calculated dose. Recovery, expressed as the measured dose divided by the calculated dose, is presented in Figure 4.2. Mean and standard deviation of recovery of spiders were 27.8 and 40 % and of collembola 5.8 and 4.6 % respectively. The frequency distributions are highly skewed to the right with median values near to zero. These results indicate that the majority of these organisms are not fully exposed to the spray.

Effect of soil moisture on availability
In all tests the soil was fairly dry and all moisture contents tested were below field capacity (pF 2.0), except for the 30 % level, which is equal to field capacity. Moisture contents of 5 and 10 % are below the wilting point (pF = 4.2). In Figure 4.3 a graphical presentation is given of the effects observed in the first toxicity test, in which captured spiders are exposed to sprayed soils of varying moisture content. Some spiders recover after an initial sublethal effect (codes 1 and 2). The toxic effects are most marked on the very dry and the very wet soils. The totals of the coded values for the effects observed in the three tests are presented in Figures 4.4 and 4.5. In Table 4.2 the results are given of testing for significance of differences between various groups of moisture classes and periods. The initial effect of the

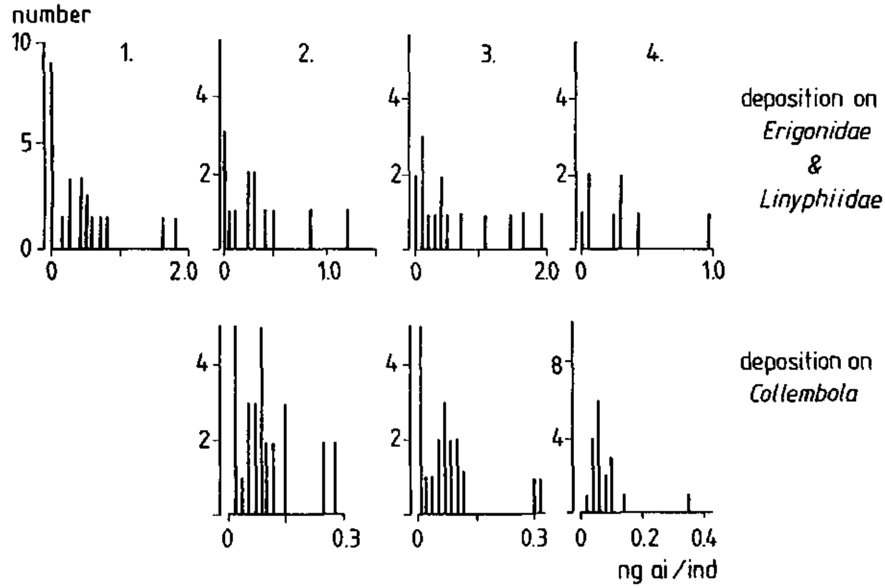


Figure 4.1 Frequency distribution of deposit of deltamethrin spray, in ng of active ingredient, on erigonid and linyphiid spiders and collembolans in four plots (1-4) in an oil-seed rape field

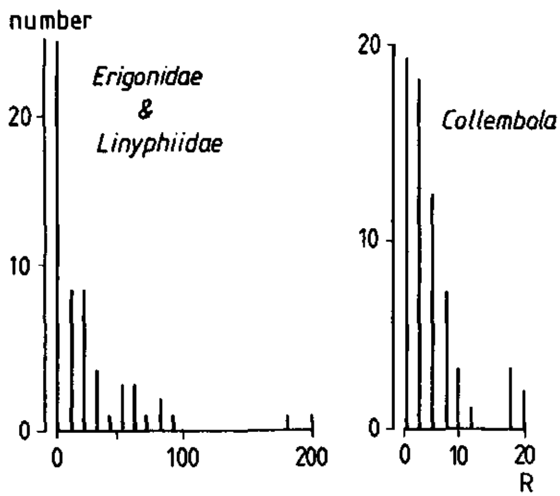


Figure 4.2 Frequency distribution of the recovery of deposit of deltamethrin spray on erigonid and linyphiid spiders and collembolans in an oil-seed rape field expressed as the percentage of the calculated maximum deposit (R).

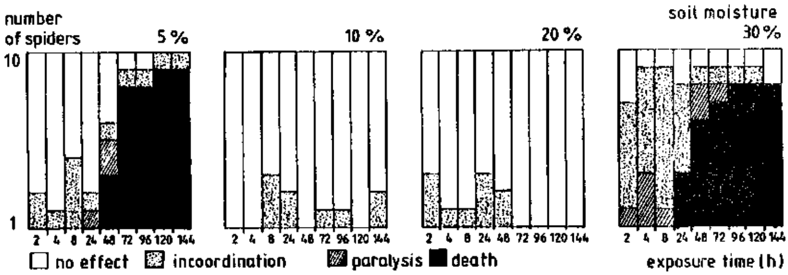


Figure 4.3 Toxic effect in time of deltamethrin sprayed on soils of different moisture content on captured *Oedothorax apicatus* (Blackwall) ♀.

residue is highest on the wettest soil (30 %), while the final effect is highest on the driest soil (5 %). In the topically treated group as well as in the control, mortality is highest at 5 % moisture content. At 20 % moisture content the reared spiders are more sensitive to the residue than the captured spiders. From the results of the reared spiders two groups of responses can be discerned: at 5 and 10 % (low initial toxicity and high final effect in the driest soil) and at 13.5 to 30 % in which a higher initial effect can be observed followed on the driest soil, by low final effect (i.e. high recovery). In the topically exposed reared spiders recovery after 48 hours is almost complete, except on the driest soil. The results of two different tests with the same soil moisture content (Figure 4.5: 20 and 30 %) are consistent.

In Table 4.3 the integrals are given of the response curves. In order to clarify the effects shown immediately after treatment the scale from 2 to 24 hours has been enlarged. The ratio topical: residual effect increases with soil moisture. The ratio is different for captured and reared spiders at comparable soil moisture classes.

The toxic effects of deltamethrin residues after a simulated rain are presented in Figure 4.6. Compared to the effects observed on not-showered surfaces of 13.5, 20 and 30 % moisture (Figure 4.5), the residual activity was reduced by 97, 92 and 97 % respectively.

Routes of uptake

The mean and standard deviation of the number of contaminated fruit flies eaten per spider after 72 hours was 15 and 4.5, respectively. The total radio activity measured after 72 hours of exposure to the different sources of contamination is given in Table 4.4. The uptake of residues from the soil accounts for more than half of the total body burden, despite the relatively high topical dose (1.4 times the equivalent of the residual dose). The contaminated food does not seem to play an important role. Details on clearance will be published elsewhere.

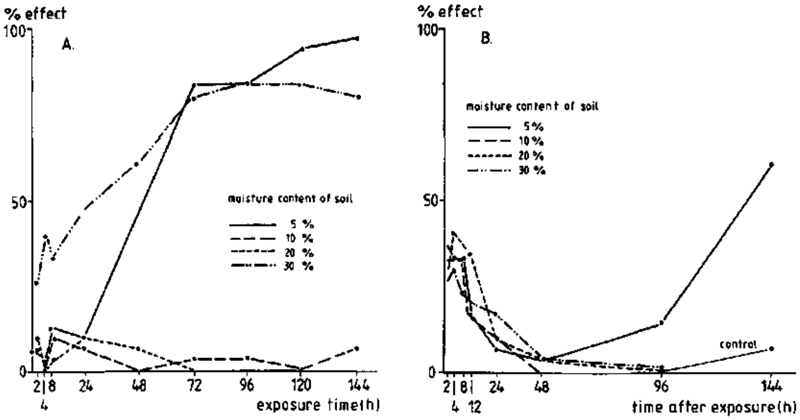


Figure 4.4 Toxic effect in time of deltamethrin sprayed on soils of different moisture content (A) and applied topically (B) on captured *Oedothorax apicatus* (Blackwall) ♀ and mortality in untreated controls

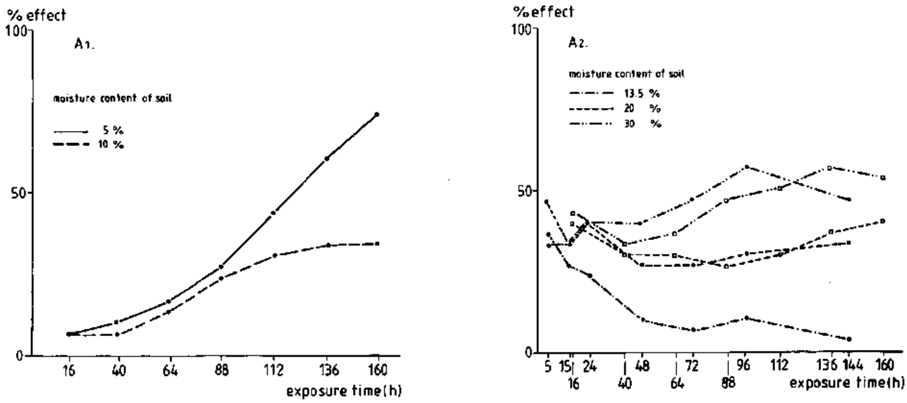
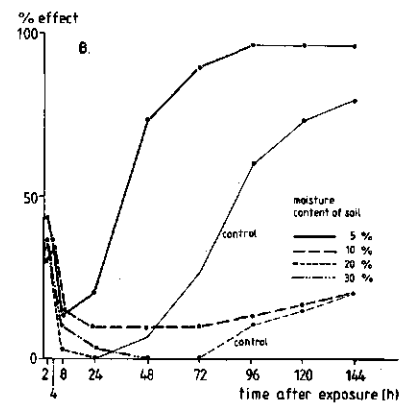


Figure 4.5 Toxic effect of deltamethrin sprayed on soils of different moisture content (A1, A2) and applied topically (B) on laboratory reared *Oedothorax apicatus* (Blackwall) ♀ and mortality in untreated controls



Discussion

The observed direct exposure to the spray in the field of both spiders and collembola was markedly lower than the estimated maximum exposure. This low recovery is assumed to be due to the behaviour of the organisms. Usually the spiders hang upside down in webs in cavities in the soil and are therefore not fully exposed to the spray reaching the soil. Furthermore, it was observed that the spiders start preening immediately after exposure to the spray.

Table 4.3 The toxic effect of Decis[R] on *Oedothorax apicatus* ♀ expressed by the integrals of the response curves
[1]r = residual; s = residual, showered; t = topical; c = control; [2]- = no observations; [3] mean values of two observations

% moisture of soil	captured spiders				reared spiders				
	r[1]	t	c	r/t	r	s	t	c	r/t
5	1309	958	245	1.4	750	-[2]	459	32	1.6
10	67	186	27	0.4	420	-	86	0	4.8
13.5	-	-	-	-	227	2	-	0	-
20	67	24	0	2.7	759[3]	22	140	0	5.4
30	1563	51	0	30.5	907[3]	10	135	0	6.7

The higher uptake, under maximum exposure conditions, from sprayed surfaces rather than from the spray, as was measured in the spiders and reflected by the difference in effect, may be attributed to the difference in exposure time. The residue on the soil may remain available for a few days in contrast to the spray.

The concentration of the Decis Flow [R] solution used for topical application is four times higher than the concentration of the field spray. Although the quantity of active ingredient is equivalent to the maximum field exposure the high lipophility of this solution compared to the field spray may give rise to a higher absorption. The possible overestimation of the effect of exposure to the spray, however, has no consequences for the conclusions drawn from the results of this study.

The effects shown in Figure 4.3 seem to be highly related to the moisture content of the soil. Direct water uptake from the soil by spiders at moisture levels below wilting point is unlikely. Direct uptake of deltamethrin combined with drought stress may therefore explain the strong effect at 5 % moist. At this moisture level, the soil may take up water from the air, and spiders may even lose water through contact with the soil. The moisture content of 20 % is still far below field capacity so water uptake from the soil was probably low. At field capacity (30 %), however, direct water uptake from the soil by the spiders may have been possible. Probably this accounts for the strong effect observed at 30 % water content.

Table 4.4 Radio-activity (dpm) measured in *Oedothorax apicatus* ♀ exposed topically, residually and orally to ^{14}C labelled deltamethrin

	Activity		% of Total Activity
Period:	72 hrs	144 hrs	72.hrs
Exposure			
Oral	2580	-	12.4
Topical	6563	9443	31.4
Residual	11744	19784	56.2

These results are in agreement with Harris and Turnbull (1978) who found an increased toxic effect of pyrethroids with increasing soil moisture content, and with the study of Van Gestel and Ma (1988) who demonstrate that the aqueous phase of the soil is the major route for the transfer of chlorophenols to living organisms.

The reduced toxic effect observed after showering was in agreement with the field observations described in Chapter II. Probably deltamethrin is washed below the soil surface by rain. This was also suggested by Hill and Schaalje (1985) who found a delayed initial dissipation in the upper 2.38 cm of soil after rain.

Exposure to residues on soil and in food depends on the walking and feeding activity of the organisms. During the study it was remarked that walking speed is affected by sublethal dosages of deltamethrin. As a consequence there may be an interference between direct and residual uptake. Another possible reason for modified exposure due to behavioral factors may be the known repellency of the compound (e.g. Atkins et al. 1978). The effect of deltamethrin on the behaviour of spiders is described in Chapter V.

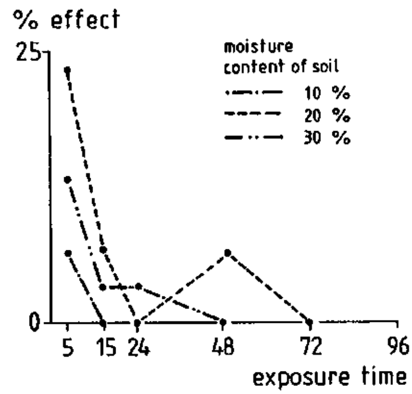


Figure 4.6 Toxic effect of deltamethrin sprayed on soils of different moisture content and showered with 4 mm water on reared *Oedothorax apicatus* (Blackwall) ♀

The difference in sensitivity between captured and reared spiders may be caused by many factors. The general condition of a field population is different from the laboratory population because the mechanisms for selection which are active in the field cannot be introduced in indoor rearing procedures. The laboratory population raised under high moisture conditions is presumably less used to (temporary) drought than the wild specimens. The reared spiders, therefore, are supposed to be more susceptible for the combined effect of drought and deltamethrin (see Chapter V) This does not explain, however, the low response at 30 % soil water content.

Conclusions

The exposure of Erigonidae and Linyphiidae and their main food items, collembolans, to Decis [R] spray in the field is considerably lower than the theoretical maximum exposure.

The toxic effect of residues of Decis [R] on the soil is strongly related to the water content of the soil. Effects are highest at water contents below wilting point and at field capacity.

Of the three main routes of uptake, i.e. consumption of contaminated prey, exposure to spray and contact with contaminated soil, the latter route is the most important, both quantitatively (highest uptake) and qualitatively (highest toxicity).

Acknowledgements

We thank the following persons for their invaluable help and advice: Mr J.H.J. van den Berg, S. van den Berg, J. Daling, Dr J. Meems, and N. Goedejohan. Procida Roussel Uclaf kindly provided the radio-labelled deltamethrin. We thank Dr. P. Oomen for his valuable comments on the manuscript.

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CHAPTER V

THE TOXIC EFFECT OF DELTAMETHRIN ON LINYPHIID AND ERIGONID SPIDERS IN CONNECTION WITH AMBIENT TEMPERATURE, HUMIDITY AND PREDATION[1]

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L. Simons, B. Aukema and J. Kammenga

Abstract

The first part of this study concerns the effect of temperature and air humidity on the toxicity of deltamethrin to the erigonid *Oedothorax apicatus*. The second part concerns the effect of deltamethrin on behaviour of linyphiids and erigonids with respect to their ability to escape from predators and to select between unfavourable (dry) and favourable (moist) habitat conditions. The toxic effect of deltamethrin was highest at the combination of high temperature and low air humidity. It was concluded that the spider's sensitivity to drought is increased by this pyrethroid. Affected spiders, however, are less able to select moist habitat conditions than unaffected ones. Walking speed of spiders was decreased by exposure to deltamethrin and their predation by carabid beetles was increased. It was concluded that the effect observed under field conditions is the result of a combination of neurological, physiological and behavioural disturbance.

[1]Accepted for publication in Archives of Environmental Contamination and Toxicology

Introduction

In a previous field study (Chapters II and III), the effect of deltamethrin on ground dwelling linyphiid and erigonid spiders appeared to depend on weather conditions. Effects were high under dry and hot and low under rainy and cold conditions. In a subsequent laboratory study (Chapter IV) it was demonstrated that deltamethrin residue was more toxic when applied to dry and very wet soils than on soils of intermediate moisture (i.e. between wilting point and field capacity). The latter was explained by the increased availability of the compound. An explanation for the increased effect on dry soils was sought in a combined effect of water stress and the toxicity of the compound. Under laboratory conditions, a large proportion of the exposed spiders recover from an initial sublethal effect. Sublethal effects (i.e. incoordinate walking or paralysis) may last up to four days. In the present study, the hypothesis is tested that a sublethal effect of deltamethrin may increase mortality due to drought and predation. These factors will affect survival if behaviour is altered to such an extent that escape from unfavorable conditions is hampered.

The study consists of two main parts:

1. The sensitivity of the spiders to deltamethrin at various temperatures and air humidity
2. The effect of deltamethrin on behavior in relation to ambient drought and predators

Material and Methods

Effect of Temperature and Air Humidity on Sensitivity to Deltamethrin

The effect of air humidity and temperature on the acute toxicity of deltamethrin was studied in two tests. In the first test, temperature was varied; no free drinking water was available. In the second test, both temperature and air humidity were varied; free drinking water was available.

The spiders used for both tests were laboratory reared *Oedothorax apicatus* (Blackwall) ♀ adults. They were kept individually in cups with a filter bottom (diameter 9 cm) and a gauze lid. The cups were kept in aquaria above aerated water or salt solutions. Air humidity was regulated by the composition of the salts, according to the method described by Winston & Bates (1960). The spiders were exposed to Decis Flow ®. 50 nl of a aqueous solution of 50 mg a.i./l (i.e. 2.5 ng deltamethrin) was applied topically to the dorsal abdomen by means of a Burkard Electric Microsyringe Applicator®. The dose used is the equivalent of a field dose of 5 g a.i./ha. The spiders's surface is about 5 mm^2. In both tests control groups were treated with 50 nl water. Responses were recorded as four distinct stages: no obvious effect, incoordinate walking, paralysis and death, coded respectively as effect 0, 1, 2 and 3. For the quantification of effects these coded values were summed over a batch of spiders. The combined effect of temperature and air humidity was analyzed by multiple regression analysis.

In the first test batches of 12 spiders were kept at 10, 20 and 30 °C in climatic chambers.

RH was maintained at 90 $\pm$ 5 %. Effects were recorded 2,3,4,5,6 and 12 hours after exposure. The test was carried out twice.

In the second test groups of 20 spiders were treated by the same methods and held at the same temperatures (10, 20 and 30 °C) but at varying RH (12, 33, 70, 85 and 100 %). Free drinking water was available to the spiders in 4 cm plastic tubes of 4 mm diameter. Effects were scored at 2,3,4,5,6,8,12,48,96 and 144 hours after treatment.

Effects on Behavior

Behavioral effects of deltamethrin in *O. apicatus* ♀ were observed in three tests. The first test examined the effect on walking speed; the second the effect on avoidance of unfavorable (i.e. dry) habitat conditions, and the third test concerned the effect on the capability to escape from predators.

In the first test the spiders' walking speed was measured in a round arena (25 cm diameter) which contained a moist cardboard bottom. Five groups of 10 laboratory reared *O. apicatus* which were acclimated to the test conditions were treated topically with 0, 0.25, 0.54, 2.5 and 5.4 ng deltamethrin. The spiders were observed individually during 3 minutes after being placed in this arena, 20 and 40 hours after treatment. Ambient temperature was 24 °C and RH was kept at 95$\pm$3 %. Behavior was recorded by a video camera. Walking patterns were redrawn on transparent paper and analyzed with an x-y tablet on a computer using a programme developed by the Department of Entomology, Agricultural University, Wageningen, The Netherlands. The response was analyzed by regression analysis.

In the second test preference for favorable moisture conditions was observed in Petri dishes (diameter 10 cm) with a clayey soil bottom. One half of the surface was moistened at 25 % wet weight and the other half was left dry. Fifteen spiders (reared *O. apicatus*) were treated topically with 0.25 ng deltamethrin and 15 spiders with water only. The dose was low to avoid mortality. Their position on the soil was observed 7 times at regular intervals during 168 hours after exposure. The difference in response between treated and untreated spiders was tested by analysis of variance.

For testing the capability of spiders to escape from predators, spiders were confronted in Petri dishes with two carabid species, *Pterostichus cupreus* and *Agonum dorsale*. In this test unidentified captured erigonids and linyphiids were used in stead of reared spiders for two reasons. Wild specimens are generally quicker than reared ones (they have been selected by predation) and the number of spiders needed for this test exceeded the rearing capacity of the laboratory. The soil in the dishes consisted of moist peat-dust; the temperature was 24 °C. Spiders were treated topically at 0.6 ng (0.25 x field dose) and the beetles (25 specimens per species per test) at 3.8 and 1.3 ng (2 x field dose) respectively, simulating low level field exposure of the spiders and high level exposure of the beetles. Four situations were created:

1. beetles untreated and spiders treated;
2. beetles treated and spiders untreated;
3. both beetles and spiders treated;
4. both untreated.

The consumption of the spiders which were fed ad libitum to the beetles was recorded during 7 days. Differences between the groups were tested by analysis of variance.

Consumption of linyphiid and erigonid spiders by *P. cupreus* was also studied in the field by gut content analysis of individuals from a sprayed and an unsprayed field in the South Flevoland Polder (The Netherlands). In two adjacent fields, one cultivated with winter wheat and the other with oilseed rape, beetles were trapped in pitfalls (20 per field in a 400 x 500 m grid). The oilseed rape field was treated with deltamethrin (5 g ai/ha). The gut contents of 10 % of the beetles of each trap, caught during the week before and one week after the treatment were analyzed for remains of linyphiids according to the method used by Hengeveld (1980).

Results

The summed effects of the treatments of the first repeated test are given in Figure 5.1. The values express the percentage of the maximum effect (i.e. all spiders dead) recorded. Although differing in absolute toxicity values, the trends are consistent. There is a positive temperature/toxicity relationship two hours after exposure. After 4 hours, however, the relationship changes: highest effect is observed at 30 °C and lowest at 20 °C with intermediate values at 10 °C. Towards the end of the test period, there is a slight recovery in the 30 °C group.

The summed results of the second test, two hours after exposure, are given in Figure 5.2 A. There is a negative relationship of toxicity with the temperature as well as with RH (Table 5.1) The effect of temperature is stronger than the effect of RH. Hundred forty four hours after exposure (Figure 5.2B), the effect at 30 °C is lowest at RH = 100 % and highest at RH ≤ 50 %. At RH = 100 % there is a negative correlation of effect and temperature ($r = -0.91$, $p < 0.05$).

Table 5.1. Multiple regression of the toxic effect of deltamethrin to *Oedothorax apicatus* ♀ at different temperatures and relative air humidity (RH). b = regression coefficient; s.e. = standard error; t = Student's t

	b	s.e. of b	t	significance of t
Temperature	-8.6	2.3	-4.7	0.0005
RH	-2.8	0.9	-3.2	0.01
constant	77.9	4.9	15.0	0.0001

F of multiple R = 14.1; $p < 0.005$

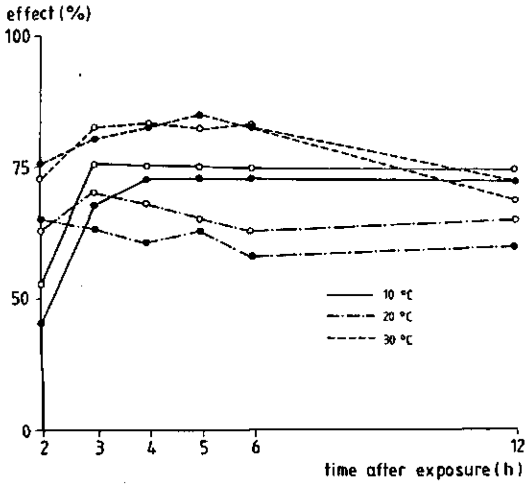
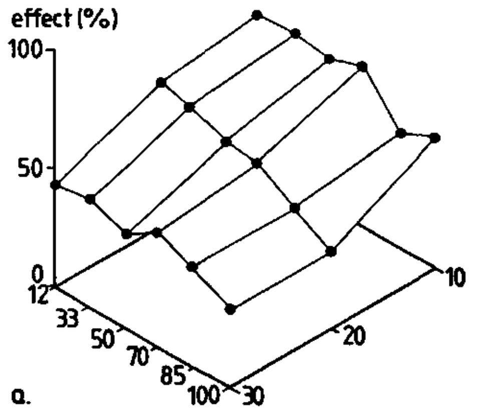


Figure 5.1. Effect (percentage of maximum) of 2.5 ng deltamethrin on *Oedothorax apicatus* ♀ at different temperatures during 12 hours of observation in two tests (black and white dots respectively).

Over the observation period, there is a faster increase in the observed toxic effect at 30 °C than at any other temperature. RH seems a major factor. In Figure 5.3 the development of the effect at 10 and 30 °C at 33 and 85% RH is given as an example. The time (i) at which the effects in both groups are inverted at the different RH values, transformed to ^{10}logi, is positively correlated with RH (r = 0.97, p < 0,01). At RH = 85 % and 100 % a relative



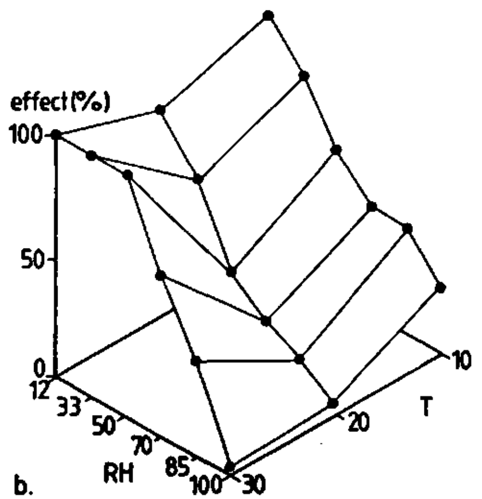


Figure 5.2. Three-dimensional presentation of the effect (percentage of maximum) of deltamethrin at different temperatures (T) and air humidity (RH) in *Oedothorax apicatus* ♀ at 2 (A) and at 144 (B) hours after exposure.

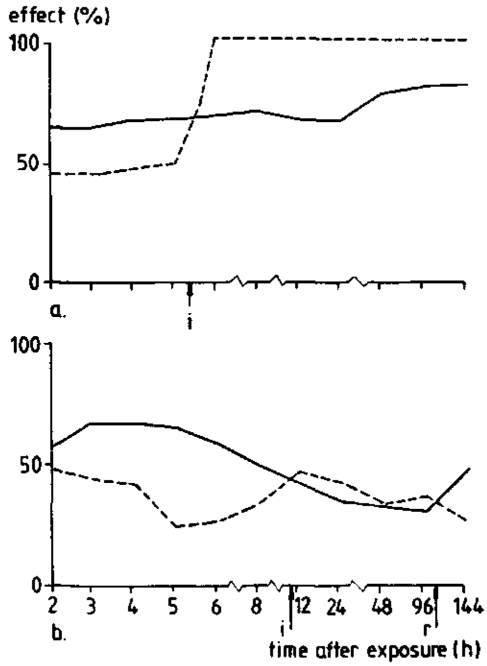


Figure 5.3. Effect (percentage of maximum) of deltamethrin in time (t in hours) on *Oedothorax apicatus* ♀ at different temperatures (T) and relative air humidity (RH). A = effect at RH 33 %; B = effect at RH 85 %. Solid line = 10 °C; broken line = 30 °C. i = inversion, r = recovery

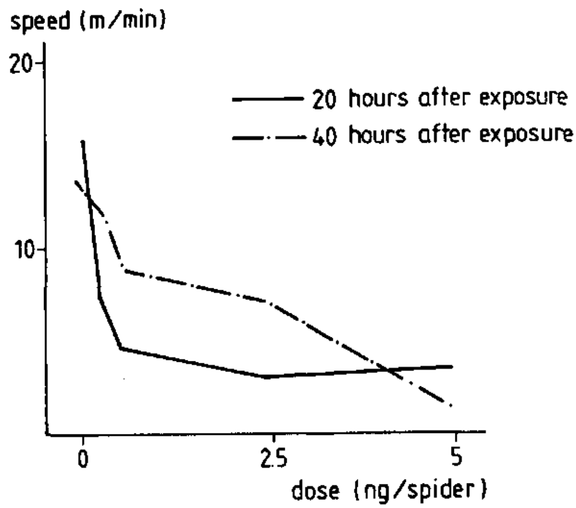


Figure 5.4. Mean walking speed in m/minute of *Oedothorax apicatus* females exposed topically to deltamethrin at two periods after exposure

recovery (r), compared to the 10 °C group, was observed in the 30 °C group 84 and 24 hours after the inversion respectively.

In Figure 5.4 the mean walking speed is given of the spiders at different dosages. Twenty hours after exposure the walking speed is significantly reduced as a function of the dose (declared variation $R^2 = 0.95$; $F = 52.5$; $p < 0.005$) and after 40 hours the decrease is significantly related to the logarithm of the dose ($R^2 = 0.82$; $F = 14.5$; $p < 0.05$).

The fraction of the spiders observed in the preference test on dry and moist soil has been presented for each effect class in Table 5.2. In the effect class 0 (i.e. no obvious effect) the preference for the moist condition is higher than the control group. In the other effect classes (paralysis and incoordination) the fraction observed at the dry site was higher. The effect of treatment was significant ($F = 1352$; $p < 0.001$).

In Figure 5.5 the number of spiders eaten by 25 carabid beetles per day is given for each group. There is a significantly higher consumption of spiders treated with deltamethrin than of untreated spiders ($F = 283$; $p < 0.0001$ in *P. cupreus* and $F = 35$; $p < 0.0001$ in *A. dorsale*). Exposed *A. dorsale* ate less contaminated spiders than unexposed individuals. In exposed beetles, the consumption of contaminated spiders decreased markedly during the observation period. A decrease was also observed in the consumption of contaminated spiders by unexposed *A. dorsale*.

The number of *P. cupreus* containing remains of linyphiids before and after a field treatment with deltamethrin are given in Table 5.3. The incidence of linyphiid and erigonid remains in the guts is low. Compared to the untreated situation, however, the consumption in the sprayed field was increased by a factor 8. During the analysis it was also observed that after treatment the guts were considerably more filled with many other prey items as well.

Table 5.2 Percentage of *Oedothorax apicatus* ♀ treated with deltamethrin and separated by effect class (mean ± standard error) observed on dry soil, in a soil moisture preference test. n = number of observations.

Effect Class	Percent on Dry Soil	n
0	2.5 ± 1.3	48
1	41.7 ± 10.3	36
2	57.0 ± 6.5	21
control	15.7 ± 5.2	105

Discussion

The data demonstrate that drought and predation alter the effect of deltamethrin on *O. apicatus*. The neurological effect of pyrethroids is negatively temperature dependent (Vijverberg et al, 1983) but in this study the negative temperature-effect relationship of deltamethrin was reversed by the effect of air-humidity and by the absence of drinking water. The observations of other authors who also observed positive temperature-effect relationships in arthropods (e.g. Sparks et al. 1982; Grafius, 1986; Schmidt and Robertson, 1986) may be explained by an effect on the water balance as well. Diuresis has been observed to increase in insects after exposure to pyrethrum (Ingram, 1955) and other insecticides (Casida and Maddrell, 1971). Gerolt (1976), however, demonstrated that contact with organophosphorous, carbamate, organochlorine and pyrethroid insecticides caused accelerated water loss from all major parts of the integument of *Musca domestica*. High humidity reduces the toxicity of a number of insecticides in *Antonomus grandis* (Gaines and Dean, 1949). Increased diuresis by anal excretion of fluid has been observed incidently during our tests. Because most terrestrial arthropods are sensitive to drought this factor may be more relevant in entomological ecotoxicology than generally accounted for.

An increased preference for moist conditions was observed in exposed spiders not showing an adverse effect (code 0). This could be explained as an increased sensitivity to drought.

A negative temperature/effect correlation was only found when free drinking water was available, shortly after exposure. After some time, (depending on RH) the effect at 30 °C increased faster than the effect at lower temperatures. In the group devoid of drinking water, however, the toxic effect at 10 °C increased faster than at the other temperatures. After 4 hours of exposure the effect at 20 °C was lowest, similar to the second test (except

Table 5.3. Number of specimens of *Pterostichus cupreus* containing remains of linyphiid spiders in sprayed and unsprayed fields. Rate = percentage positive from the sprayed field divided by the percentage positive from the unsprayed field. Effect = rate after divided by rate before spraying.

		Number Analyzed	Positive	Rate	Effect
Unsprayed	before	69	3		
	after	121	2		
Sprayed	before	52	2	0.88	
	after	62	6	7.06	8.02

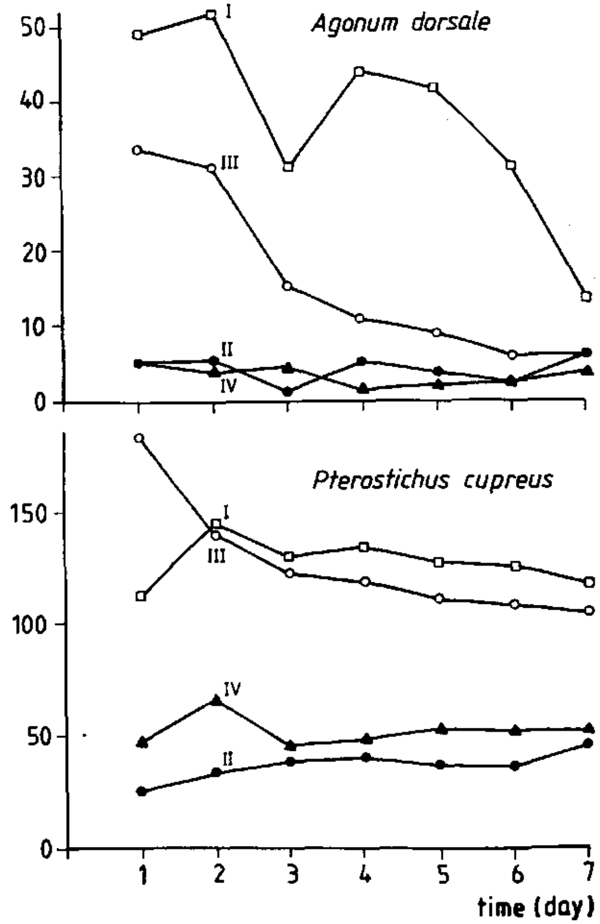


Figure 5.5. Number of linyphiid and erigonid spiders eaten per day by 25 specimens of *Agonum dorsale* and *Pterostichus cupreus* during 7 days of observation. I = beetles untreated and spiders treated; II = beetles treated and spiders untreated; III = both beetles and spiders treated; IV = both untreated.

at RH 100 %). There are apparently two processes active: the increased neurological effect at 10 °C (e.g. Vijverberg et al. 1983) and the increased effect of drought at 30 °C (Gaines & Dean 1949). A possible effect of temperature on the metabolism of the compound can be ruled out because that would have resulted in lower rather than higher effects at higher temperatures. In spiders water excretion is induced by high temperatures (Pulz, 1986). Spiders always drink when body fluids have been lost (Stewart and Martin, 1970) but at higher temperatures apparently drinking cannot compensate for the combined effect of deltamethrin and temperature. An enhanced water loss added to the reduced ability to reach the water supply would then result in stronger effects under hot and dry conditions. The recovery in the 30 °C group in both tests at high air humidity, however, may also reflect an adaptation of the spider's water balance to an initial disturbing effect of the pesticide.

Affected spiders appear to be more predated than unaffected ones. Both carabid species are very abundant in arable land in the Netherlands. Although exaggerated by the daily feeding with freshly treated spiders (in contrast to the field situation), the data suggest that consumption of contaminated spiders may give rise to a sublethal effect i.e. reduced consumption. In *A. dorsale* a combined effect of direct exposure and uptake via food was observed. Throughout the observation period, however, consumption of contaminated spiders both by treated and untreated carabids was higher than of clean spiders (except in *A. dorsale* at day 7 in group III). Although the incidence of spider parts in guts of *P. cupreus* from the field was low, the data strongly suggest the occurrence of an increased predation in a treated field. It is not known, however, to what extent predation by *P. cupreus* contributes to the mortality of *O. apicatus* in the field. Therefore, before extrapolating the data to the field situation it should be kept in mind that the test results are of a qualitative character.

The supposed effect of deltamethrin, based on the data presented thus far, is the following. Spiders which normally hang upside down in small nets in cavities and crevices in the soil are activated by the spray. (This activation shortly after spraying has been observed in the field at several occasions and is in agreement with literature data on the response of insects to pyrethroids (Ruigt 1984). During this short period of elevated activity they absorb a sublethal dose of the compound from the soil which leads to an increased sensitivity to drought but also to a reduced speed and henceforth to an increased exposure to drought and to predators.

Activity itself and, therefore, uptake of residue may be temperature dependent. However, because these spiders are normally active over a wide temperature range (4 - 30°C), the dependence on temperature of this route of exposure is expected to be limited.

The selection of microhabitats is most crucial in these web building ground spiders: thermal and hygric conditions, along with other abiotic factors and prey availability must rather precisely fit the physiological needs of the species (Pulz, 1986). The disturbance of both physiological homeostasis and the ability to select optimal microhabitats will markedly reduce their chance for survival.

Conclusions

When exposed to deltamethrin under varying temperature and air humidity conditions, *Oedothorax apicatus* ♀ show a high sensitivity at a combination of high temperature and low air humidity.

The species' sensitivity to drought is increased by deltamethrin.

Exposure of the spiders to sublethal dosages reduces their ability to escape from unfavorable (= dry) conditions and from predators.

Acknowledgments

We thank Mrs J. Eeftink and Mr T. Ariëns for their help in this project. Drs S. de Kort and H. Vijverberg are much indebted for commenting on the manuscript. We thank Mr H. van den Berg and Mrs A. Lam for technical support. The study was in part financed by the Dutch Toxicology Research Promotion Programme. We thank Prof Dr J.H.Koeman for his advise and stimulation.

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CHAPTER VI

TOXICITY OF DELTAMETHRIN TO DIFFERENT SPECIES, SEXES AND AGE CLASSES OF ERIGONIDAE AND LINYPHIIDAE

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Abstract

The toxicity of deltamethrin was examined on *Oedothorax apicatus* adults and juveniles and on *Oedothorax retusus*, *Erigone atra* and *Bathyphantes gracilis*. The spiders were exposed to sprayed surfaces (i.e. soil or glass fibre filter); to topically applied deltamethrin and orally (i.e. by offering treated prey). Juveniles were more sensitive than adults and males were more sensitive than females. Consumption of contaminated food gave rise to very slight toxic effects. The contribution of the other routes of uptake to the overall effect differed per species. The order of sensitivity, from most to least sensitive, when compared by means of the effect of exposure by both routes combined is as follows: ***O. apicatus*** ♂ > ***E. atra*** ♂ > ***E. atra*** ♀ > ***O. retusus*** ♂ = ***O. apicatus*** ♀ > ***O. retusus*** ♀. ***B. gracilis*** ♀ was as sensitive as ***O. apicatus*** ♂ when exposed to residue. This order is in agreement with field data.

Introduction

In arable land *Oedothorax apicatus* usually occurs together with *O. retusus*, *O. fuscus*, *Erigone atra*, *E. dentipalpis*, *Lepthyphantes tenuis*, *Bathyphantes gracilis*, and *Meioneta rurestris* (Nyffeler 1982, Thornhill 1983 and Chapter II). For an extrapolation of the toxicity data obtained from a single species test with *Oedothorax apicatus* to the other species the relative sensitivity of the species, sexes and age classes of this group should be tested and compared with field data. Furthermore, the females of *O. apicatus* and *O. retusus* are very similar anatomically and are therefore difficult to separate. In the field study described in Chapter II the species have been lumped together and their relative proportion has been estimated afterwards. It is questioned whether, and if so, to what extent, this lumping may affect conclusions concerning the toxicity of a compound to *O. apicatus*. In the present study, therefore, the toxicity of deltamethrin to various members of the association and to immatures is examined under laboratory conditions.

Material and Methods

Toxicity tests were carried out with spiders that were either obtained from the field or reared in the laboratory. The following tests were carried out:

1. A single dose comparison test with males and females of *E. atra* and *O. apicatus* and ♀ of *B. gracilis*, exposed via three routes: topical application, exposure to residue and oral uptake,
2. An LD_{50} test with a larval stage of *O. apicatus* exposed to residue,
3. An LD_{50} test with *O. apicatus* and *O. retusus* ♀ exposed to residue.

The spiders were kept and reared according to the methods described in Chapter IV and by Aukema et al. (in press). Captured *E. atra* and *B. gracilis* were used and reared *O. apicatus* and *O. retusus*. (*E. atra* and *B. gracilis* are difficult to grow under laboratory conditions.) In all tests 20 specimens per species and sex were used. The tests were carried out with Decis Flow ^{R}, e.c. formulation. The compound was administered to the spiders by the following techniques:

a. Topical application of 2.5 ng ai/ind applied to the dorsal abdomen by means of a Burkard electric microsyringe applicator in droplets of 50 nl.

b. Exposure to residue sprayed on soil at 50 ng ai/cm^2 in 0.05 ml solution per cm^2. The surfaces were sprayed with a Potter Precision Laboratory Spray Tower in plastic cups with a diameter of 91 mm and a gauze lid. The soil originated from the South Flevoland Polder in the Netherlands, from the chemical-free field Nz27 (Chapter II). The characteristics of the soil are given in Chapter IV. Before application the soil was air dried for 48 hours and moistened to 25 % (wet weight) water content. The cups were kept in an aquarium above aerated water, at an air humidity of 90$\pm$5 %

c. Oral administration. The spiders were fed daily with four collembolans which had been collected in the field and treated topically with 1.25 ng of deltamethrin. This quantity is equivalent to the field dose when the mean size of a collembola is 2.5 mm^2.

The following effects were distinguished: no visible effect (code 0), incoordination (code 1), paralysis (code 2), death (code 3).
The effects were recorded during 6 days.

Effects on immature *O. apicatus* were studied by exposing second instar spiders (first larval stage) to water saturated glass fibre filter (Whatman Glass Microfibre Filter GF/B), which was treated with deltamethrin according to the method described above. The filter is white and allows visual recording of effects on small specimens. Decis [R] was sprayed in the following dosages: 0.001, 0.01, 0.1, 0.25, 0.5, 1 and 2.5 ng ai/cm^2. Mortality was recorded after 7 days and LD_{50} values were calculated by probit analysis using the Statistical Analysis System (SAS Institute Inc. 1985). Parallel to this study a number of toxicity tests were carried out with adult *O. apicatus* ♀ exposed to treated glass fibre filter treated with different compounds. The results of these tests have been published by Aukema et al. (in

Table 6.1 Effect (mean coded values per spider) of deltamethrin to linyphiid spiders, exposed via different routes.
Range = classification in Duncan's Multiple Range Test. Numbers indicated by the same letter are not significantly different at $p < 0.05$. Rate = effect residual exposure: effect topical exposure

Species	Sex	Route of Exposure				
		Residual		Topical		
		Effect	Range	Effect	Range	Rate
Oedothorax apicatus	♂	6.7	ef	2.7	de	2.4
	♀	1.2	abc	0.8	bc	1.3
Oedothorax retusus	♂	0.4	a	1.6	d	0.3
	♀	0.6	ab	0.2	a	3.0
Erigone atra	♂	3.4	d	4.4	f	0.8
	♀	5.3	de	1.1	cd	3.8
Bathyphantes gracilis	♀	6.5	ef			

press), but the data on deltamethrin will be used here for a comparison of the sensitivity of the life stages.

The LD_{50} tests with ♀ of *O. apicatus* and *O. retusus* were carried out with five dosages (0.1, 0.6, 6.0, 60 and 600 ng ia/ind) and one control, applied topically to laboratory reared spiders. Mortality was recorded after 7 days. The LD_{50} values were calculated by probit analysis.

In all tests control observations were carried out with the same number of spiders as used for the different exposure routes and dosages. These spiders were treated with uncontaminated tap water.

Table 6.2 Number of spiders showing a coded effect of deltamethrin administered via food to linyphiid spiders, observed during 14 days.
t = treated with deltamethrin; c = untreated control
N = number of food items consumed per spider (*** = treated food consumed significantly less than untreated food, $p < 0.001$)

Species	Sex	Treatment	Effect Code 0	1	2	3	N
Oedothorax retusus	♂	c	260	0	0	0	23.6
		t	257	3	0	0	25.2
Oedothorax retusus	♀	c	210	0	0	0	12.4
		t	203	7	0	0	11.4
Oedothorax apicatus	♂	c	260	0	0	0	23.2
		t	272	6	0	0	22.4
Oedothorax apicatus	♀	c	263	6	2	9	10.8
		t	266	12	2	0	11.6
Erigone atra	♂	c	276	4	0	0	18.6
		t	271	8	1	0	12.3***

Results

The effects of topical application and exposure to sprayed surface observed in the single dose comparison test with four species are presented in Table 6.1. The table shows the mean effect score per spider over the observation period (mortality in the control groups was negligible in all tests). Little effect was observed in *O. retusus* and *O. apicatus* ♀ exposed to residues, while *B. gracilis* ♀ and *O. apicatus* ♂ were highly sensitive. Differences between taxa were tested by means of Duncan's Multiple Range Test (Snedecor and Cochran 1967) using the summed effect values. In Table 6.1 the values are compared. There is a significant difference in sensitivity between the species and sexes.

The order of sensitivity, from most to least sensitive, when compared by the effect of exposure by both routes combined is as follows: *O. apicatus* ♂ > *E. atra* ♂ > *E. atra* ♀ > *O. retusus* ♂ = *O. apicatus* ♀ > *O. retusus* ♀. *Bathyphantes gracilis* ♀ was as sensitive as *O. apicatus* ♂ when exposed to residue. When compared by the total effect females are less sensitive than males. When separated by route of uptake, however, there are two exceptions: females of *O. retusus* and *E. atra* exposed to residue are more sensitive than males. There is a marked difference with regard to the relative sensitivity to these two routes of uptake. The rate of residual/topical effect is highest in *E. atra* ♀ and lowest in *O. retusus* ♀.

Because the effect of the oral administration at day 7 was zero, this observation was prolonged by one week. The effects observed in this test are given in Table 6.2. Differences in behaviour and mortality in the control and the treated group have been tested by analysis of variance. There is a slight but statistically significant toxic effect of consumption of treated collembola (tested by analysis of variance; $F=15.3$ $p < 0.01$). Females consumed more than males. In one case (*E. atra* ♀) there was a reduced consumption of treated collembolans (tested by analysis of variance, $F = 22.1$, $p < 0.001$).

The LD_{50} value (7 days) of residue on glass filter for immature *O. apicatus* was 6.9 (2.4 - 19.8) ng ai/cm^2. LD_{50} (7 days) values of deltamethrin for adult *O. apicatus* ♀ vary between 47.6 and 63.3 ng ai/cm^2 (Aukema et al. in press).

The LD_{50} (7 days) values of topically applied deltamethrin to females of *O. retusus* and *O. apicatus* were 35.3 (95 % confidence limit: 14.5 - 96.5) and 3.8 (1.6 - 10.2) ng ai/ind respectively.

Discussion

The differences in sensitivity to deltamethrin between the species of these tests are generally in agreement with the field data. In Chapter II it was demonstrated that in the field *O. apicatus* ♀ are much less sensitive than *O. apicatus* ♂, *E. atra* and *B. gracilis*. This was confirmed by Thomas et al. (in press) who furthermore found *O. retusus* to be the least sensitive linyphiid in a cereal field.

The relevance of the different routes of uptake for *O. apicatus* is described in Chapter IV. The high toxic effect of deltamethrin after uptake from soil, compared to topical application has been confirmed by this study. It appears, however, that this relation varies considerably between species. Food appears to be of limited relevance as a route of exposure.

Because sex cannot be identified in the younger larval stages, the immatures used in this test were a mixture of males and females, very probably at a 50:50 rate. Males are more sensitive than females, which, in part, may have accounted for the observed high sensitivity of the immatures compared to females. The difference, however, shown between females and immatures exceeds the difference between males and females. It is, therefore, justifiable to conclude that immatures are more sensitive than adults.

The difference in sensitivity between life stages of a certain species cannot be tested in the field because of the impossibility of identifying immatures to species. In the field Thomas et al. (in press) found immature erigonids and linyphiids to be less sensitive than *O. apicatus*. The author, however, did not identify larval stages, and hatching between sampling dates may be the cause of these contradicting results.

O. retusus ♀ are less sensitive than *O. apicatus* ♀ and in the former species topical exposure is a more important uptake route than residual exposure. Conclusions, based on the effects observed in the complex *O. apicatus*/retusus ♀ may therefore be biassed with respect to the effect in either one of the species. Because in all situations hitherto studied *O. apicatus* outnumbered *O. retusus* by a factor 10 or more, the bias in conclusions regarding the former species will be limited and of a conservative nature. Depending on the characteristics of the compound to be tested, toxicity data obtained from laboratory tests with *O.*

apicatus may give rise to a slight underestimation of the effects in the field community. It is therefore recommended to include other, though less amenable species in routine testing, whenever possible.

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PART 4 : FINAL FIELD STUDY

CHAPTER VII

EFFECT OF DELTAMETHRIN ON THE ERIGONID SPIDERS *ERIGONE ATRA* AND *OEDOTHORAX APICATUS* IN OILSEED RAPE

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Abstract

In a field test, the effect was examined of Decis[R] (active ingredient deltamethrin) applied on various dosages, on erigonid spiders. The applications, on oilseed rape, were carried out in three repetitions on three dates with different weather and soil humidity conditions. Exposure was estimated by deposit measurements. The environmental factors known from previous laboratory studies to alter the toxic effect were recorded. These factors were weather, soil moisture and density of major predators (i.e. carabids). The effects on spiders were studied by pitfall trapping and by *in situ* bioassays. The results showed a stronger acute effect on *Erigona atra* than on *Oedothorax apicatus*. Recovery, however, was slower in the latter species. ED_{50} values were calculated for *E. atra*. The ED_{50} was lower on the date with low soil moisture content and low air humidity. The dose-response relationship, however, was different at the different spraying dates. The observed reduction was compared with the toxic effect predicted by a model based on previous laboratory observations. The model appeared to give a fair prediction of the observed effect.

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Introduction

The present study is the third phase in the combined field-laboratory study on the ecotoxicological effects of above-ground applications of pesticides on predatory epigeal arthropods in arable land. The purposes are:

1. to further develop the methodology for ecotoxicological field testing with this group;
2. to assess in the field the predictive value of laboratory observations on availability and toxicity of the compound studied.

In the previous field study (Chapter II) an assemblage of Linyphiidae and Erigonidae was selected as useful indicator for side-effects on predatory arthropods. Two species are specifically abundant in arable land during spring and summer, i.e. *Erigone atra* and *Oedothorax apicatus*. Species were selected with respect to their properties in field tests, i.e. abundance, regularity, sensitivity, recovery speed and amenability in *in situ* bioassays.

After a number of laboratory tests it was concluded that factors influencing the toxicity of deltamethrin are soil moisture, air humidity, temperature and predation (Chapters IV and V). In the present study these laboratory data were compared with results from the field situation. The toxicity of deltamethrin when applied to oilseed rape, was observed in three treatments carried out with different combinations of these factors.

Material and methods

The design of the test field, situated the northern part of the South Flevoland Polder, is depicted in Figure 7.1. The area is flat with clayey soil. The characteristics of the soil are the following: pH 6.6; carbonates 7 %; organic matter 4 %; clay 28 %; silt 28 % and sand 30 %. The soil is at field capacity at 30 % water content (wet weight) and at wilting point at 12 % water content. The conductivity is 50 mm/day and the bulk density is 1.2. A 75 ha field cropped to oilseed rape was divided into three parts, each divided into 9 plots. The shape of the plots, i.e. strips of 48 x 250 or 48 x 500 m was dictated by the limited manoeuvrability of the spraying equipment. Sites with obstructions such as pylons were excluded from the study area. Strips 50 to 100 m wide were left untreated on the east and west sides. In each group of 9 plots three cycles of treatments (numbered I to III, Table 7.1) were carried out with one control. The plots were treated with Decis Flow 20 (R). The treatments were carried out against *Ceutorrhynchus assimilis* (Oilseed Rape Weevil) and *Meligethes aeneus* (Oilseed Rape Glossy Bug). Treatment cycle I included one treatment which was carried out with two dosages; in cycles II and III (shown in Table 7.1) four dosages were applied in two treatments of three dosages each. The dosages varied from 2.5 to 20 g a.i./ha (5 g a.i./ha is the field dose for all treatments in oilseed rape in The Netherlands). Because the purpose of the study was to observe effects on the soil fauna, the dosages varied in accordance with the development of the covering of the soil by the crop. Therefore the dosages applied in the last test were higher than in the first test. Plots 7, 8 and 18 were untreated controls. The following variables were measured in the field: (1) density/activity of *Oedothorax apicatus* and *Erigona atra* (2) deposit of the spray on the ground in the treated field and of drift in the field down-wind (3) toxic effects in individuals exposed *in situ* bioassays (4) density of the main predator i.e. *Pterostichus cupreus* before and after the treatments.

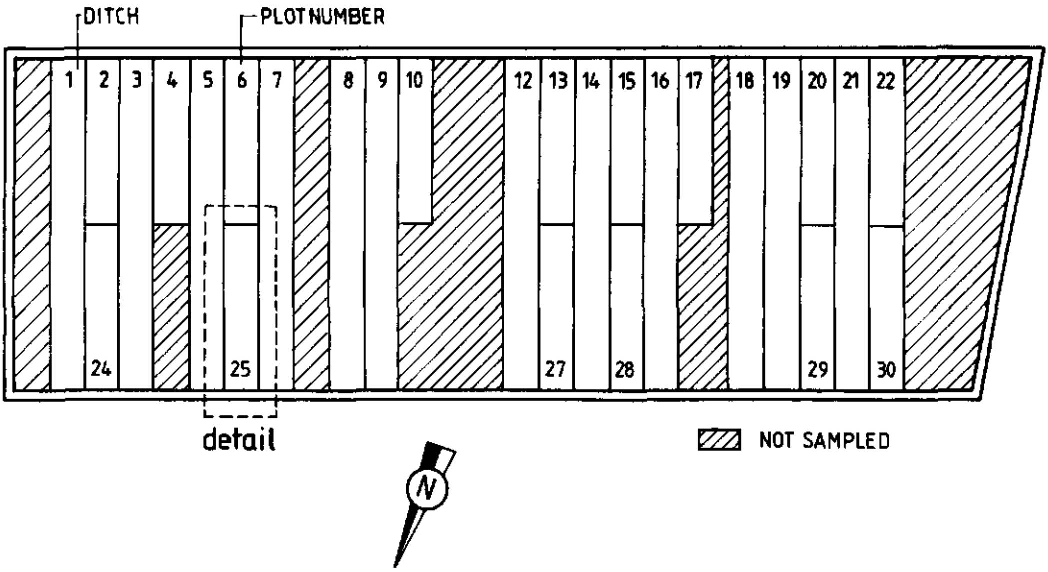


Figure 7.1 Map of the test field. Shaded areas were not sampled.

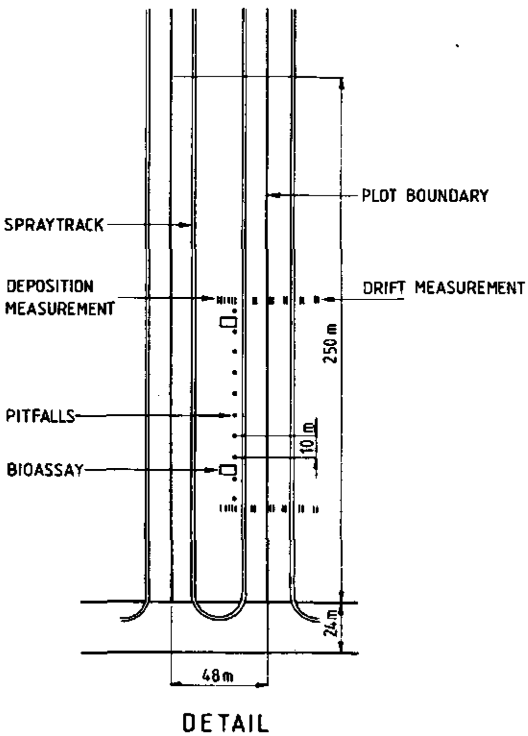


Figure 7.2 Map of a field plot

Density/activity of spiders and predators
Density/activity of linyphiids and erigonids was measured by pitfall traps. Details of the traps are given in Chapter II. Ten traps per plot were installed in a row at 10 m intervals, with the first trap 50 m from the field edge. The row was placed 1 m from a track (Figure 7.2). Traps were kept in position throughout the sampling period and were emptied at weekly intervals. *O. apicatus* and *E. atra* were identified from all samples and *P. cupreus* was also identified in the catches taken one week before and one week after each treatment.

Deposit of spray
Deposits of the spray at ground level were measured by means of water sensitive paper (Ciba-Geigy). 30 Minutes before each spraying 6 strips of 48 x 500 mm were laid on boards on the ground under the vegetation in two plots, at two sites in each plot (Figure

Table 7.1 Treatment dates, dosages applied and plots sprayed in three treatment cycles.

Cycle	Spraying date	Treatment number	Dosage (g ai/ha)	Treated plots
I	24/4	1	2.5	4; 10; 17
I	24/4	2	5.0	3; 9; 16
II	24/4	3	2.5	24; 27; 29
II	24/4	4	5.0	1; 12; 19
II	24/4	5	10.0	2; 13; 20
II	27/5	6	2.5	24; 27; 29
II	27/5	7	5.0	1; 12; 19
II	27/5	8	10.0	2; 13; 20
III	24/4	9	2.5	28; 28; 30
III	24/4	10	5.0	5; 14; 21
III	24/4	11	10.0	6; 15; 22
III	29/6	12	5.0	25; 28; 30
III	29/6	13	10.0	5; 14; 21
III	29/6	14	20.0	6; 15; 22

7.1). At the same sites two strips were exposed on stools near the top of the vegetation. In order to estimate ground deposit of drift from the sprayed plots, series of 6 pairs of strips were laid on the ground at 6 m intervals downwind from these sites. One hour after treatment the strips were collected. Number and size of droplets were measured by means of a Quantimet (R). Deposit at ground level was calculated as the fraction (%) of the deposit on vegetation top level, which was assumed to represent the field dose.

In situ *bioassays*

In situ bioassays were carried out with captive spiders, according to the method described in Chapter II. In the first treatment two containers with spiders were installed under the vegetation in two plots per dosage group (Figure 7.2) and two containers were kept at an unsprayed control site. After an apparent homogeneity in the response of the treated containers it was decided to reduce the number to three per dosage group. At the time of the first treatment only *O. apicatus/retusus* ♀ were collected. This taxon is easily recognized with the naked eye under field conditions. After the treatments the trays were covered and transported to the laboratory where they were kept at 20 °C and 90 % relative air humidity. Mortality was recorded after 4 days. At the time of the second spraying *O. apicatus* and *E. atra* were identified in the field with a microscope. This time effects were recorded daily. Because this method resulted in a high control mortality the first method was applied again in the third treatment.

Environmental factors
Data on rainfall, evaporation (Penman), temperature 150 cm above the ground and relative air humidity were obtained from the Swifterbant weather station, situated 20 km SE of the test field. The moisture content of the upper layer of the soil was estimated from rainfall, conductivity and evaporation, according to the method described by Leistra (1978) by using the crop factors given by Feddes (1987) for winter wheat.

Data processing
From catches of spiders from the 3 plots within one treatment the mean number per trap was calculated (n). For the analysis of the effect, the differences between the values from the treatment and the control area in the pre-treatment period (log(n+1)treatment - log(n+1)control) were plotted against time. Post-treatment values were then predicted from these pre-treatment data by linear regression. The differences between the observed post-treatment values and predicted ones were tested for significance by analysis of variance. The acute effect was calculated as the mean difference over three weeks after treatment (two weeks after the last treatment), expressed as a percentage of the predicted difference, in absolute numbers. The acute effect values were used for the computation of ED_{50} values for the different treatment dates. The dosages used for this computation were the values estimated from the deposit measured at ground level.

Recovery of affected populations was compared by means of the Estimated Median Recovery Time or RT_{50}. This is the time (in weeks) corresponding to the probability of 50 % of the total recovery from the reduction after treatment. This value was estimated by intrapolation from the logistic growth model:
$(\log p/(1-p))/2 + 5$ in which $p = 100 \times$ number treated/number control, per sampling date. The model was chosen for empirical reasons. For the calculation the PROBIT programme of SPSSX was used (SPSSX, 1986).

The data from previous laboratory tests were processed as follows. In order to avoid the handling of non-linear relationships, the results were selected that were obtained under conditions comparable to the field situation. From the test on soil humidity, this concerned data from the tests with 13.5, 20 and 30 % soil humidity; from tests with air humidity and temperature, data obtained at 10 and 20 °C and at RH 70, 85 and 100.
An extra factor was added to discriminate between uptake after residual and topical exposure. The value of this factor (i.e. 100 for residual and 71 for topical) was derived from results of Mullié & Everts (in prep.) who demonstrated that the toxicity of deltamethrin was increased by a factor 1.4 when absorbed via the tarsi from a surface rather than applied topically. All data used originated from tests with reared spiders exposed to an equivalent of the field dose 5 g a.i./ha. The data were integrated in a multiple regression equation calculated with SPSSX REGRESSION (SPSSX 1986). The predictiveness of this equation was tested as follows. A second regression was calculated with the data from the laboratory and the field combined. The results were compared with a third regression, also with combined results, but with an added variable U. U stands for the unknown variables not included in the regression, which may be responsible for the difference between the predicted and the observed value of the toxic effect. In the third regression the value of U was 0 in the laboratory tests and 1 in the field tests. The quotient of the mean squares of the residuals of the second and the third regression was compared with Fisher's F value at the given degrees of freedom.

Table 7.2. Reduction in _E. atra_ and _O. apicatus_ and median recovery time after deltamethrin treatments applied at different dosages and different dates (date 1 = 24/4 1987; 2 = 27/5; 3 = 29/6). n.s. = no fit to the probit model. n.r. = no recovery. [1] % of applied dose

Treatment			Dose	Deposit	Reduction (%)			RT^{50} (hours)		
					E. atra	_O. apicatus_		_E. atra_	_O. apicatus_	
Date	Number	Cycle	(g ai/ha)	(%/ g ai/ha[1])	males	males	females	males	males	females
1	1	I	2.5	94/2.3	76.5	89.0	68.2	2.8 (1.3-4.1)	5.7 (4.8-6.5)	8.0 (5.6-15.5)
1	3	II	2.5	94/2.3	78.0	95.0	63.1	2.4 (1.9-3.0)	n.s.	n.s.
1	9	III	2.5	94/2.3	84.4	94.2	65.8	2.7 (2.4-3.0)	n.s.	n.s.
2	6	II	2.5	22/0.5	33.5	n.r.	n.r.	2.1 (1.5-2.7)	-	-
1	2	I	5.0	94/4.7	91.2	94.0	91.8	4.9 (4.4-5.2)	7.9 (7.6-8.2)	9.3 (8.8-9.8)
1	4	II	5.0	94/4.7	91.5	96.8	92.3	5.4 (3.8-7.0)	n.s.	n.s.
1	10	III	5.0	94/4.7	89.1	93.6	94.5	4.6 (4.3-5.0)	n.s.	n.s.
3	12	III	5.0	32/1.6	59.5	n.r.	n.r.	-	-	-
1	5	II	10.0	94/9.4	92.1	99.2	92.9	n.s.	n.s.	n.s.
1	11	III	10.0	94/9.4	92.7	99.1	93.2	7.0 (6.1-7.9)	n.s.	n.s.
3	13	III	10.0	32/3.2	90.4	n.r.	n.r.	-	-	-
3	14	III	20.0	32/6.4	99.9	n.r.	n.r.	-	-	-

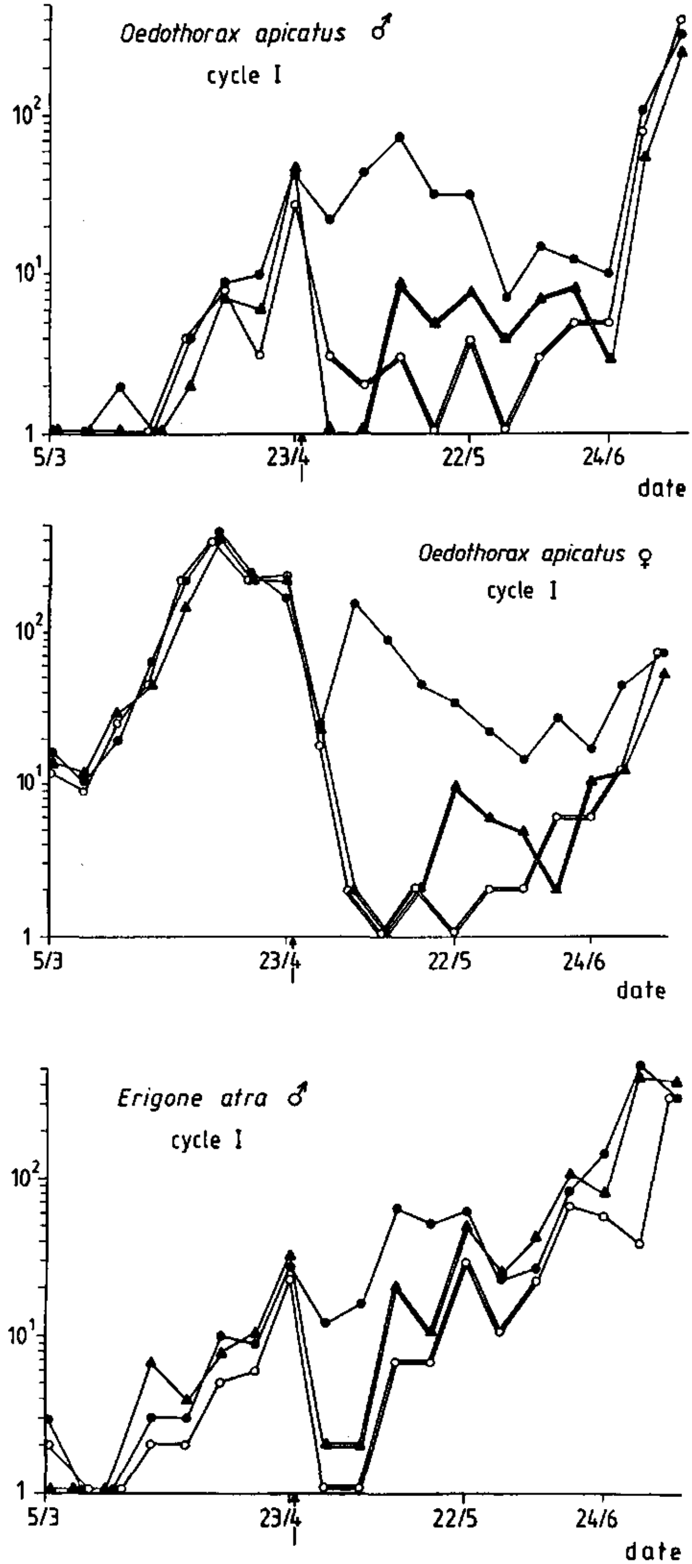
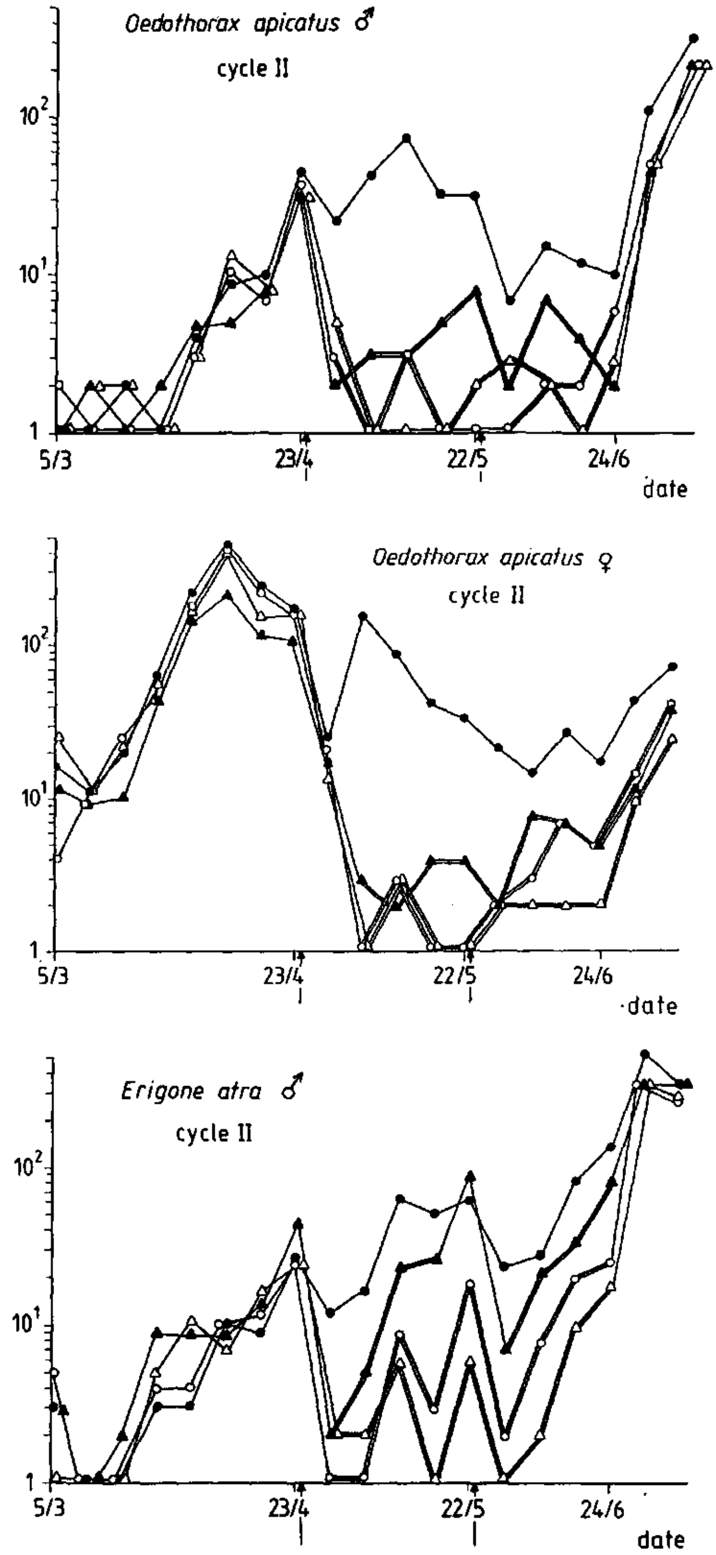


Figure 7.3 Number per three replicate plots of *Erigone atra* ♂ and *Oedothorax apicatus* ♂ and ♀ caught during treatment cycle I. The last sampling date before each treatment is indicated. Double lines indicate series that are significantly different from the control series ($p < 0.05$). (•, ▴, o and Δ ; control, 2.5, 5 and 10 g a.i./ha respectively).

Figure 7.4 Number per three replicate plots of *Erigone atra* ♂ and *Oedothorax apicatus* ♂ and ♀ caught during treatment cycle II. The last sampling date before each treatment is indicated. Double lines indicate series that are significantly different from the control series ($p < 0.05$). (•, ▲, o and Δ; control, 2.5, 5 and 10 g a.i./ha respectively).



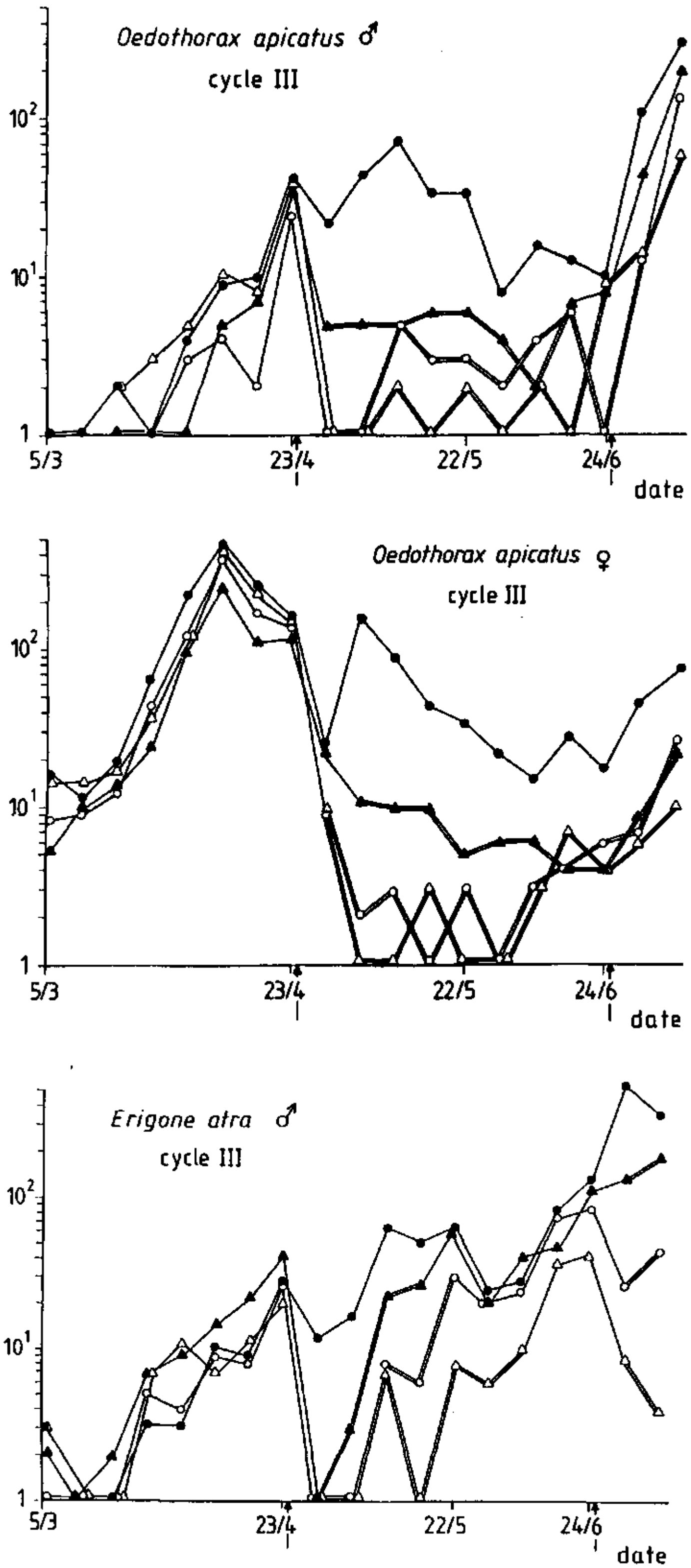


Figure 7.5 Number per three replicate plots of *Erigone atra* ♂ and *Oedothorax apicatus* ♂ and ♀ caught during treatment cycle III. The last sampling date before each treatment is indicated. Double lines indicate series that are significantly different from the control series ($p < 0.05$). (•, ▴, o and ▵ ; control, 2.5, 5 and 10 g a.i./ha on 24/4 and 5, 10 and 20 g a.i./ha on 29/6 respectively).

Results

The numbers of *O. apicatus* ♂ and ♀ and of *E. atra* ♂ per trap from the fields sprayed in treatment cycle I are given in Figure 7.3a-c. The numbers of *E. atra* ♀ caught were too low for an analysis. Before the treatment the differences between the numbers of the species in the various plots were regular, especially with regard to *O. apicatus* ♀. After treatment a dose-related response was observed. The numbers of spiders caught in treatment cycle II are given in Figure 7.4a-c. In all groups the response to the first treatment is consistent with the response observed in cycle I. At the time of the second treatment in the plots previously treated at the lowest dosage, only *E. atra* had recovered in sufficient numbers for the calculation of an effect. The effect of the second treatment was small compared to the effect of the first treatment.

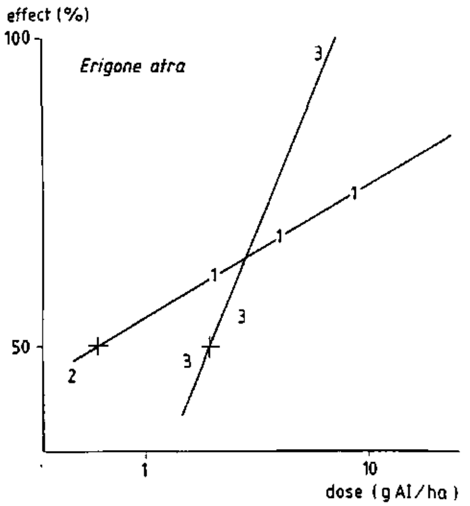


Figure 7.6 Reduction observed in *Erigone atra* ♂ after three treatments (1 to 3) at various dosages.

The effects observed in treatment cycle III (Figure 7.5a-c) on the first spraying date, correspond with the effects of cycles I and II. After the last spraying only *E. atra* ♂ showed an effect. The other taxa had not sufficiently recovered by that time.

The reduction in numbers compared to the pre-spray data and corrected for the control is given together with the RT_{50} values in Table 7.2. In *E. atra* both acute effect and RT_{50} are dose dependent. The recovery values show a high similarity at the corresponding deposit values. In *O. apicatus* ♂ the differences in response between the dosage groups are reflected in a difference in speed of recovery rather than in acute effect. Of the three taxa examined they showed the highest reduction. *O. apicatus* ♀ were least affected. At comparable dosages the RT_{50} values are lowest for *E. atra* and highest for *O. apicatus* ♀.

The calculated deposit on the soil is given in Table 7.2 (5th column). The fraction of the deposit on the soil was 94 ± 1, 22 ± 4 and 32 ± 6 % of the deposit at vegetation level (i.e. the sprayed dose) at treatment 1, 2 and 3 respectively. Deposit by drift appeared to be low. After the first treatment the highest deposit found was 10 % at one site, 6 m downwind from a sprayed plot. All other values were lower. Further than 6 m downwind no deposit was observed.

Figure 7.6 gives the reduction observed in *E. atra* at these different dosages on a log-probit scale. By the date of the second treatment *E. atra* ♂ had fully recovered at all dosages previously applied, but *O. apicatus* ♀ had only recovered at the lowest dosage. Therefore, the second treatment gave only one value. This value was in agreement with the values of the first treatment.

Table 7.3 Effective field dosages of deltamethrin (g ai/ha) on *Erigone atra* ♂, at different spraying dates. Between brackets: 95 % confidence interval.

Date:	1	3
ED_{50}	0.86 (0.63-1.09)	1.4 (1.1-1.6)
ED_{90}	5.5 (4.2-7.9)	3.0 (2.6-3.7)

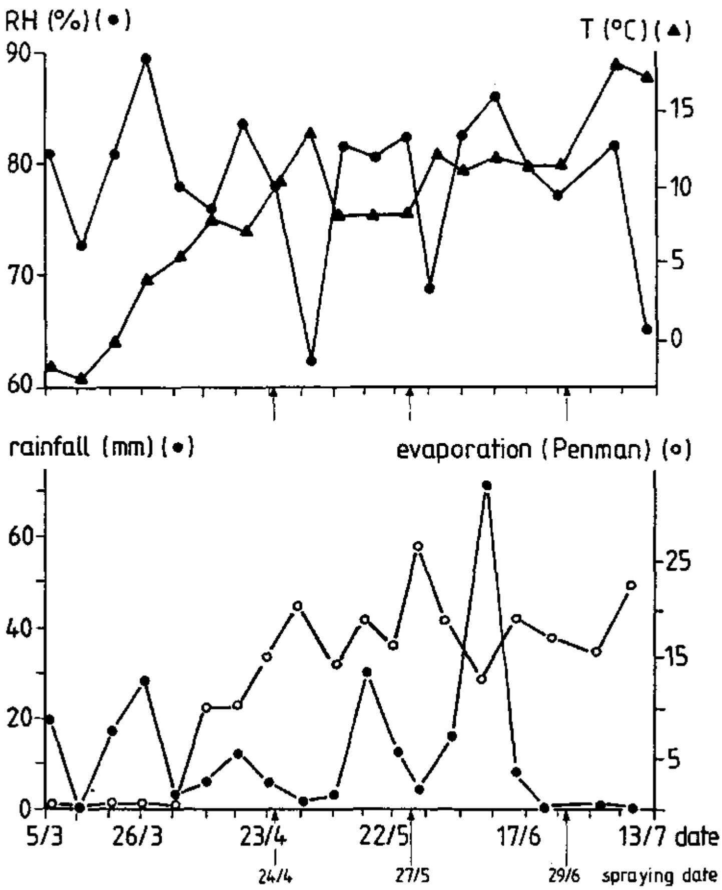


Figure 7.7 Weather data from the Swifterbant meteorological station for the sampling period. Relative air humidity (RH) and temperature (T) are given as weekly means; rainfall and evaporation as weekly totals.

The estimated ED_{50} and ED_{90} values are given in Table 7.3. The data were tested for goodness of fit to the log-probit model and for parallelism of the response curves by means of the PROBIT programme of SPSSX (SPSSX 1986). The data of the first and second treatment dates were combined. Chi-square value of the goodness of fit was 26.7 (DF = 9; $p < 0.005$) and of parallelism 16.3 (DF = 1; $p < 0.0001$), indicating a significant dose-related response but with a different slope at the different treatment dates (i.e. the first and second date versus the third date). The ED_{50} of the first treatment date is lower than that of the last one, indicating a higher efficacy during the first and second treatments in the median effective and lower dose range. The regression lines, however, have different angles; the regression line of the third date is steeper than that of the first and second dates combined. Therefore, the ED_{90} values are inverted with respect to the ED_{50} values.

In Figure 7.7 the weather data are given for the sampling period. The periods before the first two treatments were considerably drier than before the last treatment (maxima of 12 and 32 mm versus 75 mm respectively). At the time of the third treatment the air was more humid and the mean daily temperature was slightly higher (i.e. 12 versus 8 and 10 °C respectively) than after the second treatment. The calculated soil moisture contents (wet weight) were on 24/4: 26 %; on 27/5: 26 % and on 29/6: 34 %, respectively.
The numbers of the most abundant predator, *P. cupreus* are given in Table 7.4. At the time of the last treatment the numbers of this carabid were considerably higher than during the earlier treatments. At this date, however, the treatment caused a strong decrease in their numbers, which was not observed after the earlier treatments. (In the treated plots an increase in collembolans by two to three orders of magnitude was observed.)

Table 7.4 Numbers of *Pterostichus cupreus* (mean and standard error per trap) caught before (b) and one week after (a) three treatment with deltamethrin. (* = significantly different from untreated control; Student's t-test: $p < 0.01$)

Date	1		2		3	
Before/ After	b	a	b	a	b	a
Dose g ai/ha						
0	0.9(0.4)	0.8(0.3)	1.0(0.3)	1.2(0.4)	7.9(1.9)	21.4(4.2)
2.5	0.8(0.3)	0.9(0.2)	1.1(0.3)	1.1(0.4)		
5.0	0.9(0.2)	1.1(0.3)	0.8(0.3)	0.9(0.3)	6.9(2.1)	1.2(1.0)*
10.0	1.1(0.5)	1.0(0.4)	0.9(0.5)	0.9(0.4)	9.8(1.9)	0.8(0.6)*
20.0					6.1(2.0)	1.0(0.9)*

In Table 7.5 the response is given of the captured spiders exposed in the *in situ* bioassay. In the exposed containers, there was an increased mortality in all experiments, compared with the control. In the second experiment the control mortality was relatively high. In the second and third experiments the mortality was dose-related. At comparable field dosages the effect on *O. apicatus* in the first experiment was considerably higher than in the last one.

The results of the regression analyses of the laboratory data with *O. apicatus* ♀ are given in Table 7.6. The regression equation of the effect observed in reared spiders at various temperatures, relative air humidity and soil moisture classes is as follows:

$$E = -75.0 + 1.9A - 2.4T - 0.8RH + 0.7M$$

In which:

In which:	E	=	Effect, as percentage of the theoretical maximum effect
	A	=	Absorption Factor (%)
	T	=	Temperature (°C)
	RH	=	Relative Air Humidity (%)
	M	=	Soil Moisture Content (as percentage of moisture content at field capacity, wet weight)

With this formula the reduction (R, R = $\hat{E}$) observed in the field was predicted. The absorption factor (A) was calculated using the measured mean recovery of 27.8 % exposure to

Table 7.5 Mortality in *Erigone atra* and *Oedothorax apicatus* exposed to deltamethrin spray in *in situ* bioassays.
n = number of individuals exposed; m = percent mortality

Date	Dose	*E. atra*		*O. apicatus*	
1987	g ai/h	n	m	n	m
24/4	0.0			20	5
	2.5			32	84
	5.0			32	78
	10.0			32	91
27/5	0.0	5	40	7	71
	2.5	8	68	12	86
	5.0	8	87	12	92
	10.0	8	100	11	100
29/6	0.0			19	0
	5.0			26	4
	10.0			16	19
	20.0			26	87

spray (Chapter IV). Its value was 92 % for the actual field situation. T and RH are the mean values of the week after treatment, given in Figure 7.6. For the first treatment date E = 78.6 and for the third date E = 88.2. The quotient of the MS of the residuals of the second and the third regression was 1.35. $F_{0.50(12,14)} = 1.01$; $F_{0.25(12,14)} = 1.48$, indicating that the observed value of R was not significantly different from the predicted value.

Discussion

The mean reduction observed in *O. apicatus* at 4.7 g ai/ha deposit was 92.8. The intrapolated values of R for *E. atra* ♂ at 5 g ai/ha for both treatments are 88.0 (confidence interval 75.5 - 96.0) and 98.4 % (85.2 - 99.9) respectively. Although the model gives a fair prediction on the one hand, it clearly underestimates the real reduction, on the other. The difference in mean reduction, between the treatment dates (i.e. 10.4 %), however, was well predicted (9.8 %). A toxic effect (E) observed under laboratory conditions, however, is not the same as a population reduction observed in the field (R). R is related to activity in a different way from E.

The formula should be considered a first step, taking into account the rough measurements of parameters in the field compared to the laboratory; the lack of knowledge on processes occurring on the temporal and spatial scale of controlled laboratory practices, and the fact that a few parameters, such as predation and reproduction, were not included.

The possibility of selecting treatment dates with exclusive combinations of levels of the variables used in the model is a matter of mere chance. Therefore, only through numerous field observations and the continuation of analytical testing in the laboratory can a sound model for the prediction of toxic effects in the field be built.

The different angles of the dose-response regressions indicate either a different mechanism (Sprague 1969) or a different variability in sensitivity within the population. In this case it is probably a combination of both. A shallow line indicates a higher variability in the response. Variability is increased when more factors interfere with the toxicity of the compound. During the first and second sprayings, availability of the compound was low, but there may have been an effect of drought, i.e. low recovery from sublethal effects, specifically because RH values dropped dramatically after the treatments. At lower dosages the relative contribution of secondary mortality after sublethal exposure is higher than at higher dosages. At the third spraying date the residues on the soil were maximally available, but recovery under humid circumstances is high (Chapter V). The reduction observed at the third date is therefore expected to be more the result of an acute toxic effect than at the previous dates.

There is little reason to assume that the differences in response in *E. atra* ♂ related to a different physiological condition of the spiders. In contrast to females, the males are not very sensitive to seasonal changes. Moreover, the observations have been carried out in spring and early summer. During this period the population was still growing. Within this period the first offspring from over-wintering females has produced a second generation (De Keer and Maelfait 1987). The data, however, show no distinct generation peaks.

Table 7.6. Multiple regression of E = toxic effect, predicted by A = absorption factor, T = temperature, RH = relative air humidity and M = soil moisure.

Multiple R	0.97
R^2	0.93
Adjusted R^2	0.91
Standard Error	7.41

Analysis of Variance

	DF	SS	MS
Regression	4	13764	3441
Residuals	12	1185	84

F = 43.2 Significance F = 0.00001

Variables in the equation

Variable	B	SE of B	Beta	t	signif.
A	1.9	0.21	1.02	9.5	0.0000
T	-2.4	0.43	-0.47	-5.6	0.0001
RH	-0.8	0.07	-0.93	-11.1	0.0000
M	0.7	0.16	0.44	4.4	0.0009
Constant	-75.0	27.40		-2.7	0.0136

In previous work (Chapter V) it was demonstrated that the consumption of linyphiids and erigonids by *P. cupreus* is increased after exposure to deltamethrin. Although mortality of the spiders in the field by predation by carabids in not known quantitatively, it is expected that a high incidence of these predators may coincide with an increased secondary mortality after exposure to the compound. The high numbers of *P. cupreus* found before the third spraying, however, were followed by a striking reduction after the treatment. It is not known to what extent the beetles were active as predators shortly after the treatment. (An effect of deltamethrin at 5 g ai/ha on this species has not been observed earlier.)

The plots used in this test were only 48 m wide. Immigration from the untreated neighbouring fields may have affected the results. Parallel to this test a study was carried out in a collaboration with the University of Southampton, to measure in more detail the recovery speed of the most abundant erigonids and linyphiids after local depletion (Thomas et al. in press). This study was carried out near to the test field of the present study. It appeared that an exchange between a treated plot of 1.5 ha and an untreated plot of 0.5 ha was completed in 9 weeks in the virtually non-ballooning *O. apicatus* ♀. RT_{50} values at a distance of 25 m from the untreated field (i.e. half the width of our plots) were for *O. apicatus* ♀ and *E. atra* ♂ 4.0 and 6.9 respectively. The field was cultivated with winter wheat. In the present study, carried out in oilseed rape, however, *E. atra* recovered considerably quicker than *O. apicatus*. Probably the difference in vegetation structure accounts for this inconsistency.

In the present study *O. apicatus* ♀ was the least sensitive taxon. This is in agreement with all other observations, both in the field and in the laboratory.

The method for soil moisture content calculation merely provides an estimation of the real values. For the purpose of this study, however, more precise data would have been irrelevant, because detailed measurement of the moisture of the upper few millimeters of soil can only be carried out adequately in connection with a better understanding of the spider's behaviour, i.e. its preference for moisture conditions.

The method used for the calculation of deposit in active ingredient per unit of surface on the ground slightly overestimates the real deposition because the loss by drift above the vegetation is not accounted for. As a consequence, the toxic effect of deltamethrin has therefore been slightly underestimated.

Both species gave valuable data on the toxicity of deltamethrin under field conditions. The more abundant *O. apicatus*, however, recovered too slowly to allow a second observation during the same season. Its abundance, on the other hand, ensured sufficient numbers of individuals being captured for the *in situ* bioassays. By using two closely related species from the same habitat it is possible to draw conclusions for which data from one species would have been too limitative. After the third treatment, the effect observed in the *in situ* bioassays reflected fairly the effect observed in the wild population. This technique, therefore, may provide a cheap and sensitive tool for early detection of effects.

Acknowledgements

We are much indebted to Dr J Meems, J Daling and J van 't Zet (State Authority for the Lake Yssel Polders) for their help with the preparation and execution of the test. We thank Prof Dr J.H. Koeman, Dr F. Moriarty and Prof Dr L. Brussaard for their valuable comments on the manuscript. The study was financed by the Dutch Toxicology Research Promotion Programme. Roussel UCLAF kindly provided the Decis Flow[R] used for this test.

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SUMMARY

The main objective of the present study was to evaluate the possible impact of pesticides on epigeal arthropods in arable land. It was also envisaged to develop a predictive model for possible undesirable effects of pesticides on the epigeal arthropod fauna using an indicator species from the field. The strategy was the following. In the field, species were identified that were (1) sensitive to a number of pesticides, (2) abundant, (3) regular in time and space and (4) easy to sample and identify (Phase I). Two species, the erigonid spiders *Oedothorax apicatus* (Blackwall) and *Erigone atra* (Blackwall) were selected for toxicity studies in the laboratory using deltamethrin as a model compound (Phase II). The results of these studies were used to develop a predictive model which was tested under field conditions (Phase III).

Phase I: Initial Field Observations

Phase I was implemented in the South Flevoland Polder, in cooperation with the State Authority for the Development of the Lake IJssel Polders. Observations on the above-ground arthropods were conducted in 17 commercially farmed fields and in one experimental field, from March 1985 to November 1986, during two growing seasons and one winter. The arthropods were sampled by pitfall traps in oilseed rape fields which were treated with a number of insecticides and herbicides, and in cereal fields which were either treated with herbicides or weeded chemically against herbs, at recommended dosages. In the experimental field, dosages were varied and the effect of ploughing was observed. During the treatments *in situ* bioassays were carried out with captive spiders. From @ 5100 pitfall samples over 200,000 specimens were identified, belonging to 147 taxa. The observed short term effects were the following. A limited number of species were significantly affected by the treatments with insecticides. These were the carabids *Clivina fossor* after deltamethrin applications and *Trechus quadristriatus* after fenitrothion, and 7 spider species. The latter were reduced after treatments with either deltamethrin, fenitrothion or bromophos-ethyl. No effects were observed of herbicide treatments. It was also demonstrated, however, that the effect of mechanical disturbance by ploughing was comparable to the strongest effects of insecticides. It was concluded that an assemblage of spider species consisting of *Oedothorax apicatus*, *Erigone atra*, *E. dentipalpis*, *Meioneta rurestris* and *Bathyphantes gracilis* may be a sensitive indicator for early detection of side-effects of insecticides. This group represents 80-90% of the erigonid and linyphiid fauna of arable land and they are common throughout North-Western Europe. The most abundant species, *E. atra* and *O. apicatus* were selected for further study.

Possible long-term effects on the spider fauna have been studied in a transversal study in fields with different cultivation histories. The differences were related to the distance between fields rather than to cultural history. There was one exception: in cereal fields that have, since reclamation, been weeded by harrowing, the spider fauna was considerably poorer than in herbicide treated fields.

The effect of deltamethrin on the spiders was observed several times, under different weather conditions. It appeared that the toxicity was highest under hot and dry conditions and low under cold and wet circumstances. This was not in agreement with the known negative temperature-toxicity relationship of pyrethroids and the higher availablity of chemicals from wet surfaces. This relation, therefore, was studied in more detail in the following phase.

Phase II: Toxicity studies

The first step in the analytical phase was the development of new methods for rearing of and toxicity testing with the selected indicators. *O. apicatus* females appeared to be the most appropriate species both for rearing and testing.

The second step concerned the availablity of deltamethrin to this species under varying environmental conditions. Three routes of exposure were examined: topical application (simulating exposure to spray); uptake via food, and exposure to contaminated surfaces (soil or glass filter). Part of the work was carried out with radio-labelled deltamethrin. It appeared that uptake of residues from a surface by the tarsi is the most important route. Field observations carried out with a fluorescent tracer showed that the direct exposure of the spiders and their prey species to the spray, was very limited. This indicated that in the field as well, direct exposure and oral uptake are of limited importance. The availability of the residue, however, strongly depends on the water content of the soil. A decrease of the soil moisture content to 66 % of the field capacity resulted in a drop of 75 % in availability.

The third step concerned the relation between environmental factors, i.e. climate and animal antagonists, and the toxicity of deltamethrin. Toxicity tests carried out under varying moisture and temperature conditions showed that at decreasing air humidity the toxicity increased, at all temperatures tested. The compound is most toxic at a combination of a low air humidity or the absence of drinking water and a high temperature. A negative temperature-effect relationship in the range 10 - 30 °C could be observed only at maximum air humidity. The behaviour of the spiders with regard to the moisture in the environment changed after exposure to sublethal dosages of deltamethrin. Spiders not visibly affected showed an increased preference for wet conditions. Affected spiders, on the other hand, showed a reduced capacity to select moist conditions. Their capacity to escape from predators was also reduced. Analysis of gut contents of the main predator, the carabid beetle *P. cupreus*, from the fields sampled in Phase I, showed an 8-fold increase in the consumption of linyphiids and erigonids after deltamethrin spraying. A few tests were carried out with both captive and reared spiders. There appeared to be a marked difference in sensitivity between these groups. This was primarily imputed to differences in selection mechanisms between laboratory and field.
With these results a regression equation was composed which predicts the toxicity of deltamethrin to *O. apicatus* females under various weather and soil moisture conditions.

Phase III. Validation under field conditions

Similarly to Phase I, the field observations of Phase III were carried out in the South Flevoland Polder. A 75 ha field was divided into 27 test plots. These plots were treated three times at different dates, in three replicates, with three dosages of deltamethrin. The following observations were carried out: acute effects in *in situ* bio assays, activity-density of *E. atra* and *O. apicatus*; deposition of deltamethrin on the soil, on the spiders, on carabid, and on collembola (the main food items of the spiders). Meteorological data were obtained from a near-by meteo station and moisture of the top soil was calculated from data on rainfall, evaporation, and characteristics of the soil and the crop. Only *E. atra* males were abundant enough at the first and the last spraying date to allow calculation of dose-response relationships. The angles of the regressions appeared to differ for both dates. However, comparison of the observed reduction in *O. apicatus* females and *E. atra* males with the toxic effect predicted for *O. apicatus* females, showed that the laboratory based model had a fair predictive value for the given situation. Apparently erigonid spiders are good indicator species for the assessment of possible undesirable effects of insecticides.

SAMENVATTING

Het eerste oogmerk van het onderhavige onderzoek was de evaluatie van de mogelijke ongewenste bij-effekten van het gebruik van bestrijdingsmiddelen op de bovengrondse arthropodenfauna in cultuurland. Het tweede doel was de ontwikkeling van een voorspellend model voor deze effekten, gebruik makend van een indicatorsoort uit het veld. De strategie was de volgende. In het veld werden soorten onderscheiden, die (1) gevoelig waren voor een aantal bestrijdingsmiddelen, die voorts (2) abondant waren en (3) regelmatig voorkomend in tijd en ruimte, en (4) eenvoudig te bemonsteren en te determineren (Fase I). Twee soorten, de hangmatspinnen *Oedothorax apicatus* (Blackwall) en *Erigone atra* (Blackwall), werden geselecteerd voor toxiciteitsonderzoek in het laboratorium met deltamethrin als modelstof (Fase II). Op grond van de resultaten van dit onderzoek werd een voorspellend model voor de toxiciteit van deltamethrin voor hangmatspinnen opgesteld. Vervolgens werd dit model getoetst in het veld (Fase III).

Fase I. Initiëel veldonderzoek

Het veldonderzoek van Fase I is uitgevoerd in Zuidelijk Flevoland, in samenwerking met de Rijksdienst voor de IJsselmeerpolders. Gedurende twee groeiseizoenen en één winter (van maart 1985 tot november 1986) zijn op 17 bedrijfskavels en op één proefperceel de bovengrondse spinnen en insekten bemonsterd met vangpotten. De velden waren bebouwd met koolzaad of graan. Het koolzaad werd behandeld met verschillende insecticiden en herbiciden en het graan alleen met herbiciden, in de voorgeschreven doseringen. Op het proefperceel werden ook andere doseringen gebruikt en werd tevens gekeken naar het effekt van ploegen. In de ± 5.100 monsters zijn ruim 200.000 organismen gedetermineerd, waarbij 147 taxa werden onderscheiden. Veranderingen in aantallen gaven aanwijzingen voor toxische effekten. Door tevens gevangen, levende dieren aan de bespuitingen bloot te stellen kon het direkte effekt op individuen worden waargenomen. Een beperkt aantal soorten bleek te worden beinvloed door de bespuitingen. Dit waren de loopkevers *Clivina fossor*, na deltamethrin, en *Trechus quadristriatus* na fenitrothion behandelingen, en 7 spinnensoorten. Deze laatste ondervonden schade van zowel deltamethrin, fenitrothion als bromophos-ethyl bespuitingen. Herbiciden bleken geen effekt te hebben. Wel bleek dat mechanisch wieden, als alternatief voor herbicidengebruik, evenals het ploegen, de spinnenfauna sterk negatief beinvloeden. Een groep spinnen, bestaande uit *Oedothorax apicatus*, *Erigone atra*, *E. dentipalpis*, *Meioneta rurestris* en *Bathyphantes gracilis*, bleek een gevoelige indicator voor neveneffekten van insekticiden. De groep vertegenwoordigt 80 - 90 % van alle hangmatspinnen in cultuurland en is zeer algemeen in Noordwest Europa. Hiervan zijn *O. apicatus* en *E. atra* uitgekozen voor verder onderzoek.

De mogelijke lange-termijn effekten op de spinnenfauna zijn bestudeerd in een transversaal onderzoek in kavels met verschillende bespuitingsvoorgeschiedenis. De waargenomen verschillen bleken echter meer in verband te brengen met afstand tussen de kavels dan met de voorgeschiedenis, met één uitzondering. De spinnenfauna van een bedrijf, waar sinds de drooglegging geen landbouwchemicaliën zijn toegepast, bleek aanmerkelijk armer dan die van normale kavels. Dit werd geweten aan het reeds genoemde verstorende effekt van mechanisch wieden.

Van deltamethrin kon het effekt bij meerdere gelegenheden worden vastgesteld, onder verschillende weersomstandigheden. Het bleek dat het effekt sterker was bij droog en warm dan bij vochtig en koel weer. Omdat dit niet in overeenstemming was met de bekende negatieve relatie tussen de toxiciteit en de temperatuur van de stof, en ook in tegenspraak was met de bekende verhoogde beschikbaarheid van stoffen in vochtige grond, is dit verschijnsel in de volgende fase verder onderzocht.

Fase II. Toxiciteitsonderzoek

De eerste stap in de analytische fase was de ontwikkeling van nieuwe kweek- en toetsmethoden met de gekozen indicatoren. *O. apicatus* vrouwtjes bleken zich hiertoe het best te lenen.

De volgende stap betrof de beschikbaarheid van de modelstof voor deze soort onder verschillende milieuomstandigheden. Drie opnameroutes zijn onderzocht: oraal, topicaal en residueel. De eerste betreft opname via voedsel, de tweede de direkte blootstelling aan de spuitvloeistof en de derde de opname vanaf een gecontamineerd oppervlak. Bij een deel van dit onderzoek werd gebruik gemaakt van radio-aktief gelabeled deltamethrin. Het bleek dat de residuele opname de belangrijkste was. Waarnemingen in het veld, waarbij de blootstelling van spinnen en hun prooien aan de spuitvloeistof werd gemeten met behulp van een fluorescerende tracer, wezen uit dat direkte blootstelling en orale opname ook onder veldcondities van geringe betekenis zijn. Van het residu op de bodem echter, hangt de beschikbaarheid sterk af van het vochtgehalte. Een afname van het vochtgehalte tot 66 % van de veldcapaciteit veroorzaakte een afname van 75 % in het toxisch effekt.

De derde stap betrof de relatie van de toxiciteit met omgevingsfaktoren, zoals het weer en roofvijanden. Met behulp van toxiciteitstoetsen waarbij temperatuur en luchtvochtigheid werden gevarieerd, werd aangetoond dat afnemende luchtvochtigheid de gevoeligheid van de spinnen verhoogt. Dit effekt wordt versterkt door verhoging van de temperatuur en de afwezigheid van drinkwater. Een negatieve temperatuur-effekt relatie, in de range 10 - 30°C, werd alleen gevonden bij 100 % relatieve luchtvochtigheid. Het gedrag van de spinnen veranderde onder invloed van de stof. Bij een sublethale blootstelling nam de voorkeur voor vochtige omstandigheden toe bij spinnen die overigens geen duidelijk toxisch effekt vertoonden. Spinnen die wel een effekt vertoonden bleken daarentegen verminderd in staat gunstige condities te kiezen. Tevens verminderde hun vermogen te ontsnappen aan roofvijanden (loopkevers). Een mogelijke verhoogde predatiedruk onder invloed van de stof werd in het veld bevestigd door middel van analyse van maaginhouden van loopkevers vóór en nà een bespuiting. Deze wees op een achtvoudige toename van de consumptie van spinnen. Een aantal proeven werd uitgevoerd met zowel gevangen als gekweekte spinnen. Er bleek een aanmerkelijk verschil in gevoeligheid tussen beide groepen te bestaan.

Met deze resultaten werd een regressievergelijking opgesteld voor de toxiciteit van deltamethrin bij *O. apicatus* vrouwtjes, afhankelijk van opnameroute, temperatuur, luchtvochtigheid en bodemvochtgehalte.

Fase III. Veldvalidatie

Fase III werd, evenals Fase I, uitgevoerd in Zuidelijk Flevoland. Een kavel van 75 ha werd verdeeld in 27 veldjes. Hierop werd drie keer een bespuiting met deltamethrin uitgevoerd, elk in drievoud en in drie concentraties. Bij elke bespuiting werden gevangen dieren blootgesteld en werd de aktiviteits-dichtheid van de epigeïsche fauna bepaald. De depositie van de spuitvloeistof werd gemeten op de grond en op spinnen, loopkevers en spingstaarten (de voornaamste prooi van de spinnen). Weersgegevens waren afkomstig van het meteostation in Swifterbant. Het vochtgehalte van de bovengrond werd berekend met behulp van deze weergegevens en de eigenschappen van de grond en het gewas.

Zowel de reducties in aantallen als de lengte van de herstelperiode van *O. apicatus* vrouwtjes en *E. atra* mannetjes bleken gerelateerd aan de dosis. Met de laatste soort, die voldoende abondant was tijdens de eerste en de laatste behandeling, werd de voorspelling getoetst. Hierbij bleek dat het model, gebaseerd op laboratorium gegevens, voor deze twee waarnemingen een redelijke voorspellende waarde had. Blijkbaar zijn deze spinnen goede indicatorsoorten voor onderzoek naar neveneffekten van insecticiden.

CURRICULUM VITAE

James William Everts werd geboren op 12 februari 1947 te 's-Gravenhage. In 1965 behaalde hij het diploma HBS-A aan het Spinoza Lyceum te Amsterdam, in 1968 het diploma Tropische Landbouw aan de RHLS te Deventer, in 1969 gevolgd door de specialisatie Plantenziektenkunde aldaar. Hij heeft vervolgens korte tijd gewerkt bij het Biologisch Station van de Landbouwuniversiteit te Wijster, als technisch assistent. In 1970 ving hij de studie Biologie aan in Groningen, om in 1977 in Wageningen af te studeren. In dat jaar kwam hij opnieuw in dienst bij de Landbouwuniversiteit; nu bij de vakgroep Toxicologie, als uitvoerder van een vier-jarig oecotoxicologisch onderzoeksprojekt in West Afrika. Vervolgens kwam hij in vaste dienst bij dezelfde vakgroep, als wetenschappelijk medewerker Oecotoxicologie. In deze hoedanigheid heeft hij het onderwijs in dit vakgebied opgezet en een dertigtal doctoraalstudenten opgeleid. Tevens heeft hij een aantal onderzoeksprojekten in de tropen begeleid en uitgevoerd. In 1985 is hij begonnen met een gecombineerd veld-laboratorium onderzoek in Nederland, waarvan de resultaten grotendeels in dit proefschrift zijn vastgelegd.