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PREDICTING THE RISK POSED BY DELTAMETHRIN TO BENEFICIAL INVERTEBRATES IN TEMPERATE CEREAL CROPS.

by

JOHN ANTHONY WILES

Thesis submitted for the Degree of Doctor of Philosophy

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Dedicated to my loving wife Caroline and my parents and family for all their support over the last three years.

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ABSTRACT

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PREDICTING THE RISK POSED BY DELTAMETHRIN TO BENEFICIAL INVERTEBRATES IN TEMPERATE CEREAL CROPS.

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Data are presented from a three year study to evaluate the risk posed by summer applications of the synthetic pyrethroid insecticide deltamethrin to a range of polyphagous and aphid-specific predators that inhabit temperate cereal crops. The need for such a study has become evident from the difficulty in interpreting data from large-scale field trials and the general lack of quantitative data concerning the mechanisms of susceptibility and exposure that mediate the short-term effects of pesticides on these predators in cereal crops.

Initially, laboratory topical bioassays established a 300 fold range of predator susceptibility to deltamethrin, with a linyphiid spider being the most susceptible species tested. In addition, residual bioassays indicated that fresh deltamethrin residues on cereal plant foliage may be 50 to 60 times more toxic to predators than residues on a sandy loam soil. Dietary uptake studies, with a single polyphagous predator, showed that predator mortality may also result from consumption of deltamethrin contaminated prey. Following this, *in situ* bioassays determined the toxic risk posed by direct contact and exposure to realistic concentrations of deltamethrin in the field. Results indicated that low levels of mortality may occur for nocturnal ground-active predators, such as large carabid beetles, however for some plant-active diurnal predators, such as coccinellids, it may be necessary to reduce the recommended dose rate of deltamethrin by three quarters to preserve approximately 50% of the population in the crop during the 10 days after spraying.

The results are discussed in terms of how toxicological and ecological criteria can be used to predict which species are most at risk from a summer spray application and how these predicitions may be used to aid the interpretation of results from large-scale field trials. The experimental framework developed in this study may be adapted to evaluate pesticide side-effects in other crops.

CHAPTER 1

Introduction and literature review

1.1 Ecotoxicology and pesticide risk assessment

Ecotoxicology may be defined as "the science of toxic substances in the environment and their impact on living organisms" and has developed over the last twenty years from the previously independent fields of ecology and toxicology (Moriarty, 1988). The need for understanding in this field has become increasingly clear with the rapid development of the chemical industry and the lack of knowledge of the fate and effect of these chemicals in the environment. The agrochemical industry is one area where the numbers of compounds and their uses have expanded rapidly due to chemical innovation. For example Hess (1987) estimated that the use of agrochemicals has increased by 1900% between 1930 and 1980.

One of the most controversial questions that has arisen from this increased usage of chemicals is how to determine the risk posed by pesticides to organisms and the environment and how can this risk be measured and interpreted in the field (Brown, 1989a; Jepson, 1989; Jepson 1993b). Therefore there is urgent need for the development of risk assessment procedures which may be used to estimate the probabilities and magnitudes of undesired effects of these chemicals (Suter, 1990; Jepson 1993b).

In general ecotoxicological theory, several statistical extrapolation models have been developed to aid risk assessment (e.g. Kooijman, 1987; Van Straalen and Denneman, 1989). These are based on deriving concentration levels that are hazardous to the most sensitive species (Kooijman, 1987) or the most sensitive 5% of the species (Van Straalen and Denneman, 1989) in a community. However these models are only applicable to cases where the bioavailable concentration of the pollutant is constant. Therefore they are not suitable for pollutants such as pesticides, which are applied at intervals and often degrade rapidly. In order to develop models for pesticides therefore it is necessary to include other chemical factors, such as half-lives (eg. Van Straalen *et al.* 1992), and biological factors such as processes that mediate organism depletion and recovery. To date the later has not been possible because there is a lack of quantitative data concerning the importance of the mechanisms that mediate these biological processes.

This study aimed to provide an insight into the first of these biological processes, i.e. the level of depletion of organisms after a pesticide application, by determining the effects of summer-applications of the synthetic pyrethroid insecticide, deltamethrin on a range of beneficial invertebrates that inhabit temperate cereal crops. A mechanistic approach was adopted to determine the levels of mortality that may occur via likely routes of exposure, i.e. contact with deltamethrin drops during spray application, exposure to deltamethrin residues on plant and soil surfaces and by consumption of contaminated prey. Then ecological and toxicological criteria were used to determine species that may be most at risk from a deltamethrin spray. Risk in this context was defined as the probability of an organism being harmed (OECD "Organisation for Economic Co-operation and Development", 1989).

The proceding sections provide an introduction to the recognition of side-effects of pesticides and how these side-effects are currently assessed for terrestrial invertebrates in arable crops, followed by an introduction to the synthetic pyrethroid insecticides and the temperate cereal ecosystem.

1.2 Recognition of problems with pesticide usage

World agricultural productivity has increased dramatically since the so called "green revolution", i.e. the development of high yielding crop varieties, disease and pest resistant plant varieties, and the production of fertilisers and highly effective synthetic pesticides. The use of pesticides has undoubtedly become very important in world crop production, for example Finney (1990) calculated that without the use of pesticides, global crop yields would decrease by approximately 30%. However their use must be carefully managed to reduce any adverse effects in the environment.

Ripper (1944 & 1956) was one of the first to recognise that the non-specific mode of action of pesticides could lead to some potentially undesirable side-effects. However public concern over the deleterious effects of pesticides in the environment was probably first raised by Rachel Carson's book "Silent Spring" (Carson, 1962), in which she described the biomagnification of organochlorine pesticides. The biological problems that have been observed have probably also have been exacerbated by the inefficiency of pesticide application techniques in targeting the pest. For example Graham-Bryce (1987) estimated that less than 1% of the chemical that is applied contributes to the mortality of the target pest. Therefore to reduce possible undesirable side-effects it has become increasingly important to use compounds which have greater selectivity, to improve pesticide application techniques (e.g. Hall 1988 & 1991),

and to further our understanding of the mechanisms of exposure that mediate the side-effects of pesticides in the "real world".

1.3 Assessing the side-effects of pesticides on non-target terrestrial invertebrates

Assessment of the side-effects of pesticides on organisms may be carried out on several experimental scales, ranging from small scale laboratory bioassays (often at individual species level), to area-wide field studies (assessing effects on populations and communities). Progression from one scale to another, i.e. from laboratory to field studies, will increase realism but reduce control of experimental variables and will also increase the cost of the work (Jepson, 1989; Croft, 1990a).

Over the past few decades a wealth of test methodologies have been developed for testing the side-effects of pesticides on terrestrial invertebrates (see Jepson 1993a for a review of tests carried out on insects, spiders and mites). The methodologies include laboratory tests (sometimes called Tier 1 tests), such as topical, residual, dietary and behavioural bioassays, semi-field tests (sometimes called Tier 2 tests) such as *in situ* bioassays, the use of field cages and barriered plots, and field tests (sometimes called Tier 3 tests), such as large, open plot trials or whole-field trials. This diversity reflects the ingenuity of ecotoxicologists to design bioassays for specific purposes on each experimental scale, however it often causes problems in comparing results between studies within a given experimental scale due to differences in factors such as treatment and exposure procedures, test conditions and experimental design. Therefore it has become clear that where results need to be comparable, i.e. for registration testing of pesticides, standardised protocols are required to produce quantitative, repeatable and cost-effective testing methods at each scale of investigation (Jepson, 1987; Sotherton *et al.* 1988; Brown *et al.* 1990). An example of such standardized protocols are those developed for laboratory and field-based studies by the International Organisation on Biological Control (IOBC) "Working group on pesticides and beneficial invertebrates" (e.g. Hassan, 1989 & 1992). These are currently used to provide a robust screen of the toxicity of large numbers of agrochemicals to a range of beneficial invertebrates.

The results of laboratory and semi-field bioassays may be useful in their own right but may be of even greater value if they can be used 1) to gain further insight into the mechanisms that mediate side-effects in the field and 2) to make predictions of possible effects. To do this it is necessary to understand spatial and temporal factors

which are important in determining the nature of effects and the biological and operational factors with affect the level and duration of the effects.

A conceptual framework upon which such methodologies could be based was suggested by Jepson (1989) (Figure 1.1). Jepson defined three temporal/spatial scales on which side-effects could be assessed. These were the "micro" scale (which determines the level of initial uptake and toxicity of the pesticide by individuals in the crop during and after spray application), the "meso" scale (which determines within year effects on individuals and populations within the treated plot(s)), and the "macro" scale (which determines effects on populations in whole-field studies, between fields and between seasons up to commercial use). The arrows at the top of Figure 1.1 (from left to right) give a chronological sequence of the processes of environmental

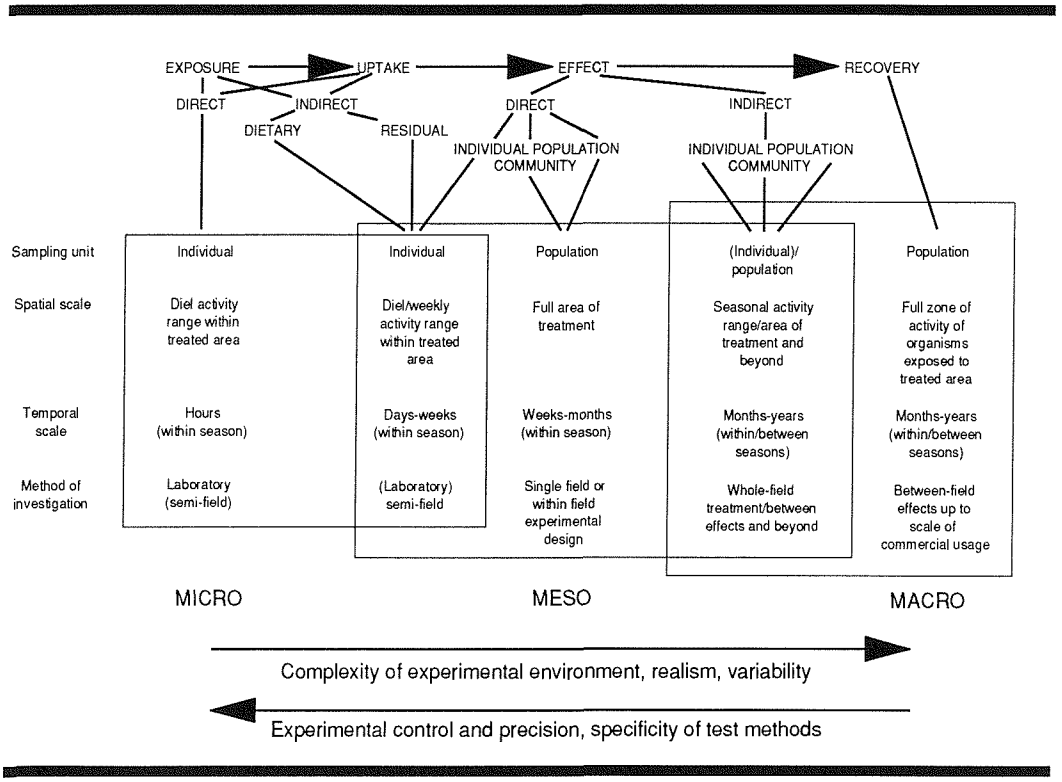

Figure 1.1 The temporal and spatial scales over which the processes of contamination and biological impact of pesticides act on non-target invertebrates in arable crops. (adapted from Jepson, 1989 and Croft, 1990a).

contamination leading to the effects of a pesticide on non-target invertebrates. This includes all of the processes from initial exposure to final recovery. The interlocking boxes indicate the three temporal/spatial scales. The areas falling wholly within one box have independent methodologies associated with them and cannot be investigated using the techniques associated with a preceding temporal or spatial scale. An

example of this are direct toxic effects, which occur immediately after spray application and depend upon short temporal and spatial scales related largely to the diel activity cycle of the species and it's distribution in the crop.

The level and duration of pesticide side-effects will be mediated by a range of biological and operational factors (Table 1.1). The short-term effects, i.e. the initial depletion of invertebrate populations by the pesticide, will be a function of susceptibility and exposure whereas the long-term effects, i.e. the probability of the population being harmed, will depend on the ability of the species to recover which will be mediated by it's life-history strategy and dispersal capacity (Jepson, 1989) and on the scale of

Table 1.1 Factors affecting the level and duration of pesticide impact on non-target invertebrates. (from Jepson, 1988)

Biological factors	Operational factors
Exposure to pesticides	
***At the time of spraying*;**	
proportion of population in the sprayed area	application volume
degree of protection by crop canopy or soil refuges	nozzle parameters and droplet spectrum
droplet capture efficiency	application frequency
***Following spraying*;**	
residual exposure: distribution pattern and diel cycle	persistence and breakdown of active ingredient formulation
dietary exposure: availability of contaminated prey	environmental influences on bioavailability
Susceptibility	
genetic, structural, & physiological factors	intrinsic toxicity
environmental factors	application rate
Recovery/Reinvasion	
***Direct ecological factors*;**	
mobility/dispersal	product persistence
voltinism	
proximity of reservoir	
***Indirect ecological factors*;**	
degree of oligophagy/polyphagy	toxic effects on alternative prey items
extent of depletion of preferred prey	
***Sub-lethal effects*;**	
repellency	
behavioural activation	

pesticide treatment. The later has been shown by a number of recent studies in cereal crops in which recovery rate decreased as the area treated with pesticide increased (Jepson and Thacker, 1990; Thacker, 1991; Duffield 1991). Because of the diverse methodology and different temporal and spatial scales used to assess pesticide side-effects it is clear that great care is required when interpreting the results of studies (Brown and Sharpe, 1988) and extrapolating effects at one level to another (Jepson, 1988a & b; Everts, 1990; Thacker and Jepson, 1990; Duffield, 1991; Thacker, 1991). It is clear that if this is going to be possible in the future understanding of the mechanisms that mediate both short-term and long-term side-effects are necessary.

1.4 The synthetic pyrethroid insecticides

1.4.1 The worldwide success of pyrethroid insecticides

Currently at least 27 different synthetic pyrethroids are marketed around the world (AGROW, 1991a). However in the next few years the world market for pyrethroids is likely to become increasingly competitive because of impending patent expires of some of the most widely used pyrethroids such as cypermethrin, permethrin and deltamethrin (AGROW, 1991b).

In 1989 the pyrethroids comprised approximately a quarter of all foliar insecticides used in agriculture worldwide and were sprayed over an estimated 100 million hectares (Cox, 1990). Properties such as broad spectrum activity, high levels of control at low application rates, high toxicity to insects, low toxicity to mammals, rapid knockdown, antifeedant and repellent effects in insects, and lack of persistence (i.e. rapid biodegradability) in the environment have led to this success (Cox, 1990). However due to their broad-spectrum nature, they also have the draw back that they also tend to be toxic to non-target insects, many of which are important natural enemies of pests (Croft, 1990a).

1.4.2 The development of pyrethroid insecticides

The pyrethroid insecticides are derivatives of the pyrethrins, a group of esters which occur naturally in the flowers of a number of species of Chrysanthemum, including *C. cinerariaefolium* and *C. roseum* (Compositae) (Staudinger and Ruzicka, 1924; cited from Leahey, 1985). Leahey (1985) gives a full historical introduction. The elucidation of the structure of the pyrethrins has also been exhaustively reviewed by Crombie and

(1985).

1.4.3 The use of pyrethroid insecticides in agriculture

The natural pyrethrins had the advantage of low mammalian toxicity but the disadvantage of low photostability (Bullivant and Pattenden, 1976; Ruzo and Casida, 1981) a factor which limited their effectiveness as agricultural insecticides in field crops. The first photostable synthetic analogue (pyrethroid), permethrin, was developed at Rothamsted Research Station in the U.K. (Elliot *et al.* 1973a & b). By the late 1970's many more photostable synthetic pyrethroids had been discovered, such as cypermethrin (Breese and Highwood, 1977), deltamethrin (Hervé *et al.* 1977) and fenvalerate (Mowlam *et al.* 1977). These showed increased insecticidal activity, and offered great potential for public health, veterinary and agricultural use (Breese, 1977; Elliot *et al.*, 1978; Ruscoe, 1979) due to their broad-spectrum activity against a range of lepidopteran, coleopteran, homopteran, heteropteran, dipteran, orthopteran and thysanopteran pests (Hirano, 1989; Cox, 1990). Because of their highly lipophilic nature and low volatility, pyrethroids act as contact insecticides and are not systemic (Graham-Bryce, 1987) and therefore the main routes of intoxication are by being directly contacted during spraying, contact with residual deposits or consumption of contaminated substrates (Hirano, 1989). They are fast acting causing rapid knockdown and have also shown antifeeding and repellent activity against some arthropods (David and Somasundarum, 1985).

1.4.4 The environmental fate of pyrethroid insecticides

Due to the high insecticidal activity of pyrethroids, commercial formulations of some of the most active compounds such as deltamethrin, cypermethrin, and lambda-cyhalothrin are effective at very low field dosage rates, i.e. 5 to 25 g AI ha^{-1}. In the field, the photostable pyrethroids persist on crops for 7 to 30 days (Elliot, 1989). Upon contact with soil, residues of most pyrethroids are strongly adsorbed to colloids (particularly organic matter) where they are further metabolized by microorganisms (Elliot 1989). Due to this binding to the soil leaching is of little concern as they often remain in the top few centimetres of the soil (Demoute, 1989).

The behaviour of pyrethroids in the soil is related to factors such as temperature, soil type and soil moisture (Demoute, 1989). For example Hill and Schaalje (1985) found deltamethrin had a soil half-life of 10 to 11 weeks at 10°C and 5 to 6 weeks at 20°C. Most pyrethroids also have a negative temperature coefficient (i.e. the toxicity increases as temperature decreases) (Harris and Kinoshita, 1977; Sparks *et al.* 1983).

increases as temperature decreases) (Harris and Kinoshita, 1977; Sparks *et al.* 1983). Unlike some of the chlorinated hydrocarbon insecticides, which are significantly volatile and dispersed in air currents, most pyrethroids used in crop protection have low vapour pressures and do not disperse (Demoute, 1989). The pyrethrins are toxic to fish in the laboratory as they are strongly adsorbed by the gills. However in practice their impact is less than predicted due to adsorption to competing lipophilic material in river banks, pond sediments and organic matter (Elliot *et al.* 1978). An overview of the toxicity of pyrethroids to aquatic organisms is given by Coats *et al.* (1989).

1.4.5. The mode of action of pyrethroid insecticides

The precise mode of action of synthetic pyrethroid insecticides is still debated and varies between different pyrethroids and organisms, however the symptoms exhibited in both vertebrates and invertebrates are indicative of a neurotoxic effect. Many researchers have studied the biochemical (see Ruigt, 1985; Leahey, 1985; Soderland and Bloomquist, 1989) and the kinetic (e.g. Ford *et al.* 1981; Greenwood *et al.* 1990) processes involved in the mode of action of pyrethroids in a range of organisms. It is accepted that the sodium channel is the primary target for the neurotoxic action of pyrethroids (Soderland and Bloomquist 1989). The progression from irritation and hyperactivity to knockdown and mortality observed in intoxicated insects is indicative of a sequential poisoning from the peripheral, sensory system to the central nervous system. Treatment of houseflies, *Musca domestica* (L.), with pyrethroids results in the uncoupling of the usually coordinated flight activity prior to the final inhibition of motor activity, a fact which supports the idea of an initial bias towards peripheral sites of action (Adams and Miller, 1980).

The toxicity of pyrethroids to insects is variable (Croft, 1990a). Physiological resistance or tolerance to pyrethroids is not only related to species-dependent differences in the site of primary lesion but is also governed by the ability of the organism to detoxify the insecticide e.g. by esterase activity or hydrolysis in tissues such as the cuticle and gut (Soderland and Bloomquist, 1989). Pyrethroids are of low toxicity to mammals because they are largely converted, by hydrolytic or oxidative attack, to polar metabolites which are then eliminated in the faeces and urine, unchanged or as conjugates, before sensitive sites are reached (Litchfield, 1985).

1.4.6 The development of pest resistance to pyrethroid insecticides

Pest resistance and cross-resistance to pyrethroids has developed widely, particularly in tropical climates where repeated insecticide applications have resulted in severe

be resistant to pyrethroids (Georghiou, 1986). Resistance is being combated by improving understanding of the genetics involved (e.g. Roush and McKenzie, 1987; Tabashnik, 1990; Tabashnik *et al.* 1990; Devonshire and Field, 1991) and the development of integrated resistance management programmes (Tabashnik and Croft, 1982; Tabashnik, 1989 & 1990; Croft 1990b; Denholm and Rowland, 1992). Elliot (1989) pointed out that strategies to guard against resistance developing and to diminish adverse effects to biological systems by choice of appropriate compound, timing, and site of application are increasingly recognized as of outstanding importance. He also suggested that the prospect of developing pyrethroids with properties appropriate for particular insect control applications were excellent. New groups of pyrethroids are being developed (for example ethofenprox, which is not an ester and has no centres of symmetry). However Elliot (1989) stated that whether these products will be able to compete with well-established products is largely a question of economics.

In the context of this study the risk of resistance developing in cereal aphids to pyrethroids in the U.K. cereals is thought to be minimal because insecticidal spray applications are usually only made once or possibly twice per season. These sprays are several months apart and chiefly aimed at different species of cereal aphids and therefore selection pressure for resistance is likely to be low. The potential for development of resistance in relation to the use of reduced-dose rates for pest management is discussed in Chapter 2 (2.4.2).

1.4.7 The effects of pyrethroid insecticides on non-target invertebrates

A large amount of information is available concerning the side-effects of synthetic pyrethroids on non-target organisms in a wide range of crops around the world. Therefore for brevity only general trends of the toxicity of pyrethoids to non-target invertebrates are discussed here, using data from the SELCTV database held by Professor Brian Croft at Oregon State University, Corvallis. For comprehensive reviews of the side-effects of pyrethroids on beneficial invertebrates see Croft and Whalon (1982), Hill (1985), Smith and Stratton (1986), Inglesfield (1989) and Croft (1990a).

Analysis of toxicity trends from records on the SELCTV database by Theiling (1987) and Theiling and Croft (1988) has led these authors to suggest that the pyrethroids are probably the most toxic class of organic insecticides to beneficial invertebrates developed to date. This trend is exemplified by comparison of mean toxicity ratings to beneficial invertebrates obtained from the SELCTV database concerning all records

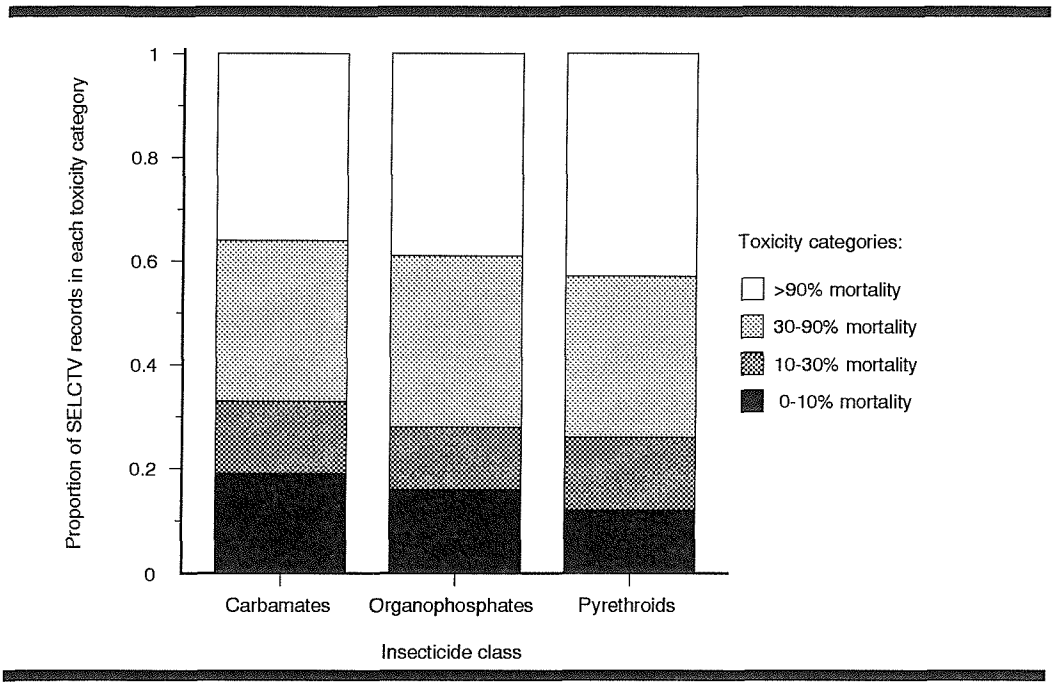

Figure 1.2 Comparison of proportions of records in different toxicity categories for carbamate, organophosphate and pyrethroid insecticides to beneficial invertebrates from the SELCTV database. (adapted from Theiling, 1987 and Croft, 1990a)

of the toxicity of three of the major classes of insecticides, (i.e. the organophosphates, the carbamates and the pyrethroids) (Figure 1.2). The number of records in the database for each insecticide class were 5089, 1353 and 701 respectively. The pyrethroids were shown to have a larger proportion of records (approx. 43%) in the highest toxicity category (i.e. causing >90% mortality) than the organophosphates (approx. 38%) and the carbamates (approx. 36%). The pyrethroids also had a smaller proportion of records (approx. 13%) in the lowest toxicity category (i.e. causing 0-10% mortality) compared to approximately 17% for the organophosphates and 19% for the carbamates.

Despite this high overall toxicity however, there is some evidence in the literature to suggest that the toxicity of individual pyrethroids can be variable within different predatory groups (Croft and Whalon, 1982). Several pyrethroids have been shown to exhibit physiological selectivity to certain species of parasitoids and predators (e.g. *Venturia canescens* (L.) and *Chrysoperla carnea* (Stephens)) (Croft, 1990a). One of the reasons for this is thought to be highly active esterase detoxification mechanisms that are present in some species of predators (Rajakulendran and Plapp, 1982; Croft and Mullin, 1984). However even though selectivity has been shown in some cases there is little evidence to suggest any overall differences in selectivity between different

pyrethroid compounds. Theiling and Croft (1988) compared toxicity trends between different pyrethroids using the SELCTV database and were unable to identify any individual compounds that showed a consistent pattern of physiological or broad selectivity. However some authors believe (e.g. Elliot, 1989) that the potential to develop selective pyrethroids may be increased in the future due to advances in pyrethroid chemistry.

1.5 The effects of pyrethroid insecticides on aphid predators in U.K. cereals : A case study

1.5.1 The pest status of cereal aphids (Homoptera: Aphididae) in U.K. cereals

Cereal aphids have been recorded in the U.K. since the eighteenth century (Marsham, 1798, cited from Dixon, 1987). However they were not given pest status until the early 1950's when they were found to be vectors of Barley Yellow Dwarf Virus (BYDV) (Kendall *et al.* 1984). There are three common species, the grain aphid (*Sitobion avenae* (F.)), the rose-grain aphid (*Metopolophium dirhodum* (Walk.)), and the bird-cherry oat aphid (*Rhopalosiphum padi* (L.)), although several other species have been recorded and may occasionally be common. For the biology, life-cycles and pest status of cereal aphids see reviews by Vickerman and Wratten (1979), Carter *et al.* (1980), Dixon (1987) and Burn (1987).

There are two main periods when aphids invade cereals. *R. padi* is the principle BYDV vector in the autumn and spring, which can cause severe stunting of infected plants (Kendall *et al.* 1984), whereas *S. avenae* and *M. dirhodum* cause damage in the summer by direct feeding and honeydew production (Rabbinge and Carter, 1983; Vereijken, 1979) which can reduce grain weight and quality (Lee *et al.* 1981). In the U.K., where approximately 2 million hectares of wheat are grown each year, cereal aphids have a high pest status in the autumn and summer, even though outbreaks are sporadic.

1.5.2 Controlling cereal aphids with insecticides

Aphid populations may be controlled by insecticides in two ways, by treating crops routinely (prophylactically) with an aphicide or by monitoring aphid numbers in the crop and using threshold levels to decide when spraying is necessary. Field trials measuring yield losses due to cereal aphids in the U.K. have led ADAS (the Agricultural Development and Advisory Service, M.A.F.F.) to advise farmers to spray cereals with

an aphicide in the summer if populations of *S. avenae* are increasing between flowering and the milky ripe stage (decimal growth stages 61 to 75, Zadoks *et al.* 1974) and exceed 5 per ear (George, 1975; George and Gair, 1979) or when 66% of ears are infested. For *M. dirhodum* the threshold is 30 or more aphids per flag leaf during the same growth stages. George and Gair (1979) estimated 10 to 20% yield losses due to direct damage by *S. avenae* in winter wheat whereas Vereijken (1979) concluded that in the absence of a fungicide treatment, losses caused by fungi growing on aphid honeydew may cause up to 50% yield loss.

The problem with applying insecticides prophylactically is that it is uneconomic to spray when aphids are not causing economic damage. Also if a non-selective aphicide is used it may kill beneficial non-target species including indigenous natural enemies of aphids which may lead to a latter aphid outbreak if favourable conditions occur (Powell *et al.* 1985). The advantage with prophylactic spraying however, from the farmers point of view, is that it acts as an insurance policy and does not have the risks of monitoring and forecasting.

To aid forecasting of likely damage by aphid populations and the use of economic thresholds more sophisticated computer models have been developed, taking into account pest numbers, crop growth stage, and economics of spraying (e.g. Mann and Wratten, 1986). These models have been shown to perform as well or better than simple threshold advice (Mann and Wratten, 1991) and can lead to economic benefits for the farmer.

1.5.3 Insecticide usage in U.K. cereals

Between 1988 and 1990 the area of cereals in the U.K. treated with insecticides increased by 139% (Davis *et al.* 1990). This rise was almost entirely due to a sevenfold increase in the area treated with pyrethroids. The majority of the spraying took place with cypermethrin, deltamethrin and fenvalerate to control aphids in the autumn. The use of insecticides to control aphids in the summer has also increased over recent years (e.g. Sly, 1986; Rands *et al.* 1988). In a recent survey of the use of insecticides by farmers to control cereal aphids in the U.K., Wratten and Mann (1988) found the most common active ingredients applied as cereal aphicides were the organophosphates, dimethoate and demeton-S-methyl, and the carbamate, pirimicarb. They found that over 55% of the 115,000 ha surveyed were treated with summer aphicides and over 60% of the treated area was sprayed with broad-spectrum compounds often at levels of aphid infestation below ADAS damage thresholds.

1.5.4 The use of summer-applied synthetic pyrethroids in U.K. cereals.

Before 1990 application of synthetic pyrethroids was restricted to the autumn only where they have been shown to provide good control of BYDV (Gibson *et al.* 1982; Perrin, 1986) and may have limited effects on many of the important polyphagous predators which will have dispersed to field boundaries to overwinter (Sotherton, 1984 & 1985). In 1990 the moratorium placed on the summer use of synthetic pyrethroid insecticides by the U.K. Advisory Committee on Pesticides since 1982, was partially lifted for two products, deltamethrin and alphacypermethrin. Deltamethrin was approved to be applied at a rate of 6.25 g AI ha^{-1} and alphacypermethrin at a rate of 16.5 g AI ha^{-1}. Both products were initially given 12 months provisional approval for summer use. They could be applied between the onset of flowering and the milky ripe stage, i.e. between decimal crop growth stages 61 to 73 (Zadoks *et al.* 1974). The moratorium was initially imposed due to lack of data on the spectrum of activity on non-target invertebrates, fears of the aquatic toxicity, and cross resistance potential with currently used organophosphate compounds. However it was lifted for deltamethrin largely because of the results of summer field trials carried out by Fischer and Chambon (1987) and Vickerman *et al.* (1987a & b). The findings from these studies will be reviewed later (section 1.6.2).

It is likely that the number of synthetic pyrethroids as summer applied aphicides in cereals will increase in the future particularly as they are very effective aphicides and public concern is pressing for a reduction in the organophosphate compounds currently in use because of properties such as high mammalian toxicity.

1.5.5 Approaches to reduce insecticide inputs in cereals

1.5.5.1 "Conservation Headlands"

In the last few years "Conservation Headlands" have become more prominent around cereal fields in the U.K. as a result of research by The Game Conservancy Trust. The Game Conservancy has proposed a selective spraying programme over six metre headland strips at crucial times of the year to encourage farmland wildlife such as butterflies, beneficial invertebrates such as carabid and staphylinid beetles and hoverflies which act as aphid predators, and insects such as leaf beetles, weevils and sawfly larvae which provide an important food source for gamebird chicks (Sotherton, 1990). The guidelines produced by The Game Conservancy inform farmers which insecticides, fungicides, growth regulators and herbicides may be applied to the headland strips and at what time of the season. In the case of insecticides the

guidelines advise that autumn applications may be made provided spray drift into hedgerows is avoided. Spring applications of insecticides should be avoided after 15th March.

1.5.5.2 Use of reduced-dose rates

The impact of broad-spectrum pesticides on beneficial invertebrates in cereals have been shown by the findings of farm-scale studies such as the Boxworth Project (Grieg-Smith *at al.* 1992). Results from Boxworth indicated that pesticides applied under intensive treatment regimes to cereals may reduce the capacity of predators to limit prey populations (Burn, 1992). Also results from within-field experiments (Duffield and Baker, 1990; Duffield, 1991) suggest that aphid resurgence may occur in the centre of cereal plots treated with the broad-spectrum insecticides as predators may take several days or weeks to reinvade the centre of the crop.

In order to reduce these problems, rational decisions need to be taken when sprays are required to control pest outbreaks. These may include the choice of suitable compounds and/or dose-rates to reduce the impact of the spray on beneficial invertebrates. This may be possible by using a "soft" or selective compound, or possibly by exploiting differences in susceptibilities of pests and predators by reducing dose-rates. The most selective insecticide available for use in U.K. cereals is probably the carbamate pirimicarb (e.g. Brown 1989b; Entwistle 1989). In a recent pesticide usage survey summer-applied aphicides Wratten and Mann (1988) found that pirimicarb was applied to a larger area of cereals (37%) than the broad-spectrum organophosphate insecticides dimethoate (31%) and demeton-S-methyl (30%). However this still indicates that overall a large area was sprayed with broad-spectrum insecticides.

If broad-spectrum insecticides are used, it may be possible to use them at reduced-dose rates. The aim of this approach is that the dose applied must still remain effective against the pest while enabling a proportion of the predators to survive. The result ideally being that these predators may then remain in the crop, providing that some food is available, and prevent the pest from resurging. Studies by Poehling (1988 & 1989) with the aphid *M. dirhodum* and predators such as syrphid larvae, coccinellids and chrysopids have indicated that it may be possible to increase the selectivity of compounds such as pirimicarb and the pyrethroid fenvalerate to predators by reducing dose rates. However whether this approach can be exploited or not is likely to be a question of economics. Studies carried by Mann *et al.* (1991) to determine the

economics of full-rate and reduced-rate summer spray applications of pirimicarb and the synthetic pyrethriod fenvalerate to control cereal aphids have suggested that under certain circumstances the high efficiencies of these insecticides at full dose-rate were not necessary for effective aphid control and that better economic returns could be made by reducing the quantity of active ingredient per hectare. They found that the relative effects between dose rates varied according to crop growth stage and the size of aphid populations at the time of spraying. For example reduced-dose rates were more profitable than full-rate applications when aphid populations were low or developed late in the season.

1.5.5.3 Enhancing natural enemies

The third approach for reducing pesticide inputs is by manipulating or enhancing numbers of beneficial invertebrates in cereals crops and therefore increasing their potential to control aphid numbers. Two approaches are currently being researched in U.K. cereals. The first is the creation of "island" habitats (earth-ridges sown with hedgerow grass species) in cereal crops which may act as over-wintering sites for a range of polyphagous predators. Creating these earth-ridges in the field may redistribute or enhance predator populations (Thomas, 1989; Thomas, 1990; Thomas *et al.* 1992). Full details of how to create these ridges and the costs involved are given in Thomas *et al.* (1991). The second method involves sowing strips of flowering plants along field margins. These act as pollen and nectar resources for beneficial invertebrates such as hoverflies and honey bees. One of the plants used so far, the North American *Phacelia tanacetifolia* (a member of the Hydrophyllaceae family), has provided promising results with hoverflies. In a recent study in maize on the Isle of White hoverflies in fields with borders of *Phacelia* laid twice as many eggs per aphid as those in control fields (Hickman and Wratten, 1993).

These approaches need to be integrated in the future so that pesticides may be used rationally and the potential of biological control agents can be maximised. The prospects of achieving this may be promising as lower profit margins on cereal crops are likely to encourage farmers to implement these approaches to reduce their variable costs.

1.5.6 The role of natural enemies in aphid control in temperate cereal ecosystems

Concern over the environmental effects of pesticides has generated much interest in the natural enemies of cereal aphids in the last 10 to 15 years. These natural enemies

can be placed into three main categories; polyphagous predators, aphid-specific predators and parasitoids. Wratten (1987) and Wratten and Powell (1991) reported that approximately 400 species of beneficial arthropods inhabit cereal fields in the south of England. Some of the most abundant species are listed in Table 1.2.

Table 1.2 Common invertebrate natural enemies of cereal aphids that inhabit temperate cereal crops.

POLYPHAGOUS PREDATORS

Coleoptera: Carabidae
Agonum dorsale (Pontoppidan)
Bembidion lampros (Herbst)
B. obtusum (Serville)
Calathus fuscipes (Goeze)
Demetrias atricapillus (L.)
Harpalus rufipes (Degeer)
H. affinis (Shrank)
L. pilicornis (F.)
Nebria brevicollis (F.)
Notiophilus biguttatus (F.)
Pterostichus melanarius (Ill.)
P. madidus (F.)
Trechus quadristriatus (Shrank)

Coleoptera: Staphylinidae
Philonthus cognatus (Stephens)
Tachyporus chyrsomelinus (L.)
T. dispar (Paykull)
T. hypnorum (F.)
T. obtusus (L.)

Araneae: Linyphiidae
Bathyphantes gracillus (Blackwall)
Erigone atra (Blackwall)
Lepthyphantes tenuis (Blackwall)
Meioeta rurestris (Blackwall)
Oedothorax apicatus (Blackwall)
O. fuscus (Blackwall)
O. retusus (Westring)

Araneae: Lycosidae
Pardosa pullata (Clerck)

Dermaptera
Forficula auricularia (L.)

Diptera: Empididae
Empis livida (Meig.)
Platypalpus minutus (Meig.)
P. pallidiventri (Meig.)

Diptera: Dolichopodidae
Sciapus platyterus (F.)

APHID-SPECIFIC PREDATORS

Coleoptera: Coccinellidae
Adalia bipunctata (L.)
Coccinella septempunctata (L.)
Propylea quadridecempunctata (L.)

Diptera: Syrphidae
Episyrphus balteatus (Degeer)
Melanostoma mellinum (L.)
Metasyrphus corollae (L.)
Syrphus vitripennis (Meig.)

Hemiptera: Anthocoridae
Anthocoris spp.

Neuroptera: Chrysopidae
Chrysoperla carnea (Stephens)

PARASITOIDS

Hymenoptera: Braconidae
Aphidius ervi (Haliday)
A. picipes (Nees)
A. rhopalosiphi (De Stefani-Perez)
Praon volucre (Haliday)
Toxares deltiger (Haliday)

(Adapted from Jepson, 1989)

1.5.6.1 Polyphagous predators

The majority of aphid predators found in the cereal ecosystem are polyphagous. More than 100 species may be common in the summer months (Sunderland *et al.* 1986). The most important groups are the carabid beetles (Coleoptera: Caradidae), the staphylinid beetles (Coleoptera: Staphylinidae), the linyphiid spiders (Arachnida: Araneae: Linyphiidae), the earwigs (Dermaptera: Forficulidae) and predatory flies (Diptera: Empididae and Dolichopodidae) (Edwards *et al.* 1979).

The most efficient means of restricting aphid outbreaks is the elimination of immigrants before they are able to begin reproduction (Potts, 1977). Although polyphagous predators consume prey other than cereal aphids, such as Collembola, mites and Diptera larvae, they are present in the field during the crucial establishment phase of aphids and some have been shown to consume aphids at low densities (Sunderland and Vickerman, 1980). It is for this reason and their widespread abundance that they have received much attention.

Potts and Vickerman (1974) were the first to show significant negative relationships between cereal aphid numbers and the proportion of predatory arthropods in different fields. This was later shown again by Chambers *et al.* (1982) and in Sweden by Ekbom and Wiktelius (1985). Many researchers have manipulated polyphagous predator abundance by excluding them with barriers dug into the soil, and have found an increase in aphid numbers when no predators are present (Edwards *et al.* 1979; De Clerq and Pietraszko, 1983; Chiverton, 1986; Winder, 1990).

Sunderland (1975) and Sunderland and Vickerman (1980) proved that polyphagous predators feed on aphids by finding aphid remains in the dissected guts of many species of polyphagous predators. However as many aphid predators are fluid feeders gut dissection is not always helpful and so Crook and Sunderland (1984) developed an enzyme-linked immunosorbent assay (ELISA) to detect aphid remains in predators. They produced an antiserum which was relatively specific to *S. avenae* and was sensitive to 0.01 of an adult aphid. Sopp (1987) used this technique and found high aphid levels in four carabid, one staphylinid, and one linyphiid spider species, collected from cereal fields when aphid densities were low. Chiverton (1986) carried out similar studies to identify those polyphagous predators which fed upon *R. padi*.

Numerous studies have been carried to evaluate the ecology and role of polyphagous predators in reducing aphid numbers in cereal crops. These include studies of

abundance and density (eg. Sunderland and Vickerman, 1980), consumption rates and feeding (eg. Pearson, 1980; Griffiths, 1983; Scheller, 1984; Loughridge and Luff, 1983; Sopp and Wratten, 1986; Coombes, 1987; Winder, 1990; Mauremootoo, 1991; Dennis and Wratten, 1991), foraging behaviour (eg. Halsall and Wratten, 1988; Halsall, 1990), overwintering sites (eg. Sotherton, 1984 & 1985), phenology in relation to that of aphids (eg. Coombes and Sotherton, 1986), response to prey heterogeneity (eg. Bryan and Wratten, 1984), as well as the possible effects that cultural practices (eg. Shires, 1980; Powell *et al.* 1985) may have upon them.

1.5.6.2 Aphid-specific predators

The aphid-specific predators include coccinellid larvae and adults (e.g. *C. septempunctata*), syrphid larvae (e.g. *E. balteatus*) and lacewing larvae (e.g. *C. carnea*) (Vickerman and Wratten, 1979). The value of these predators as aphid control agents has been widely reported (Rabbinge *et al.* 1979; Chambers *et al.* 1983; Sunderland *et al.* 1986). These predators, especially the larvae, are very voracious (Chambers and Adams, 1986). The adults and larvae can locate aphids at low densities, for example syrphid adults lay eggs in response to aphid densities of 0.4 to 0.5 aphids per shoot, and the larvae develop quickly (Chambers, 1988). However aphid-specific predators often reproduce when aphids are abundant and well into the establishment phase to ensure an adequate food supply for their offspring and therefore the aphids may have already caused economic damage (Chambers *et al.* 1983).

1.5.6.3 Parasitoids

Aphid parasitoids belong to two families of Hymenoptera, the Aphelinidae and the Aphidiidae, which are the most common in the U.K. (Powell, 1982). They lay a single egg in aphids which subsequently develops into a larva and kills its host. The most common species, *A. ervi*, *A. rhopalosiphi*, *A. picipes*, and *P. volucre* have been shown to influence the population growth of *S. avenae* (Ankersmit, 1982). Parasitoids can be very abundant, are mobile between fields, and can be active in the early season (Powell, 1983; Vorley, 1986). Chambers *et al.* (1983) have stated that parasitoids have major effects in the early stages of aphid population development when densities are as low as 0.1 aphid per shoot. Aphid parasitoids tend to emigrate from crops early in the season (Vorley, 1986) which may be a strategy to reduce loss of offspring to late-season aphid predation and hyperparasitism. This may mean that in years when late-season aphid outbreaks occur (i.e. after cool springs), parasitoid activity may have declined while there is still significant aphid immigration into the crop. However Carter

et al. (1982) showed by simulation studies that parasitoids reduce the rate of population growth of *S. avenae*. Likewise Vorley and Wratten (1985) have predicted, using a simulation model, that aphids in the absence of parasitism would increase at flowering at a rate seven times than that in the presence of parasitism.

1.5.7 Studies of the effects of synthetic pyrethroids on non-target terrestrial invertebrates in temperate crops

The non-target terrestrial organisms that have received most attention when testing pyrethroid insecticides in temperate crops are, 1) soil organisms, 2) honey bees and 3) predators and parasites. The first two groups are discussed briefly in this section and the third group are discussed in section 1.6.2 in the context of this project. For full reviews of the effects of pyrethroids on these organisms see Smith and Stratton (1986), Hill (1985), and Inglesfield (1989).

1.5.7.1 Soil organisms

The soil community consists of two important components, the microflora (fungi, bacteria and algae) and invertebrates (from microscopic Tardigrada, Rotifera, Nematoda, and Turbellaria to the larger Collembola, Acari, Myriapoda and the annelid worms). All of these organisms have a vital role in the maintenance of soil fertility (Richards, 1974). Inglesfield (1989) summarised work by numerous authors on a range of pyrethroids and unpublished data from Shell Research Ltd. and suggested that they have shown no adverse effects on the activities of soil microflora. Studies reported by Hill (1985) and Inglesfield (1984) have also shown no significant effects of pyrethroids on earthworms. However laboratory studies by Curl *et al.* (1987), using radio-labelled cypermethrin, demonstrated that radio-active pyrethroid metabolites were accumulated by earthworms. Feeding studies demonstrated that no further accumulation occurred in birds and mammals.

1.5.7.2 Honey bees

Honey bees are highly valued by farmers as pollinators and honey producers worldwide (eg. Southwick and Southwick, 1992). Acute laboratory toxicity tests with a range of pyrethroids have shown them to be highly toxic to bees (Smart and Stevenson, 1982; Murray, 1985) with LD_{50} values as low as 0.03μg bee^{-1} (Murray, 1985). However field studies have shown pyrethroids present little or no hazard to honey bees (*Apis mellifera* L.) when used at field rates (Pike *et al.* 1982; Shires *et al.* 1984). Pyrethroid treatment causes a reduction in bee foraging in the 24 hours after spraying (Shires *et al.* 1984). This reduction in activity was attributed to repellent

effects of the formulation by Delabie *et al.* (1985) who carried out oral exposure experiments with technical grade and formulated cypermethrin. However Rieth and Levin (1988) have challenged this finding and suggest that the oral route will not be the principle mechanism of exposure in the field. They found that pyrethroid active ingredients act as contact repellents and sub-lethal dose causes transient inhibition of activity.

Pyrethroids are considered safe to bees and are used widely in crops such as oilseed rape. However recently concern has been raised over reports of bee deaths in oilseed rape. Research has recently shown that this could be due to synergism between pyrethroid insecticides and ergosterol biosynthesis inhibitor fungicides which may increase pyrethroid toxicity to bees several fold depending on the compounds concerned (Pilling, 1992).

1.6 Aims of the study

A mechanistic approach was adopted in this study to predict the risk posed by deltamethrin to aphid predators and parasitoids that inhabit temperate cereal crops. The need for such an approach to be taken for summer-applied synthetic pyrethroids in temperate cereal crops has become clear from the difficulty in interpreting results from field trial experiments (see section 1.6.2) and the lack of quantitative data concerning the importance of the different mechanisms that mediate pesticide side-effects on beneficial invertebrates.

Because of the absence of evidence for differences in selectivity between the different pyrethroids currently in use (see 1.4.7) it was decided to use a single compound, deltamethrin, as a model to develop a testing framework to aid understanding of the pesticide side-effects on the beneficial invertebrates in temperate cereals.

1.6.1 The selection of deltamethrin as the test pyrethroid

Deltamethrin was chosen as the test pyrethroid for several reasons. Firstly because it was one of the first synthetic pyrethroid insecticides to be given provisional approval for use as a summer-applied aphicide in U.K. cereal crops. Secondly because most of the published field trial data available from summer applications of pyrethroids in temperate cereals concerns this product. Thirdly because it is known to be one of the most toxic pyrethroid compounds (Figure 1.3) and fourthly because it is the most widely used pyrethroid worldwide, with an approximate 30% share of pyrethroid market and is registered in over 100 countries around the world (AGROW, 1991b). Therefore

there is already a relatively large base of information available on it's side-effects and behaviour in other ecosystems.

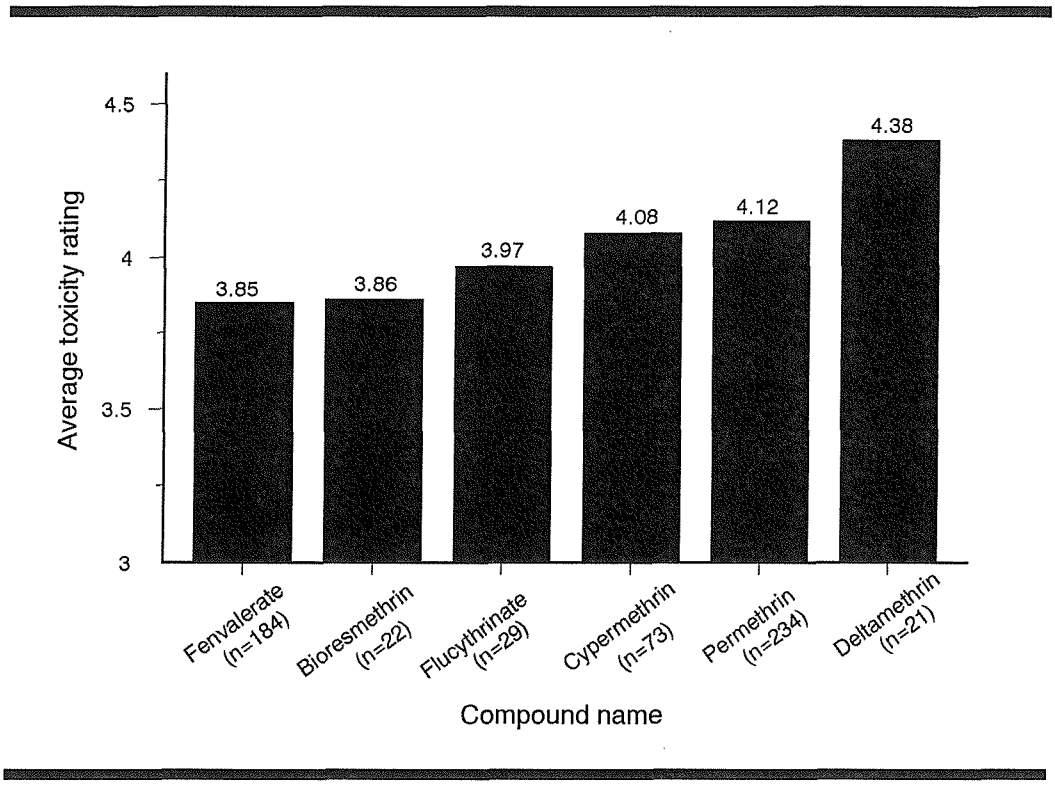

Figure 1.3 Toxicity ratings for six synthetic pyrethroid insecticides to beneficial invertebrates from the SELCTV database. (Adapted from Croft, 1990a).
Toxicity ratings based on a scale of 1 to 5: 1 = no effect on beneficials, 2 = <10% effect, 3 = 10 to 30% effect, 4 = 31 to 90% effect, 5 = >90% effect. n = number of records.

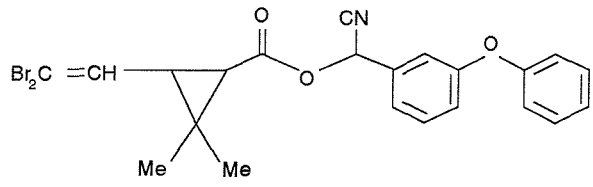

Figure 1.4 The chemical structure of deltamethrin

The chemical structure and and a summary of the properties of deltamethrin are given in Figure 1.4 and Table 1.3. Formulated deltamethrin 2.5% E.C. with xylene, toluene, ethyl and propyl benzenes (Decis 25 g l^{-1}, Hoechst U.K. Ltd.) obtained from a commercial supplier was used in all experiments in this study.

Table 1.3 A profile of the chemical properties of deltamethrin
(From The Pesticide Manual (1983) and AGROW (1991a)).

Common name :	Deltamethrin
Code names :	NRDC 161 (Licensed to Roussel Uclaf) CODEX 135 OMS 1998
Chemical name : (IUPAC)	(s)-α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropan-1-carboxylate
Empirical formula :	$C_{22}H_{19}Br_2NO_3$
Properties :	Technical grade (Roussel Uclaf) ≥ 98.5% deltamethrin m/m; Colourless crystalline powder; Melting point 98-101°C; Vapour pressure 2μPa at 25°C; Solubility at 20°C,< 2 μg l^{-1} in water, 500 g l^{-1} in acetone. Stable to air. More stable in acid than alkaline media.

1.6.2 Field studies on the effects of deitamethrin on aphid predators in temperate cereals

Much of the information available on the side-effects of summer applications of deltamethrin on non-target invertebrates in temperate cereals has come from four large scale field experiments carried out in the 1980's (Table 1.4). The results from these studies have indicated that summer applications of deltamethrin may adversely affect some groups of beneficial invertebrates, such as linyphiid spiders (e.g. Fischer and Chambon, 1987), staphylinid beetles (e.g. Basedow *et al.* 1985; Vickerman *et al.* 1987b), empid flies and coccinellid larvae (e.g Vickerman *et al.* 1987a).

The toxicological implications of these results are difficult to interpret however, as they are primarily based on numerical reductions in trap catches. Therefore the chance of detecting these changes will be affected by the limitations of each sampling method and the experimental design. For example pitfall trap capture efficiency is known to be species-dependent (Curtis, 1980, Halsall and Wratten, 1988; Topping and Sunderland, 1992) and can be affected by the surrounding environment, such as soil type and vegetation (Speight and Lawton 1976; Adis, 1979). Surface searching is time consuming and will underestimate species if they burrow underground. Whereas D-vac

Table 1.4 Summaries of field studies on the side-effects of summer-applied deltamethrin sprays on communities of aphid predators in temperate cereal crops

Author & chemical application details (mass & volume)	Crop, decimal growth stage & date of spray	Design & plot size	Assessment methods	Summary of results and conclusions
Basedow *et al.* (1985) Deltamethrin E.C. 7.5 g AI ha^{-1} in 300 l ha^{-1} Toxic standards: None	W. wheat (Growth stage not given). Spray date 18th June 1980 & 1981	Sprayed plots 72 x 250 m Control plots 72 x 72 m	Pitfall traps 20 per treatment 10 m apart	Carabid species breeding in spring/early summer were rare in this study. One spring-breeder (*Loricera pilicornis*) and three autumn-breeders, (*P. melanarius*, *P. niger*, & *T. quadristriatus*) were not effected significantly. Numbers increased after spray application in treated and control plots. At the spray dates the autumn breeders were hidden in the soil as pupae. Three species of spring breeding staphylinids, *T. hypnorum*, *L. fulvipenne*, & *T. rufipes* seemed to be strongly reduced in numbers. Linyphiid spiders were affected most heavily (reduced by 92%). Adverse effects were still evident 60 days after treatment.
Vickerman *et al.* (1987a) Deltamethrin E.C. 6.23 g AI ha^{-1} in 220 l ha^{-1} Toxic standards: Dimethoate 399 g AI ha^{-1} Pirimicarb 139 g AI ha^{-1}	W. wheat G.S. 60 to 63 Spray date 21st June 1984	40.5 ha field 2 Reps. 4 randomised 3.9 to 5.1 ha plots	D-Vac 10 samples/plot/date Total area = 4.6 m^2 To 11 weeks post-treatment	Deltamethrin did not affect the total numbers of non-target arthropods but effects were found on some predator groups. Empididae were reduced by 56%. Dolichopididae were more numerous in treated than control plots (which may be attributed to scarsity at time of application). Coccinellid larvae were reduced by 65%. *Aphidius* spp. parasitoids increased in deltamethrin plots 2 weeks post-treatment.

Table 1.4 (cont.)

Author & chemical application details (mass & volume)	Crop, decimal growth stage & date of spray	Design & plot size	Assessment methods	Summary of results and conclusions
Vickerman *et al.* (1987b) Deltamethrin E.C. 6.23 g Al ha^{-1} in 220 l Ha^{-1} Toxic standards: Dimethoate 399 g Al ha^{-1} Pirimicarb 139 g Al ha^{-1}	W. wheat G.S. 60 to 63 Spray date 21st June 1984	40.5 ha field Each assessment 2 reps. 4 randomised plots 3.9 to 5.1 ha	D-Vac 10 samples/plot/date Total area = 4.6 m^2 To 75 days post-treatment Quadrats 0.1 m^2 5 samples/plot 10 m intervals	Deltamethrin reduced the numbers of Carabidae by 22% and Staphylinidae by 20% during the post-treatment period in the D-vac samples. Numbers of *D. atricapillus* (adults and larvae) and *Tachyporus spp.* (adults and larvae) were reduced significantly by deltamethrin. In the quadrats the Carabidae were reduced by 4%.
Fischer & Chambon (1987) Deltamethrin E.C. 6.25 g Al ha^{-1} in 300 l ha^{-1} 1983 in 200 l ha^{-1} 1984,1985 Toxic standards: Dimethoate 400 g Al ha^{-1} Phosalone 600 g Al ha^{-1}	W. wheat G.S. 60-62 & 63-65 Spray date 20th June in 1983 & 1985, 19th June in 1984	18.5 ha field 3 plots of 6 ha, each divided into 3 sub-plots	Pitfall traps (2/sub-plot) Water traps (4/sub-plot) D-Vac (33 samples/sub-plot) Ear sampling (25-33/sub-plot)	Action was noticed on predatory Diptera (Empididae and Dolichopididae) and on spiders (Erigonidae, Lycosidae, Linyphiidae, and Theridiidae). The detritiphagous insects Sciaridae and Chironomidae), the Carabidae and Staphylinidae and most micro-Hymenoptera showed little or no difference after treatment.

sampling, whilst providing an estimate of the absolute numbers at a given time, is inefficient at sampling larger predator species and sampling would have to be carried out at night to determine effects on nocturnal species. Even given that these sampling methods may be the best available and that their limitations have been minimised, significant numerical differences between catches will only indicate changes in abundance and activity of species and cannot be used to infer direct toxicological effects. This difficulty is exemplified in the two studies by Vickerman *et al.* (1987a & b). They suggested that reductions in the numbers of coccinellid larvae may have been due to starvation rather than direct mortality from deltamethrin and that reductions in numbers of plant-active predators such as *T. hypnorum* and *D. atricapillus* were possibly because these predators were exposed to higher levels of residues on the cereal plants. These suggestions can only be verified by detailed information on the relative toxicities of deltamethrin to different predator species and detailed information concerning the relative toxicity of deltamethrin residues on different substrates.

Therefore because of the lack of toxicological data on mechanisms of toxicity of deltamethrin to beneficial invertebrates in temperate cereals, with the exception of linyphiid spiders which have been shown to be sensitive to deltamethrin (Everts, 1990; Thomas *et al.* 1990; Everts *et al.* 1991; Mullié and Everts, 1991; Jagers op Akkerhuis and Van der Voet, 1992; Jagers op Akkerhuis and Hamers, 1992), a rigorous experimental approach was adopted to establish the susceptibility of a range predators to deltamethrin via their likely routes of exposure.

1.6.3 The experimental framework and goals of the project

Laboratory bioassays were used to determine the topical, residual and dietary toxicity of deltamethrin to predators and to establish the susceptibility spectra exhibited by a wide taxonomic and size range of beneficial species. Also *in situ* bioassays were carried out to determine the levels of mortality that may occur from realistic concentrations of deltamethrin in the field.

The goals of the study were to establish susceptibility rankings of species to determine which may be at greatest risk from deltamethrin sprays, to predict levels of mortality that may result from field rate applications of deltamethrin and to predict dose rates which may minimise mortality of the predators whilst still providing aphid control. A flow chart indicating the experimental framework of the study and how bioassay results were integrated to make predictions of risk is shown in Figure 1.5.

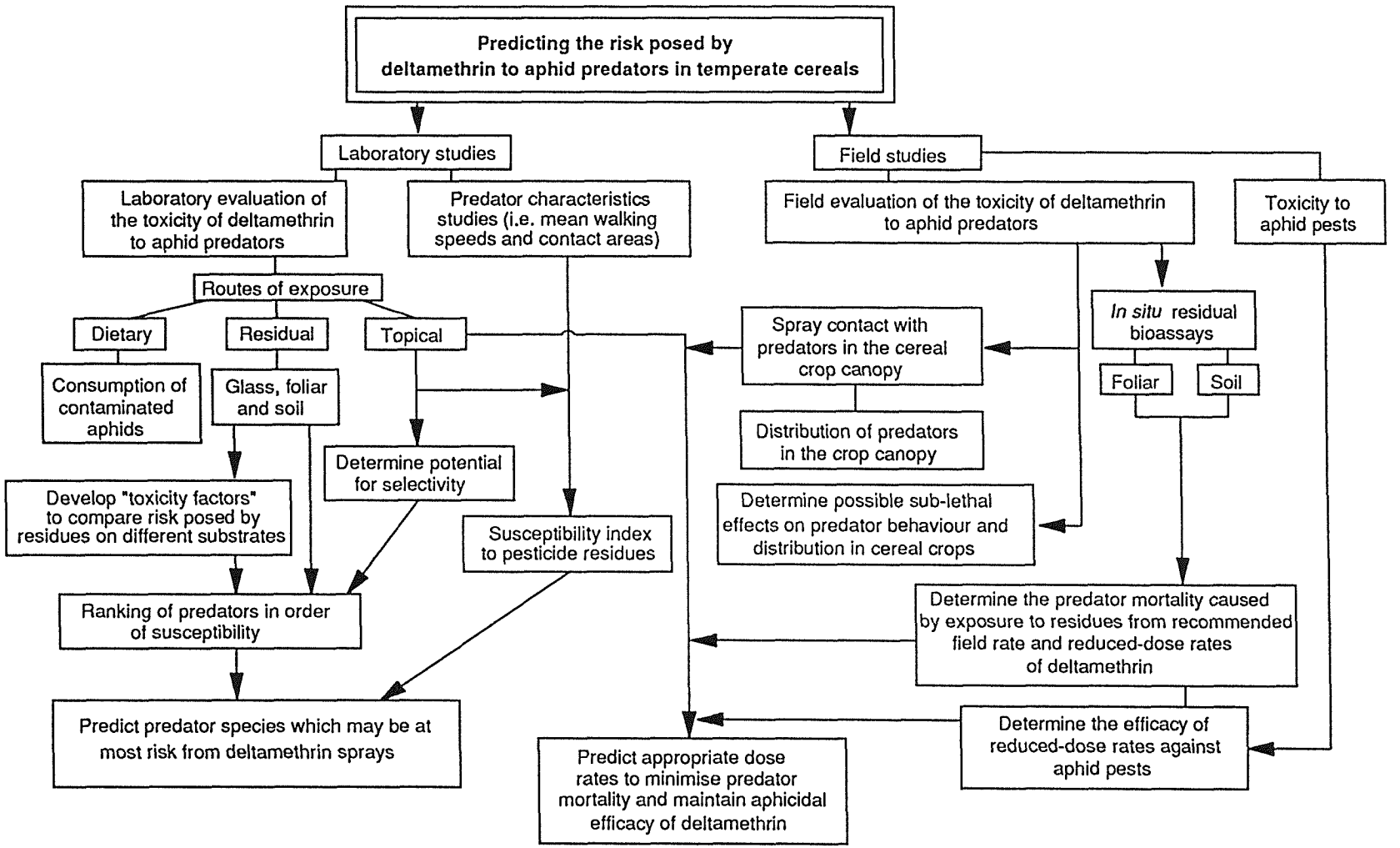

Figure 1.5 Flow chart indicating the mechanistic approach taken in this study and how results were integrated to make risk predictions

CHAPTER 2

The susceptibility of the grain aphid *Sitobion avenae* (F.) (Homoptera: Aphididae) and it's natural enemies to deltamethrin.

2.1 INTRODUCTION

The toxicity of pyrethroid insecticides to arthropod natural enemies and their potential for achieving selectivity has received much attention, particularly in crops such as apples and cotton (Croft and Whalon, 1982; Theiling and Croft, 1988; Pickett, 1988). In order to exploit the potential for selectivity in integrated pest management systems detailed ecotoxicological studies of pest and natural enemy communities are required. This chapter aims to measure the susceptibility of the grain aphid *S. avenae* and a range of it's important natural enemies to deltamethrin by laboratory topical bioassay to enable comparisons to be made between species on the basis of their intrinsic susceptibility.

The most commonly used index of susceptibility is the LD_{50}, an estimate of the median lethal dose, normally obtained from analysis of dose-response data (Finney, 1971; Busvine, 1971; Robertson and Preisler, 1992). The LD_{50} may be expressed in terms of dose per arthropod, which gives an indication of susceptibility in the field, or in terms of dose per unit body weight, which gives a measure of the intrinsic susceptibility of the species to the toxicant. Both measurements are considered in this chapter. Many factors may influence the susceptibility of individual arthropods to pesticides. These include the characteristics of the organism tested (eg. lifestage, age, size, weight, or sex), the environmental conditions (eg. temperature and humidity), and also the nature of the pesticide concerned, (eg. active ingredient, application method, diluent and formulation) (Jepson, 1989; Croft, 1990a). For comparability of results therefore these factors must be controlled as far as possible. To achieve this, it is an advantage to undertake controlled-dose tests under constant laboratory conditions and to use laboratory cultured insects, identical in age and physiological condition. Many of the predators used in these bioassays however had not previously been cultured and the field capture of active individuals at their peak of seasonal activity was relied upon.

By comparing the susceptibilities of a range of co-existing predators and an aphid pest

to deltamethrin this chapter aimed to address the following questions:

1. Does susceptibility vary significantly between pests and predators in cereals?
2. Can any variation in susceptibility be accounted for in terms of differing body mass or by separating the groups taxonomically?
3. Is there any evidence for selectivity between pest and natural enemy species?

2.2 EXPERIMENTAL METHODS

2.2.1 Test invertebrates

The pest species used in these bioassays was the grain aphid, *S. avenae*. Many of the predators that were tested were highly ranked in importance as aphid predators (Sunderland and Vickerman, 1980; Sopp and Wratten, 1986). They included polyphagous predators such as the large carabid beetles *P. melanarius*, *H. rufipes* and *N. brevicollis*, the medium sized carabid beetle *A. dorsale* and the small carabid beetles *D. atricapillus*, *T. quadristriatus*, *B. obtusum* and *B. lampros*, the small staphylinid beetle *T. hypnorum*, and females of the linyphiid spider *E. atra*. Adults and larvae of the aphid-specific coccinellid *C. septempunctata* were also tested. The adult coccinellids were collected in May, denoted by (1), and July, denoted by (2), and were from two separate generations. The 4th instar coccinellid larvae, denoted by (L), were also collected in July. The two species of *Bembidion* were tested because they differ in body size, habitat preference and exposure to pesticides. *B. obtusum* is a field-overwintering species and is exposed to autumn and summer pyrethroid treatments whereas *B. lampros* overwinters in field boundaries and therefore avoids autumn sprays (Sotherton 1984 & 1985; Jepson, 1989). *E. atra* females alone were tested because spider catches mainly consisted of females of this species.

The predators were captured between October 1989 and August 1990 in cereal fields and field margins at Leckford, near Stockbridge, Hampshire, by dry pitfall trapping, Dietrick vacuum suction sampling, hand-held air aspirator and surface searching (Southwood, 1987). The Carabidae and Staphylinidae were kept in plastic aquaria, containing a layer of moist soil. They were fed on ground, moist cat biscuits ("Delicat"-Quaker Latz GmbH). The coccinellids were placed in perspex boxes with barley plants infested with *S. avenae*. The linyphiid spiders were kept in perspex boxes with moist tissue paper and were provided with live fruit flies (*Drosphila* spp.) as food. The aphid, *S. avenae* was cultured on barley seedlings. All test invertebrates were kept in a controlled environment room in an insectary, maintained at 19-22°C, 55-70 % relative

humidity, and photoperiod 16:8 L:D, prior to treatment.

After treatment, all test invertebrates were placed in clean, ventilated, containers with food and returned to the insectary where responses were recorded at 24 h intervals for the next four days. Individuals were classified as unaffected (moving as normal), or affected either knocked down (with moving antennae, mandibles, and/or legs but unable right themselves permanently) or dead (with no response to stimulation).

2.2.2 Topical application procedure

Formulated deltamethrin (2.5% E.C.) was used in all bioassays. Distilled water was used as the diluent and for control treatments. Standard stock solutions were prepared immediately before each test from which the appropriate dilutions were made.

Topical applications were performed using a 250 µl Hamilton gas-tight syringe mounted in either a Burkard hand or automatic microapplicator (Burkard Manufacturing Co. Ltd.)(Arnold, 1967). The syringe was calibrated to deliver drops of 0.1, 0.5 or 1 µl (depending on size of test organism). The syringe and needle were cleaned with detergent ("Decon 90" - Decon Manufacturing Ltd.) and thoroughly rinsed in tap and then distilled water between treatments. Prior to treatment, test organisms were anaesthetised with CO_2 from a cylinder supply. The period of exposure to CO_2 was between 10 to 60 seconds, again depending on the size of the test species. Pesticide drops were placed at the junction of the pronotum and elytra for the beetles and on the abdomen of spiders. Initially, range-finding tests were carried out, with five logarithmically-spaced doses and a water control, and five to ten test organisms per dose. From the results of these treatments, a definitive dose-range was determined with four to seven doses. Before treatment, thirty individuals of each species were weighed to determine mean body mass for later analysis. The number of individuals of each species tested per pesticide dose was dependent on their abundance in the field: *S. avenae* (n=30), *P. melanarius* (n=30), *N. brevicollis* (n=30), *H. rufipes* (n=20), *A. dorsale* (n=30), *D. atricapillus* (n=30), *T. quadristriatus* (n=40), *B. lampros* (n=20), *B. obtusum* (n=20), *T. hypnorum* (n=30), *E. atra* (♀) (n=10), *C. septempunctata* (1) (n=30), *C. septempunctata* (L) (n=20), and *C. septempunctata* (2) (n=30). The dose-response data from the definitive bioassays are given in Appendix 1.

2.2.3 Assessment of knockdown time

The mean time to knockdown was assessed for six species of predators after topical treatment with deltamethrin to compare rates of intoxication between species and

estimate percent survival from doses causing knockdown. The predators tested represented a range of different taxonomic, size and susceptibility groups, i.e. a large carabid beetle (*N. brevicollis*), susceptible (*B. obtusum*, *T. quadristriatus*) and tolerant (*D. atricapillus*) small carabid beetles, a stapylinid beetle (*T. hypnorum*), and females of a linyphiid spider (*E. atra*). The topical dosing procedure was as described in 2.2.2 and the treatment doses were selected from the definitive dose-range for each species. Batches of five individuals were treated per dose and the time from dosing to knockdown (defined as when the arthropods were unable to right themselves) was recorded on a stop watch for each individual.

2.2.4 Statistical analyses

Probit analysis was carried out on the 72 h dose-response data to obtain dose-response statistics (Finney, 1971). Abbotts formula was used to correct the data for control mortality (Abbott, 1925). Only dead arthropods were included in the calculations, although after 72 h few individuals remained knocked down. The slopes and positions of the probit lines were compared for different species using maximum likelihood procedures (Ross, 1987). A pairwise testing procedure was used to compare all the species. These tests were used to infer patterns of susceptibility within and between taxonomic groups and size groups. Linear regression analyses were carried out to determine overall susceptibility and tolerance relationships to deltamethrin within and between predatory groups.

2.3 RESULTS

2.3.1 Determination of the end-point of toxic effect

The 24, 48, 72, and 96 h LD_{50} values given by probit analysis were plotted against time for all species to determine the end-point of the toxic effect. Figure 2.1 shows plots for six of the test species including representatives from the different taxonomic groups and size groups. These include the aphid (*S. avenae*), a large carabid (*N. brevicollis*), a small carabid (*B. obtusum*), the staphylinid beetle (*T. hypnorum*), a coccinellid adult (*C. septempunctata*)(1) and the linyphiid spider (*E. atra* (♀)). The plots showed a similar trend of decline in LD_{50} value over time for all six species over the first 96 hours after treatment, although the curves indicate different rates of poisoning. In all cases the LD_{50} values approached a stable end-point, though low level mortality may have continued for a longer period. The 72 h assessment was chosen for comparison of susceptibilities.

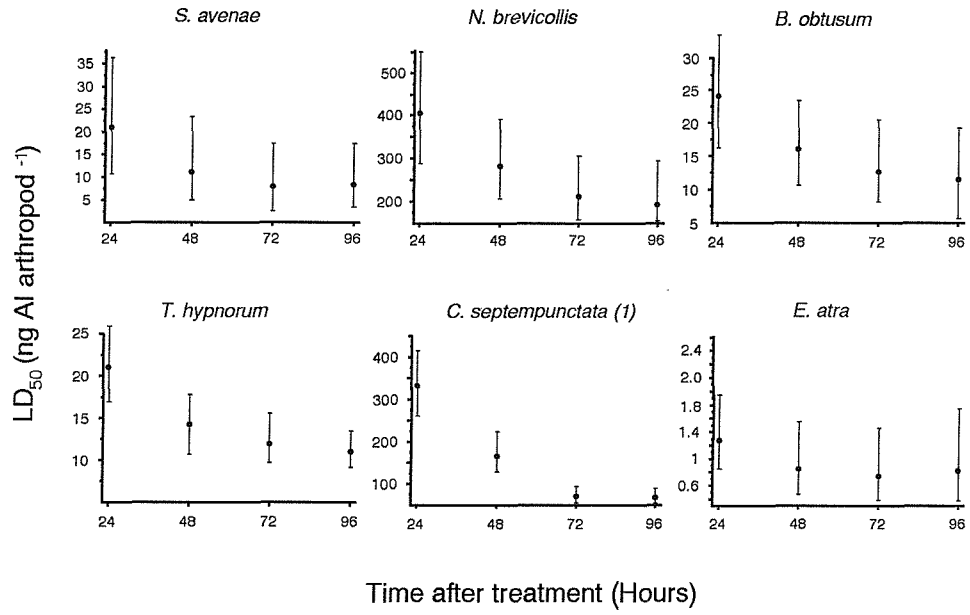

Figure 2.1 Variation in topical LD_{50} (ng AI arthropod^{-1}) with time after treatment for deltamethrin applied to an aphid and five natural enemies. Bars indicate 95% fiducial limits.

2.3.2 Analysis of dose-response relationships

The summary statistics from probit analysis of the 72 h dose-response data are given in Table 2.1. Only the χ^2 statistic for *E. atra* (♀) indicated significant heterogeneity. This was probably a result of the low number of individuals tested per treatment dose (n=10). The range of LD_{50} values varied between 0.8 and 232 ng AI arthropod^{-1} and 0.8 and 66.2 μg AI g body weight^{-1} for the species tested. The susceptibility ranking in terms of ng AI arthropod^{-1} closely followed body size rather than taxonomic grouping. The smallest species such as *E. atra* and *S. avenae* were the most susceptible and the large carabid beetles were the least susceptible. The only exception was the small carabid *D. atricapillus* which was less susceptible than some of the larger coccinellid and carabid beetles tested. When susceptibility was expressed as μg AI g body weight^{-1} there was less difference between the taxonomic groups. The linyphiid spider *E. atra* (♀) again had the lowest LD_{50} value (0.76 μg AI g body weight^{-1}). However, the susceptibility ranking was reversed for most of the beetle species, i.e. the large beetles were intrinsically more susceptible than the small beetle species. The aphid had a higher LD_{50} value (13.93 μg AI g body weight^{-1}) than all of the predators except the small carabid beetle *D. atricapillus* (66.17 μg AI g body

Table 2.1 72 h probit statistics of a cereal aphid and eleven species of natural enemies that inhabit temperate cereal crops for deltamethrin.

Family *Species*	Probit slope	LD_{50} (& s.e.) (Detransformed) (ng AI arthropod^{-1})	Mean Body Weight (& s.e.) (mg)	LD_{50} (µg AI g body weight^{-1})	Heterogeneity χ^2 (d.f.) Signif.
Aphididae					
S.avenae	1.52	8.6 (3.0)	0.62 (0.03)	13.93	2.67 (2) ns
Carabidae					
P.melanarius	1.60	135.8 (30.5)	154.10 (3.47)	0.88	0.32 (4) ns
H.rufipes	2.21	147.5 (28.1)	72.89 (1.36)	2.02	0.42 (2) ns
N.brevicollis	1.71	218.8 (36.0)	62.67 (1.85)	3.49	1.22 (3) ns
A.dorsale	2.00	83.6 (10.8)	12.00 (0.22)	6.97	0.43 (3) ns
D.atricapillus	1.78	231.6 (29.8)	3.50 (0.04)	66.17	0.66 (4) ns
T.quadristriatus	2.87	15.4 (1.6)	2.52 (0.02)	6.10	0.89 (2) ns
B.obtusum	1.52	12.7 (2.9)	1.76 (0.05)	7.22	0.65 (2) ns
B.lampros	2.63	12.9 (2.1)	2.55 (0.14)	5.07	3.27 (2) ns
Staphylinidae					
T.hypnorum	2.70	12.5 (1.4)	1.64 (0.04)	7.62	0.85 (2) ns
Coccinellidae					
C.septempunctata (1)	1.77	71.8 (9.8)	34.92 (0.79)	2.06	5.57 (5) ns
C.septempunctata (L)	2.32	31.2 (4.8)	39.40 (0.58)	0.80	2.94 (2) ns
C.septempunctata (2)	1.80	99.4 (19.4)	42.23 (0.50)	2.35	0.10 (3) ns
Linyphiidae					
E.atra ♀	4.64	0.8 (0.1)	1.05 (0.03)	0.76	8.41 (2) *

Signif. = Significance level; ns = not significant, * = $P<0.05$.

weight^{-1}) which was the least susceptible species tested.

The summary statistics from maximum likelihood analyses of 72 h dose-mortality statistics are given in the matrices in Tables 2.2, 2.3 and 2.4. These compare the position and parallelism of the probit lines for all pairs of test species, in terms of ng AI arthropod^{-1} and µg AI g body weight^{-1}. None of the tests gave significant heterogeneity ($P>0.05$) for the dose-response data included. These matrices may be used to answer specific questions concerning the relative susceptibilities of the organisms concerned and the evidence for selectivity between pest and predator species. At a given level of exposure in the field, the risk posed by a pesticide to an arthropod species will be a function of susceptibility in terms of active ingredient per arthropod (Table 2.2). Thus, if the position of the dose-response curves of the different species are considered, differences in susceptibility may be inferred for those pairwise comparisons that give significant separations. Using this criterion for selectivity, no significant differences could be detected between *S. avenae* and *E. atra* (♀), *T. hypnorum*, *B. obtusum*, *B. lampros*, or *T. quadristriatus*. Several species were however significantly more tolerant to deltamethrin than the aphid species. These included *C. septempunctata* adults and larvae, *P. melanarius*, *N. brevicollis*, *H. rufipes*, *A. dorsale* and *D. atricapillus*. The basis for any selectivity is further explored in Table 2.4 which determines the significance of any separations between dose-response curves along the dose axis, once the effect of body weight is excluded. Significant pairwise separations here would indicate possible physiological differences between predators and prey. In this case however the only species of predator that was significantly more tolerant to deltamethrin than *S. avenae* was *D. atricapillus*, the other species fell into one homogenous group.

A second set of questions concern patterns of tolerance within the predators themselves. Table 2.2 indicates that *E. atra* (♀) was significantly less tolerant than all species tested, except *S. avenae*. Whereas *D. atricapillus* was significantly more tolerant than all other species, except *N. brevicollis*. There was also evidence that the small and large carabids tended to separate into two distinct groups. When the effect of body weight was taken into account in the analysis (Table 2.4), a large number of significant separations remained, indicating physiological differences in the response of the different species. *E. atra* (♀) was still the least tolerant species overall and *D. atricapillus* was the most tolerant species. The small Carabidae and Staphylinidae again tended to form a homogenous group, however amongst the large Carabidae, *P. melanarius* was significantly less tolerant than most species. The coccinellid larvae

Species	S.a.	C.s. (2)	C.s. (L)	C.s. (1)	E.a.	T.h.	B.o.	B.l.	T.q.	D.a.	A.d.	H.r.	N.b.
P.m.	19.3 ***	2.2 ns	2.0 ns	4.3 *	6.3 *	2.2 ns	2.3 ns	1.8 ns	8.5 **	5.8 *	2.1 ns	0.5 ns	7.3 **
N.b.	35.9 ***	5.4 *	7.7 **	13.8 ***	14.2 ***	8.6 **	8.5 **	7.4 **	20.4 ***	0.2 ns	10.1 **	7.3 **	
H.r.	23.6 ***	0.6 ns	7.3 **	6.4 *	9.4 **	7.5 **	6.9 **	7.1 **	14.4 ***	7.2 **	5.0 *		
A.d.	37.2 ***	2.1 ns	7.9 **	1.4 ns	14.0 ***	11.2 ***	9.5 **	9.4 **	23.9 ***	13.5 ***			
D.a.	41.6 ***	14.9 ***	10.3 **	19.4 ***	16.3 ***	11.2 ***	10.9 ***	9.7 **	24.5 ***				
T.q.	0.2 ns	17.9 ***	13.0 ***	56.5 ***	20.4 ***	1.2 ns	0.3 ns	0.1 ns					
B.l.	2.5 ns	5.8 *	4.0 *	16.6 ***	19.2 ***	0.7 ns	0.1 ns						
B.o.	2.4 ns	6.6 *	3.9 *	25.0 ***	10.0 **	0.4 ns							
T.h.	0.9 ns	7.0 **	9.2 **	30.1 ***	29.4 ***								
E.a.	1.2 ns	12.3 ***	15.2 ***	35.0 ***									
C.s.(1)	71.1 ***	1.1 ns	18.4 ***										
C.s.(L)	10.9 ***	5.4 *											
C.s.(2)	31.7 ***												

Table 2.2 Matrix of χ^2 statistics from pairwise maximum likelihood analyses for differences in position of 72 h probit lines of the test species (ng AI deltamethrin arthropod^{-1}).

Values in boxes give χ^2 statistic (d.f.1) and level of significance: ns = not significant, * = P<0.05, ** = P<0.01, *** = P<0.001.

Key to test organisms :- S.a.- *S. avenae*, C.s.(2)- *C. septempunctata* (July adults), C.s.(L)- *C. septempunctata* (4th instar larvae), C.s.(1)- *C. septempunctata* (May adults), E.a.- *E. atra* (♀), T.h.- *T. hypnorum*, B.o.- *B. obtusum*, B.l.- *B. lampros*, T.q.- *T. quadristriatus*, D.a.- *D. atricapillus*, A.d.- *A. dorsale*, H.r.- *H. rufipes*, N.b.- *N. brevicollis*, P.m.- *P. melanarius*.

Species	S.a.	C.s. (2)	C.s. (L)	C.s. (1)	E.a.	T.h.	B.o.	B.l.	T.q.	D.a.	A.d.	H.r.	N.b.
P.m.	9.4 **	3.8 ns	14.1 ***	28.7 ***	9.1 **	37.7 ***	10.1 **	23.8 ***	33.4 ***	0.1 ns	6.9 **	2.9 ns	0.1 ns
N.b.	9.8 **	13.1 ***	14.5 ***	34.8 ***	9.1 **	37.9 ***	10.2 **	24.6 ***	33.8 ***	0.4 ns	7.8 **	3.7 ns	
H.r.	10.6 **	0.1 ns	10.3 **	5.0 *	9.1 **	34.6 ***	8.0 **	20.5 ***	29.7 ***	2.2 ns	1.3 ns		
A.d.	8.8 **	2.3 ns	7.0 **	0.4 ns	9.0 **	31.8 ***	6.1 *	17.3 ***	26.0 ***	6.1 *			
D.a.	9.0 **	2.7 ns	13.7 ***	25.3 ***	9.1 **	37.4 ***	9.8 **	23.4 ***	33.0 ***				
T.q.	14.3 ***	30.7 ***	8.7 **	25.3 ***	8.2 **	3.3 ns	4.6 *	0.4 ns					
B.l.	8.4 **	21.3 ***	4.4 *	16.6 ***	8.4 **	1.9 ns	2.0 ns						
B.o.	1.7 ns	8.5 **	0.3 ns	5.4 *	8.7 **	10.6 **							
T.h.	21.5 ***	35.5 ***	15.7 ***	31.0 ***	7.9 **								
E.a.	8.9 **	9.1 **	8.8 **	9.0 **									
C.s.(1)	3.1 ns	10.0 **	6.2 *										
C.s.(L)	10.0 ***	11.2 ***											
C.s.(2)	6.4 *												

Table 2.3 Matrix of χ^2 statistics from pairwise maximum likelihood analyses for differences in parallelism of 72 h probit lines of the test species (ng AI deltamethrin arthropod^{-1}).

Values in boxes give χ^2 statistic (d.f.1) and level of significance: ns = not significant, * = P<0.05, ** = P<0.01, *** = P<0.001.

Key to test organisms :- S.a.- *S. avenae*, C.s.(2)- *C. septempunctata* (July adults), C.s.(L)- *C. septempunctata* (4th instar larvae), C.s.(1)- *C. septempunctata* (May adults), E.a.- *E. atra* (♀), T.h.- *T. hypnorum*, B.o.- *B. obtusum*, B.l.- *B. lampros*, T.q.- *T. quadristriatus*, D.a.- *D. atricapillus*, A.d.- *A. dorsale*, H.r.- *H. rufipes*, N.b.- *N. brevicollis*, P.m.- *P. melanarius*.

Species	S.a.	C.s. (2)	C.s. (L)	C.s. (1)	E.a.	T.h.	B.o.	B.l.	T.q.	D.a.	A.d.	H.r.	N.b.
P.m.	2.8 ns	17.5 ***	0.3 ns	37.2 ***	2.2 ns	52.2 ***	19.9 ***	33.7 ***	35.4 ***	29.3 ***	28.7 ***	10.7 **	25.6 ***
N.b.	0.5 ns	4.7 *	7.5 **	4.9 *	12.4 ***	14.5 ***	7.4 **	3.7 ns	3.9 *	19.5 ***	7.2 **	7.3 **	
H.r.	1.0 ns	1.3 ns	7.1 **	1.3 ns	8.9 **	28.8 ***	10.6 **	15.2 ***	17.1 ***	19.3 ***	14.8 ***		
A.d.	0.0 ns	18.3 ***	8.9 **	19.6 ***	13.7 ***	1.0 ns	1.1 ns	0.1 ns	1.7 ns	15.0 ***			
D.a.	28.1 ***	22.5 ***	9.8 **	12.8 ***	16.2 ***	12.2 ***	10.9 ***	9.9 **	25.3 ***				
T.q.	1.3 ns	16.6 ***	14.7 ***	20.9 ***	19.5 ***	4.7 *	5.0 *	0.1 ns					
B.l.	0.1 ns	13.2 ***	13.2 ***	13.7 ***	18.3 ***	1.9 ns	0.8 ns						
B.o.	0.1 ns	13.4 ***	5.0 *	11.0 ***	9.9 **	0.1 ns							
T.h.	0.0 ns	31.3 ***	23.6 ***	34.3 ***	28.8 ***								
E.a.	1.2 ns	9.6 **	0.4 ns	23.5 ***									
C.s.(1)	0.0 ns	0.0 ns	19.9 ***										
C.s.(L)	0.0 ns	5.3 *											
C.s.(2)	1.1 ns												

Table 2.4 Matrix of χ^2 statistics from pairwise maximum likelihood analyses for differences In position of 72 h probit lines of the test species (μg Al deltamethrin g body weight^{-1}).

Values in boxes give χ^2 statistic (d.f.1) and level of significance: ns = not significant, * = P<0.05, ** = P<0.01, *** = P<0.001.

Key to test organisms :- S.a.- *S. avenae*, C.s.(2)- *C. septempunctata* (July adults), C.s.(L)- *C. septempunctata* (4th instar larvae), C.s.(1)- *C. septempunctata* (May adults), E.a.- *E. atra* (♀), T.h.- *T. hypnorum*, B.o.- *B. obtusum*, B.l.- *B. lampros*, T.q.- *T. quadristriatus*, D.a.- *D. atricapillus*, A.d.- *A. dorsale*, H.r.- *H. rufipes*, N.b.- *N. brevicollis*, P.m.- *P. melanarius*.

were less tolerant than adult coccinellid beetles from either generation, indicating possible physiological differences between adult and larval stages.

A linear regression model was fitted to data for Log 72 h LD_{50} (µg AI g body weight^{-1}) against mean body weight (mg) for the coleopteran predators (Figure 2.2) giving a significant negative correlation (r^2 = 81.9; F = 36.2***; d.f.=1,8; P<0.001) between susceptibility and body weight. The smaller beetles were less susceptible to deltamethrin per unit body weight that the larger beetles. Both *D. atricapillus* and *C. septempunctata* (4th instar larvae) were excluded from the analysis because of the apparent physiological differences previously mentioned.

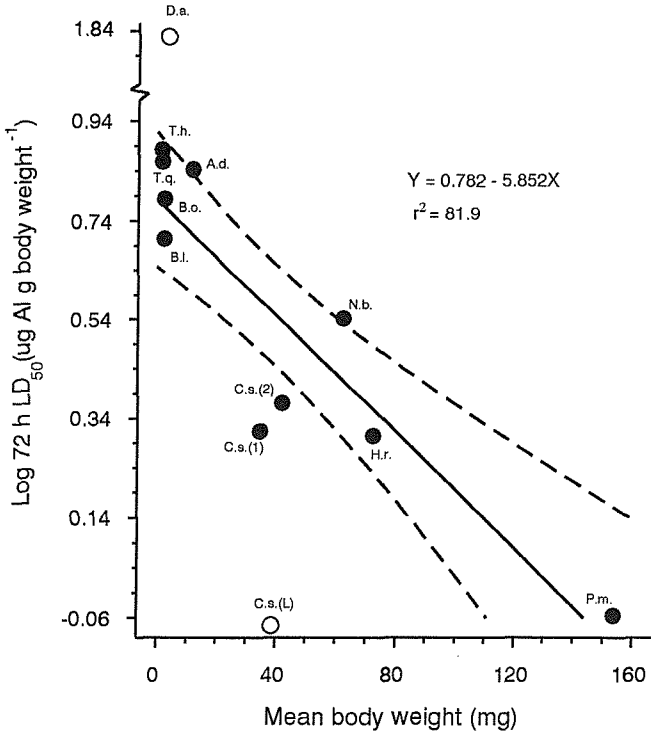

Figure 2.2 Correlation between Log 72 h LD_{50} (µg AI g body weight^{-1}) for deltamethrin and mean body weight (mg) for ten species of coleopteran predators. Dashed lines indicate 95% confidence intervals. The small carabid *D. atricapillus* and the coccinellid larvae *C. septempunctata* (L) (signified by the open circles) were excluded from the regression due to possible physiological differences in tolerance from the other species.

Key to test organisms for Figures 2.2 and 2.3 :- S.a.- *S. avenae*, C.s.(2)- *C. septempunctata* (July adults), C.s.(L)- *C. septempunctata* (4th instar larvae), C.s.(1)- *C. septempunctata* (May adults), E.a.- *E. atra* (♀), T.h.- *T. hypnorum*, B.o.- *B. obtusum*, B.l.- *B. lampros*, T.q.- *T. quadristriatus*, D.a.- *D. atricapillus*, A.d.- *A. dorsale*, H.r.- *H. rufipes*, N.b.- *N. brevicollis*, P.m.- *P. melanarius*.

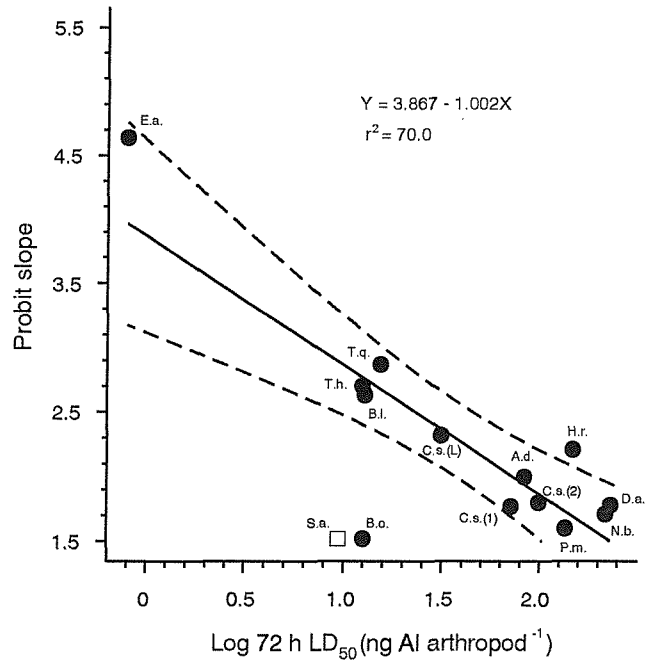

Figure 2.3 Correlation between probit slope and Log 72 h LD_{50} (ng AI arthropod^{-1}) for thirteen natural enemies to deltamethrin. Dashed lines indicate 95% confidence intervals.
The aphid (signified by the open square) was excluded from the regression due to possible differences in physiology.

The interpretation of differences in the form of the tolerance distributions and the slopes of the dose response curves for the different arthropods is more difficult. There was evidence that the herbivore *S. avenae* had a significantly lower slope than many of the carnivorous species (Table 2.3). *E. atra* (♀) also had a significantly steeper slope than any other species indicating a fundamental difference in the form of it's response to deltamethrin. A linear regression model was fitted to data of probit slope against Log 72 h LD_{50} (ng AI arthropod^{-1}) for the natural enemy species (Figure 2.3) giving a significant negative correlation (r^2 = 70.0; F = 25.7***; d.f.=1,11; P<0.001) between tolerance distribution and predator susceptibility. The linyphiid spider *E. atra* (♀) was the most susceptible species and had the steepest probit slope, whereas amongst the Coleoptera the smaller species tended to have a steeper slope than the larger species. The aphid pest was excluded from the analysis because of suspected differences in metabolising the pesticide from the predators previously suggested.

2.3.3 Mean knockdown time

The mean time to knockdown (secs) was plotted against topical dose (ng AI

arthropod^{-1}) (Figure 2.4) giving asymptotic curves for the six predator species. The

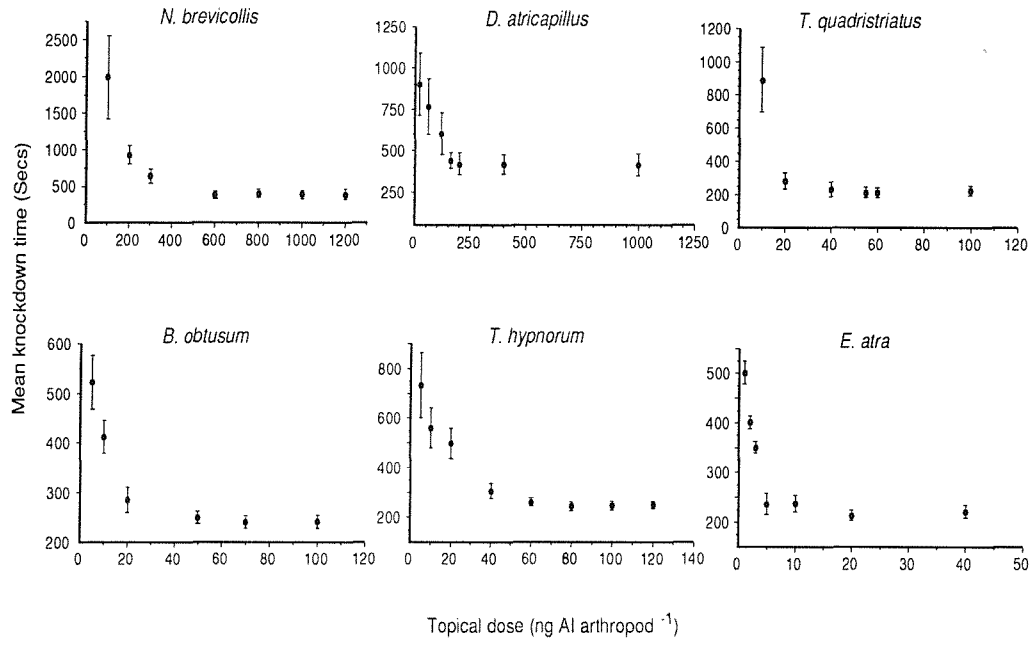

Figure 2.4 Variation of mean knockdown time (secs) with topical dose of deltamethrin (ng AI arthropod^{-1}) for six species of natural enemies. Bars indicate 95% confidence limits.

asymptotic knockdown time varied from 210 seconds for *T. quadristriatus* and 213 for *E. atra*, to 413 seconds for *D. atricapillus*. The asymptote values on the dose axis

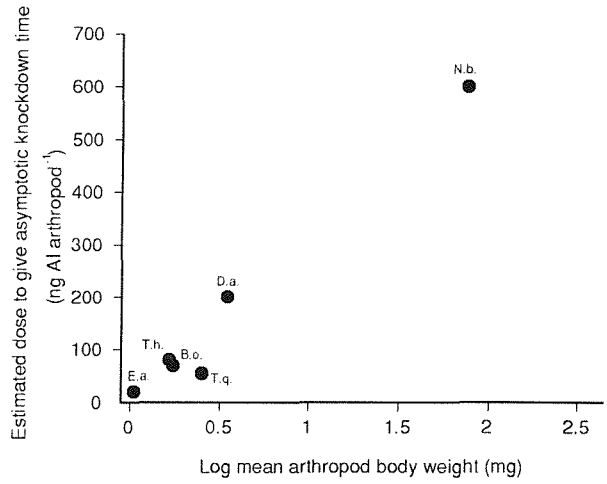

Figure 2.5 Comparison of estimated deltamethrin dose (ng AI arthropod^{-1}) to give asymptotic knockdown time and Log mean arthropod body weight (mg) for six species of predators.
Key to test organisms for Figures 2.5 and 2.6 :- E.a.- *E. atra* (♀), T.h.- *T. hypnorum*, B.o.- *B. obtusum*, T.q.- *T. quadristriatus*, D.a.- *D. atricapillus*, N.b.- *N. brevicollis*.

represent the dose where the chemical supply is no longer limiting and therefore enable exploration of between-species differences in the dynamics and kinetics of deltamethrin activity. The estimated dose (ng AI arthropod^{-1}) to give the asymptotic knockdown time, i.e. causing the lowest mean knockdown time, varied from 20 ng AI for *E. atra* to 600 ng AI for *N. brevicollis*. These doses were plotted against log mean body weights (mg) of the six predator species in Figure 2.5 producing a trend of increasing dose with predator weight.

The asymptotic time to knockdown was plotted against the predicted percent survival of each predator species from the minimum dose giving asymptotic knockdown time in Figure 2.6. The predicted percent survival was calculated by substituting the dose to give asymptotic knockdown time into the 72 h dose-response equations given in Table 2.1. *Demetrias atricapillus* was predicted to have a much greater survival rate (54%) after receiving this dose of deltamethrin than the large carabid beetle *N. brevicollis* (23%), the small carabid and staphylinid beetle species *B. obtusum*, *T.*

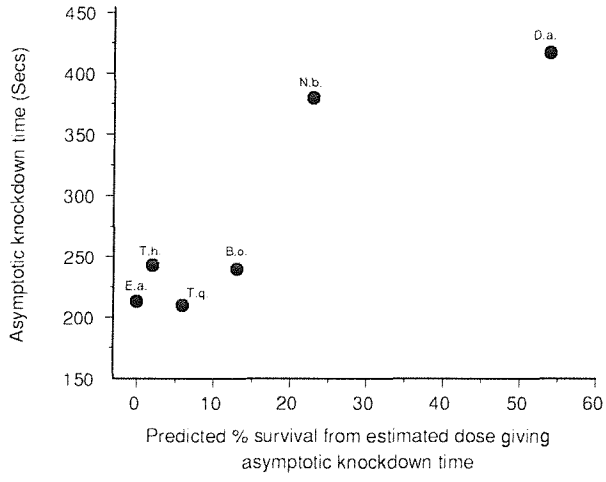

Figure 2.6 The predicted percent survival from the minimum deltamethrin dose giving asymptotic knockdown time for six species of predators.

quadristriatus and *T. hypnorum* (13% to 2%) and the linyphiid spider *E. atra* (0%). Figure 2.6 also shows that *D. atricapillus* had the greatest asymptotic knockdown time of the species tested suggesting that a structural or physiological feature, unique to this species was affecting it's tolerance.

2.4 DISCUSSION

2.4.1 Susceptibility trends

Significant differences in susceptibility and tolerance distribution were found for species within different orders, i.e. the Araneae, Hemiptera and Coleoptera and trends between susceptibility and body size were evident within the Coleoptera. The order of susceptibility for LD_{50} values in terms ng AI arthropod^{-1} closely followed body size, the larger species being least susceptible to deltamethrin, with the exception of *D. atricapillus*. These trends broadly agree with the results of Theiling and Croft (1988), who produced mean toxicity values for pyrethroid insecticides to orders and families of arthropod natural enemies from their SELCTV database. They found that pyrethroids were more toxic to the Araneae and Coleoptera than the Hemiptera. Within the Coleoptera they found pyrethroids to be more toxic to the Staphylinidae than the Coccinellidae, and less toxic to the Carabidae.

The most susceptible predator tested was the linyphiid spider *E. atra* (♀). It's high susceptibility may be partly attributed to it's low body weight. However the fact that it had a significantly narrower tolerance distribution to deltamethrin than the other test species indicated that this may not be the sole reason for it's high susceptibility. Other differences, such as it's soft bodied nature or basic physiology may also be important.

The least intrinsically susceptible species was the small carabid *D. atricapillus*. Knockdown time studies and observations during bioassay assessments indicated that this species had a greater ability to recover from deltamethrin poisoning under the given laboratory conditions than the other species of predators tested. The tolerance mechanism of this species is unknown. It may be related to nerve insensitivity or decreased availability of the pyrethroid at the primary site of action mediated by factors such as decreased cuticular penetration, enhanced detoxification, storage in insensitive tissue or increased elimination (Ruight, 1985; Soderlund and Bloomquist, 1990; Greenwood *et al.* 1990). Tolerance mechanisms to pyrethroids are not uncommon and have previously been reported in other species of natural enemies, such as chrysopids and parasitoids (Ishaaya and Casida, 1981; Chang and Plapp, 1983; Bashir and Crowder, 1983; Feng and Wang, 1984).

The susceptibility of the aphid *S. avenae* did not differ significantly, in terms of ng AI arthropod^{-1}, from the small carabid beetles, the staphylinid beetle and the linyphiid

spider. It was however significantly more susceptible than the large and medium carabid beetles, the small carabid *D. atricapillus* and the coccinellid adults and larvae. This suggested that the small carabids, the staphylinid and the linyphiid spider may be at greater risk from a pyrethroid spray than the large carabids and adult coccinellids. This ranking agrees with results from several field studies, which have shown that linyphiid spiders and small beetle species are affected by pyrethroid sprays (Basedow *et al.* 1985; Vickerman *et al.* 1987a & b; Brown *et al.* 1988; Everts, 1990). However it is unwise to make predictions of effects in the field from susceptibility data without considering the likely exposure of the organisms to the chemical (section 4.3).

2.4.2 The potential for selectivity

The aphid *S. avenae* had a significantly shallower probit slope than most of the predators, indicating that it had a relatively wide tolerance distribution to deltamethrin. This difference, between herbivorous and carnivorous arthropods, has been attributed to the armoury of detoxifying enzymes either present or inducible in herbivores to deal with plant chemical defences (Dodd, 1973; Plapp, 1981; van Emden, 1988). Because of this there may be scope for determining reduced dose-rates, which are selective in favour of the predators (van Emden, 1988). Four of the predator species tested, the small carabids *T. quadristriatus* and *B. lampros*, the staphylinid *T. hypnorum* and the linyphiid spider *E. atra*, had significantly steeper slopes than the aphid and thus might escape effects at doses which still kill *S. avenae*. It is unlikely however, that selective doses could be found for linyphiid spiders, because of their high susceptibility. The implications of using reduced dose-rates in terms of selection for pest resistance have yet to be fully resolved. In theory high, rather than low, dose-rates could be used to prevent resistance development by killing all individuals (Tabashnik and Croft, 1982; Denholme and Rowland, 1992). Even if all the conditions for the target pest subjected to the high dose strategy (eg. immigration of susceptible genotypes that are able to mate with resistant homozygotes, susceptibility of heterozygotes to the insecticide and low resistance gene frequency etc.) are fully met however, threats to resistance in other pests and disruption of control by natural enemies may carry too great a cost (Tabashnik, 1990). Although the role of natural enemies has not been explicitly considered within the modelling and evaluation of resistance management tactics, low dose-rates which encourage the survival of natural enemies are likely to be of net benefit in integrated pest control and resistance management strategies (Roush, 1989; Croft, 1990b; Tabashnik, 1990).

2.4.3 Risk assessment using toxicological statistics

The dose-response data have indicated that determining overall susceptibility trends between different taxonomic groups may be complicated by differences in physiology. However the regression analyses have suggested that it may be possible to determine susceptibility relationships within taxonomic groups, such as within the Coleoptera in this study, and, once validated, these may be useful in risk assessment procedures as it may be possible to estimate species susceptibilities of closely related groups of predators to a compound from simple measurements such as body weight.

In the field predatory arthropods will be exposed to pesticides by several routes including direct contact with spray drops during spraying, contact with pesticide residues on soil or plant surfaces, and possibly by consumption of contaminated prey. It is therefore likely that the species under test in this study will differ in exposure level. For example coccinellid adults and larvae are plant-active and diurnal and are therefore likely to be contacted directly by spray drops during spraying and also exposed to relatively high concentrations of pesticide on plant surfaces after spraying, whereas many of the carabid beetles are ground-active and nocturnal and may therefore be hidden in refuges during spraying thus avoiding direct contact with spray and when active during the night they may be exposed to relatively low concentrations of pesticide on the soil. Therefore the use of laboratory derived susceptibility data alone is unlikely to be sufficient to predict field effects. This data may however, be used to rank organisms in order of their susceptibility to a chemical, which may help selection of organisms for registration testing, or aid the interpretation of semi-field and field studies (Jepson, 1993b). Also the toxicological statistics may be incorporated with estimates of exposure, and used to develop simple models to aid risk assessment (Jepson *et al.* 1990a; Jepson, 1993b). This approach will be explored further in Chapter 3.

CHAPTER 3

An Index of the intrinsic susceptibility of aphid predators to residual deposits of deltamethrin.

3.1 INTRODUCTION

One of the most important routes of exposure to pesticides for many predatory invertebrates is by contact with pesticide residues after a spray application (Croft, 1990a; Mullié and Everts, 1991). The level of exposure of a given species is likely to depend upon species-specific intrinsic factors, such as the degree of contact with pesticide treated substrates and the susceptibility of the species to the pesticide, and extrinsic factors, such as the pesticide deposition rate on any given substrate, substrate-dependent interactions of the pesticide which mediate the bioavailability and toxicity of the compound and environmental factors, such as temperature and humidity.

The index described in this chapter aimed to predict the relative susceptibilities of seven species of aphid predators, that inhabit temperate cereal crops, to deltamethrin residues. Intrinsic species characteristics, such as walking speed and contact area, were measured and used to derive a function of exposure which was then used as a correction factor for susceptibility data to predict the relative susceptibilities of different predator species to deltamethrin residues. This approach may provide an insight into the reasons why some species may be at greater risk from pesticide residues than others. This would be useful in aiding selection of organisms for registration testing and helping the interpretation of field studies.

3.1.1 Development of the susceptibility index

Salt and Ford (1984) investigated factors that determine the residual toxicity of pesticides to insects using a stochastic simulation model. Their model simulates the encounter and transfer of insecticide from treated plant surfaces to lepidopteran larvae and predicts the proportion of insects responding. Jepson *et al.* (1990a) developed a reductionist approach based upon a sensitivity analysis of this model for short-term hazard prediction for terrestrial invertebrates exposed to pesticides. They postulated that the walking velocity of the insect, the proportion of pesticide transferred per encounter and the insects' area of contact with the treated surface had an important influence on its' susceptibility to pesticide residues. The index (given below), proposed

by Jepson *et al.* (1990a), consists of the ratio between an exposure function

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Susceptibility Index = (v x w x a)/LD_{50}

Susceptibility parameter: Topical LD_{50} (µg AI $insect^{-1}$)

Exposure function parameters:

v = mean walking speed (cm sec^{-1})
w = mean track width (distance between tarsi)(cm)
a = mean contact area (proportion of area covered by insect that is contacted)

(based on walking track width,walking speed and the proportion of the area, covered by the insect, that is contacted) and susceptibility (expressed as the species' topical tolerance distribution at end-point). Susceptibility is measured by the topical LD_{50} in terms of dose per insect and not dose per unit body weight, to correct for variations in body size between species. The susceptibility index value gives an estimate of the dose encountered per unit tolerance of the species. A high index value would indicate that lethal doses may be readily acquired by that species, which may therefore be highly susceptible to pesticide residues, whereas a relatively low susceptibility index value may indicate slower uptake and thus lower susceptibility. The index only provides a comparative measure of susceptibility under the given experimental conditions and is not intended to be used to predict effects in the field as these will be related to complex biological and operational factors such as the behaviour and distribution of the organisms, the environmental conditions and the nature of the pesticide (Jepson, 1989; Everts, 1990). The index may however be used to compare groups of organisms with similar habits. It is an intrinsic characteristic that modifies basic susceptibility measurements by a function of potential exposure.

3.2 EXPERIMENTAL METHODS

3.2.2 Test invertebrates

The predators that were tested included polyphagous predators such as the large carabid beetles *P. melanarius* and *N. brevicollis*, the medium sized carabid beetle *A. dorsale*, and the small carabid beetles *D. atricapillus* and *B. lampros*. Also included were the small staphylinid beetle *T. hypnorum* and the aphid-specific coccinellid *C.*

septempunctata.

The predators were captured in cereal fields and field-margins at Leckford, near Stockbridge, Hampshire, UK, using the methods described in Chapter 2 (2.2.1). The Carabidae and Staphylinidae were kept in plastic aquaria, containing a layer of moist soil. They were fed on ground, moist cat biscuits ("Delicat"). The coccinellids were placed in perspex boxes with barley plants infested with *S. avenae*. All invertebrates were kept in a controlled environment room in an insectary, maintained at 19-22°C, 55-70 % relative humidity and photoperiod 16:8 L:D, prior to treatment.

3.2.3 Determination of model parameters

i) Topical bioassays to determine species susceptibility to deltamethrin

These were described in Chapter 2 (2.2.2). The 72 h LD_{50} values were used as a measure of the susceptibility of the seven predator species to deltamethrin at end-point.

ii) Exposure parameters

a) Mean walking speed

A Panosonic video camera (WVP-A1E) and video cassette recorder were used to record the speed of movement of the seven coleopteran species on a lightly compacted, sieved, sandy loam soil surface. Video recordings were made for two batches of five individuals of each species in a plastic arena 56cm x 29cm x 9cm. The sides of the arena were coated with Fluon, polytetrafluoroethylene (PTFE), (Whitford Plastics, Runcorn, Cheshire, UK) to prevent beetles from climbing the arena sides. The video recordings were made in light for the diurnal species (*C. septempunctata*, *D. atricapillus*, and *B. lampros*) and in the dark with a red light source for the nocturnal species (*P. melanarius*, *N. brevicollis*, *A. dorsale* and *T. hypnorum*) in a controlled environment room in an insectary, maintained at 19-22°C, 40-60 % relative humidity, and photoperiod 16:8 L:D. Griffiths *et al.* (1985) have shown that red light does not effect the nocturnal activity of *A. dorsale*. An estimate of the walking speed of each species was obtained by analysing a one hour period of the video recording for each species. A time was chosen when all beetle species were active. This was from 11-00 to 12-00 hours Greenwich Mean Time (GMT) for diurnal species and 23-00 to 00-00 hours GMT for nocturnal species. Five walking tracks were measured for individuals of each species at 10 minute intervals by tracing the walking path of insects on a sheet of acetate overlying the monitor screen. An ipsometer (map measurer) was calibrated, allowing for screen curvature, to measure the length of path (cm). The time taken to

cover the distance was found from the stop-watch on the screen and an estimate of the mean walking speed was calculated for each species. A total of fifty readings were made per species. These measurements of mean walking speed were later verified by Micromeasure (Wye College Software) which is a computerised system enabling analysis of video recordings of insect behaviour.

b) Measurement of contact area and track width.

White kymograph paper (R.A. Brand Ltd., Paper Manufacturers, Hedge End, Hampshire, U.K.) was attached to a kymograph apparatus (Jackson, 1917) in a fume cupboard. The paper was coated with a layer of soot by igniting gas passed through a Drechsel bottle assembly (Quickfit Ltd.) containing toluene (General Purpose Reagent: GPR) (Merck Ltd.) connected to a Johnson burner. The paper was smoked uniformly by rotating the kymograph drum at a constant rate over the burner. After it had been smoked, the paper was carefully removed from the drum and attached to a flat surface. Individual test invertebrates were allowed to walk across the paper sweeping away the layer of soot on the areas they contacted and leaving a track. Clearer tracks were obtained when the paper was coated with a thin uniform soot layer. The tracks were semi-permanently fixed through immersion in a 2% solution of Shellac (Merck Ltd.) in methanol GPR (Merck.). An IBAS image analysis computer (Kontron Ltd.) was calibrated to measure the proportion that the area of smoke removed by the insect occupied within the its walking track. A total of thirty tracks were measured for each species.

3.2.4 Validation of index predictions

Laboratory residual bioassays were carried out to test the predictions of the relative susceptibilities of the beetle species to deltamethrin. Only five of the seven beetle species were tested because of a lack of field availability at the time of the test. These included four species of carabids, *P. melanarius*, *N. brevicollis*, *B. lampros*, and *D. atricapillus* and one staphylinid *T. hypnorum*. Batches of 14 to 16 beetles of each species were exposed to deltamethrin deposits in all three bioassays and batches of 10 insects were used in the controls. Plastic trays (56cm x 29cm x 9 cm) with Fluon (PTFE) coated sides were used as arenas. The tops of each arena were covered with a glass sheet to prevent beetles that were capable of flying from escaping. The chambers were ventilated via hypodermic needles placed under the glass sheet and connected to an aquarium pump via rubber tubing. Bioassays were for 2 hours on glass and 24 hours on soil. The same arenas were used in both types of bioassay. In the first tests a sheet of glass was fitted to the bottom of the arena and in the second

set of tests a 2cm deep layer of sieved sandy loam soil was lightly compacted into the base of the arena. The surfaces were then sprayed with deltamethrin at a rate of 6.25g AI ha^{-1} and a volume of 200 l ha^{-1} with a CP15 knapsack sprayer (Cooper Pegler Ltd.) fitted with a 1 m boom and three Lurmark FO2-80 nozzles. The spray deposit was allowed to dry for approximately 30 minutes before the beetles were introduced to the arenas. The control arenas remained untreated. After exposure the beetles were removed from the test arenas and placed in clean, ventilated, containers with food and kept in the insectary where responses were recorded at 24 h intervals for the next four days. Individuals were classified as unaffected (moving as normal), or affected, either knocked-down or dead. Individuals that displayed uncoordinated movement when stimulated were recorded as knocked-down and individuals that remain immobile and showed no response to stimulation were recorded as dead. Treatment mortality was corrected for control mortality using Abbott's formula (Abbott, 1925).

3.3 RESULTS

The parameters for the susceptibility index are given in Table 3.1. The mean walking speed of the beetle species varied from 0.46 cm sec^{-1} for *C. septempunctata* to 2.87 cm sec^{-1} for *A. dorsale.* Frequency distributions of walking speeds are plotted for each species in Figure 3.1. The frequency distributions obtained for all species were found to be normally distributed (χ^2 test for goodness of fit, $P>0.05$). The small staphylinid beetle *T. hypnorum* and the small carabid beetle *B. lampros* had the narrowest mean track widths (0.23 and 0.21 cm respectively), and the large carabid *P. melanarius* had the widest mean track width (1.41 cm). The species with the largest mean proportional contact area with the surface were the large carabid *P. melanarius* and the small staphylinid *T. hypnorum* contacting 6% and 5.9% of their body areas (Table 3.1). The species that had the lowest mean proportional contact areas were the small carabid *D. atricapillus* (1.3%) and the ladybird *C. septempunctata* (1.7%). Examples of the walking track patterns for three of the test species can be seen in Figure 3.2 (Plates a to c). The track patterns varied considerably between the species tested. For example, the ground beetle *P. melanarius* tended to drag its tarsi over the surface (Figure 3.2 Plate a), whereas the rove beetle *T. hypnorum* tended to contact the surface with its abdomen and the ladybird *C. septempunctata* (Figure 3.2 Plate c) tended to leave a more delicate track and did not drag its' tarsi or contact the surface with its' abdomen. The differences in track patterns and contact areas between the beetle species may be related to differences in body posture and walking action and differences in leg morphology (Forsythe, 1981 & 1983) for example between those

species which are able to climb plants and those that are not.

Table 3.1 Parameters for the estimation of the exposure of seven species of cereal aphid predators to pesticide residues.

Family Species	Mean walking speed ($cm\ sec^{-1}$) (& 95% Confidence Limits)	Mean track width (cm) (& 95% CL)	Mean proportional contact area (& 95% CL)
Carabidae			
P.melanarius	1.85 (1.76-1.94)	1.41 (1.36-1.46)	0.060 (0.057-0.062)
N.brevicollis	2.12 (2.04-2.19)	1.37 (1.32-1.41)	0.039 (0.038-0.041)
A.dorsale	2.87 (2.80-2.95)	0.73 (0.71-0.75)	0.024 (0.021-0.026)
D.atricapillus	1.51 (1.47-1.56)	0.36 (0.34-0.38)	0.013 (0.011-0.015)
B.lampros	1.68 (1.63-1.72)	0.21 (0.19-0.23)	0.031 (0.029-0.033)
Staphylinidae			
T.hypnorum	1.85 (1.78-1.92)	0.23 (0.21-0.24)	0.059 (0.056-0.062)
Coccinellidae			
C.septempunctata	0.46 (0.40-0.51)	0.77 (0.74-0.80)	0.017 (0.014-0.020)

The susceptibilities and calculated exposure functions and susceptibility indices for the seven species of coleopteran aphid predators are given in Table 3.2. The most susceptible species to deltamethrin were the small carabid *B. lampros* and the staphylinid *T. hypnorum* which had 72 h LD_{50} values of 0.013 µg AI $insect^{-1}$. The least susceptible species were the large carabid *N. brevicollis* and the small carabid *D. atricapillus* which had 72 h LD_{50} values of 0.219 and 0.232 µg AI $insect^{-1}$. The values for the exposure function were greatest for the larger beetles than for the smaller

Table 3.2 Exposure and susceptibility indices for seven species of cereal aphid predators to deltamethrin residues.

Family Species	72 h Topical LD_{50} for deltamethrin (µg AI $Insect^{-1}$) (& 95% Fiducial Limits)	Exposure function (v x w x a) (& 95% C.L.)	Susceptibility Index $(v \times w \times a)/LD_{50}$ (& 95% C.L.)	Rank *
Carabidae				
P.melanarius	0.14 (0.09-0.21)	0.16 (0.14-0.18)	1.15 (0.67-2.00)	2
N.brevicollis	0.22 (0.16-0.31)	0.11 (0.10-0.13)	0.52 (0.33-0.82)	5
A.dorsale	0.08 (0.06-0.11)	0.05 (0.04-0.06)	0.60 (0.36-0.96)	4
D.atricapillus	0.23 (0.18-0.30)	0.007 (0.005-0.009)	0.03 (0.02-0.05)	7
B.lampros	0.013 (0.009-0.018)	0.011 (0.009-0.013)	0.84 (0.53-1.44)	3
Staphylinidae				
T.hypnorum	0.013 (0.010-0.016)	0.025 (0.021-0.029)	1.93 (1.43-2.96)	1
Coccinellidae				
C.septempunctata	0.10 (0.07-0.14)	0.006 (0.004-0.008)	0.06 (0.03-0.12)	6

* - rank = 1, predicted to be most susceptible, rank = 7, predicted to be least susceptible.

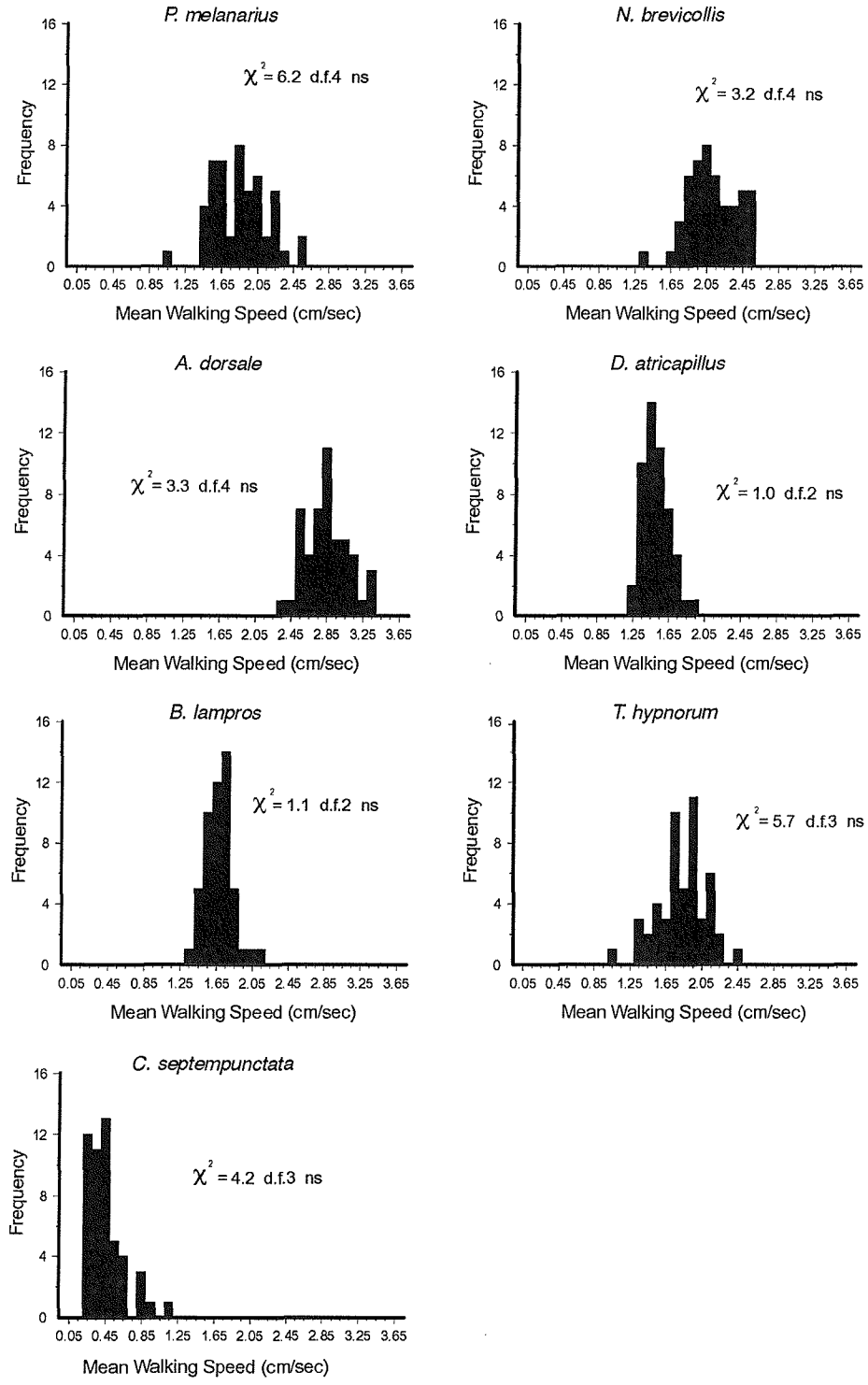


Figure 3.1 Frequency distributions of the walking speeds of seven species of aphid predators. χ^2 values indicate goodness of fit to normal distribution; ns = P>0.05.

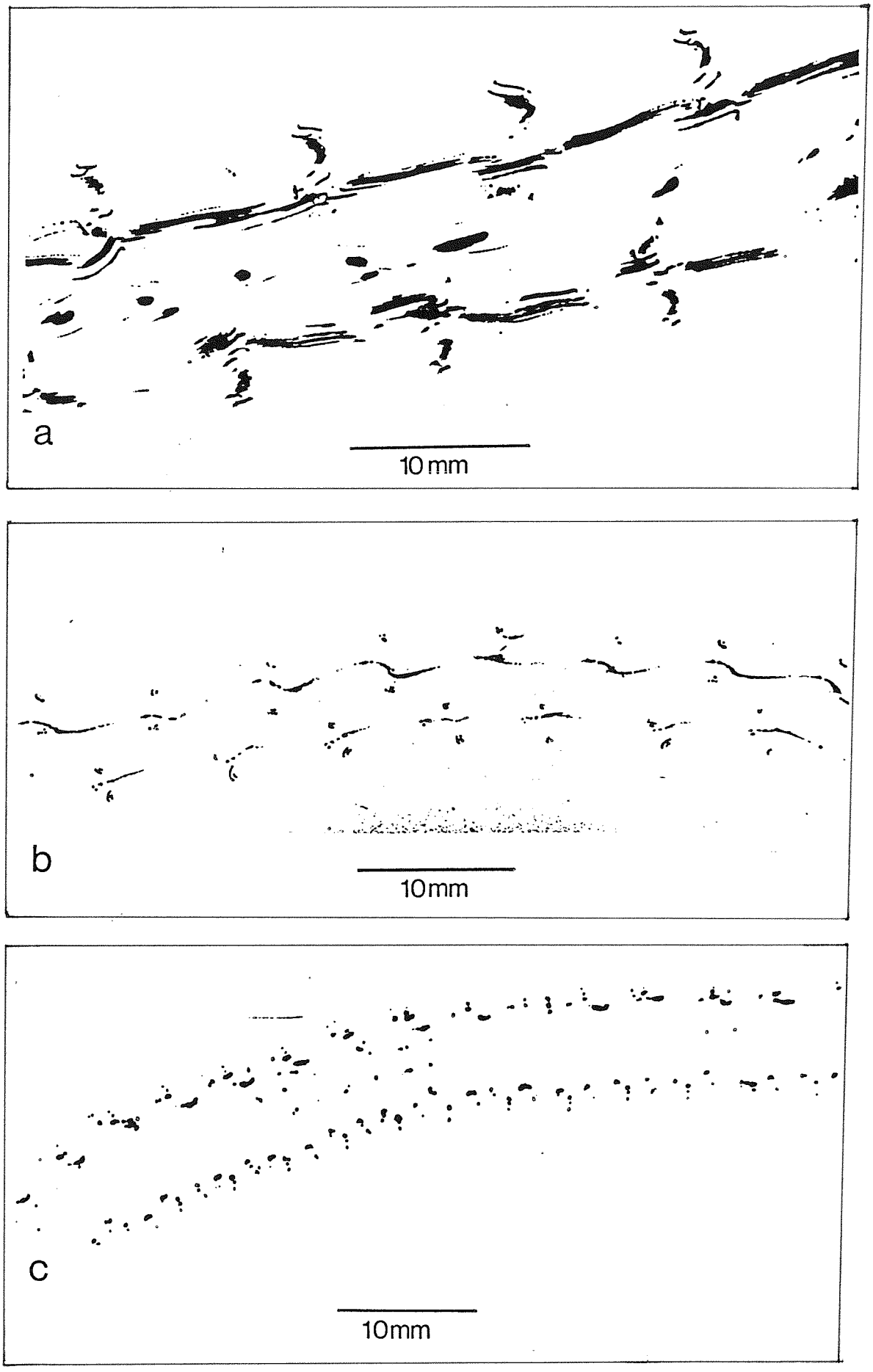


Figure 3.2 Example kymograph walking tracks for three species of aphid predator.

a) *P. melanarius*, b) *A. dorsale*, c) *C. septempunctata*

beetles with the exception of the ladybird *C. septempunctata* which had the lowest exposure function of all.

When the exposure function was divided by the susceptibility to give the susceptibility index prediction (Table 3.2), the small staphylinid *T. hypnorum* had the largest value (1.93) which indicated that it may be the most susceptible species to deltamethrin residues. The second highest susceptibility index value was that of the large carabid *P. melanarius* (1.15), which had the largest exposure function value, due to its size and high proportional contact area. The small carabid *D. atricapillus* had the lowest susceptibility index value (0.03) indicating that this species may be least at risk from deltamethrin residues of the seven tested.

The mortality rankings from the two bioassays to test the predictions of the susceptibility index are given in Table 3.3. The mortality rankings for the five species tested agree with the susceptibility indices predictions for the most susceptible species *T. hypnorum* and the least susceptible species *D. atricapillus*. The intermediate rankings were as predicted by the susceptibility in the 2 h glass bioassay however they were not exactly as predicted in the 24 h soil bioassay. This may be a result of differences in behaviour on the different substrates, for example some beetles may burrow into the soil and therefore be exposed to less pesticide residues or differences in species activity patterns during the longer period of exposure.

Table 3.3 Predicted and actual rankings of the susceptibilities of five species of aphid predators to deltamethrin residues.

Family Species	Susceptibility Index *	Mortality ranking from 2 h glass bioassay (% mortality in parenthesis) *	Mortality ranking from 24 h soil bioassay (% mortality in parenthesis) *
Carabidae			
P.melanarius	2	2 (60)	3 (13)
N.brevicollis	4	4 (38)	4 (0)
D.atricapillus	5	5 (20)	4 (0)
B.lampros	3	3 (57)	2 (27)
Staphylinidae			
T.hypnorum	1	1 (71)	1 (38)

* - rank 1 = most susceptible, rank 5 = least susceptible.

3.4 DISCUSSION

The susceptibility index (Table 3.2) indicated that, under the given conditions, the staphylinid beetle *T. hypnorum* would be the most susceptible (rank = 1) to deltamethrin residues, because it had a high exposure index and was relatively susceptible to the deltamethrin. The index predicted that the small carabid *D. atricapillus* would be least susceptible to deltamethrin residues (rank = 7) because it had a low proportional contact area and a high LD_{50} value. This may suggest that the susceptibility of the species to residues is strongly related to the intrinsic susceptibility of the insect to the pesticide, however trends within the five carabid species tested indicated that susceptibility to pesticide residues may not be so obvious, as a large carabid *P. melanarius* was predicted to be more susceptible than several smaller, more susceptible species.

The index predictions of the most and least susceptible species agreed well with the mortalities observed in the two residual bioassays. However the species falling between these two extremes agreed less consistently with the predictions. The 95% confidence intervals of the susceptibility indices for some of the species tested showed considerable overlap, indicating that the intermediate rankings may be sensitive to small variations in susceptibility or walking speed. When the mean walking speeds were excluded from the exposure function however, the susceptibility values still matched the ranking from the two hour glass bioassay. This may indicate that saturation points for the uptake of deltamethrin from the glass surface were reached for the species tested. Saturation of pyrethroid uptake with distance moved was not found to occur with lepidopteran larvae by Salt and Ford (1984) but has been found to occur in a short period of time with linyphiid spiders by Jagers op Akkerhuis and Hamers (1992).

The validation of the susceptibility index is not conclusive. However the model appears to be capable of predicting extremes of susceptibility. This may be useful for regulatory bodies who wish to select the species most at risk for further registration testing and also for the interpretation of field trial results.

The susceptibility index is applied to plant and soil surface active invertebrates only and not subterranean species. Loose or fissured soil, or soil with a surface flora are likely to present different risks of contamination and toxic effects. Thus the nature of the index as a corrective factor for laboratory bioassay data must be emphasized. The

index does not take into account the following factors which will also affect the impact of a given residual pesticide deposit in the field:

a) the species diel activity cycles (i.e. one species may walk fast but may only be active for a short period per day and may therefore pick up a small amount of pesticide: a slower moving species may spend a larger proportion of the day active and pick up a larger amount of pesticide).

b) the distribution of the species through the crop (i.e. some species may be active on plant surfaces where the pesticide may be at higher residue levels or be more available than on the soil).

c) the proportion of the population that are likely to be in the crop during and shortly after spraying.

d) behavioural responses, such as activation or repellency which may modify exposure dramatically.

To develop models that may accurately predict mortality resulting from exposure to residual deposits of pesticides it is necessary to quantify the amount of pesticide picked up in a given exposure period. Salt and Ford (1984) and Jepson *et al.* (1990a) established that the toxicity of residual deposits of pesticides against crawling insects is influenced by the droplet size, density and mass of active ingredient and concentration-dependent behavioural responses. The extent of pesticide transfer will depend on the probability of the insect encountering the pesticide and the proportion of the insecticide which adheres to the insect cuticle (Ford and Salt, 1987). To attempt an analysis of all these parameters for numerous species would be extremely time consuming. Therefore simple indices, of the form described in this chapter, may be used in testing frameworks to select species for *in situ* bioassays within the appropriate crop (Jepson *et al.* 1990b). These may then feed into more complicated models of hazard, which could be verified with semi-field trials if safety criteria are not met.

CHAPTER 4

Substrate-mediated toxicity of deltamethrin residues to aphid predators: The estimation of "toxicity factors" to aid risk assessment.

4.1 INTRODUCTION

The risk posed by pesticide residues to natural enemies will not only depend upon species dependent biological factors such as activity patterns, behaviour and contact area with the surface, which were discussed in the previous chapter, but also on the interaction between physical and chemical factors which determine the duration of bioavailability of the compound on any given substrate. These factors include processes of sorption (adsorption and desorption), volatilization, temperature and humidity, droplet characteristics and chemical formulation (Hartley and Graham-Bryce, 1980; Ford and Salt, 1987; Hall, 1987 & 1988; Felsot and Lew, 1989; Arnold and Briggs, 1990; Gerstl, 1991). It is only by combining these factors that the relative risks posed by residues on different substrates may be predicted.

In general the bioavailability of a pesticide on soil (Briggs, 1973) and plant surfaces (Ford and Salt, 1987) is negatively correlated with it's octanol/water partitioning coefficient. However it will also be affected by factors such the thickness and architecture of the wax layer in plant material (Adams *et al.* 1987) and factors such as organic matter (Harris, 1967; Briggs, 1981) and clay content (Arnold and Briggs, 1990) in soils. These sorption processes and degradation by microorganisms are known to restrict the toxicity of most pyrethroids against soil organisms (Elliot *et al.* 1978).

Because uptake and the resultant toxicity of pesticide residues to an organism are mediated by the bioavailability of the compound on any given substrate, measurements of pesticide persistence are likely to be of less value for risk assessment than measurements of bioavailability. For example studies concerning residue analysis of mineral soils have reported half-lives of between three and eight weeks for deltamethrin (Chapman *et al.* 1981; Miyamoto and Mikami, 1983; Hill, 1983; Hill and Schaalje, 1985) whereas bioavailability studies by Mullié and Everts (1991) using radio-labelled ^{14}C deltamethrin and linyphiid spiders have indicated that deltamethrin may have a bioavailable half-life of only 42 hours on a humic clay soil. The risk posed by deltamethrin residues to invertebrates on the soil may therefore be much lower than residue studies suggest.

Aphid predators were used as indicators of the bioavailability of deltamethrin residues on soil and cereal foliage in the bioassays described in this chapter in order to predict the relative risk posed by these residues to invertebrates in cereal crops and to determine the more general applicability of Mullié and Everts (1991) findings.

Standardised procedures for testing the side-effects of pesticide residues on beneficial invertebrates have been developed over the last 15 years by organisations such as the IOBC "Pesticides and Beneficial Invertebrates Working Group" (Hassan *et al.* 1987 & 1991,; Hassan, 1985, 1989 & 1992; Samsoe-Peterson, 1985). These procedures give guidance concerning the choice of test organism and substrate for different crop/pest categories. The tests provide a robust screen of a large number of compounds and act as the first stage in a step-wise testing procedure. This approach may be useful in ranking the toxicity of pesticides to beneficial invertebrates but may be of limited use in interpreting and predicting risk posed by the pesticides to beneficial species in the field as the tests often only measure responses on a single, unrealistic substrate i.e. glass, and to a single pesticide dose, i.e. the recommended field rate of the pesticide. Two points arise from this. Firstly it would be useful to be able to correct mortality data from bioassays on an unrealistic surface to a more realistic surface, i.e. foliage and soil, and secondly, while it is accepted that it is only possible to use single doses when screening large numbers of pesticides, it may be more useful to explore dose-response relationships for pest management purposes.

The series of laboratory bioassays described in this chapter aimed to develop a more predictive approach to aid risk assessment for single compounds by quantify dose-response relationships for a wide taxonomic and size range of important aphid predators found in cereals to deltamethrin residues. Brown, Lawton and Shires (1983) have previously carried out laboratory bioassays to determine the residual toxicity of three aphicides, including the pyrethroid cypermethrin, to a similar set of predators. They tested low numbers of predators per dose (n=10) however and their rankings only permitted susceptibility comparisons between species on a glass substrate. The bioassays in this study determined the relative risks posed by deltamethrin residues to aphid predators on the substrates they are likely to encounter in the field; wheat foliage and soil. By doing this the second aim was to investigate substrate-mediated toxicity of deltamethrin, by comparing the relative effects of deltamethrin residues on foliar and soil substrates for given invertebrate species. Estimates of the bioavailability (expressed as bioavailable half-life) of deltamethrin were obtained from *in situ* bioassay in cereal crops, in which insects were used as indicators of chemical availability. These

were then incorporated into the risk estimates to improve the relevance of the predictions to the "real world".

4.2 EXPERIMENTAL METHODS

4.2.1 Test invertebrates

The invertebrates tested in these bioassays included polyphagous and aphid-specific predators. The polyphagous predators were the large carabid beetles *P. melanarius* and *N. brevicollis*, the small carabid beetles *D. atricapillus* and *B. obtusum* and the small staphylinid beetle *T. hypnorum*. The aphid-specific predators were the coccinellid *C. septempunctata*, the syrphid *E. balteatus* and the parasitoid *A. rhopalosiphi*.

Adult individuals of each species were tested, with the exception of the coccinellid *C. septempunctata*, where adults and 4th instar larvae were tested in the foliar bioassays. Adult hoverflies are not aphidophagous themselves however they are likely to come into contact with pesticide residues in the field when searching foliage.

In the field these beneficial invertebrates partition themselves throughout the crop canopy and on the soil surface (Vickerman and Sunderland, 1975). Some are mainly associated with plant foliage, e.g. hymenopteran parasitoids, syrphids and coccinellid larvae, some with the soil, e.g. the larger Carabidae, while others spend varying proportions of their time on both substrates. The species tested in these bioassays therefore represented the taxonomic and crop distribution range of predators and parasitoids active in temperate cereals infested by aphids (Table 4.1).

Table 4.1 Crop activity categories for the test invertebrates.

	Crop activity category		
	Plant active	Soil active	Plant and soil active
Species tested	*A. rhopalosiphi* *E. balteatus* *C. septempunctata* (L)*	*N. brevicollis* *B. obtusum* *P. melanarius*	*D. atricapillus* *C. septempunctata* (A)* *T. hypnorum*

C. septempunctata (L) indicates 4th instar larvae and *C. septempunctata* (A) indicates adults.

A series of short-term residual exposure bioassays were carried out for each species on one or both substrates. The duration of the initial bioassays was limited to two hours to avoid complications arising from differential residue decay rates and to minimise the effects of differences in behaviour and activity levels between test organisms. A further series of soil bioassays, with a 72 hour exposure period, were carried out to obtain a better separation of species susceptibilities on this substrate as the two hour bioassays were of insufficient duration to give toxic effects for the carabid beetle species tested.

Glass was used as a third substrate to act as a standard. The four species of predator tested on this substrate were, *N. brevicollis*, *D. atricapillus*, *T. hypnorum* and *C. septempunctata.* Many standard tests are undertaken on glass (Jepson, 1993a) and the inclusion of this substrate therefore permitted toxicity data from these tests to be compared with data obtained on more natural substrates.

4.2.2 Capture and maintenance of test invertebrates

Field capture of active individuals at their peak of seasonal activity was relied upon to provide the experimental invertebrates. The coleopteran predators were captured between October 1989 and 1990 in cereal fields and field margins on the Leckford Estate, Stockbridge, Hampshire, using the methods described in Chapter 2 (2.2.1). Adult hoverflies were captured in June and July 1990 from field-margins using butterfly nets and hand-held aspirators and parasitoids were obtained from laboratory cultures.

Prior to the bioassays all test invertebrates were kept in a controlled environment room in an insectary, maintained at 19-22°C, 55-70 % relative humidity, and photoperiod 16:8 L:D. The Carabidae and Staphylinidae were maintained and fed as described in Chapter 2 (2.2.1). The coccinellids and parasitoids were kept in perspex boxes with barley plants infested with the grain aphid *S. avenae* and the hoverflies were kept in ventilated perspex boxes and provided with a honey and water solution.

4.2.3 Residual bioassay techniques

i) Two hour bioassays on cereal flag leaves

Foliar bioassays were carried out using a similar technique to that described by Efe (1991). Clean glass plates (12cm x 12cm) covered with freshly excised flag leaves from field-grown, untreated, winter wheat plants, cv. Galahad, at decimal growth stage 59 (Zadoks *et al.* 1974). These were attached to the glass via strips of double-sided adhesive tape. Leaves were placed in parallel, base to tip, on each plate with their

adaxial surface exposed, ensuring that the glass plate was completely covered.

Exposure chambers, consisting of a section of plastic drainpipe (9.5 cm diameter and 5cm high), were placed over the flag leaf-covered plates after treatment. The chambers had ventilation holes cut into the sides which were covered with fine gauze and their inner walls had previously been coated with a suspension of Fluon (PTFE) to prevent insects from climbing the sides. A clean glass plate was placed over the top of each chamber and the chambers were ventilated with humidified air via tubes connected to an aquarium pump.

ii) Two and seventy-two hour bioassays on soil

The soil used in the bioassays was dug from a depth of 15-20 cm from an untreated field boundary on the Leckford Estate, Hampshire. Large stones were removed by hand and smaller stones were removed with a 2 mm mesh sieve. Mineral composition analysis in the laboratory indicated that it was a sandy loam (55% sand, 24% silt, 14% clay and 7% organic matter; mean pH = 6.8 in a 1:1 soil/water slurry). The test soil had a mean moisture content of 22% (w/w).

The soil exposure chambers consisted of plastic tubs (9.5 x 5 cm) containing a 30 ± 2g sample of the soil lightly compacted in the base. The sides of the tubs had again been coated with PTFE to prevent test invertebrates from climbing the sides. A plastic inlay, consisting of a tub with the bottom removed, was placed in each tub before spraying to avoid contamination of the chamber sides. The inlays were removed immediately after spraying and the chambers were ventilated in a similar manner to the flag leaf chambers.

iii) Two hour bioassay on glass.

These bioassays were carried out using the same procedure as the flag leaf bioassays. New glass plates (12cm x 12cm) that had been cleaned with the detergent ("Decon 90" - Decon Manufacturing Ltd.) and rinsed with distilled water were used as the substrate for exposure. The exposure chambers were identical to those used in the flag leaf bioassays.

4.2.4 Treatment procedure

Stock solutions of deltamethrin were prepared immediately before each experiment from formulated deltamethrin (2.5% E.C.). Distilled water was used as the diluent and for the control treatment. The test substrates were sprayed under a Potter Tower

(Potter, 1952) calibrated to deliver spray at a volume of 200 l ha^{-1}. The tower was thoroughly cleaned and flushed with acetone and water between treatments. Initially, range-finding bioassays were carried out using five to ten insects per dose and three to four logarithmically spaced doses. From these definitive ranges of between five and seven doses were selected. After treatment the chambers were returned to the insectary and the deposits were allowed to dry for approximately 30 minutes before the test organisms were introduced. Five test invertebrates were exposed per chamber. Nocturnal species, such as *P. melanarius*, *N. brevicollis*, and *T. hypnorum*, were exposed in darkness and the diurnal species, such as *B. obtusum*, *D. atricapillus*, *C. septempunctata*, *E. balteatus* and *A. rhopalosiphi*, were exposed under artificial light. The area of exposure was 70.9 cm^2 in all bioassays, except for the flag leaf bioassays with *E. balteatus* and *A. rhopalosiphi*. These two organisms tended to walk on the upper surface of the chambers and therefore flag leaf-covered plates were placed on the top as well as the bottom of chambers to ensure exposure. A light source was placed over these chambers to ensure adequate illumination through the gause covered ventilation holes in the sides of the chambers. The treated surface area in these chambers was 141.8 cm^2 because two plates were used however the mean pesticide deposit per unit area was the same as in the other bioassays.

The number of invertebrates of each species tested per dose in the definitive bioassays were as follows: 2 h flag leaf exposure - *D. atricapillus* (n=20), *T. hypnorum* (n=20), *C. septempunctata* (n=20), *C. septempunctata* 4th instar larvae (n=20), *E. balteatus* (n=20), and *A. rhopalosiphi* (n=30); 2 h soil exposure, *N. brevicollis* (n=20), *D. atricapillus* (n=20), *T. hypnorum* (n=20) and *C. septempunctata* (n=20); 72 h soil exposure, *P. melanarius* (n=30), *N. brevicollis* (n=30), *D. atricapillus* (n=30), *B.obtusum* (n=30), *T. hypnorum* (n=30), and *C. septempunctata* (n=30); and 2 h glass exposure, *N. brevicollis* (n=20), *D. atricapillus* (n=30), *T. hypnorum* (n=20) and *C. septempunctata* (n=20).

After exposure, test invertebrates were placed in clean, ventilated, containers with food and responses were recorded at 24 h intervals for the next four days. Individuals were classified as unaffected, moving as normal, or affected, i.e. either knocked down, with moving antennae, mouthparts and/or legs, or dead, with no response after stimulation. Raw dose-response data for all definitive bioassays are given in Appendix 2.

4.2.5 Statistical analyses

Probit analysis was performed on 72 h mortality data from the two hour bioassays and

144 h mortality data (i.e. 72 h after exposure) in the 72 h bioassays to obtain dose-response statistics (Finney, 1971). These data were chosen for analysis because few individuals remained knocked down after this time and thus the mortality response appeared to be near end-point. Abbott's formula was used to correct the mortality data for control effects (Abbott, 1925). The slopes and positions of the probit lines were compared between species using maximum likelihood procedures (Ross, 1987). A pairwise testing procedure was undertaken to compare all the species and to infer patterns of susceptibility of predators to deltamethrin residues within and between substrates.

4.3 RESULTS

4.3.1 The susceptibility of plant active predators and parasitoids to deltamethrin residues on flag leaves

The summary statistics from probit analysis of the 2 h flag leaf dose-mortality data are given in Table 4.2. There were no indications of heterogeneity in the data sets (P>0.05).

Table 4.2 72 h probit statistics for 2 h flag leaf residual bioassays.

Family *Species*	Probit slope	LD_{50} (& s.e.) (Detransformed) (g AI ha^{-1})	Heterogeneity χ^2(d.f.)Signif. ns = P>0.05
Carabidae			
D.atricapillus		> 50*	
Staphylinidae			
T.hypnorum	2.73	1.2 (0.15)	2.91 (2)ns
Coccinellidae			
C.septempunctata (A)	2.50	2.0 (0.29)	0.13 (2)ns
C.septempunctata (L)	1.58	0.4 (0.10)	1.99 (2)ns
Syrphidae			
E.balteatus	2.16	4.8 (0.96)	2.24 (2)ns
Braconidae			
A.rhopalosiphi	2.31	7.1 (0.86)	0.16 (2)ns

Signif. = Significance level.

* = maximum mortality obtained at the highest dose test for *D. atricapillus* (50.8 g AI ha^{-1}) was 10%.

C. septempunctata (A) = Adults; *C. septempunctata* (L) = 4th Instar larvae.

Probit analysis was not possible for *D. atricapillus* because mortality remained low at highest deltamethrin concentration tested. The range of LD_{50} values varied from >50 g AI ha^{-1} to 0.4 g AI ha^{-1}. The ranking of susceptibility for the species tested (from most to least susceptible) was as follows *C. septempunctata* (L) > *T. hypnorum* > *C. septempunctata* (A) > *E. balteatus* > *A. rhopalosiphi* > *D. atricapillus*.

Table 4.3 Matrix of χ^2 statistics from pairwise maximum likelihood analyses for differences in i) position and ii) parallelism of 72 h probit lines of the test species.

i) Position

Species	C.s.(L)	A.r.	E.b.	T.h.
C.s.(A)	13.1 ***	18.0 ***	5.7 *	5.5 *
T.h.	15.1 ***	12.6 ***	3.9 *	
E.b.	5.8 *	2.8 ns		
A.r.	17.6 ***			

ii) Parallelism

Species	C.s.(L)	A.r.	E.b.	T.h.
C.s.(A)	10.8 ***	8.5 **	9.5 **	2.2 ns
T.h.	10.1 ns	8.7 **	9.1 **	
E.b.	13.9 ***	0.2 ns		
A.r.	13.2 ***			

Values in boxes give χ^2 statistic (d.f.1) and level of significance; ns = not significant, * = P<0.05, ** = P<0.01, *** = P<0.001. Key to test species: C.s.(A) - *C. septempunctata* (Adults), E.b. - *E. balteatus*, A.r. - *A. rhopalosiphi*, C.s.(L) - *C. septempunctata* (4th instar larvae)

Three of the four coleopteran predators tested were more susceptible to foliar deltamethrin residues than the syrphid *E. balteatus* and the parasitoid *A. rhopalosiphi*. Pairwise maximum likelihood analyses indicated significant differences in susceptibility between all species except *E. balteatus* and *A. rhopalosiphi* (Table 4.3). A large number of significant separations were also evident for parallelism of probit lines. *C. septempunctata* (L) had a significantly shallower slope than all of the other species tested whereas *C. septempunctata* (A) and *T. hypnorum* had significantly steeper probit slopes than the other species. No significant difference in slopes was evident between *E. balteatus* and *A. rhopalosiphi* or *C. septempunctata* (A) and *T. hypnorum*.

4.3.2 The susceptibility of soil active predators to deltamethrin residues on a sandy loam soil

The summary statistics from probit analysis of the 2 and 72 h soil dose-mortality data

are given in Tables 4.4 and 4.5. There were no indications of heterogeneity in the data sets (P>0.05).

Table 4.4 72 h probit statistics for 2 h soil residual bioassays.

Family *Species*	Probit slope	LD_{50} (& s.e.) (Detransformed) (g AI ha^{-1})	Heterogeneity χ^2 (d.f.) Signif. ns = P>0.05
Carabidae			
N. brevicollis		> 170*	
D. atricapillus		> 500*	
Staphylinidae			
T. hypnorum	1.36	52.8 (12.0)	0.65 (3) ns
Coccinellidae			
C. septempunctata (A)	1.95	97.8 (18.1)	0.37 (2) ns

Signif. = Significance level.

* = maximum mortality obtained at the highest dose test for *N. brevicollis* (169.7 g AI ha^{-1}) was 20%; and maximum mortality obtained at the highest dose test for *D. atricapillus* (499.7 g AI ha^{-1}) was 10%.

Probit analysis for dose-response data of *N. brevicollis* or *D. atricapillus* was not possible in the 2 h bioassays because mortality remained low at highest deltamethrin concentrations tested (Table 4.4). Maximum likelihood procedures indicated that the staphylinid *T. hypnorum* and the coccinellid *C. septempunctata* did not differ in susceptibility (χ^2 = 2.0 ns, d.f.1, P>0.05) or tolerance distribution (χ^2 = 1.7ns, d.f.1, P>0.05) in the 2 h bioassays.

The 72 h bioassays enabled separation of susceptibilities. The LD_{50} values of the six species tested varied between 267 g AI ha^{-1} and 4.2 g AI ha^{-1} (Table 4.5). The susceptibility ranking of the species tested (from most to least susceptible) was *T. hypnorum* > *B. obtusum* > *C. septempunctata* (A) > *P. melanarius* > *N. brevicollis* > *D. atricapillus*. The χ^2 statistics from pairwise maximum likelihood analyses indicated only one non-significant difference in susceptibility which was between the large

Table 4.5 72 h probit statistics for 72 h soil residual bioassays.

Family *Species*	Probit slope	LD_{50} (& s.e.) (Detransformed) (g AI ha^{-1})	Heterogeneity χ^2(d.f.)Signif. ns = P>0.05
Carabidae			
P.melanarius	*2.13*	*52.3 (6.28)*	*0.43 (3)ns*
N.brevicollis	*1.89*	*53.2 (7.08)*	*1.20 (3)ns*
D.atricapillus	*2.07*	*267.3 (36.1)*	*0.91 (3)ns*
B.obtusum	*2.14*	*7.8 (0.88)*	*5.41 (4)ns*
Staphylinidae			
T.hypnorum	*2.52*	*4.2 (0.50)*	*0.78 (2)ns*
Coccinellidae			
C.septempunctata(A)	*1.96*	*16.6 (2.05)*	*0.45 (4)ns*

Signif. = Significance level

carabid beetles *P. melanarius* and *N. brevicollis* (Table 4.6). Large numbers of significant separations were evident in parallelism. *T. hypnorum* had the steepest probit slope and *N. brevicollis* the shallowest probit slope of the species tested.

Table 4.6 Matrix of χ^2 statistics from pairwise maximum likelihood analyses for differences in i) position and ii) parallelism of 72 h probit lines of the test species.

i) Position

Species	C.s.(A)	T.h.	B.o.	D.a.	N.b.
P.m.	19.2 ***	25.5 ***	23.8 ***	29.5 ***	0.1 ns
N.b.	19.4 ***	24.6 ***	23.2 ***	27.1 ***	
D.a.	25.8 ***	27.0 ***	23.2 ***		
B.o.	13.1 ***	20.3 ***			
T.h.	22.7 ***				

ii) Parallelism

Species	C.s.(A)	T.h.	B.o.	D.a.	N.b.
P.m.	19.6 ***	31.6 ***	46.2 ***	19.8 ***	0.1 ns
N.b.	10.7 ***	31.2 ***	47.1 ***	17.1 ***	
D.a.	36.6 ***	34.5 ***	59.4 ***		
B.o.	28.6 ***	13.3 ***			
T.h.	24.5 ***				

Values in boxes give χ^2 statistic, d.f.1, and level of significance; ns = not significant, *** = P<0.001. Key to test species: P.m. - *P. melanarius*, N.b. - *N. brevicollis*, D.a. - *D. atricapillus*, B.o. - *B. obtusum*, T.h.- *T. hypnorum*, C.s.(A) - *C. septempunctata* (Adults)

4.3.3 The susceptibility of aphid predators to deltamethrin residues on glass

Probit analysis was not possible for *N. brevicollis* and *D. atricapillus* as mortality remained low at the highest deltamethrin concentrations tested (Table 4.7). Maximum

likelihood analysis indicated that the positions of the probit lines of the two other species were similar (χ^2=0.2 ns, P>0.05) but that *T. hypnorum* had a significantly steeper probit slope than the *C. septempunctata* adults (χ^2=21.2 ***, P<0.001). There were no indications of heterogeneity in the data sets (P>0.05).

Table 4.7 72 h probit statistics for 2 h glass residual assays.

Family *Species*	Probit slope	LD_{50} (& s.e) (Detransformed) (g AI ha^{-1})	Heterogeneity χ^2 (d.f.) Signif. ns = P>0.05
Carabidae			
N.brevicollis		> 37*	
D.atricapillus		> 37*	
Staphylinidae			
T.hypnorum	2.88	1.22 (0.15)	1.15 (2)ns
Coccinellidae			
C.septempunctata(A)	2.57	1.66 (0.21)	2.91 (3)ns

Signif. = Significance level.

* = maximum mortality obtained at the highest dose test for *N. brevicollis* (36.7 g AI ha^{-1}) was 45%; and maximum mortality obtained at the highest dose test for *D. atricapillus* (36.7 g AI ha^{-1}) was 33%.

4.3.5 Estimation of "toxicity factors" to aid the prediction of risk

Pairwise maximum likelihood analyses to compare individual differences in position and parallelism of the probit lines for *C. septempunctata* adults and *T. hypnorum* on the three substrates tested in the 2 h bioassays indicated no significant differences in position and parallelism between the probit lines on the glass and flag leaf surfaces for either *C. septempunctata* or *T. hypnorum* (Table 4.8). However the probit lines differed significantly in both position and parallelism between glass and soil and flag leaf and soil for *C. septempunctata* and *T. hypnorum*. This indicated that both beetles were significantly more susceptible to deltamethrin residues on glass or flag leaves than on the soil and that they both had a significantly narrower tolerance distribution on glass and flag leaf surfaces than on the test soil. The toxicity of deltamethrin residues to *T. hypnorum* and *C. septempunctata* adults on the three test substrates were compared by iterating a sequence of lethal dose ratios calculated from dose-response statistics for each pair of substrates. The sequence of doses selected represented responses between LD_{10} and LD_{90} (Table 4.9) to allow for differences between probit slopes. The mean values obtained were called "toxicity factors" (Tf).

These have no units and give an estimate of the relative toxicity of deltamethrin residues to a given organism on the two substrates that are compared.

Table 4.8 Matrix of χ^2 statistics from pairwise maximum likelihood analyses for differences in i) position and ii) parallelism of 72 h probit lines of two species of predators on three test substrates.

i) Position

Species	T.h.(G)	T.h.(F)
T.h.(S)	8.0 **	6.0 **
T.h.(F)	0.1 ns	

i) Position

Species	C.s.(G)	C.s.(F)
C.s.(S)	5.4 *	7.6 **
C.s.(F)	1.5 ns	

ii) Parallelism

Species	T.h.(G)	T.h.(F)
T.h.(S)	19.6 ***	12.6 ***
T.h.(F)	0.1 ns	

ii) Parallelism

Species	C.s.(G)	C.s.(F)
C.s.(S)	30.4 ***	20.0 ***
C.s.(F)	1.2 ns	

Values in boxes give χ^2 statistic, d.f.1, and significance; ns = not significant, * = P<0.05, ** = P<0.01, *** = P<0.01. Key to test species: T.h. - *T. hypnorum*, C.s. - *C. septempunctata*. Key to substrates: (G) - Glass, (F) - Flag leaf, (S) - Soil

The Tf values were similar for both species of predators in all substrate comparisons (Table 4.9). The values indicated that the toxicity of deltamethrin residues on glass and flag leaf surfaces were very similar (Tf approximately 1). The Tf values for

Table 4.9 Toxicity factors comparing the relative bioavailability of deltamethrin residues to *T. hypnorum* and *C. septempunctata* adults on glass, flag leaf and soil substrates.

Test species	Toxicity factors (Tf) calculated from substrate comparisons					
	Glass/Flag leaf		Glass/Sandy loam soil		Flag leaf/Sandy loam soil	
	Mean	(95% C.L.)	Mean	(95% C.L.)	Mean	(95% C.L.)
T. hypnorum	0.98	(0.93-1.03)	53.6	(5.9-101.3)	57.6	(5.0-110.2)
C. septempunctata (A)	1.23	(1.19-1.27)	63.7	(45.4-82.0)	60.6	(41.4-79.8)

The values given in the table were obtained from iteration of the ratios of LD_{10}, DL_{30}, LD_{50}, LD_{70}, and LD_{90} doses for pairs of substrates.

comparisons between glass and soil and flag leaf and soil substrates however indicated that deltamethrin residues on glass and flag leaf surfaces were approximately 50 to 60 times more toxic to *T. hypnorum* and *C. septempunctata* than residues on the sandy loam soil.

4.3.6 Modification of "toxicity factors" to predict the relative risk posed by deltamethrin residues on flag leaves and soil to predators in the field

Toxicity factors may be useful as a correction factor for laboratory bioassay data from standard substrates (see 4.4.3). In order to estimate the relative risk posed by pesticide residues to beneficial invertebrates in the field however, further correction for the duration of bioavailability of the deposits on each given substrate may be necessary. The relative risk posed by deltamethrin residues to *T. hypnorum* and *C. septempunctata* adults on soil and flag leaf substrates in the field was therefore estimated using Equation 1 below:

$$\text{Relative risk} = Tf_{A/B} \times BHI_{A/B} \qquad \text{(Eq.1)}$$

where $Tf_{A/B}$ = the toxicity factor, i.e. the ratio of the iterated dose-mortality responses of the invertebrates on substrates A (flag leaves) and B (soil), and $BHI_{A/B}$ = an estimate of the relative bioavailable half-lives of deltamethrin on the same substrates under field conditions. The relative risk values obtained have units.

Values of bioavailable half-lives (BHI) were obtained for deltamethrin on flag leaf and soil substrates in a mature winter wheat crop using mortality data from 24 h exposure *in situ* bioassays from two separate studies (Table 4.10). Both studies were carried out at the same field site, in different seasons, and therefore the soil types are likely to have been similar. Also this field site was where the soil used in these bioassays was collected from. In both studies fresh batches of insects were exposed to deltamethrin residues on flag leaves and soil on each day after spray application. The bioavailable half-life of deltamethrin on the substrates was estimated as the number of days taken for the mortality of the test invertebrates to fall to 50% of the initial mortality observed.

Table 4.10 Estimates of the bioavailable half-lives of deltamethrin residues on soil and flag leaves in a mature cereal crop.

Substrate type	Estimated bioavailable half-lives from *in situ* bioassay data (Days)	
	(Unal and Jepson, 1991)[1]	(This study - see Appendix 3)[2]
Flag leaves	6.0	4.5
Soil	2.4	1.6

[1] - *B. lampros* was used as the test species, [2] - *C. septempunctata* was used as the test species

Table 4.11 Estimation of the relative risk posed by deltamethrin residues to *T. hypnorum* and *C. septempunctata* on flag leaf and soil substrates in the field.

Predator species	Toxicity factors (Tf) comparing the toxicity of deltamethrin residues residues on flag leaf and soil (95% C.L.)	Estimated mean bioavailable half-life (BHl) for deltamethrin on flag leaves relative to soil (Days)	Predicted relative risk posed by deltamethrin residues on flag leaves compared to soil
T. hypnorum	57.6 (5.0 - 110.2)	2.68	154.3 (13.4 - 295.3)
C. septempunctata	60.6 (41.4 - 79.8)		162.4 (111.0 -213.9)

The relative bioavailable half-lives of deltamethrin residues on flag leaves compared with soil was estimated by calculating the ratio of bioavailable half-lives from the two studies (Table 4.10). These ratios were 2.54 (from Unal and Jepson, 1991) and 2.81 (from the study in Appendix 3). As these values were in close agreement a mean was taken (2.68) to act as an estimate of the relative bioavailable half-lives (Table 4.11). This estimate was then substituted into equation 1 together with the toxicity factors (Table 4.10) to give predictions of the relative risk posed by deltamethrin residues on flag leaves relative to residues on the soil (Table 4.11). The mean values of relative risk were 154.3 for *T. hypnorum* and 162.4 for *C. septempunctata*.

4.4 DISCUSSION

4.4.1 The susceptibility of plant active predators and parasitoids to deltamethrin residues on flag leaves

The five species of predators and parasitoids tested in the flag leaf bioassays showed a 100 fold range of susceptibility to deltamethrin residues. The small carabid beetle *D. atricapillus* was the least susceptible of the predators tested, with doses in excess of 8 times current recommended field rate of deltamethrin in U.K. cereals causing only low levels of mortality. Knockdown symptoms were observed during exposure to deltamethrin residues but *D. atricapillus* was able to recover shortly afterwards. The reasons for this may have been physiological, i.e. it may possess innate tolerance mechanisms (as discussed in Chapter 2 (2.4.1)), and/or behavioural/morphological as this beetle tends to walk relatively slowly and has a relatively low contact area with the substrate (see Chapter 3 (3.4)). The other species tested had LD_{50} values similar to or lower than the recommended field rate of deltamethrin (i.e. 6.25 g AI ha^{-1}) indicating that mortality may occur in the field from residual uptake. The most susceptible predator was *C. septempunctata* 4th instar larvae which had an LD_{50} value of approximately 0.06 of field rate. The reason for this high susceptibility may be because

the larvae tend to contact the substrate with their abdomen and are therefore likely to have a high contact area with the substrate which may increase pesticide uptake. Also sub-lethal poisoning effects appeared to reduce searching and feeding efficiency on the days after exposure. The small staphylinid beetle *T. hypnorum* was the second most susceptible species to flag leaf deposits. This may be because of it's relatively high contact area with the substrate (Chapter 3 (3.4)). *C. septempunctata* adults were less susceptible to foliar deltamethrin residues than *T. hypnorum* but more susceptible than either the adult hoverfly *E. balteatus* or the parasitoid *A. rhopalosiphi*. This may be related to differences in activity during the bioassay. The coccinellids appeared to be more active than either of these species during the period of exposure and therefore deltamethrin uptake may have been proportionally greater.

4.4.2 The susceptibility of soil active predators to deltamethrin residues on a sandy loam soil

The LD_{50} values from the 2 h soil bioassays ranged from 8.4 to >80 times the recommended field rate of deltamethrin for cereals for the four species tested. This duration of exposure was insufficient to separate species susceptibilities. The 72 h bioassays enabled comparisons of susceptibility between species and gave LD_{50} values which varied from 0.7 to 42.8 times field rate. The susceptibility ranking for the six predators tested broadly agreed with the predictions given in Chapter 3 (3.3) for a similar set of test species. The staphylinid *T. hypnorum* was the most susceptible species and the carabid *D. atricapillus* was the least susceptible. A large number of significant differences were evident between the slopes of the probit lines for the species tested however interpretation of these differences was difficult because these responses are not only likely to be related to the intrinsic susceptibility and innate characteristics of the species but also species-specific behaviour and activity patterns. Observations made during the bioassays indicated that the staphylinid *T. hypnorum* and the coccinellid *C. septempunctata* were generally active for a higher proportion of the period of exposure than the carabid beetles. This, together with *T. hypnorum*'s high contact area, may partly explain why the staphylinid beetle was found to be more susceptible to deltamethrin residues on soil than all of the other species tested in these bioassays, including the small carabid *B. obtusum*, which has a similar intrinsic susceptibility to deltamethrin (Chapter 2 (2.3.2)).

4.4.3 Substrate-mediated toxicity of deltamethrin residues to aphid predators and the use of toxicity factors in risk assessment

Comparisons of the toxicity of deltamethrin residues on the three test substrates were

made for two predator species, *T. hypnorum* and *C. septempunctata*, giving similar toxicity factor values. These values indicated that the toxicity of fresh deltamethrin residues to beneficial invertebrates on glass and winter wheat flag leaves were similar, under the given conditions. This may be because penetration of deltamethrin into the epicuticular wax layer of the wheat flag leaves was relatively slow and therefore a large proportion of the deposit remained available on the leaf surface during the 2 h bioassay. However care must be taken in assuming that no interactions occurred between the glass substrate and deltamethrin. The glass plates used in these bioassays were carefully cleaned before use but no deactivation procedures were carried out.

The toxicity of deltamethrin residues to *T. hypnorum* and *C. septempunctata* on the glass and flag leaves was approximately 50 to 60 times greater than on the sandy loam soil. When the 95% confidence limits were accounted for the predicted values indicated that deltamethrin was 5 to 110 times more toxic on the glass and flag leaves than on the soil indicating large differences in bioavailability. Toxicity factors such as these, if validated for a larger number of species, may be useful correction factors for bioassay data. For example glass is widely used as a test substrate in bioassays (Jepson, 1993a). If reliable corrections could be made to data from glass bioassays to allow extrapolation of results to more natural substrates the numbers of bioassays and hence testing costs would be reduced. The toxicity factors may also be used in risk assessment. For example the staphylinid beetle *T. hypnorum* was found to be of similar intrinsic susceptibility to deltamethrin as the small carabid beetle *B. obtusum* (Chapter 2 (2.3.2)), but the fact that the staphylinid beetle is plant-active whereas the carabid beetle is mainly ground-active would indicate that the risk posed by contact with spray residues may be much greater for the staphylinid, assuming that the two beetles had similar exposure.

The limitations of "toxicity factors" must also be realised however. Values will vary according to different substrates (i.e. leaves with different wax properties or thicknesses and soil with different mineral compositions), different bioassay conditions and durations of exposure, and between test organisms with different habits. Therefore to be of general value standardised test conditions and exposure methods need to be followed for groups of organisms with similar habits.

4.4.4 Predicting the relative risk posed by deltamethrin residues on flag leaves and soil to predators in the field

The incorporation of estimates of the bioavailable half-life of deltamethrin on wheat flag leaves and soil may improve the relevance of predictions to the field. The estimates of bioavailable half-life of deltamethrin on flag leaves was approximately two to three times that on soil. Therefore this approximately tripled the estimated risk posed by deltamethrin residues on the flag leaf to predators relative to the soil (95% confidence limits were 13.4 to 295.3 for *T. hypnorum*). It is difficult to validate these predictions however. Some evidence that differences of this magnitude are likely to exist has been provided by studies by Jagers op Akkerhuis and Hamers (1992) concerning differences in bioavailability of ^{14}C deltamethrin to a linyphiid spider. They have shown that soil covered with fungi and moss could increase the bioavailability of deltamethrin to a spider by a factor of approximately 100 compared to soil with no such cover.

The risk predictions suggest that after a deltamethrin spray application plant-active predators may be at a much greater risk from suffering effects from exposure to deltamethrin residues in a cereal crop than the predators that remain on the soil. Plant-active predator species, such as the small staphylinid *T. hypnorum* and adults and larvae of the coccinellid *C. septempunctata*, are likely to be most affected (4.4.1). These predictions agree with the results of several field trials which have shown that the abundance of predators such as staphylinids, for example *Tachyporus* spp. (Basedow *et al.* 1985; Vickerman *et al.* 1987b), and coccinellids, for example *C. septempunctata* (Vickerman *et al.* 1987a), was reduced in cereal crops after treatment with deltamethrin whereas ground-active species were less affected.

The predictions may therefore be useful to identify species which may be most at risk from suffering effects from exposure to residues because of the substrate they inhabit. Until factors such as bioavailability and uptake by organisms can be quantified more easily and precisely, approaches such as the one outlined in this chapter may offer the most readily obtainable insight into predicting substrate-mediated risk via residual exposure.

CHAPTER 5

The dietary toxicity of deltamethrin to *Nebria brevicollis* (F.) (Coleoptera: Carabidae).

5.1 INTRODUCTION

The main routes of exposure of predatory invertebrates to pesticides are by direct and residual contact with spray droplets and by the consumption of pesticide contaminated prey (Croft and Brown, 1975; Croft, 1977; Jepson, 1989; Everts *et al.* 1991). Of these three routes of exposure the dietary route has probably received the least attention even though several researchers have shown that predator mortality can occur via dietary intake of pesticide contaminated prey (Ahmed, 1955; Satpathy *et al.* 1968; Kabacik-Wasylik and Jaworska, 1973; Gholson *et al.* 1978; Dixon and McKinlay, 1988). This chapter concerns the dietary exposure of the carabid beetle *N. brevicollis* to cereal aphid prey contaminated with deltamethrin.

Dietary uptake may be an important short-term source of exposure to pesticides for these predators because a large number of spray contaminated prey may fall to the ground after a spray application where they may be discovered and possibly consumed. For example in a field study in potatoes Dixon and McKinlay (1992) found higher proportions of the carabid *Pterostichus madidus* (F.) containing aphid remains in plots treated with demeton-S-methyl than in untreated plots 24 h after spray application. If consumption does occur, the rate of ingestion will depend upon temperature, the level of hunger of the beetle and the prey species (Theile, 1977; Mols, 1987 & 1988) as well as the level of pesticide contamination. Everts *et al.* (1991) have shown that linyphiid spiders will consume deltamethrin contaminated prey although this route was considered to be relatively unimportant by Mullié and Everts (1991) who found that under intoxication, the dietary intake was reduced.

The carabid beetle *N. brevicollis* was chosen as the test species in this study because it is present in cereal crops in the early summer and in the autumn and is an active aphid predator at times when pyrethroids are used against cereal aphids (Sopp *et al.* 1987; Winder, 1990). It has also been shown to suffer reduced population levels following autumn pyrethroid applications (Pullen *et al.* 1992) and seems to be sensitive to suffering local population extinctions in intensive spray regimes (Burn, 1992).

The experiments were undertaken to answer the following questions concerning possible effects of the dietary exposure of *N. brevicollis* to aphid prey contaminated with deltamethrin;

1) Will beetles consume aphids contaminated with deltamethrin ?

2) Does the level of hunger have an effect on the number of contaminated aphids consumed ?

3) What are the toxic effects of dietary uptake ?

5.2 EXPERIMENTAL METHODS

5.2.1 Test invertebrates

Adult *N. brevicollis* were captured using the same techniques described in Chapter 2 (2.2.1). The aphid species used as prey in these experiments was the rose-grain aphid *M. dirhodum*.

Prior to the experiments all invertebrates were kept in a controlled environment room, maintained at 19-22°C and 55-70 % relative humidity with a 16:8 L:D photoperiod. The beetles were kept in plastic aquaria containing a layer of moist soil and were fed on ground, moist, cat biscuits ("Delicat"- Quaker Latz). The aphids were obtained from laboratory-held populations and were cultured continuously on barley seedlings in a pesticide-free environment.

Adults aphids were removed from the cultures and freeze-killed in Petri dishes 24 hours before each experiment. The freezing procedure was carefully monitored to avoid freeze-drying the aphids. It was known that predators such as carabid beetles will consume dead prey items providing they have not decayed or desiccated too much (Theile, 1977). Preliminary experiments with *N. brevicollis* confirmed this.

5.2.2 Treatment of Aphids

Formulated deltamethrin (2.5% E.C.) was used as the test chemical, with distilled water as the diluent and the control treatment. Stock solutions of deltamethrin were prepared before each experiment from which serial dilutions were made to obtain the required test concentration of deltamethrin (30 ng μl^{-1}). Topical application to the abdomen of freeze killed aphids was carried out using a 250 µl Hamilton gas-tight syringe mounted in a Burkard hand microapplicator (Burkard Manufacturing Co. Ltd.) (Arnold, 1967). The syringe was calibrated to deliver drops of 1 µl of deltamethrin (30 ng μl^{-1}) to each aphid. This concentration was chosen as it approximates to the

recommended field concentration of deltamethrin (31.25 ng μl^{-1}, i.e. 6.25 g AI ha^{-1} in 200 l water) for use as a summer cereal aphicide. The 1 µl droplet size was chosen to ensure a complete covering of spray over the aphid. Control aphids were dosed with a 1 µl droplet of water. Care was taken to avoid droplet run-off from the aphid and the drops were allowed to dry before the aphids were moved.

Fifteen aphids were placed in 9 cm diameter plastic tubs with a single beetle. A total of thirty beetles were used in each experiment, half were provided with deltamethrin treated aphids and half were given water-treated control aphids. The beetles were then kept under constant conditions of 19-22°C and 55-70% humidity for the duration of the observations.

The experimental procedure was repeated four times with beetles that had been provided with food 24, 48, 72 and 120 hours prior to the experiment. The numbers of contaminated or control aphids eaten by each beetle over a 24 hour period was recorded and observations of poisoning responses were made. The beetles were then removed and placed in clean, ventilated, tubs with fifteen untreated aphids. The response of the beetles, i.e. moving as normal, knocked down (with antennae, mandibles or legs moving but unable to right themselves permanently) or dead (showing no response to stimulation), and the numbers of aphids eaten were recorded at 24 hour intervals for the following six days. All beetles were transferred into clean, ventilated, tubs with fresh batches of fifteen, untreated, freeze-killed aphids each day.

5.2.3 Statistical analyses

Log (x+1) transformed aphid consumption data were analysed for differences between control and exposed beetle consumption rates and between the starvation treatment for each day of the experiment by two-way ANOVA using Tukeys HSD test (Tukey, 1949) to determine the significance of differences between individual means. Probit analysis was performed on 72 h mortality data to obtain dose-response statistics (Finney, 1971). The 72 h mortality data were chosen for analysis because no individuals remained knocked down after this time and therefore it was concluded that the response was at end-point. The slope and position of the dietary probit line was compared with a 72 h topical probit line for deltamethrin and *N. brevicollis* (obtained from Chapter 2) by maximum likelihood procedures (Ross, 1987).

5.3 RESULTS

The mean numbers of contaminated aphids eaten during the 24 h exposure period and the estimated mean dietary uptake of deltamethrin per beetle, calculated by multiplying the mean number of aphids consumed by the dose of deltamethrin per aphid (i.e. 30 ng AI aphid^{-1}), for each hunger treatment are given in Table 5.1. The mean numbers of contaminated aphids consumed per beetle varied from 2.5 for the beetles that had

Table 5.1 Mean consumption of aphids treated with deltamethrin by *N. brevicollis* for the four hunger treatments. (Control beetles were provided with untreated aphids)

Hunger treatment (Time of last feeding prior to exposure)	Mean number of aphids eaten per beetle (± 95% C.L.)	Estimated mean dietary uptake of deltamethrin per beetle (ng AI Arthropod^{-1}) (± 95% C.L.)	% of beetles regurgitating
24 h	2.5 (± 0.9)	75 (± 27)	60
Control	6.5 (± 1.0)	-	0
48 h	2.8 (± 0.8)	84 (± 24)	67
Control	6.7 (± 1.0)	-	0
72 h	3.8 (± 0.9)	114 (± 27)	53
Control	8.7 (± 1.1)	-	0
120 h	4.5 (± 1.0)	135 (± 30)	80
Control	10.2 (± 1.3)	-	0

been fed 24 hours prior to exposure, to 4.5 for the beetles that had been fed 120 hours prior to the experiment. Direct observation of the beetles during the first two hours after exposure indicated that between 53 and 80% of beetles that had consumed contaminated prey showed a regurgitation response (Table 5.1). The mean consumption rate of uncontaminated aphids per control beetle varied from 6.5, for the beetles that had been provided with food 24 hours before exposure, to 10.2 for the beetles that had been provided with food 120 hours prior to exposure. The numbers of contaminated aphids consumed by the beetles were significantly less ($P<0.001$) than the uncontaminated aphids consumed by the control beetles in all four starvation treatments (Table 5.2). Significant differences in aphid consumption ($P<0.001$) were also evident due to beetle hunger level (Table 5.2). Tukey's HSD test indicated that significantly less aphids were consumed by beetles that had been provided with food 24 and 48 hours before the experiment than those that had been provided with food 72 and 120 hours before the experiment.

Table 5.2 Results from two-way ANOVA of the consumption of aphids by *N. brevicollis* in all treatments on each day of the experiment.

Day 0 = Day of exposure to treated aphids; Days 1-6 = Days after initial exposure, with untreated aphids provided; Signif. = Significance level; *** = P<0.001, ** = P<0.01, ns = not significant (P>0.05)

Day number	Differences in aphid consumption between exposed and control beetles	Differences in aphid consumption between beetles in different starvation treatments	Interactions
	F-ratio (d.f.)Signif.	F-ratio (d.f.)Signif.	
0	135.2 (1,112)***	10.8 (3,112)***	0.2 (3,112)ns
1	149.7 (1,94)***	0.9 (3, 94)ns	4.7 (3, 94)**
2	62.6 (1,101)***	0.5 (3,101)ns	1.0 (3,101)ns
3	1.6 (1,101)ns	0.5 (3,101)ns	0.9 (3,101)ns
4	0.1 (1,101)ns	0.7 (3,101)ns	0.5 (3,101)ns
5	3.0 (1,101)ns	0.1 (3,101)ns	1.0 (3,101)ns
6	0.1 (1,101)ns	0.5 (3,101)ns	0.3 (3,101)ns

The observed percentage mortalities of *N. brevicollis* resulting from consumption of contaminated prey in the four hunger treatments are given in Figure 5.1. The mortality

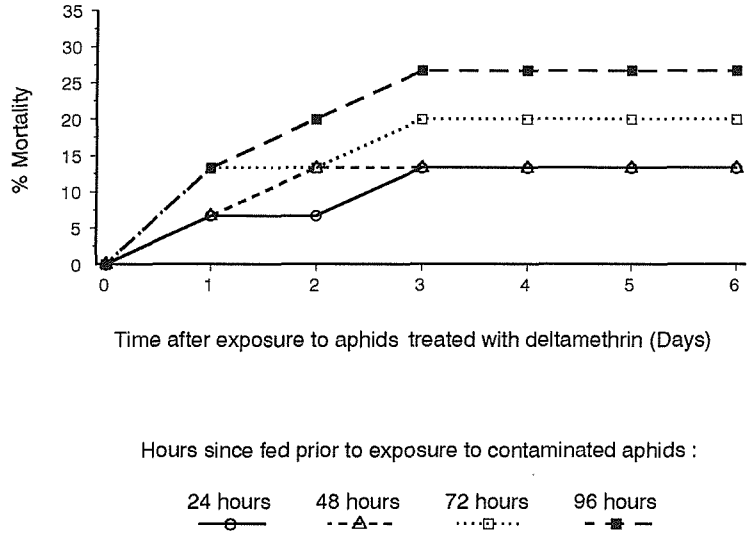


Figure 5.1 Percentage mortality of *N. brevicollis* in the four hunger treatments on the six days after consuming aphids treated with deltamethrin.

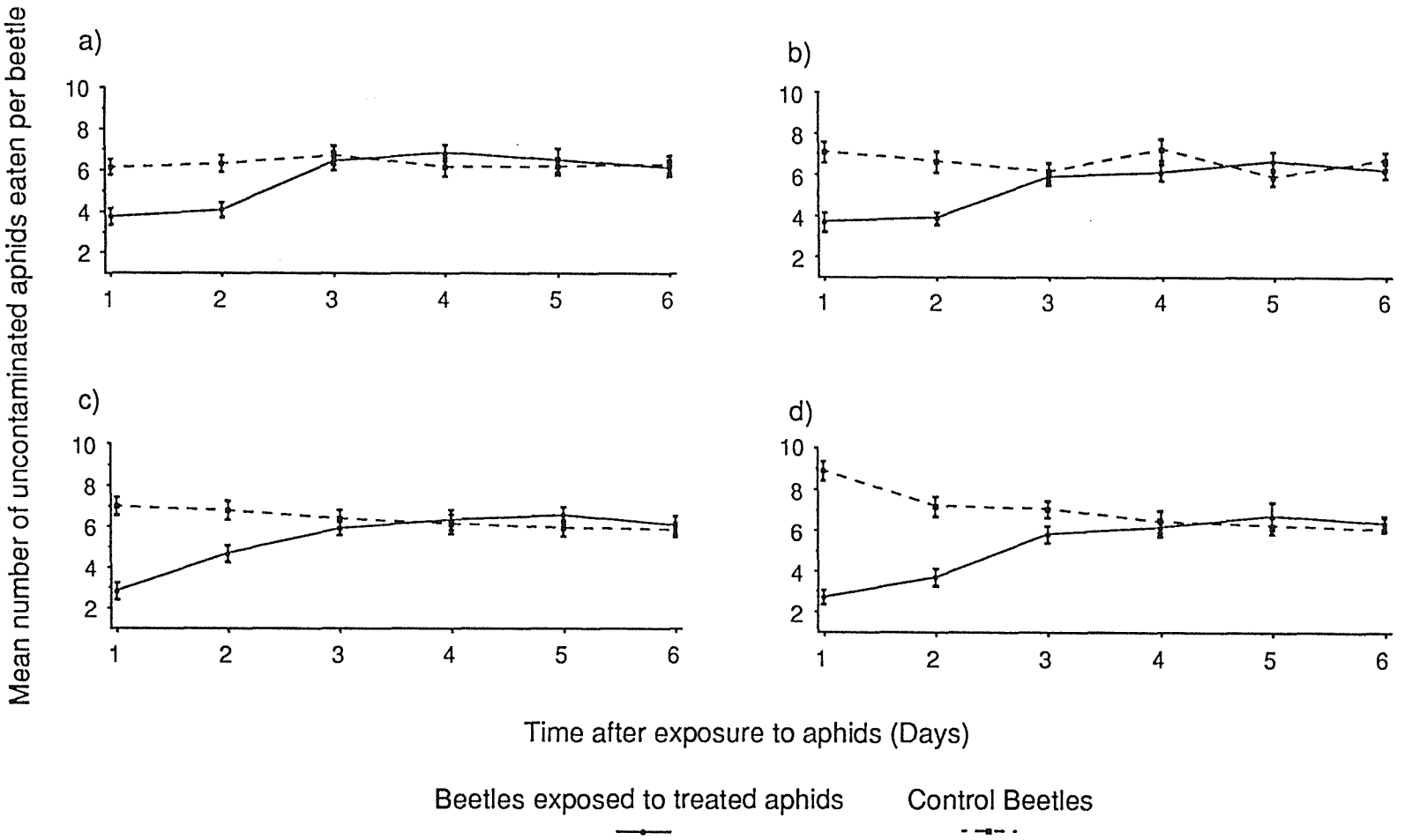


Figure 5.2 The mean numbers of untreated aphids consumed by *N. brevicollis* beetles on the six days following exposure to treated or untreated aphids. Batches of beetles had been provided with food a) 24 hours, b) 48 hours, c) 72 hours and d) 120 hours before exposure. Error bars indicate standard errors.

varied between 13% for those beetles that had been fed 24 or 48 hours before exposure, to 27% for beetles that had been starved for 120 hours prior to exposure. No mortality occurred in the control beetles.

The mean numbers of untreated aphids eaten per day on the six days after initial exposure to contaminated or uncontaminated aphids are given for the four experiments in Figure 5.2 (a to d). The beetles that survived after consuming contaminated prey ate significantly fewer untreated aphids ($P<0.001$) than the control beetles on the two days after exposure to contaminated aphids (Table 5.2). By the third day after exposure there were no significant differences ($P>0.05$) between the consumption rates of the beetles that had consumed contaminated aphids and the control beetles (Table 5.2). A significant interaction ($P<0.01$) was evident between beetles in the different starvation treatments and aphid consumption by exposed and control beetles on Day 1 of the experiment (i.e. the first day after initial exposure to contaminated or uncontaminated prey) (Table 5.2). Aphid consumption by the control beetles increased with starvation level whereas aphid consumption by the beetles that had been exposed to deltamethrin contaminated aphids declined with starvation level. This difference may have been due to compensation feeding by the control beetles that had been starved the longest and a reduction in aphid consumption by the beetles that had received the highest dietary dose of deltamethrin.

The dietary dose received by individual beetles in all of the starvation treatments was calculated by multiplying the number of aphids consumed per beetle by the dose of deltamethrin per aphid (i.e. 30 ng AI aphid^{-1}). Data for beetles consuming the same numbers of aphids were then grouped to obtain an estimate of mortality for dose-response analysis. No mortality occurred for beetles that had consumed three treated aphids or less, although some of these beetles exhibited knockdown symptoms. Mortality occurred in one of the nine beetles that had consumed four treated aphids, two of the seven beetles that had consumed five treated aphids and three of the four beetles that had consumed six aphids. All four of the beetles that had consumed more than six aphids died. Even though numbers were low it was possible to establish a 72 h dose-response relationship for *N. brevicollis* and deltamethrin by probit analysis. The 72 h dietary and topical dose-response data were compared to investigate the relative toxicity of deltamethrin to *N. brevicollis* by the two routes of exposure. The probit statistics are given in Figure 5.3. Maximum likelihood procedures were used to compare the position and parallelism of the two probit lines and indicated that the

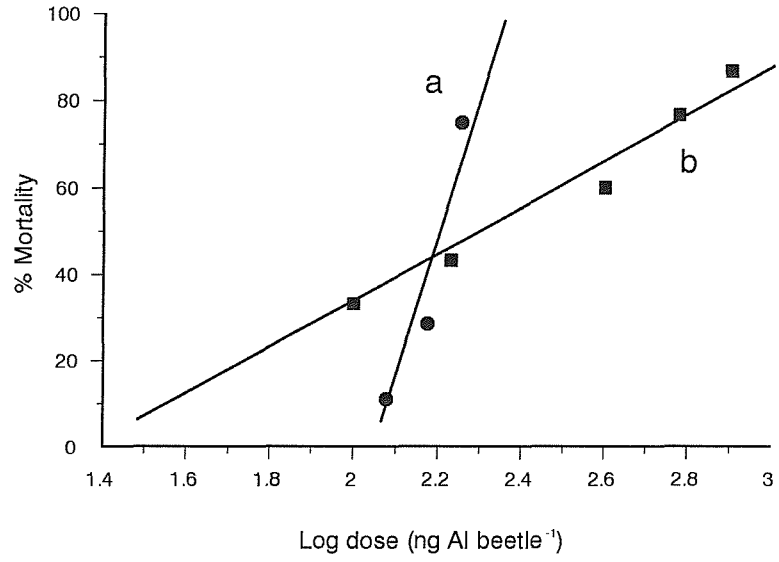


Figure 5.3 72 h dietary and topical probit lines for *N. brevicollis* and deltamethrin.
Line a = dietary exposure probit line, y = 10.4x - 18.0, χ^2 = 0.4ns, d.f.1, P>0.05; Line b = topical exposure probit line, y = 1.7x + 0.99, χ^2 = 1.2ns, d.f.3, P>0.05.

dietary exposure probit line had a significantly steeper slope than the topical exposure probit line (χ^2= 4.34*, d.f.1, P<0.05) but that the lines did not differ significantly in position along the dose axis (χ^2= 0.39ns, d.f.1, P>0.05). The dietary and topical 72 h LD_{50} values (with 95% fiducial limits) were 161.8 (128.3 to 203.9) and 218.8 (156.4 to 306.2) ng AI beetle^{-1} respectively.

5.4 DISCUSSION

The results provided little evidence to suggest that deltamethrin contaminated prey had a repellent effect on *N. brevicollis* because only two beetles of the sixty exposed in the four hunger treatments failed to consume at least one contaminated aphid. The number of contaminated aphids consumed and the percentage mortality that occurred increased with beetle hunger. A high proportion of the beetles that consumed contaminated aphids demonstrated a regurgitation response within a short time. This response to deltamethrin poisoning via dietary uptake may partly explain the steeper slope of the dietary exposure probit line compared with the topical exposure probit line. The regurgitation response may have caused a significant loss of fluid leading to dehydration and thus increased mortality. The possible role of water loss in poisoning

by pyrethroids has been reported for lepidopteran larvae by Greenwood *et al.* (1990) and Broderick *et al.* (1991) in relation to weight loss and changes in haemolymph volumes. Both authors found complex dose-dependent relationships between the rate of water loss and the rate at which larvae died.

Some sub-lethal effects resulted from the consumption of contaminated prey. Beetles that survived after consuming contaminated aphids ate significantly fewer untreated aphids in the two days following pesticide uptake than the control beetles. This will result in a short-term reduction in predatory efficiency of beetles that consume contaminated prey. It may also however reduce the subsequent uptake of contaminated prey as decreased feeding may reduce the chance of the beetle receiving further dietary doses. This has implications for modelling pesticide uptake and effects in the period following spray application. It also has implications for the predatory potential of beetles in reduced-dose pesticide application regimes, where low doses are intended to preserve and maintain pest limitation by natural enemies (Poehling, 1989).

These experiments have shown that the carabid beetle *N. brevicollis* will consume deltamethrin contaminated aphids, resulting in both lethal and sub-lethal effects. However several questions still need to be addressed in order to be able to evaluate the importance of the dietary route of exposure to pyrethroid insecticides in the field. The period that dead aphids remain suitable for predator consumption when on the soil surface must be determined because desiccation may render them unsuitable as prey items within hours of death. In addition it is not known whether predatory beetles show any preference between uncontaminated and contaminated prey if both are available. Finally it is unclear whether sub-lethal doses received by other routes of exposure, such as by direct contact or residual routes, will reduce feeding rates and hence the level of dietary exposure.

CHAPTER 6

The risk posed by direct contact with deltamethrin to aphid predators in cereal crops.

6.1 INTRODUCTION

The most immediate route of exposure of beneficial invertebrates to pesticides is by direct contact with pesticide droplets during a spray application. At this time the toxic risk posed by the formulated pesticide will be at its' greatest, for the given deposition rate, because the active ingredient will be unaffected by environmental factors which mediate adsorption or breakdown processes (Jepson, 1989; Cilgi and Jepson, 1992). Therefore the probability of any given organism being harmed via this route of exposure will be a function of the organisms' susceptibility to the active ingredient and the volume and concentration of the spray formulation to which it is exposed (Jepson, 1989; Jepson, 1993b).

The chance of direct exposure to spray, for a given invertebrate species, will be a function of biological factors, such as the proportion of the population in the sprayed area and the degree of protection offered by the crop canopy or soil refuges and operational factors, such as the volume, mass application rates, droplet size spectrum and the vertical distribution of the pesticide spray (Jepson, 1989). Therefore, within cereal crops, it may be reasonable to assume that nocturnal and/or ground-active organisms may be at lower risk from direct exposure to spray than diurnal and/or plant-active species. Amongst the diurnal plant-active species, predators which spend a high proportion of their time in the crop canopy, for example coccinellid larvae and adults, are likely to be at greater risk for direct exposure to pesticides that the more dispersive species, such as adult syrphids and parasitoids, which may avoid direct exposure altogether because crop disturbance by the sprayer before the spray pass may enable them to fly away.

This chapter describes a series of experiments to quantify spray deposition on predators which occupy plant and ground levels in the cereal crop canopy and to estimate the levels of mortality that may result from deltamethrin sprayed at a rate of 6.25 g AI ha^{-1} in 200 l water. The range of species chosen for the experiments represented a wide size and susceptibility range of predators that inhabit temperate cereal crops (Table 6.1).

Table 6.1 Predator species tested in the experiments

	Position of exposure	
	Crop canopy and ground level	Ground level only
Test species	*C. septempunctata* (A) *C. septempunctata* (L) *D. atricapillus*	*P. melanarius* *H. rufipes* *B. lampros*

C. septempunctata (A) - adults, *C. septempunctata* (L) - 4th instar larvae.

It is difficult to collect live predators immediately after a spray application and determine the affects of direct exposure on them while ensuring they may have not been exposed by other routes, i.e. to residues. Therefore the mean volumetric spray deposition on the predators tested in this study was determined by placing dead individuals in positions which approximated to their realistic positions in the crop canopy and measuring the volume of spray on the predators using a fluorescent tracer technique developed by Jepson *et al.* (1987) and Cilgi (1988). Spray deposition data were then combined with susceptibility data (Chapter 2 (2.3.2)) to predict the possible levels of mortality that would result from the estimated mean deltamethrin dose received. In addition, studies of the natural distribution of a population of adult *C. septempunctata* in a mature cereal crop infested with cereal aphids were carried out in order to apply mortality predictions of beetles occupying given crop strata to a realistic predator population distribution in a cereal crop. From this the overall levels of mortality that may result in a coccinellid population in a cereal crop by direct exposure to deltamethrin were estimated.

6.2 EXPERIMENTAL METHODS

6.2.1 Test invertebrates

The predator species used in these experiments (Table 6.1) were captured from cereal fields and field margins on the Leckford Estate, near Stockbridge, Hampshire, using the methods described in Chapter 2 (2.2.1). They were maintained in an insectary prior to the experiments and then freeze-killed 24 to 48 hours before use. Handling was minimal during this period to prevent damage or removal of cuticular wax layers which may affect spray retention.

6.2.2 Ground-active predators

Experiments to quantify the volumetric spray deposition on all six predator species at ground level under a cereal crop canopy were carried out in June 1990 in a 20 m^2 plot of a winter wheat crop, cv. Galahad. The crop was at decimal growth stages 71 to 73 (Zadoks *et al.* 1974) and had a density of 410 to 430 tillers per square metre. Predators were placed on the ground beneath marked wheat plants selected at random in the plot. The numbers of predators of each species placed in the plot were; *P. melanarius* (n=42), *H. rufipes* (n=28), *B. lampros* (n=32), *D. atricapillus* (n=22), *C. septempunctata* adults (n=30) and *C. septempunctata* 4th instar larvae (n=16). The plot was then sprayed with fluorescent tracer (see 6.2.4).

6.2.3 Plant-active predators

Spray deposition experiments in the crop canopy, with the three diurnal plant-active predators, *C. septempunctata* (adults), *C. septempunctata* (4th instar larvae) and *D. atricapillus*, were carried out in July 1991 in a plot of the same size and same wheat variety as above. This crop was at decimal growth stages 73 to 75 and had a density of 380 to 400 tillers per square metre. Twenty *C. septempunctata* (adults), five *C. septempunctata* (4th instar larvae) and ten *D. atricapillus* were exposed per crop stratum in the sprayed plot and the unsprayed control plot. Six crop strata were chosen as attachment sites for the plant-active predators. These included vertical attachment midway up the ear, horizontal attachment in the centre of the adaxial surface of the flag and first leaves, and horizontal attachment in the centre of the abaxial surfaces of flag and first leaves. Only *C. septempunctata* (adults) were attached to the abaxial leaf surface of the first leaf due to lack of availability of the other predators.

The predators were attached to plant surfaces prior to spraying via small squares of double-sided adhesive tape, approximately 0.25 cm^2 in size. The squares of tape were placed on the plant at the appropriate stratum and then individual predators were carefully placed on the squares of tape using forceps. The bond was sufficiently strong to hold the predators in place during spraying but weak enough to ensure that the predators could easily be removed with forceps without damage after spray application.

6.2.4 Quantification of volumetric spray deposition on aphid predators in a cereal crop canopy using a fluorescent tracer

The deposition of the spray on the predators in or under the cereal crop canopy was measured using a fluorescent tracer "Fluorescein" (Acid yellow 73, Aldrich) as a 0.05%

(w/v) solution with 0.1% wetting agent using a procedure similar to those described by Cilgi (1988) and Cilgi and Jepson (1992). An Oxford Precision sprayer fitted with a dry boom with four Lurmark 02-F80 nozzles (50cm spacing) and operating at 2 bar pressure was used to apply a water and tracer spray mixture at a rate of 200 l ha^{-1} in both experiments. The wheat crops in both studies were at growth stages where commercial aphicide applications would be recommended in an aphid infested crop.

After application spray deposits were allowed to dry for approximately 30 minutes. The predators in both experiments were then carefully removed from their position in the crop using forceps and were immediately placed individually in vials containing 10 ml of phosphate buffer solution (pH 6.8, 0.1M anhydrous di-sodium hydrogen orthophosphate with sodium dihydrogenorthophosphate dihydrate). To avoid contamination the forceps were cleaned between handling each predator. The vials were returned to the laboratory and stored in cold, dark conditions until analysis.

A standard calibration curve was obtained from measured volumes of the original spray formulation added to 10 ml of buffer via a microapplicator. A 3 ml aliquot was taken from each vial and the volume of tracer was determined by analysis in a Perkin-Elmer LS-3B flourescence spectrometer operating at 490 nm excitation and 515 nm emission. These readings were corrected for control readings, taken from buffer solutions containing unexposed beetles, and the volume of spray formulation, in microlitres, was calculated in terms of μl per beetle for each crop position and predator species.

6.2.5 Predicting the level of mortality from the mean volumetric deposition data.
The estimated dose of deltamethrin that the predators would have received from direct exposure at each given position in the crop canopy were calculated using the following expression (adapted from Jepson 1993b),

$$Dt = Vf \times C$$

where Dt is the dose received (e.g. ng AI $arthropod^{-1}$), Vf is the mean volume of formulation impinging upon arthropods at a given position in the crop (μl $arthropod^{-1}$) and C is the concentration of active ingredient (AI) in the field formulation of deltamethrin (i.e. 31.25 ng AI μl^{-1}). The dose (Dt) was then substituted into the 72 h probit equations obtained in Chapter 2 (2.3.2) in order to calculate the expected levels of mortality that would result for each predator species for its' given position in the

cereal crop.

6.2.6 The distribution of adult *C. septempunctata* in an aphid infested cereal crop

A cubic cage with 2 metre sides was erected over a plot of winter wheat cv. Galahad at decimal growth stage 69 to 71 (Zadoks *et al.* 1974). The cage consisted of steel scaffolding poles covered by a purpose-made cover of "Tygan" netting (1mm mesh size). The crop within the cage was artificially infested with the cereal aphids *S. avenae* and *M. dirhodum* several weeks prior to the experiment and during the experiment the level of aphid infestation was recorded on twenty marked ears and flag leaves in the cage. Initially 100 adult ladybirds were released at ground level in the cage and were left undisturbed for two days to establish themselves in the crop. This number of ladybirds was chosen for practical reasons because it was a manageable population size for monitoring in a 4 m^2 area of crop. Assessments of the ladybird distribution in the crop canopy were made on five separate days (27th, 28th, 30th June and 1st and 2nd July 1991). The positions of ladybirds at nine crop strata were recorded by entering the cage at two hourly intervals throughout each day between 09-00 and 19-00 British Summer Time (BST) to determine crop distribution patterns. The nine crop strata positions were the; ear, stem (from ground to ear level), adaxial flag leaf, abaxial flag leaf, adaxial first leaf, abaxial first leaf, adaxial second leaf, abaxial second leaf and ground level.

6.3 RESULTS

6.3.1 Volumetric spray deposition and estimated levels of mortality of predators on the ground under a cereal crop

The mean volumetric spray deposition rates on predators on the ground under the cereal crop canopy in the 1990 study varied between 0.03 μl $beetle^{-1}$, for the small carabid beetle *B. lampros*, to 0.83 μl $beetle^{-1}$, for the relatively large carabid beetle *P. melanarius* (Table 6.2). The estimated mean deltamethrin doses received by the six predators tested varied between 0.9 and 25.9 ng AI $beetle^{-1}$. The 4th instar coccinellid larvae and the large carabid beetle *P. melanarius* were predicted to suffer the highest mortality, 17% and 12% respectively, from the mean doses they received. The predicted mortalities of the other predator species were low however, varying between 0% and 4% (Table 6.2).

Table 6.2 Mean volumetric spray deposition on predators on the ground in a mature cereal crop and the predicted mortality that may result from a deltamethrin spray at 6.25 g AI ha^{-1}.

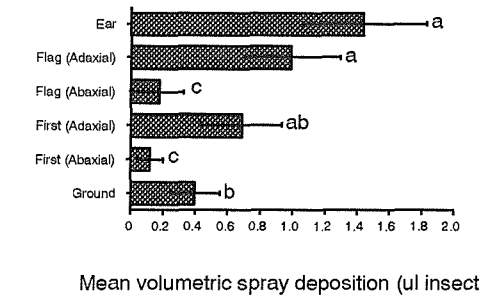
Test species	Mean spray deposition per beetle (& 95% C.L.) (µl $beetle^{-1}$)	Estimated deltamethrin dose received per beetle (& 95% C.L.) (ng AI $beetle^{-1}$)	Predicted % mortality from mean dose received (& 95% C.L.)
P. melanarius	0.83 (0.43-1.23)	25.9 (13.4-38.4)	12 (5-19)
H. rufipes	0.63 (0.27-0.99)	19.7 (8.4-30.9)	2 (0-5)
B. lampros	0.03 (0.021-0.039)	0.9 (0.7- 1.2)	0
D. atricapillus	0.22 (0.07-0.43)	6.9 (2.2-13.4)	0 (0-1)
C. septempunctata (A)	0.34 (0.06-0.62)	10.6 (1.9-19.4)	4 (0-10)
C. septempunctata (L)	0.39 (0.03-0.75)	12.2 (0.9-23.4)	17 (0-38)

C. septempunctata (A) = Adults; *C. septempunctata* (L) = 4th instar larvae

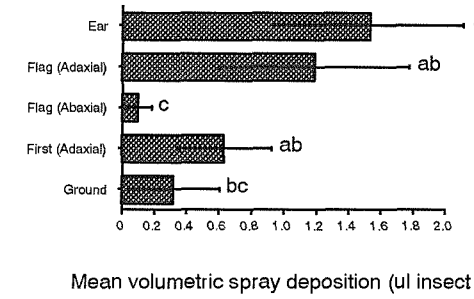
6.3.2 Volumetric spray deposition on predators in the cereal crop canopy

Similar patterns of volumetric spray deposition were evident for the three predators exposed to spray in the cereal crop canopy in the 1991 study (Figure 6.1). One-way ANOVA on log (x+1) transformed spray deposition data showed significant differences in volumetric spray depositions between positions in the crop canopy for *C. septempunctata* (Adults) ($F = 33.6^{***}$, d.f.= 5,114, $P<0.001$), *C. septempunctata* (4th instar larvae) ($F = 12.8^{***}$, d.f.= 4,20, $P<0.001$) and *D. atricapillus* ($F = 12.7^{***}$, d.f.= 4,45, $P<0.001$). Significantly higher spray depositions occurred on all three predators on the ear compared to those on the abaxial leaf surfaces or on the ground. There were however no significant differences between deposition rates on predators on the ear and adaxial surfaces on the flag and first leaves although a trend of declining deposition rate was evident through the crop canopy. The lowest spray deposition rates occurred on predators attached to the abaxial leaf surfaces.

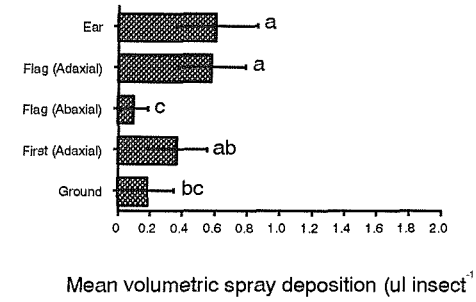
Both *C. septempuncatata* adults and 4th instar larvae received higher mean spray deposition rates in the cereal crop canopy than the carabid beetle *D. atricapillus* and therefore received higher doses of deltamethrin (Figure 6.1). The highest mean levels of mortality were predicted for *C. septempunctata* 4th instar larvae, which varied from 66% on the ear to 1% on the abaxial surface of the flag leaf (Figure 6.1). The predicted mean mortality levels for *C. septempunctata* adults ranged from 26% on the ear to 0% on the abaxial surface of the first leaf, whereas the highest level of mortality predicted for *D. atricapillus* was 2% on the adaxial surface of flag leaves or on the ear.



Estimated mean deltamethrin dose received (ng AI insect^{-1}) & 95% C.L. at recommended field rate (i.e. 6.25 g AI ha^{-1})	Predicted % mortality & 95% C.L. from the dose received
45.0 (33.1 - 56.8)	26 (19 - 33)
30.9 (21.9 - 40.0)	18 (11 - 23)
5.6 (0.9 - 10.3)	1 (0 - 3)
21.6 (13.8 - 29.4)	11 (6 - 16)
3.8 (1.3 - 6.3)	0 (0 - 1)
12.5 (7.8 - 17.2)	5 (2 - 8)



47.8 (29.4 - 66.3)	66 (47 - 77)
37.2 (18.8 - 55.6)	56 (30 - 72)
3.1 (0.6 - 5.6)	1 (0 - 4)
19.7 (10.9 -28.4)	32 (14 - 46)
10.0 (1.3 - 18.8)	12 (0 - 30)



19.1 (11.3 - 26.9)	2 (0 - 4)
18.1 (11.2 - 24.4)	2 (0 - 4)
3.1 (0.3 - 5.9)	0
11.6 (5.9 - 17.2)	1 (0 - 2)
5.9 (0.9 - 10.9)	0

Figure 6.1 Mean volumetric spray deposition on a) *C. septempunctata* adults, b) *C. septempunctata* 4th instar larvae and c) *D. atricapillus* in a mature cereal crop and the predicted mortality that may result from deltamethrin sprayed at a rate of 6.25 g AI ha^{-1} in 200 l water.

Bars indicate 95% confidence limits. Different letters (a, b, c & d) indicate significant differences in mean spray deposition rates between crop strata for each predator species according to Tukeys' HSD test (Sokal and Rohlf, 1981).

6.3.3 The distribution of adult *C. septempunctata* in an aphid infested cereal crop

The crop distributions of ladybirds in the field cage on the five days of assessment are given in Figure 6.2. During the experiment between 82% and 100% of the ladybirds were present in the crop at the times when distributions were assessed. Beetles were occasionally observed on the sides of the cage, however at the end of the experiment only three of the one hundred beetles released were missing.

Similar ladybird distribution trends were evident in the cereal plot between the five days of assessment. The highest numbers of ladybirds were observed on the ears and flag leaves, which accounted for between 50% and 84% of the ladybirds observed at any one time. On average less than 10% of the ladybird population were on the stem, the abaxial surface of the first leaf and on the second leaf during each assessment. During the period of assessment the mean numbers of aphids on the marked ears showed a net decline from 29 aphids per ear to 18 aphids per ear, whereas the numbers of aphids on the flag leaves remained relatively constant (Figure 6.3).

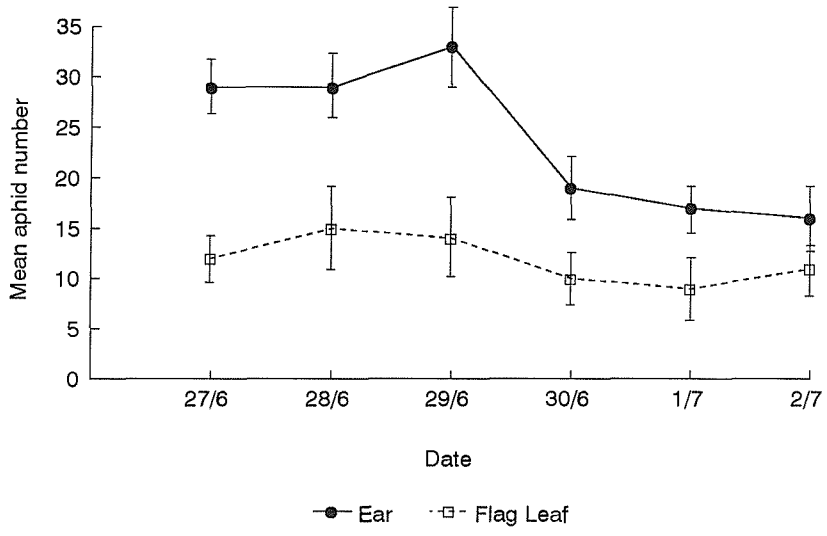


Figure 6.3 Mean aphid numbers on 20 marked ears and flag leaves in the field cage during the period of observation. Bars indicate standard errors.

Patterns of ladybird distributions in the cereal crop where also evident between different assessment times within each day, particularly at the ear and ground levels of the crop (Figure 6.2). The proportion of ladybirds on the ear tended to increase between 09-00 and 15-00 and decline after this time whereas the opposite trend

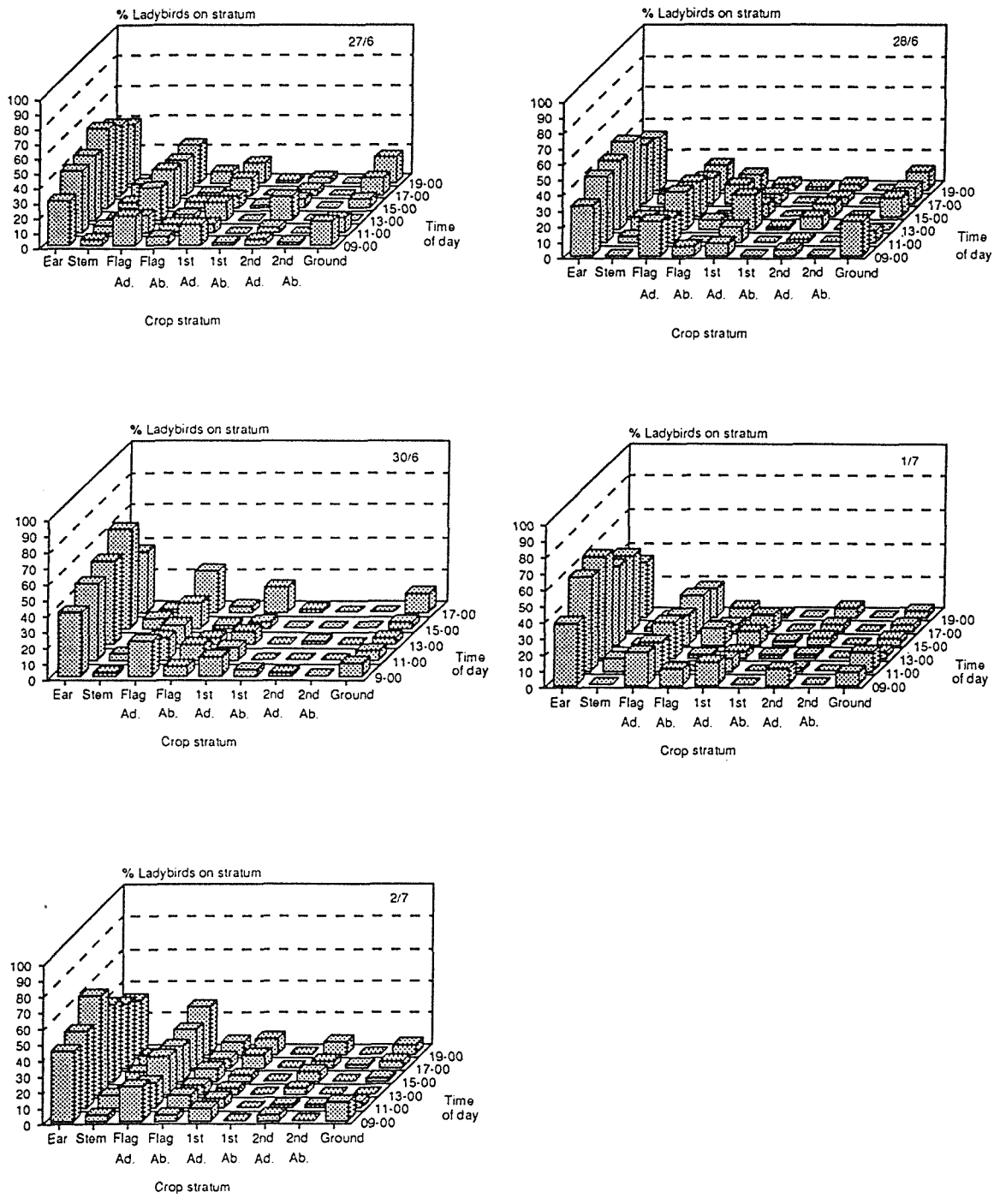


Figure 6.2 The distribution of *C. septempunctata* adults in a wheat crop infested with cereal aphids during five days of assessment in a field cage.

Table 6.3 Comparison between the numbers of *C. septempunctata* adults observed on wheat ears and those in the rest of the cereal crop for the assessment times showing the largest differences in ladybird distributions.

Date of assessments	Assessment times between which differences in ladybird numbers on the ear relative to rest of the crop canopy were compared	χ^2 (d.f.) Significance
27/6	09-00 v 15-00	11.0 (d.f.1) ***
28/6	09-00 v 15-00	4.3 (d.f.1) *
30/6	17-00 v 15-00	15.1 (d.f.1) ***
01/7	19-00 v 13-00	15.7 (d.f.1) ***
02/7	19-00 v 13-00	12.9 (d.f.1) ***

*** = P<0.001, * = P<0.05. Yate's correction was applied as d.f. = 1.

occurred for the number of ladybirds observed on the ground (Figure 6.2). Contingency chi squared analysis was carried out to compare the observed ladybird numbers on the ear (Table 6.2) and the soil (Table 6.3) for time intervals showing the greatest differences in ladybird distributions. Significantly more ladybirds were observed on the ear at 13-00 and 15-00 hours compared to the 09-00 hours (27/6 and 28/6) and 17-00 and 19-00 hours (30/6, 1/7 and 2/7) whereas significantly less ladybirds were observed on the soil at 13-00 and 15-00 hours that at 09-00 (27/6, 28/6, 1/7 and 2/7) and 17-00 hours (30/6).

Table 6.4 Comparison between the numbers of *C. septempunctata* adults observed on the ground and those in the rest of the cereal crop for the assessment times showing the largest differences in ladybird distributions.

Date of assessments	Assessment times between which differences in ladybird numbers on the ground relative to rest of the crop canopy were compared	χ^2 (d.f.) Significance
27/6	19-00 v 13-00	19.8 (d.f.1) ***
28/6	09-00 v 13-00	25.0 (d.f.1) ***
30/6	17-00 v 13-00	3.9 (d.f.1) *
01/7	09-00 v 13-00	4.4 (d.f.1) *
02/7	09-00 v 13-00	13.2 (d.f.1) ***

*** = P<0.001, * = P<0.05. Yate's correction was applied as d.f. = 1.

6.3.4 Predicted levels of mortality of populations of *C. septempunctata* in a cereal crop from direct contact with a deltamethrin spray

Mean ladybird distributions were calculated for each assessment time between 09-00 and 19-00 hours by combining data from all five days of observation. Data concerning the numbers of ladybirds on the stem and the second leaf were excluded because the volumetric spray deposition on ladybirds had not be quantified for these crop strata. The numbers of ladybirds observed at these levels accounted for between 6% and 8% of the total observations and were therefore unlikely to affect mortality predictions greatly. The distribution of a hypothetical population of 1000 ladybirds was calculated from the overall proportions of ladybirds for six crop strata (Table 6.5). Mortality predictions were then made for this population due to direct contact with deltamethrin spray at a rate of 6.25 g AI ha^{-1} in 200 l water using the volumetric deposition data

Table 6.5 Estimated distribution of a population of 1000 *C. septempunctata* adults in a cereal crop between 09-00 and 19-00 hours.

Crop strata	Time (BST)					
	09-00	11-00	13-00	15-00	17-00	19-00
Ear	410	572	584	575	472	401
Flag leaf (Adaxial)	235	182	230	230	251	254
Flag leaf (Abaxial)	71	58	48	53	63	91
First leaf (Adaxial)	124	91	111	71	116	130
First leaf (Abaxial)	13	0	9	9	4	11
Ground	147	97	18	62	94	113

from section 6.3.2. The predictions indicated than the population of ladybirds would suffer a mean reduction of between 17% and 20% from direct contact depending on the time of day at which the spray was applied (Figure 6.4). Marginally lower levels of mortality were predicted if the spray was applied at 09-00 or 19-00 as lower numbers of ladybirds were present at ear level at these times.

A similar prediction was made for a population of 1000 *C. septempunctata* 4th instar larvae (Figure 6.4). This was based on the assumption that the coccinellid larvae had the same distribution as adults in the crop. The data from the numbers of ladybirds on the stem, abaxial surface of the first leaf and the adaxial and abaxial surfaces of second leaf were excluded as the volumetric spray deposition had not been measured

for these strata (6.2.3). However the numbers of ladybirds at these levels only accounted for between 7% to 9% of the observations. The predicted mortality levels of *C. septempunctata* 4th instar larvae via direct contact with deltamethrin sprayed at recommended field rate varied between 48% and 56% again depending on the time of day of the spray application (Figure 6.4).

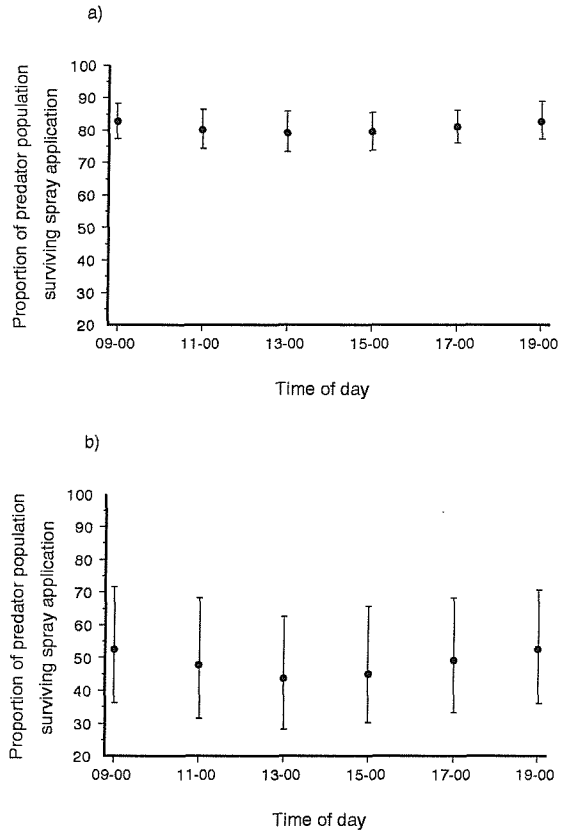


Figure 6.4 Predicted survival of a population of a) *C. septempunctata* adults and b) *C. septempunctata* 4th instar larvae from direct contact exposure to deltamethrin sprayed at a rate of 6.25 g AI ha^{-1} in a cereal crop.

6.4 DISCUSSION

6.4.1 Predicted levels of mortality for predators in a cereal crop from direct exposure to a deltamethrin spray

The volumetric spray deposition on *C. septempunctata* adults and 4th instar larvae and on *D. atricapillus* showed a pattern of spray stratification through the cereal crop canopy, with predators present at the top of the crop canopy, i.e. on the ear and adaxial flag leaf surface, receiving the highest amount of spray. This was consistent with spray deposition patterns found on *C. septempunctata* adults in the ceral crop

canopy at a range of crop growth stages by Cilgi and Jepson (1992). Predators that were present on the abaxial leaf surfaces received the lowest volumes of spray which was probably because the foliage provided sheltered from the spray. The mean spray deposition on *C. septempunctata* adults and 4th instar larvae were similar through the crop canopy, however because the larvae were more susceptible to deltamethrin than the adults (Chapter 2 (2.3.2)) much higher levels of mortality were predicted for the larvae at each crop stratum. The predicted mortality of the carabid beetle *D. atricapillus* from direct exposure to deltamethrin was lower than for the coccinellids. *D. atricapillus* received a lower volume of spray then the coccinellids probably because it has a smaller surface area than the coccinellids and because it is also much less susceptible to deltamethrin (Chapter 2 (2.3.2)).

At ground level, beneath the cereal crop canopy, spray deposition rates on predators closely followed predator body size. Relatively large predators which had the highest surface area, i.e. the large carabids *P. melanarius* and *H. rufipes*, received a higher volumetric spray deposition than relatively small predator species such as *B. lampros*. This also agreed with the findings of Cilgi and Jepson (1992) for a similar set of predators. The mean spray depositions on the three predators tested at ground level in both the 1990 and 1991 studies were also very similar and provided some evidence to validate the mortality predictions obtained for the given crop densities and growth stages.

Overall the experiments indicated that plant-active predators, such as coccinellid larvae, that are present on flag leaves and ears during a deltamethrin spray application are likely to suffer between 30 and 77% mortality whereas coccinellid adults may suffer between 11 and 33% mortality on the same crop strata. The small carabid *D. atricapillus* seemed to be at low risk from direct exposure to a deltamethrin spray because of it's small body size and relatively low susceptibility to deltamethrin. Levels of mortality of between 0 and 19% were predicted for ground-active predators such as the carabid beetles. The larger species were at greater risk from exposure than the small species due to their higher surface areas. However many of these large carabid species are nocturnal and therefore these levels of mortality are likely to be an over estimate because a proportion of the predators may be hidden in soil refuges during the day and therefore avoid direct contact with spray.

6.4.2 The distribution of adult *C. septempunctata* in an aphid infested cereal crop

The field cage study of ladybird distribution in the cereal crop canopy indicated that a

high proportion of ladybirds may be present on flag leaves and wheat ears during the day and may therefore be at a high risk from direct contact with a pesticide spray. This distribution is likely to be related to the fact that coccinellids are known to be positively phototactic (Majerus and Kearns, 1989) and are therefore often found at the apex of plants. It may also be because aphids were present on the ears and flag leaves and therefore contact with prey and honeydew may have provided an arrestant stimulus for the coccinellid predators on these strata (eg. Carter and Dixon, 1984). The fact that aphid numbers declined on the ear during the assessment period may indicate that some degree of aphid control had occured, however the reduction in aphid numbers on the ear may also have been due to a population crash as the aphid densities peaked on the 29th June and then fell suddenly. Changes in ladybird distribution patterns were evident in the crop within days, for example the higher numbers of ladybirds observed on wheat ears in the middle of the day compared to the beginning and end of the day. This may have been related to thermoregulatory behaviour of the beetles mediated by environmental factors, such as changes of microclimate or light intensity (eg. Honek, 1983).

6.4.3 Predicted levels of mortality of populations of *C. septempunctata* in a cereal crop from direct contact with a deltamethrin spray

The results indicated that populations of *C. septempunctata* adults, distributed in the crop in a similar way to the population observed in the field cage, would suffer mortality levels of between 17 and 20% by direct contact with deltamethrin sprayed at the current recommended field rate in U.K. cereals. The predicted levels of mortality for populations of *C. septempunctata* 4th instar larvae, with the same distribution in the crop canopy, were much greater than for the adults, varying between 48 and 56%, because of the higher susceptibility of the larvae to deltamethrin (Chapter 2 (2.3.2)). The variations of predicted mortalities at different times of the day may have implications for minimising the impact of sprays on predator populations. Mortality trends indicated that lower levels of direct contact mortality would result if sprays were applied in the morning or evening when a higher proportion of predators were on the ground and therefore offered greater protection from the spray.

The predicted levels of mortality of *C. septempunctata* adults may be acceptable from a pest management point of view as approximately 80% of the predator population would survive the initial spray application. However the affects of other routes of exposure, such as contact with residues, must be also be evaluated to determine the

overall impact of the spray on predator populations. The effects of exposure of *C. septempunctata* to deltamethrin residues in the field are explored further in Chapter 7.

CHAPTER 7

The toxicity of deltamethrin sprays to aphids and predators in cereal crops.

7.1 INTRODUCTION

Predators are often exposed to pesticide deposits while foraging in the crop or on the ground after a spray application (Croft, 1990a). The factors which mediate the risk posed by these residues to invertebrates, such as intrinsic species characteristics and interactions of the pesticide with given substrates have been discussed in detail in Chapters 3 and 4. The experiments described in this chapter aimed to determine the levels of mortality that may result from the exposure of plant-active predators to realistic concentrations of deltamethrin applied at the recommended field rate (i.e. 6.25 g AI ha^{-1} in 200 l water) in a cereal crop. Plant-active predators were chosen for this study to compare the relative risks posed by deltamethrin residues to predators on cereal plant foliage and on the soil in the field.

The toxicity of deltamethrin residues to the predators was assessed using *in situ* bioassays to continuously confine batches of predators on deltamethrin treated flag leaves or soil over a 10 day period after a spray application. The plant-active predators tested in the bioassays included the coccinellid beetle *C. septempunctata*, the staphylinid beetle *T. hypnorum* and the carabid beetle *D. atricapillus*. Only adult life stages were tested. These species were chosen because they represented a wide susceptibility range to deltamethrin (Chapter 2 and Chapter 4), they differed in their intrinsic susceptibilities to pesticide residues (Chapter 3) and because they had all been shown to suffer reductions in numbers after deltamethrin applications in field trials (eg. Vickerman *et al.* 1987a & b). The flag leaf and soil surfaces were chosen as the test crop strata because they were likely to cover the extremes of toxic risk posed by deltamethrin residues to predators in a cereal crop. For example predators may be at relatively low risk when exposed to residues on the soil compared to flag leaves because the bioavailability of deltamethrin on this substrate is much lower than on plant foliage (Chapter 4) whereas deltamethrin residues on flag leaves may pose a relatively high risk to predators because spray deposition rates are known to be greatest towards the top of crop canopy (Cilgi, 1988; Cilgi and Jepson, 1992; Chapter 6).

The second aim of the study was to investigate dose-response relationships of

predators and the aphid pest in the field in order to determine dose rates which may minimise predator mortality while still providing adequate levels of aphid control. Only *C. septempunctata* were used in reduced-dose tests due to the lack of abundance of the other predators. The reduced-dose rates of deltamethrin tested in these bioassays were half recommended field rate and a quarter recommended field rate. These application rates were selected on the basis of mortality levels observed in preliminary *in situ* bioassays with *C. septempunctata* exposed to field rate and half field rate applications of deltamethrin. The ladybirds were exposed to flag leaf and soil residues, as in the field rate bioassays, to determine mortality levels that may result for continuous exposure to residues on these crop strata. In addition, the mortality levels from continuous residual exposure of *C. septempunctata* to deltamethrin residues, at the given application rates, were combined with predictions of direct contact mortality (Chapter 6), for the same application rates, to predict overall levels of mortality for *C. septempunctata* populations in a cereal crop via these two routes of exposure during the 10 days after a deltamethrin spray application.

7.2 EXPERIMENTAL METHODS

7.2.1 Test invertebrates

Adult *C. septempunctata*, *T. hypnorum* and *D. atricapillus* were collected in May and June 1991 in cereal fields and field margins at Damerham, near Fordingbridge, Hampshire by a hand-held air aspirator and surface searching (Southwood, 1987). After capture all predators were returned to an insectary and were maintained and fed as described in Chapter 2 (2.2.1) prior to the experiments.

7.2.2 Experimental plots

The experiments were carried out in July 1991 in a winter wheat crop cv. Apollo. The crop had a mean density of 412 tillers per square metre and was at decimal growth stages 70 to 71 (Zadoks *et al.* 1974) at the time of spray application. The experimental plots each measured 5m x 2m and were arranged in a randomized block design with four plots per treatment and four treatments. The treatments were recommended field rate of deltamethrin (6.25 g Al ha^{-1} in 200 l water), half recommended field rate of deltamethrin (3.13 g Al ha^{-1} in 200 l water), quarter recommended field rate of deltamethrin (1.56 g Al ha^{-1} in 200 l water) and unsprayed controls. The area of crop selected for the plots contained natural infestations of the aphid *S. avenae*. Aphid numbers were assessed on ten marked ears in all plots for each treatment prior to

spray application and on the 12 days after spray application to compare the aphicidal efficacy of the three deltamethrin dose rates tested.

The plots were sprayed using an Oxford precision hand-held sprayer fitted with a dry boom with four Lurmark 02-F80 nozzles (50 cm spacing) and operated at 2 bar pressure. The sprayer was calibrated to deliver spray at a volume rate of 200 l ha^{-1} and was carefully cleaned and flushed with water before use. The doses of deltamethrin were applied to the plots in ascending order and the sprayer was flushed with water between application of different dose rates. After spraying the spray deposits were allowed to dry for 30 minutes before the test invertebrates were exposed.

Mean spray deposition rates were measured in all of the experimental plots using a fluorescent tracer "Fluorescein" to verify that similar volumes of spray had been applied to each plot, to ensure that no spray drifted into the control plots and to quantify the levels of residues that the beetles had been exposed to. The tracer was applied as a 0.05% solution in the spray mixture using the same procedure as described in Chapter 6 (6.2.4). Spray deposition rates were measured on the flag leaves by collecting 10 flag leaves at random from each plot. Spray deposition at ground level was measured by placing 5.25cm diameter discs of filter paper at ground level before spray application and collecting them afterwards. All of the samples were placed individually in vials containing 10ml of buffer solution (see 6.2.4) and were returned to the laboratory where the tracer deposition rates were quantified using the procedure described in Chapter 6 (6.2.4). The deposition data were corrected in terms of $\mu l/cm^2$ for the flag leaves, by estimating the area of photocopied paper silhouettes of the leaves, and the soil, using the area of the filter paper.

7.2.3 Exposure studies

Three types of exposure studies were carried out during the experiments. These included;

1) Continuous exposure of predators on flag leaves until the endpoint of response was reached. Assessments were terminated if 100% mortality occurred. The purpose of these studies were to determine the toxic risk posed by deltamethrin residues on flag leaves to predators remaining in the crop canopy after a spray application.

2) Exposure of predators on flag leaves for 24 h after spray application before they were transferred into soil exposure chambers under the crop canopy where they were continuously exposed for the duration of the experiment. These studies will be referred

to later in the text as "soil transferal bioassays". They aimed to determine the toxic risk posed by residues to individuals that had moved from the plant to the soil after a spray application either to avoid further contact with the spray or because of a lack of food. 3) Exposure of predators to weathered residues in the crop two and four days after spray application. These studies aimed to determine the persistence of toxic effects and to determine the effects of residues on predators that may be reinvading the crop.

Totals of 80 *C. septempunctata* (20 per plot), 60 *T. hypnorum* (15 per plot) and 40 *D. atricapillus* (10 per plot) were used in experiments 1) and 2) and a total of 80 *C. septempunctata* (20 per plot) and 40 *T. hypnorum* (10 per plot) were tested in experiment 3). A total of 80 *C. septempunctata* (20 per plot) was also used for both of the reduced-dose rates tested. During the experiments *T. hypnorum* and *D. atricapillus* were provided with ground, moist cat biscuits every two days whereas *C. septempunctata* were provided with aphids approximately 10 aphids on each day.

7.2.4 *In situ* bioassay chambers

Multiple-leaf bioassay chambers and soil exposure bioassay chambers were used to confine batches of predators on the adaxial surfaces of flag leaves and on the soil in the experiments.

7.2.4.1 Multiple-leaf bioassay chambers

These consisted of a glass plate (12cm x 12cm) completely covered with flag leaves from individual plots. The flag leaves were excised from random plants in each plot and attached carefully to the glass plates in parallel, base to tip, adaxial surface upwards, via strips of double-sided adhesive tape. Batches of five *C. septempunctata*, *T. hypnorum* or *D. atricapillus* were released onto the flag leaves and chambers were immediately placed over the plates and secured with adhesive tape. The chambers used to confine *C. septempunctata* consisted of plastic tubs (9.5cm in diameter and 6.5cm high) with Fluon-coated sides and with gause covered ventilation holes in the top. They provided an exposure area of 70.9 cm^2. See Appendix 3 for a full description and diagram of the multiple-leaf bioassay chambers used for *C. septempunctata*. Modified chambers were used to confine the smaller predators, *T. hypnorum* and *D. atricapillus* on the flag leaves. These consisted of a perspex sheet base which had a 5cm diameter circle cut into the centre to accommodated small perspex chambers (5cm diameter and 3cm high). The perspex sheet was placed over the flag leaf-covered plates and secured via metal clips at each side. The insects were released

into the circle and the chambers were put into place. The chambers had Fluon-coated sides and ventilation holes in the top and provided an exposure area of 19.6 cm^2.

After the beetles had been introduced all of the chambers were placed in the crop within their respective plots and were left to experience field conditions. New plates, with fresh flag leaves, were prepared on each day of the experiment and the batches of beetles were transferred to the fresh surface. This was necessary as the excised leaves tended to become desiccated after 24 h and it also ensured that the deltamethrin residues on the new leaves harvested from each plot had been weathered in their natural positions in the crop.

7.2.4.2 Soil exposure bioassay chambers

These chambers consisted of plastic tubs (9.5cm diameter x 6.5cm high) with Fluon-coated sides that had been sunk to a depth of 1 to 2cm into the soil surface under the crop canopy in the experimental plots. The chambers were filled with a layer of soil approximately 1cm deep. The soil was taken from the field site and was lightly compacted into the bottom of the chamber making sure that any stones were removed. Plastic inlays, consisting of identical tubs with their bottoms removed, were placed in each chamber prior to spray application. The purpose of these was to avoid spray contamination of the sides of the chambers above the ground surface. The inlays were removed immediately after the spray application. Batches of five *C. septempunctata*, *T. hypnorum* and *D. atricapillus* were transferred from the flag leaf exposure chambers, after a 24 hour initial exposure period, to the soil exposure chambers. Lids with gause covered ventilation holes were then placed over each chamber to prevent the beetles from flying out of the chambers and also to reduce the chance of beetles being lost due to predation by birds. The beetles remained in the same chambers for the duration of the assessments. See Appendix 3 for a full description and diagram of the soil exposure bioassay chambers.

7.2.5 Mortality assessments

The responses of the predators in each bioassay chamber were assessed in the field at 24 h intervals during the period of exposure. The responses were recorded as unaffected, i.e. moving as normal, or affected, either knocked down or dead. Mortality values were calculated for the total numbers of individuals of each species that were recorded as dead for each given exposure bioassay and treatment. These values were corrected for control mortality using Abbott's formula (Abbott, 1925).

7.3 RESULTS

7.3.1 Volumetric spray deposition in the experimental plots

The mean spray deposition rates on the flag leaves and on the soil in the sprayed plots showed no significant heterogeneity between the experimental plots (Table 7.1). The mean spray deposition rates were higher on the flag leaves than on the soil and

Table 7.1 Mean fluorescent tracer deposition rates at flag leaf and ground levels in the sprayed experimental plots.

Treatment	Plot number	Mean spray deposition on the flag leaves (± s.e.) (μl/cm^2)	Mean spray deposition on the soil (± s.e.) (μl/cm^2)
Field rate	1	0.28 ± 0.02	0.26 ± 0.02
	2	0.30 ± 0.02	0.20 ± 0.02
	3	0.26 ± 0.04	0.22 ± 0.03
	4	0.31 ± 0.02	0.23 ± 0.01
Half field rate	1	0.29 ± 0.02	0.23 ± 0.03
	2	0.33 ± 0.02	0.25 ± 0.02
	3	0.31 ± 0.03	0.23 ± 0.02
	4	0.33 ± 0.02	0.24 ± 0.03
Quarter field rate	1	0.32 ± 0.03	0.21 ± 0.02
	2	0.29 ± 0.02	0.25 ± 0.03
	3	0.28 ± 0.01	0.23 ± 0.02
	4	0.29 ± 0.02	0.22 ± 0.02
One-way analysis of variance			
F		1.38 ns (P>0.05)	1.03 ns (P>0.05)
d.f.		11,108	11,108

ns = not significant

varied between 0.26 and 0.33 μl/cm^2, whereas mean deposition rates on the soil varied from 0.20 to 0.26 μl/cm^2. Samples of flag leaves and filter paper on the soil in the control plots were found to have no tracer deposited on them indicating that no spray drift had occurred into the control plots.

7.3.2 Comparing the risk posed by continuous exposure on flag leaves and on the soil

Because there were no differences in spray deposition rates in the treated plots, insect mortalities from plots of the same treatment were totalled to compare predator

mortalities at each 24 h assessment time between the flag leaf and soil transferal bioassays. Similar trends were evident between the overall percentage mortalities of *C. septempunctata*, *T. hypnorum* and *D. atricapillus* from continuous exposure to flag leaf residues and 24 h exposure to flag leaf residues followed by continuous exposure to soil residues (Figure 7.1) with higher levels of mortality being recorded in the flag leaf bioassays. The predator that suffered the highest mortality during the bioassays was the staphylinid beetle *T. hypnorum*. High levels of mortality also occurred in the coccinellid *C. septempunctata* whereas the small carabid *D. atricapillus* suffered

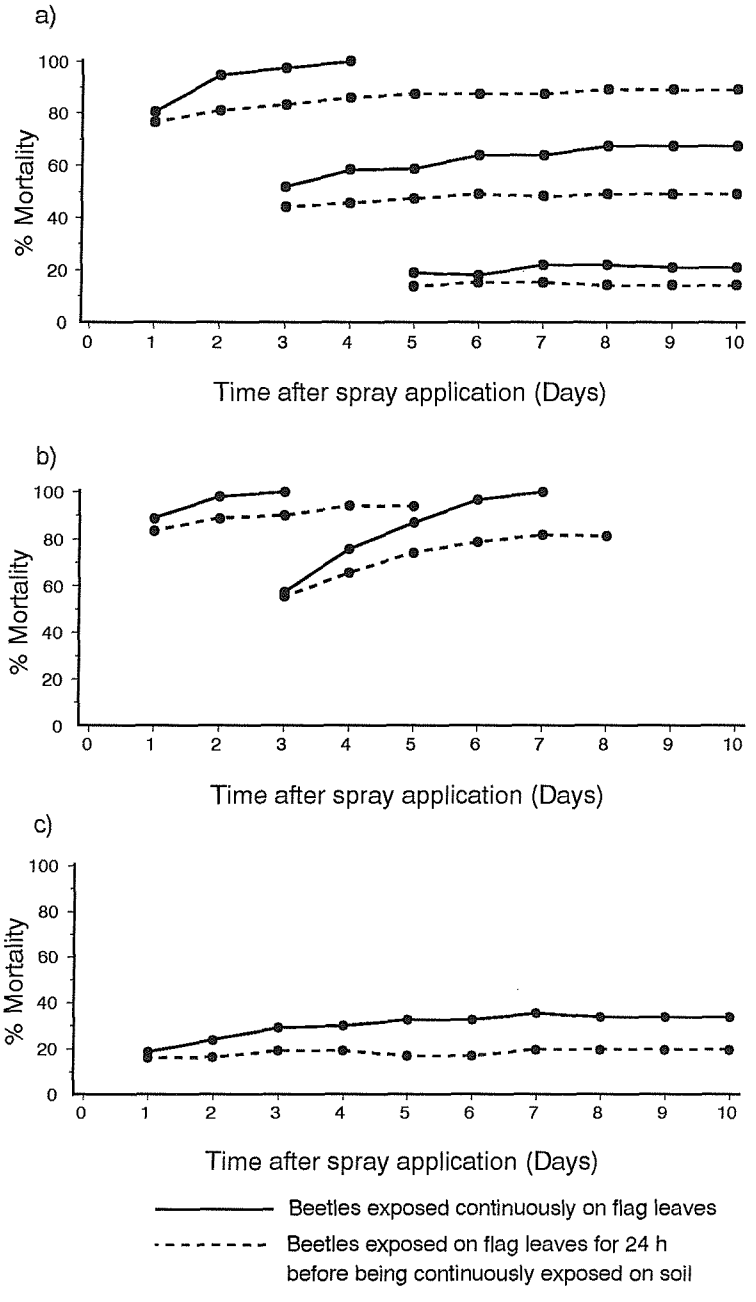


Figure 7.1 Percentage mortalities of a) *C. septempunctata*, b) *T. hypnorum* and c) *D. atricapillus* exposed continuously on flag leaves or exposed for 24 h on flag leaves followed by continuous exposure on the soil for plots treated with deltamethrin at a rate of 6.25 g AI ha^{-1}. Values corrected for control mortality.

relatively low mortality levels. The mortality from the initial 24 h of exposure on flag leaves were similar in all of the bioassays and varied between 84% and 89% mortality for *T. hypnorum*, 77% and 80% mortality for *C. septempunctata* and 16% to 18% mortality for *D. atricapillus*. Mortality levels increased rapidly for *T. hypnorum* and *C. septempunctata*, reaching 100% for *T. hypnorum* after 3 days of continuous exposure to deltamethrin residues on flag leaves and reaching 100% for *C. septempunctata* after 4 days whereas mortality levels for *D. atricapillus* showed increases of only 11% in the continuous flag leaf bioassays and an increase of 4% mortality in the soil transferal bioassay over the whole 10 day assessment period (Figure 7.1). Mortality levels of *T. hypnorum* were only recorded for between four and five days as after this time control mortality increased to between 18% and 25%. The maximum observed control mortalities in the bioassays with *C. septempunctata* and *D. atricapillus* were 8% and 10% respectively over the 10 day assessment period.

Mortality was observed for *T. hypnorum* introduced onto flag leaf residues two days after the deltamethrin spray application and for *C. septempunctata* that were introduced two and four days after the spray application (Figure 7.1). Mortality levels of *T. hypnorum* increased from 57% to 100% in the four days after initial exposure in the continuous flag leaf exposure bioassays and rose from 56% to 81% during five days after initial exposure in the soil transferal bioassays. Mortality levels of between 44% and 51% occurred over an eight day exposure period for *C. septempunctata* introduced 2 days after the spray application and between 11% and 14% mortality occurred for beetles exposed to four day old residues in the flag leaf and the soil transferal bioassays respectively.

7.3.3 The effect of reduced-dose rates on *C. septempunctata* and the a cereal aphid pest

The levels of ladybird mortality observed in the flag leaf exposure bioassays and the soil transferal bioassays were similar in the first 24 h of exposure on the flag leaves (Figure 7.2 a & b) within each dose rate treatment. *C. septempunctata* mortality levels varied between 77% and 81% in the plots treated with the recommended field rate of deltamethrin (6.25 g Al ha^{-1}), 49% and 56% in the plots treated with half recommended field rate (3.13 g Al ha^{-1}) and 20% to 21% in the plots treated with a quarter of the recommended field rate (1.56 g Al ha^{-1}). The rates of increase of ladybird mortality in the flag leaf bioassays were greater than in the soil transferal bioassays (Figure 7.2). Mortality levels increased by approximately 43% and 18% in the plots treated with half and a quarter recommended field rate in the flag leaf bioassays over the 10 day

assessment period compared to increases of between 8 and 12% mortality for the three rates in the soil transferal bioassays over the same period. In both exposure studies mortality values appeared to reach stable endpoints by the end of the 10 days of continuous exposure.

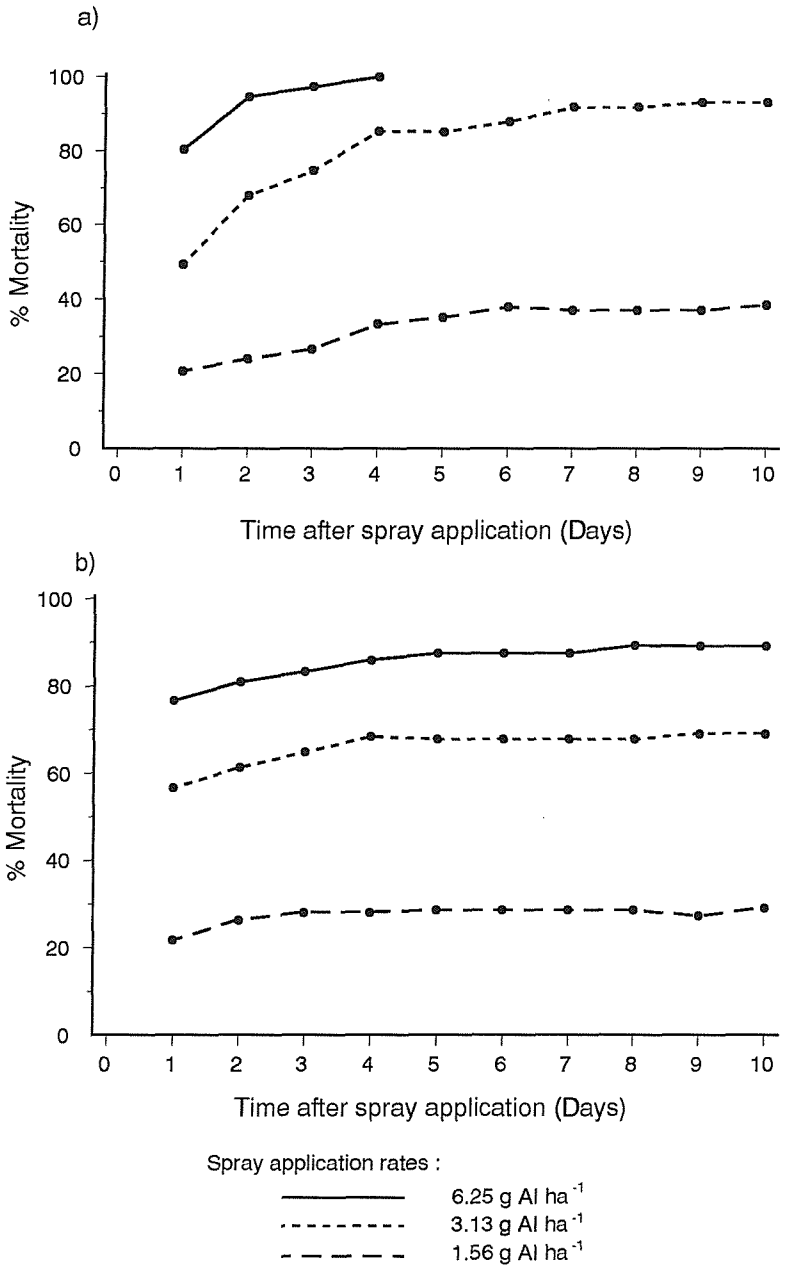


Figure 7.2 Percentage mortalities recorded each day for *C. septempunctata* that were, a) exposed continuously on flag leaves and b) exposed for 24 h on flag leaves before being transferred to treated soil, in plots sprayed with deltamethrin at recommended field rate, half recommended field rate and a quarter recommended field rate. Values corrected for control mortality.

The mean aphid densities from the ear counts in the plots for each treatment indicated that all three deltamethrin dose rates provided aphid control (Figure 7.3). Mean aphid

numbers in the pre-spray period were similar in all of the experimental plots, ranging between 13 and 16 aphids/ear. On the first day after spray application mean aphid numbers had been reduced to 2.2 aphids/ear in the field rate treated plots (sprayed with 6.25 g AI ha^{-1}), 4.3 aphids/ear in the half field rate treated plots (sprayed with 3.13 g AI ha^{-1}) and 3.7 aphids/ear in the quater field rate treated plots (sprayed with 1.56 g AI ha^{-1}). Mean aphid numbers on the ears declined further in all of the sprayed plots over the following three days to levels of approximately 1 aphid/ear. In the plots treated with recommended field rate of deltamethrin mean aphid numbers remained

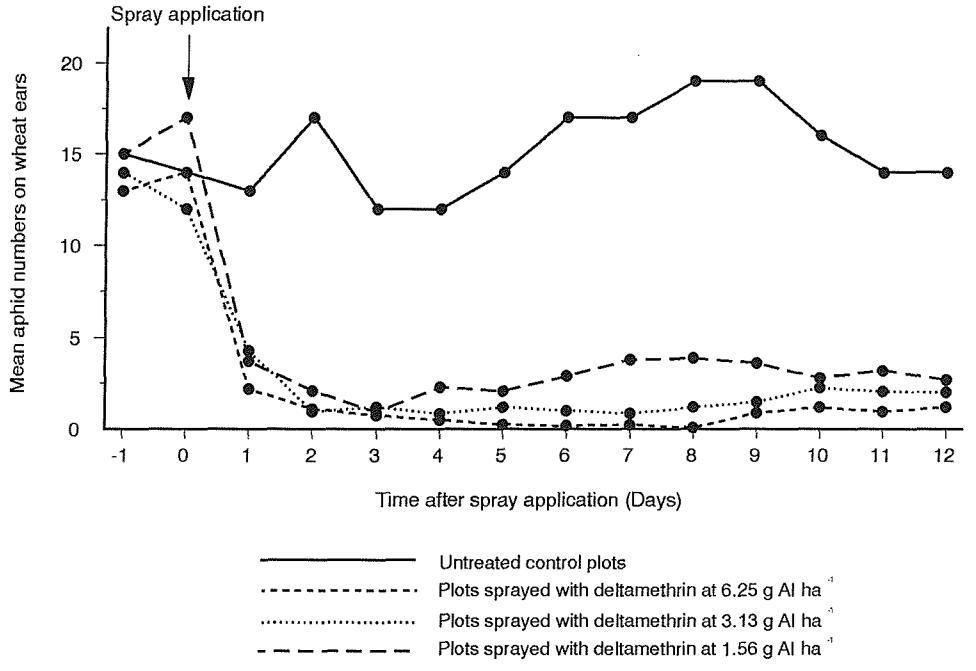


Figure 7.3 Mean aphid densities on wheat ears in the control and plots sprayed with recommended field rate (6.25 g AI ha^{-1}), half recommended field rate (3.13 g AI ha^{-1}) and a quarter recommended field rate (1.56 g AI ha^{-1}) of deltamethrin.

at less than one aphid/ear up to 12 days after spray application. The mean aphid numbers in the plots treated with half recommended field rate rose to approximately 2 aphids/ear during this period whereas the mean number of aphids in the plots treated with a quarter of the recommended field rate rose to approximately 4 aphids/ear eight days after spray application before declining again.

7.3.4 Predicting reduced-dose rates to minimise mortality of *C. septempunctata*

In order to make predictions of mortality levels for *C. septempunctata* after given periods of exposure and to account for any variations in these data a non-linear

regression was fitted to the continuous exposure mortality data (Table 7.2). A hyperbola of form (y = mx/(b+x)) was found to provide the best fit to these data and curves were fitted using Sigma Plot v4.1 (Jandel Corporation). Example curves from the continuous flag leaf exposure bioassays are given in Figure 7.4.

Table 7.2 Slope and intercept parameters from non-linear regressions of the form y = mx/(b+x) fitted to *C. septempunctata* mortality data from continuous exposure to deltamethrin residues on flag leaves and soil.

Surface of exposure	Deltamethrin application rate (g AI ha^{-1})	Value of slope (m) (± S.D.)	Value of intercept (b) (± S.D.)
Flag leaf	6.25	109.1 (± 0.32)	0.345 (± 0.066)
	3.13	104.6 (± 1.29)	1.099 (± 0.061)
	1.56	43.9 (± 0.45)	1.396 (± 0.014)
24 h on flag leaf before transferal to soil	6.25	90.5 (± 0.51)	0.198 (± 0.018)
	3.13	70.9 (± 0.50)	0.260 (± 0.024)
	1.56	30.0 (± 0.47)	0.328 (± 0.006)

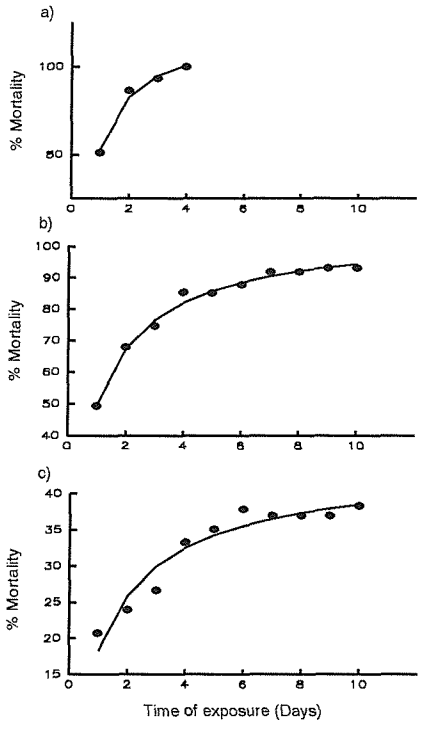


Figure 7.4 Example curves (y = mx/(b+x)) fitted to mortality data from the continuous exposure of *C. septempunctata* on flag leaves in plots that had been sprayed with deltamethrin at a) 6.25 g AI ha^{-1}, b) 3.13 g AI ha^{-1} and c) 1.56 g AI ha^{-1}.

Predicted mortality values were calculated for 1, 5 and 10 days of continuous exposure of *C. septempunctata* in the flag leaf bioassays and the soil transferal bioassays. These were chosen to represent mortality levels recorded at the beginning, middle and end of the exposure period. The predicted percentage mortality values were plotted

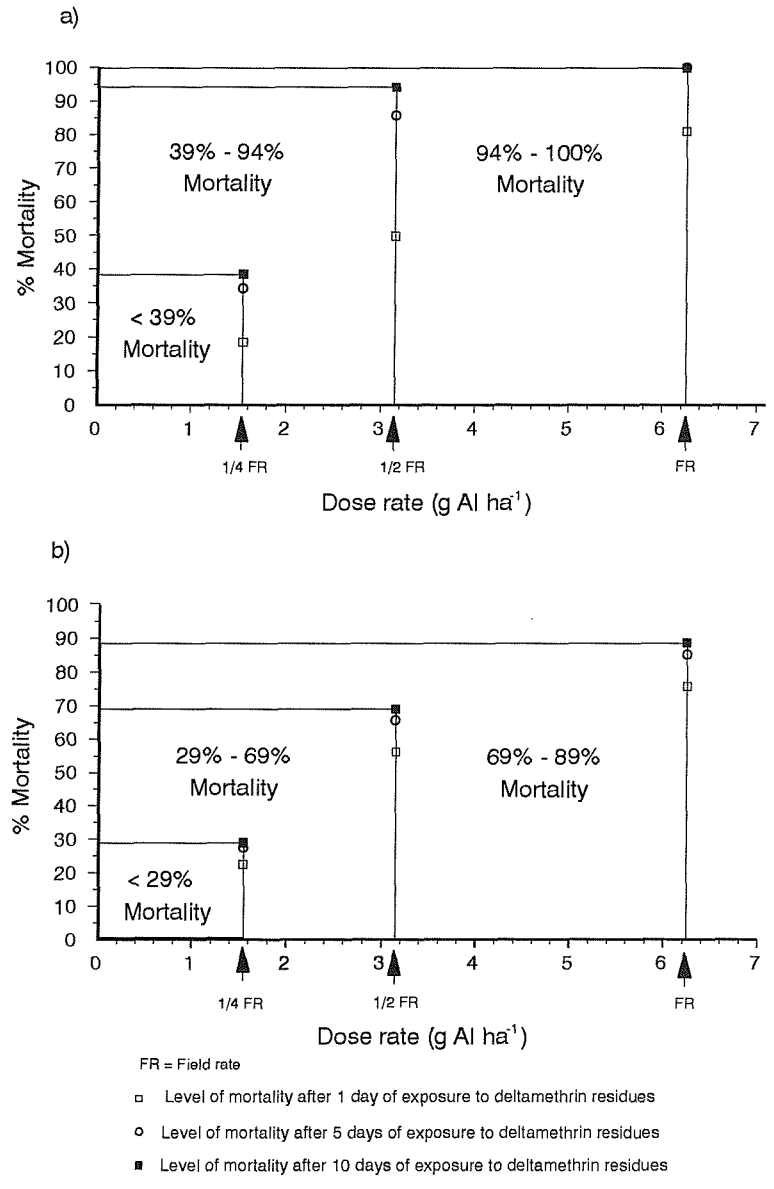


Figure 7.5 Dose-response predictions for *C. septempunctata* a) exposed continuously to deltamethrin residues on flag leaves and b) exposed to deltamethrin residues on flag leaves for 24 h followed by exposure to deltamethrin residues on soil. Values corrected for control mortality.

against the deltamethrin dose rate that had been applied and these were then joined within boxes for each given dose rate to produce zones of predicted mortality for *C. septempunctata* exposed continuously to deltamethrin residues in a cereal crop for 10 days after spray application (Figure 7.5). The predicted mortality zones suggested that

ladybirds exposed to deltamethrin residues continuously on flag leaves for 10 days after a deltamethrin spray of between half (3.13 g AI ha^{-1}) and full recommended field rate (6.25 g AI ha^{-1}) may suffer between 94 and 100% mortality. Deltamethrin spray applications of between half recommended field rate (3.13 g AI ha^{-1}) and a quarter recommended field rate (1.56 g AI ha^{-1}) were predicted to cause between 39 and 94% mortality of ladybirds continuously exposed on the flag leaves over the same period whereas doses less than a quarter of the recommended field rate (1.56 g AI ha^{-1}) were predicted to result in less than 39% ladybird mortality (Figure 7.5a). The predicted zones of mortality from the soil transfer bioassays indicated that ladybirds would suffer lower affects from deltamethrin if they moved from the foliage to the ground. The mortality zones predicted that between 69% and 89% mortality would occur for ladybirds exposed to deltamethrin residues sprayed at rates between recommended field rate (6.25g AI ha^{-1}) and half recommended field rate (3.13 g AI ha^{-1}), between 29% and 69% ladybird mortality would occur between half recommended field rate (3.13 g AI ha^{-1}) and a quarter of the recommended field rate (1.56 g AI ha^{-1}) and that less than 29% mortality would occur for ladybirds exposed to deltamethrin spray residues applied at rates lower than a quarter of the recommended field rate (Figure 7.5b).

In the field *C. septempunctata* adults will not only be exposed to spray residues in a cereal crop but are also likely to suffer mortality from direct exposure to deltamethrin during a spray application (Chapter 6). Therefore, in order to predict the combined toxic risk posed by both of these routes of exposure, mortality levels predicted from exposure to the three dose rates (i.e. 6.25 g AI ha^{-1}, 3.13 g AI ha^{-1}, 1.56 g AI ha^{-1}) for both routes of exposure were added (Figure 7.6). This was based on the assumption that the toxic effects of doses received by different routes of exposure were additive. The direct contact mortalities were calculated by taking a mean population distribution of ladybirds in the cereal crop (Chapter 6 (6.3.4)). The predicted levels of direct contact mortality were approximately 19% at 6.25 g AI ha^{-1}, 8% at 3.13 g AI ha^{-1} and 3% at 1.56 g AI ha^{-1}. When these were added to the mortalities from exposure to residues on the flag leaves the overall mortality predictions varied from 42% to 100% for dose rates between half recommended field rate (3.13 g AI ha^{-1}) and a quarter recommended field rate (1.56 g AI ha^{-1}) and predicted an overall mortality of less than 42% for dose rates of less than a quarter of the recommended field rate (1.56 g AI ha^{-1}) (Figure 7.6a). The overall levels of predicted mortalities for beetles than had been exposed to residues on flag leaves and transferred to the soil varied from 77% to 100% for dose rates between recommended field rate (6.25 g AI ha^{-1}) and half

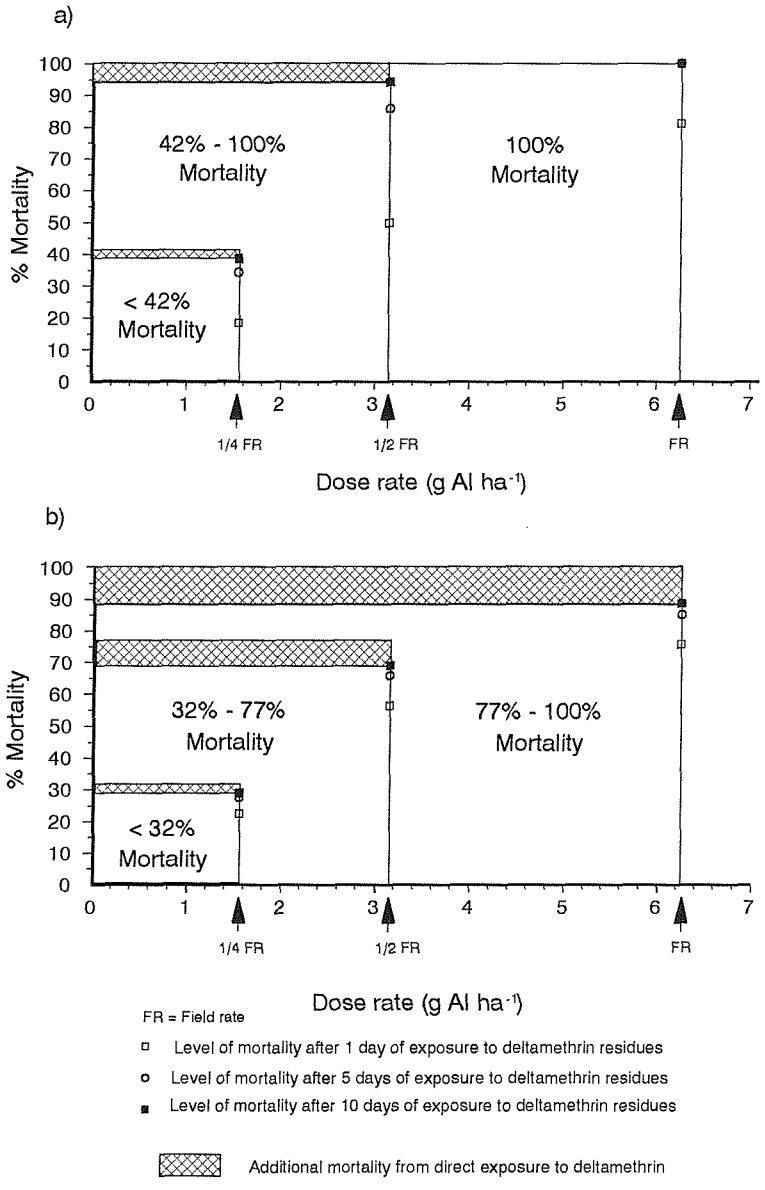


Figure 7.6 Dose-response predictions for *C. septempunctata* a) exposed directly to deltamethrin spray and continuously to deltamethrin residues on flag leaves and b) exposed directly to deltamethrin spray and to deltamethrin residues on flag leaves for 24 h followed by exposure to deltamethrin residues on soil. Values corrected for control mortality.

recommended field rate (3.13 g AI ha^{-1}), from 32% to 77% for dose rates between half field rate (3.13 g AI ha^{-1}) and a quarter field rate (1.56 g AI ha^{-1}) and less than 32% mortality for dose rates lower than a quarter of the recommended field rate (1.56 g AI ha^{-1}).

7.4 DISCUSSION

7.4.1 The toxic risk posed by recommended field rate applications of deltamethrin to *C. septempuncata*, *T. hypnorum* and *D. atricapillus* on flag leaves and the soil in a cereal crop

All three species of plant-active predator tested in these *in situ* bioassays suffered mortality from the exposure to residues from deltamethrin sprayed at current recommended field rate in U.K. cereals (i.e. 6.25 g AI ha^{-1}). Of the three predators, the staphylinid beetle *T. hypnorum* suffered the highest levels of mortality in both the continuous flag leaf exposure bioassays and the soil transferal bioassays, with responses ranging from 79% to 100% mortality. This may have been due to its' relatively high susceptibility to deltamethrin (Chapter 2 and Chapter 4) and because it is known to have a high contact area with substrates and therefore may pick up proportionally higher doses of pesticide than other species (Chapter 3). The coccinellid beetle *C. septempunctata* also suffered high levels of mortality in both exposure bioassays. These beetles were observed to be more active than the other predator species tested and therefore even though they are known to have a low contact area with the substrate (Chapter 3) their relatively high activity may have increased the rate at which they picked up the pesticide. The carabid beetle *D. atricapillus* suffered the lowest low levels of mortality. This may be because this predator is relatively tolerant to deltamethrin (Chapter 2) and also has a relatively low contact area with the substrate compared to other predators (Chapter 3). These results suggest that the reductions in catch numbers of *Tachyporus* spp., *D. atricapillus* and coccinellids found by Vickerman *et al.* (1987a & b) were likely to have been indicative of toxic effects. However the results from this study have indicated that the impact of a deltamethrin spray is likely to be far more severe on predators such as *T. hypnorum* and *C. septempunctata* that *D. atricapillus*.

Differences were evident between the toxic risk posed by deltamethrin residues on the flag leaf compared to the soil. Mortality levels increased very little over the exposure period for *C. septempunctata* and *D. atricapillus* transferred to the soil. This may indicate that deltamethrin had been adsorbed onto the soil and may have become less available to the predators within a short time whereas residues on the flag leaves remained available for a longer period. Differences in the bioavailability of deltamethrin in terms of estimates of its' bioavailable half-life on flag leaves and a sandy loam soil have been discussed in Chapter 4. Comparisons of the toxicity of deltamethrin to invertebrates on these substrates using mortality data from 24 h *in situ* bioassays with

B. lampros (Unal and Jepson, 1991) and *C. septempunctata* (Appendix 3) indicated that the bioavailable half-life of deltamethrin on flag leaves may be between 4.5 and 6.0 days whereas it may only be between 1.6 and 2.4 days on a sandy loam soil.

Residue studies to determine the persistence of deltamethrin sprays on wheat foliage by Hill and Inaba (1990) have shown that the detectable residues of deltamethrin declined by 50% in approximately 5 days after spray application. In this study the staphylinid beetle *T. hypnorum* still suffered high levels of mortality when exposed to deltamethrin residues two days after spray application, however the coccinellid beetle *C. septempunctata* suffered reduced levels of mortality when exposed to deltamethrin deposits two and four days after the spray application. Reductions in the levels of mortality suffered by predators entering the crop over five to ten days after a spray application have also been shown by Unal and Jepson (1991) and by the data given in Appendix 3. These results suggest that deltamethrin residues are likely to pose a reduced risk to predators entering the crop approximately one week after a spray application.

7.4.2 The aphicidal efficacy of reduced-dose rates of deltamethrin

The aphicidal efficacy of the three deltamethrin dose rates tested in this study (6.25 g Al ha^{-1}, 3.13 g Al ha^{-1} and 1.56 g Al ha^{-1}) were similar, causing reductions in mean aphid numbers of 95%, 90% and 95% respectively from their pre-spray levels during the three days after spray application. These results agreed with field studies by Turner (1992) who found similar reductions in aphid numbers in plots sprayed with deltamethrin at recommended field rate, a third of the recommended field rate and a fifth of the recommended field rate. The mean aphid numbers in the plots sprayed at recommended field rate remained lower than those in the other plots during the post-spray period. This may indicate that deltamethrin sprayed at higher rates had a longer residual activity against the aphids. The mean aphid numbers on the ears in the plots treated with a quarter of the recommended field rate began to increase four days after the spray application which mirrored an increase in the mean aphid numbers on the ears in the control plots over the same period. This may indicate that the residual activity of deltamethrin applied at a quarter of the recommended field rate was of relatively short duration.

The economic threshold for cereal aphids on wheat ears is 5 aphids/ear (George and Gair, 1979). The mean numbers of aphids in the plots treated with a quarter of the recommended field rate of deltamethrin reached approximately 4 aphids/ear eight days

after the spray application. This may indicate that a further aphid outbreak was occurring. If the reduced-dose rate applied however enabled the preservation of a large proportion of a given predator population in the crop, these may, in theory, remain in the crop and suppress the growth of the pest population. The reliability and effectiveness of predators in providing control of the remaining pest populations after a spray application has yet to be established however.

7.4.3 Predicting reduced-dose rates to minimise mortality of *C. septempuncata* in a cereal crop

The mortality predictions have indicated that *C. septempunctata* adults exposed to spray residues in a cereal crop in the 10 days after spray application may suffer high levels of mortality from continuous exposure to deltamethrin residues sprayed at doses of between half and full recommended field rate. Dose reductions of between half and three quarters of the recommended field rate of deltamethrin may be required to preserve between 6 and 71% of the population depending upon whether they were exposed to residues continuously on flag leaves or initially on flag leaves followed by the soil.

The predictions of mortality resulting from the combined direct and residual routes of exposure suggested firstly, that exposure to residues may be a more important route of pesticide uptake for *C. septempunctata* than direct exposure to spray and secondly that the recommended field rate of deltamethrin may need to be reduced by as much as three quarters to preserve more than 50% of the adult coccinellid population present in the crop at the time of spraying and the following 10 days. These results also have implications for the effects of deltamethrin sprays on coccinellid larvae. Given the greater susceptibility of coccinellid larvae to deltamethrin (e.g. 4th instar larvae tested in Chapter 2 and Chapter 4) it is likely that even greater reductions in dose rates would be required to preserve populations of larvae in the crop.

These predictions were based on the assumption effects of doses received by the different routes of exposure were additive. Preliminary laboratory investigations carried out during this study with *C. septempunctata* have suggested that the effects of doses from these routes of exposure may be additive however the toxic interactions between doses received by different routes of exposure requires further investigation. Also it was only possible to test three dose rates in these bioassays because of limitations of the numbers of predators available. The accuracy of predictions may be improved by evaluating responses to a larger number of dose rates.

Overall these experiments have provided a first attempt to develop a method for predicting reduced-dose rates that may preserve predators in the crop after spraying. The results suggest that there may be some scope for the use of reduced-dose rates of deltamethrin for pest management in cereals. Dose rates of approximately a quater of the recommended field rate (i.e. 1.56 g AI ha^{-1}) appeared to offer reasonable levels of aphid control (7.4.2) and were predicted to cause less than 50% mortality of a population of adult ladybirds in the crop at the time of spraying and the following 10 days.

In order to be of value for pest management purposes the predictions require validation in the field with natural populations to ascertain a) if predators are preserved by these reduced-dose rates, b) if the surviving predators remain in the sprayed crop after spraying and c) if the predators are capable of suppressing any residual populations of aphids that remain in the crop after the spray. One aspect of this, i.e. the effects of deltamethrin residues on the behaviour and distribution of adult *C. septempunctata* in cereal crops after a spray application, will be explored further in Chapter 8.

CHAPTER 8

Sub-lethal effects of deltamethrin residues on the behaviour and distribution of *C. septempunctata* in cereals.

8.1 INTRODUCTION

The risk posed by pesticides to beneficial invertebrates should not only be considered in terms of direct mortality but also in terms of sub-lethal effects which may lead to reduced effectiveness in suppressing pest populations. The previous chapters have described experiments to determine the lethal effects of deltamethrin on beneficial invertebrates that inhabit temperate cereal crops via the three main routes of uptake, i.e. direct contact, contact with residues and dietary intake. This chapter describes a field study undertaken to investigate the sub-lethal effects of deltamethrin residues on the foraging behaviour and distribution of adult *C. septempunctata* in a mature winter wheat crop.

Predators such as *C. septempunctata* will undertake a wide range of behaviours on cereal plants. These include location of food and mates, finding oviposition sites and the location of refugia to escape adverse conditions. These behaviours are mediated by the biological characteristics of the organism, such as locomotory patterns and sensory perception, internal factors, such as hunger and reproductive state (Carter and Dixon, 1982 & 1984; Rhamhalinghan, 1987) and external environmental factors, such as climatic conditions (Nakamuta, 1987), habitat quality (Honek, 1982 & 1983; Carter and Dixon, 1982) and possibly exposure to pesticides.

If sub-lethal doses of pesticides are picked up by predators they may cause behavioural changes, such as altered foraging patterns, disrupted sexual communication or host recognition (Elzen, 1989), and/or physiological changes, such as altered reproduction, reduced longevity, egg viability or fitness (Moriarty, 1969). It is therefore important to understand these changes if we are to exploit biological control and augmentation of natural enemies for pest management purposes.

Coccinellids are one of the most intensively studied groups of predators and since early studies by Fleschner (1950), Banks (1954, 1957) and Dixon (1959) on their searching behaviour, numerous authors have studied their biology and ecology (Frazer, 1988). Many authors have also determined the toxicity of pesticides to

coccinellids in the laboratory and field (i.e. Coats *et al.* 1979; Poehling *et al.* 1985; Vickerman *et al.* 1987a; Poehling, 1988; Zoebelein, 1988) but few have looked at the more subtle, sub-lethal influence that contact with pesticide residues may have upon their behaviour. Such effects are well documented for the synthetic pyrethroid insecticides and common poisoning symptoms include a rapid excitory action in many invertebrates (Naumann, 1990). Examples of these sub-lethal effects include the repellent/irritant responses shown by aphids (Highwood, 1979; Rice *et al.* 1983; Lowery and Boiteau, 1988; Adams and Hall, 1990), mites (Iftner and Hall, 1983; Penman and Chapman, 1983; Berry *et al.* 1990) and honeybees (Delabie *et al.* 1985) and also antifeedant responses shown by Lepidoptera (Tan, 1981 & 1982) and Coleoptera (Hajjar and Ford, 1990). Relatively little information is available however, concerning the possible sub-lethal effects of pyrethroids on predators in the field.

The main aims of the study were; 1) to determine if exposure to deltamethrin residues affected the foraging behaviour of *C. septempunctata* adults; 2) to determine if exposure affected their distribution in the cereal crop canopy and 3) to determine if there was any evidence of repellency.

8.2 EXPERIMENTAL METHODS

8.2.1 Test invertebrates

Adult *C. septempunctata* were collected from unsprayed hedgerows and field verges on the Leckford Estates, Stockbridge, Hampshire and from the Allenford Farm, Damerham, Hampshire with a hand-held aspirator in May and June 1991. They were kept in ventilated boxes in an insectary, maintained at 19-22°C, 55-70% humidity and a photoperiod of 16:8 L:D and were provided with barley plants infested with cereal aphids prior to the experiment. On the day of the experiment the boxes were removed and taken to the field site. All the coccinellid beetles used during the study were provided with food in the 24 hours prior to the experiments.

8.2.2 Test plots

The test plots consisted of two 2 x 15 m areas of winter wheat cv. Apollo, at decimal growth stage 69 to 71 (Zadoks *et al.* 1974). The plots had a mean crop density of 412 tillers per square metre and contained natural infestations of the cereal aphids *S. avenae* and *M. dirhodum*. Mean aphid numbers were recorded on ears and flag leaves from twenty marked tillers within each plot throughout the experimental period. One plot was sprayed with deltamethrin, using an Oxford precision hand-held sprayer fitted

with a dry boom with four Lurmark 02-F80 nozzles (50 cm spacing) and operated at 2 bar pressure. The sprayer was calibrated to deliver spray at a rate equivalent to the recommended field rate of deltamethrin in cereals (i.e. 6.25 g AI ha^{-1} in 200 l water). The other plot remained unsprayed. The treated plot was 20 metres away from the untreated plot to avoid contamination of the control plot by spray drift and spray deposits were allowed to dry in the treated plot before the experimental introductions and observations began.

8.2.3 Observation procedure

Individual ladybirds were released on the ground in the centres of the control or treated plot and observations began one minute later. The behaviour of the ladybird and it's position in the crop canopy were recorded at 30 second intervals for a period of 15 minutes. The test ladybird was then removed from the plot and placed in a separate container. New individuals were used in each test. The relatively short 15 minute observation period was chosen because observations in treated and control areas could not be made concurrently. Ladybirds were therefore released alternately in the control and treatment plots and the short observation period enabled higher levels of replication. In addition, preliminary studies had shown that knockdown, resulting from pesticide uptake was unlikely to occur during the 15 minute observation period. The 30 second recording interval was chosen because continuous recording of behaviour changes was difficult in the crop canopy.

A total of 80 ladybirds were used in the experiment, eight in the control plot and eight in the treated plot per day. Observations continued for four days after the deltamethrin treated plot had been sprayed (Day 1 being the day of spraying and Day 5 being the fourth day after spraying). All observations took place between 10-00 and 16-00 BST (British Summer Time). Maximum daily temperatures were recorded in the plots for the duration of the experiment.

8.2.4 Behaviour categories

Four behavioural categories were chosen for the experiment from preliminary behavioural observations of adult *C. septempunctata*. These were defined as;

1) Walking - any ambulatory movement.
2) Resting - remaining motionless with no visible movement of body parts.
3) Feeding - handling or consumption of prey items.
4) Grooming - rubbing motions of the legs over the body surface and/or wings.

Flying was recorded in four of the 80 individuals observed. Observations were

terminated if the ladybird flew out of the experimental area.

8.2.5 Canopy distribution

The observations of beetle position were classified into six crop strata. These were the ear, flag leaf, stem (from ear to ground level), first leaf, second leaf (which was partly desiccated) and the ground. The observations on the leaves were further divided into adaxial and abaxial leaf surfaces. These canopy distribution categories were chosen because pesticide residues and their effects on non-target invertebrates are known to partition out between these crop strata (i.e. Chapter 6; Cilgi and Jepson, 1992; Unal and Jepson, 1991).

8.2.6 Statistical analyses

Behaviour category count data for the ladybirds in control and treated plots were compared on each day of the experiment by contingency chi squared analysis using Yates' correction when the degrees of freedom were equal to 1 (Cohen, 1988). The observed behavioural transition probabilities for the 30 second observation intervals were calculated by first-order Markov chain analysis using purpose written computer software. Overall trends in the observed distributions of ladybirds in the cereal crop canopy were compared between the control and treated plots for each day of the experiment by contingency chi squared analysis.

8.3 RESULTS

8.3.1 Ladybird behaviour

No ladybirds were knocked down during the period of observation in the treated plot however, four ladybirds exhibited flight behaviour during observation and flew out of the experimental plot. These were therefore excluded from the analysis. All four ladybirds flew out of the deltamethrin treated plot, two flying on day 1 (the day of spray application) and the other two flying on day 4 (three days after application).

The proportion of observations of ladybirds that fell into each of the four behaviour categories in the treated and untreated plots over the five days of testing are given in Figure 8.1. Observations of *C. septempunctata* walking behaviour during the five days accounted for between 36 and 82% of the observations in the treated plot and between 14 and 58% of the observations in the untreated plot. Resting behaviour

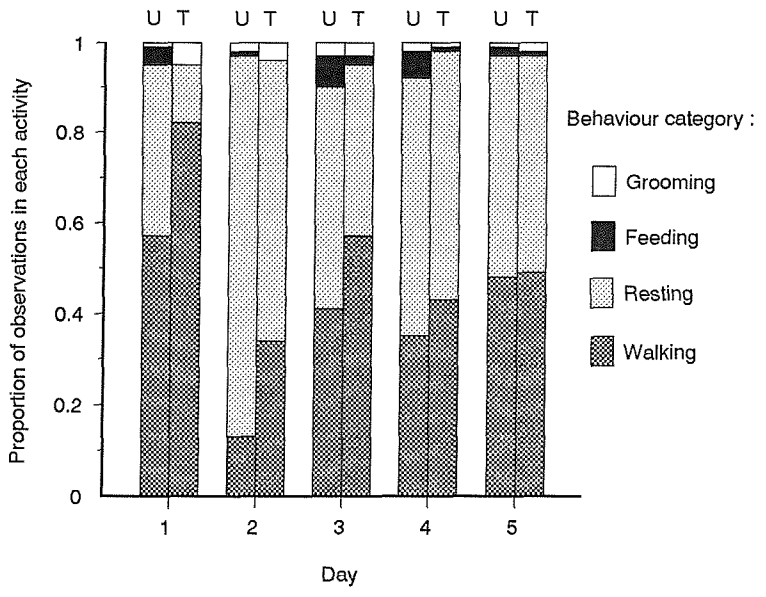


Figure 8.1 Proportions of observations of *C. septempunctata* adults in each behaviour category in the untreated (U) and deltamethrin treated (T) wheat plots during the five day experimental period.
Day 1 = Day of spray application.

varied between 12 and 60% of the observations in the treated plot and 85 and 38% in the untreated plot. Feeding and grooming behaviour observations accounted for between 0 and 3% and 1 and 5% respectively in the treated plot and between 1 and 7% and 1 and 3% in the untreated plot.

Contingency chi squared analysis of observation count data gave significant differences in the overall patterns of behaviour of the ladybirds between the untreated and treated plots on Day 1 (χ^2=48.0***, d.f.3, P<0.001), Day 2 (χ^2=33.9***, d.f.3, P<0.001), Day 3 (χ^2=48.7***, d.f.3, P<0.001) and Day 4 (χ^2=10.7*, d.f.3, P<0.05). There were however, no significant differences in the overall patterns of ladybird behaviour in the untreated and treated plots on Day 5 (χ^2=0.7ns, d.f.3, P>0.05). Comparisons between individual behaviours (Table 8.1) indicated that ladybirds in the treated plot walked significantly more and rested significantly less than those in the untreated plot on Days 1, 2 and 3. Ladybirds in the treated plot also groomed significantly more than those in the untreated plot on Day 1. Although no feeding was observed for ladybirds in the treated plot on the first two days, they fed significantly less on Days 3 and 4 than ladybirds in the treated untreated plot. These differences were also indicated in the transition probabilities given in Table 8.2. On the first two days, the ladybirds showed a higher probability of walking on consecutive observations

in the deltamethrin treated plot than in the untreated plot. The ladybirds also showed

Table 8.1 Table of χ^2 statistics comparing numbers of observations within each behaviour category on each day for ladybirds in treated and untreated wheat plots.
- = Test could not be performed; Degrees of freedom = 1; *** = P>0.001, ** = P<0.01, * - P<0.05, ns = not significant. See Figure 8.1 for directions of differences.

Behaviour category	Comparisons between observations in each behaviour category for ladybirds in treated and untreated wheat plots on each day of the experiment				
	Day 1	Day 2	Day 3	Day 4	Day 5
Walking	30.1***	30.1***	11.1***	3.2ns	0.1ns
Resting	33.1***	30.8***	5.4*	0.2ns	0.1ns
Feeding	-	-	5.3ns	8.4**	0.3ns
Grooming	6.9**	1.8ns	0.1ns	0.8ns	0.6ns

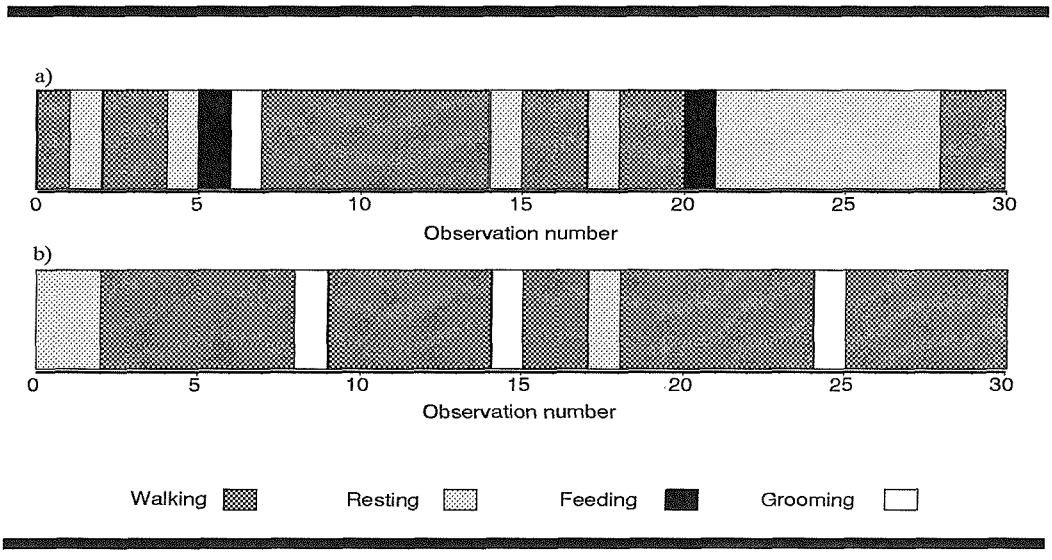


Figure 8.2 Example behavioural sequences for a) a ladybird in the untreated plot and b) a ladybird in the deltamethrin treated plot on the first day of the experiment.

a higher probability of walking being followed by grooming or grooming followed by walking in the treated plot than in the untreated plot on all five days. Examples of behaviour sequences for individual ladybirds in the treated and untreated plots which typified the trends on Day 1 of the experiment are given in Figure 8.2.

Control plot					Deltamethrin treated plot				
	Day 1					Day 1			
From/To	w	r	f	g	From/To	w	r	f	g
w	0.703	0.256	0.042	0.000	w	0.826	0.138	0.000	0.037
r	0.345	0.643	0.012	0.000	r	0.680	0.160	0.000	0.160
f	0.111	0.222	0.333	0.222	f	0.000	0.000	0.000	0.000
g	0.000	1.000	0.000	0.000	g	0.800	0.200	0.000	0.000
	Day 2					Day 2			
From/To	w	r	f	g	From/To	w	r	f	g
w	0.556	0.444	0.000	0.000	w	0.625	0.346	0.000	0.029
r	0.124	0.840	0.000	0.036	r	0.200	0.753	0.000	0.047
f	0.000	1.000	0.000	0.000	f	0.000	0.000	0.000	0.000
g	0.167	0.833	0.000	0.000	g	0.429	0.571	0.000	0.000
	Day 3					Day 3			
From/To	w	r	f	g	From/To	w	r	f	g
w	0.750	0.185	0.065	0.000	w	0.708	0.232	0.036	0.024
r	0.205	0.741	0.045	0.009	r	0.333	0.655	0.011	0.000
f	0.176	0.353	0.235	0.235	f	0.250	0.000	0.000	0.750
g	0.000	0.800	0.200	0.000	g	0.666	0.333	0.000	0.000
	Day 4					Day 4			
From/To	w	r	f	g	From/To	w	r	f	g
w	0.566	0.374	0.051	0.010	w	0.598	0.374	0.009	0.017
r	0.322	0.617	0.061	0.000	r	0.277	0.708	0.015	0.000
f	0.267	0.333	0.200	0.200	f	0.333	0.666	0.000	0.000
g	0.250	0.750	0.000	0.000	g	1.000	0.000	0.000	0.000
	Day 5					Day 5			
From/To	w	r	f	g	From/To	w	r	f	g
w	0.586	0.397	0.017	0.000	w	0.612	0.320	0.017	0.051
r	0.407	0.537	0.056	0.000	r	0.294	0.667	0.039	0.000
f	0.250	0.250	0.000	0.500	f	0.333	0.333	0.000	0.333
g	0.666	0.333	0.000	0.000	g	0.800	0.200	0.000	0.000

Table 8.2 Behavioural transition probabilities for *C. septempunctata* adults in untreated and deltamethrin treated wheat plots during the five days of assessment. Behaviour categories; w = walking, r = resting, f = feeding, g = grooming.

The sensitivity of the 30 second observation period for detecting differences in behaviour was estimated by calculating the mean consecutive time spent in each behaviour by beetles in the treated and untreated plots for each day of the experiment (Table 8.3). The results indicated that the 30 second period was adequate for detecting differences in walking and resting behaviour because the mean times spent in these behaviours were greater than the assessment period. The 30 second assessment period was however less sensitive to detecting differences in feeding and grooming

behaviours. The values in Table 8.3 indicated that consecutive observations in these behaviours were rare, as most estimated mean times were equal to 30 seconds. Therefore their frequencies may have been under-estimated, however relative differences are still likely to be indicative of changes in behaviour patterns.

Table 8.3 Estimated consecutive time spent in each behaviour by ladybirds in a) untreated and b) deltamethrin treated wheat plots during the experiment.
- = Behaviour not observed, 30* = no consecutive observations occurred.

a)

Behaviour category	Estimated mean consecutive time spent in each behaviour (secs) by the ladybirds on each day of the experiment				
	Day 1	Day 2	Day 3	Day 4	Day 5
	Mean +/- 95% C.L.	Mean +/- 95% C.L.	Mean +/- 95% C.L.	Mean +/- 95% C.L.	Mean +/- 95% C.L.
Walking	112.2 +/- 24.0	66.3 +/- 21.6	105.0 +/- 25.8	76.5 +/- 17.4	73.2 +/- 16.8
Resting	85.2 +/- 45.6	159.9 +/- 79.8	101.4 +/- 31.2	78.9 +/- 15.0	76.5 +/- 15.9
Feeding	33.3 +/- 7.5	30*	31.8 +/- 3.9	30*	30*
Grooming	-	30*	35.9 +/- 12.9	30*	30*

b)

Behaviour category	Estimated mean consecutive time spent in each behaviour (secs) by the ladybirds on each day of the experiment				
	Day 1	Day 2	Day 3	Day 4	Day 5
	Mean +/- 95% C.L.	Mean +/- 95% C.L.	Mean +/- 95% C.L.	Mean +/- 95% C.L.	Mean +/- 95% C.L.
Walking	147.2 +/- 38.1	68.4 +/- 19.1	79.2 +/- 20.1	85.8 +/- 22.0	86.4 +/- 21.4
Resting	36.0 +/- 6.3	126.9 +/- 41.0	81.4 +/- 27.1	107.4 +/- 36.3	83.4 +/- 13.5
Feeding	-	30*	30*	30*	30*
Grooming	30*	30*	30*	30*	30*

Overall differences in the level of activity between days may be attributable to variations in environmental conditions. The ladybirds were less active in the untreated and treated on the second day of the experiment when the lowest temperature was recorded (Table 8.4) and in general, activity trends appeared to correlate well with the maximum daily temperature. Variation of temperatures within days were not measured.

Table 8.4 Maximum daily temperatures in the wheat crop canopy during the experiment.

	Day				
	1	2	3	4	5
Temp. (°C)	21	18	23	19	24

Food availability may also have influenced ladybird behaviour. The mean aphid numbers on the marked ears and flag leaves in the untreated and treated plot are

given in Figure 8.3. Aphid numbers in the deltamethrin treated plot declined from a mean of 16 aphids/ear to 1.2 aphids/ear and from 5.5 aphids/flag leaf to 0.7 aphids/flag leaf after spraying and the numbers remained low for the duration of the experimental period. This compares with densities of approximately 14 aphids/ear and 6 aphids/flag leaf at the onset in the control plot which remained reasonably constant throughout the experimental period. Behaviour category data (Figure 8.1) indicated that no feeding was observed for beetles in the treated plot on the first two days of the experiment however some feeding was observed in the final three days whereas feeding was observed on each day for beetles in the control plot.

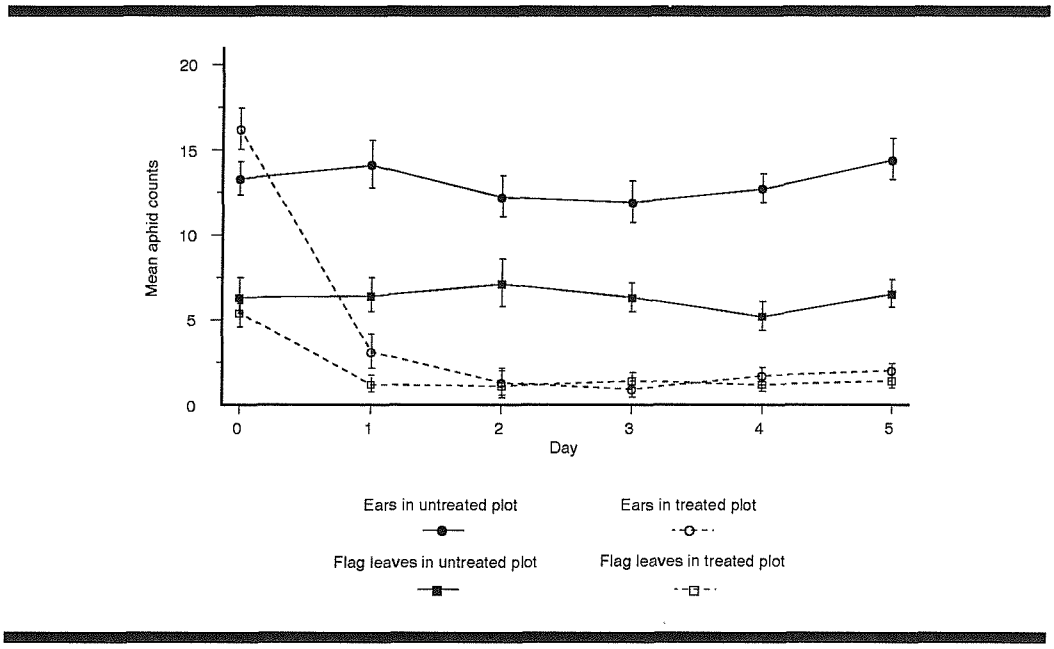


Figure 8.3 Mean aphid numbers on ears and flag leaves in the untreated and deltamethrin treated plots. Error bars indicate 95% confidence intervals.

8.3.2 Ladybird distribution

The observed proportions of ladybirds in the untreated and deltamethrin treated wheat crop canopies are given for each of the five days of the experiment in Figure 8.4. *C. septempunctata* distributions in the untreated plot appeared similar on all five days, with a higher proportion of observations on the ear and flag leaves than on the stem, first and second leaf and ground. Contingency chi squared analysis was used to compare overall numbers of ladybird observations on the given crop strata between the deltamethrin treated plot and the control plot within each day. Significant differences in observed ladybird distributions were evident between the deltamethrin treated and the untreated plots on Day 1 (χ^2 = 14.5*, d.f.5, P<0.05) and Day 2 (χ^2 =

55.6***, d.f.5, P<0.001) but no overall differences in ladybird distributions were found on Day 3 (χ^2 = 6.1ns, d.f.5, P>0.05), Day 4 (χ^2 = 6.3ns, d.f.5, P>0.05) and Day 5 (χ^2 = 3.4ns, d.f.5, P>0.05). Trends in the observed ladybird distributions on the first two days of the experiment indicated that fewer observations of ladybirds occurred on the ears and flag leaves in the deltamethrin treated plot compared with the untreated plot. Also a higher number of observations of ladybirds were evident on the first leaves and the ground in the deltamethrin treated plot than the control plot.

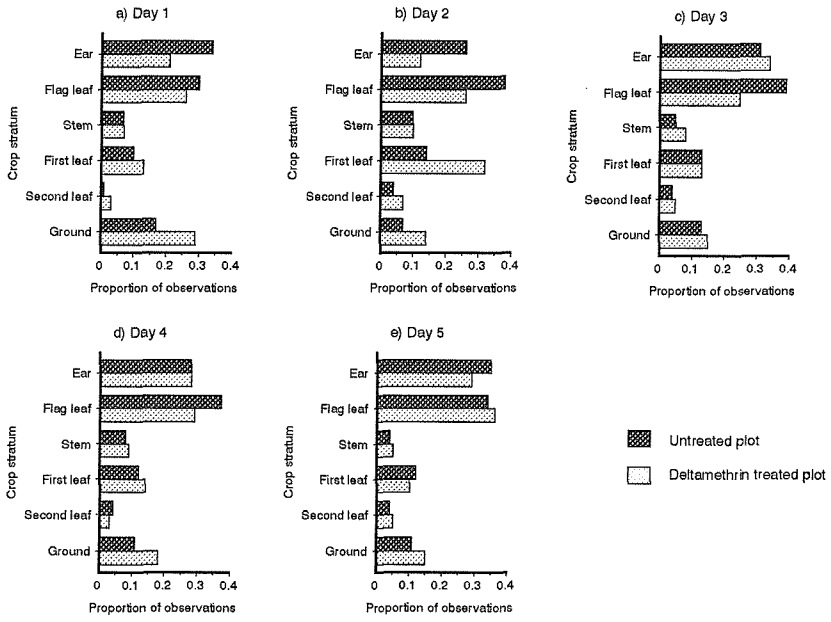


Figure 8.4 Proportions of observations of *C. septempunctata* on each crop stratum in the untreated and deltamethrin treated wheat plots on the day of treatment and the following four days.

The proportion of observations on the flag and first leaf adaxial surfaces were lower in the treated plot than the untreated plot on the first three days of the experiment, varying between 48 and 68% and 61 and 86% respectively (Figure 8.5). These distribution trends were investigated further by comparing total numbers of ladybird observations using contingency chi squared analysis. Second leaf data was not included because of low numbers of observations. Significant differences were found between the proportions of ladybird observations on the abaxial leaf surfaces in the treated plot compared to the control plot on Day 1 (χ^2=19.2***, d.f.3, P<0.001), Day 2 (χ^2=29.6***, d.f.3, P<0.001) and Day 3 (χ^2=13.4**, d.f.3, P<0.01), however no significant differences were found on Day 4 (χ^2=1.9 ns, d.f.3, P>0.05) or Day 5 (χ^2=5.3

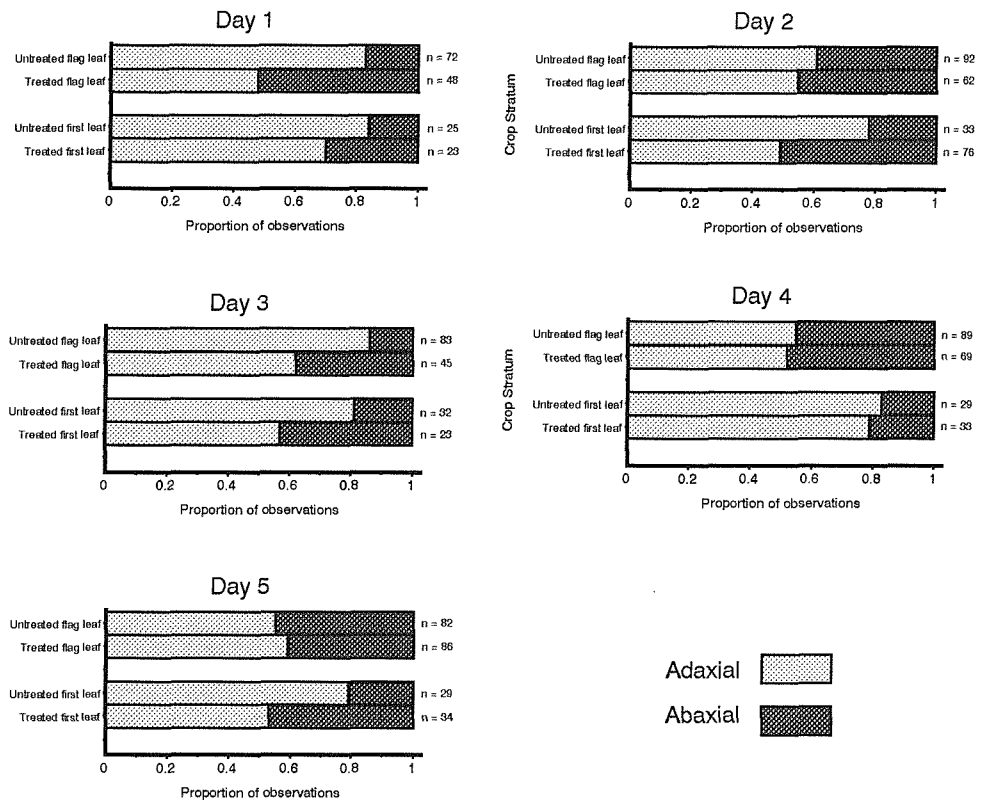


Figure 8.5 Proportions of observations of *C. septempunctata* on adaxial and abaxial flag and first leaf surfaces in the untreated and deltamethrin treated plots during the experimental period.

ns, d.f.3, P>0.05). This indicated that ladybirds were observed more frequently on the adaxial leaf surface in the untreated plot on the first three days of the experiment compared with the ladybirds in the deltamethrin treated plot.

8.4 DISCUSSION

8.4.1 Differences between ladybird behaviour in treated and untreated crops

Significant differences in overall behaviour patterns were evident between ladybirds in the untreated and deltamethrin treated plots on the day of spray application and on the following three days. Trends between individual behaviours indicated significant increases in walking and grooming behaviour and significant decreases in resting and feeding behaviour for ladybirds in the deltamethrin treated plot compared with those

in the untreated plot. This may indicate a sub-lethal irritant effect or hyperactivity caused by deltamethrin uptake. The higher proportion of observations of walking behaviour in the treated plot may also have been a result of increased searching for food which is known to be dependent upon hunger level in coccinellids (Frazer and Gilbert, 1976; Carter and Dixon, 1982). All of the ladybirds used in these experiments were provided with food prior to observations in order to reduce this effect however it's importance during the 15 minute observation period could not be determined.

The patterns of behavioural transition probabilities confirmed that ladybird beetles in the deltamethrin treated plot had a higher probability of walking continuously or of walking and then grooming or vice versa than ladybird beetles in the untreated plot. Grooming behaviour is a reflex action (Eisner, 1961) and is probably initiated by an irritant on chemoreceptors located on the insect body. Grooming behaviour is shown during pyrethroid poisoning in house flies (Golenda and Forgash, 1986) and therefore increased grooming behaviour by the ladybirds in the treated plot is likely to have been a symptom of pesticide uptake. Grooming may also be an important route of contamination by insecticide residues as the ladybirds may transfer pesticide from its' appendages to its' body surfaces and even mouthparts.

Less feeding was observed in the treated plot than in the control plot. This may have been a result of reduced prey availability and/or reduced stimuli from aphid honeydew in the treated plot however there may also have been more subtle interactions between sub-lethal poisoning effects on the ladybirds and feeding. For example the dietary experiments in Chapter 5 have shown that aphid consumption by the carabid beetle *N. brevicollis* may be reduced significantly for several days after sub-lethal poisoning by deltamethrin. Decreased feeding after sub-lethal exposure to pesticides has also been shown by several other authors (e.g. Dempster, 1968; Grapel, 1982; Brust *et al.* 1986).

8.4.2 The effect of deltamethrin on ladybird distribution in a wheat crop canopy

Coccinellids are positively phototactic and negatively geotactic. This is why they are often found at the apex of plants (Majerus and Kearns, 1989). The plant apex also tends to be the growing point of the plants where their prey, such as aphids, often feed. This may explain why more ladybirds were observed on the ear and flag leaves than lower in the crop canopy during the experiment in the untreated plot. The significant differences in ladybird crop distribution between the deltamethrin treated plot and the control plot on the first two days of the experiment suggested that exposure

to deltamethrin residues may cause a redistribution of ladybirds down the crop canopy towards the ground. This redistribution may have been mediated by the increased walking behaviour shown on these days which may have been caused by the sub-lethal poisoning effects of deltamethrin or decreased food availability in the treated plot.

Fewer ladybirds were found on the adaxial leaf surface than the abaxial surface on days 1 to 4. This may indicate that deltamethrin had a repellent effect on the ladybirds because spray deposits are known to be lower on the abaxial plant surface than the adaxial surface (Cilgi and Jepson, 1992). This effect may also have been caused by increased walking activity in the treated plot resulting from hunger. However all ladybirds were provided with food before the experiment, in order to reduce hunger effects, and the alterations in behaviour were reduced by days 4 and 5 despite the fact that aphids numbers were still low in the treated plot. This may indicate that deltamethrin causes a short-term irritant/repellent effect.

Although the effects of hunger should not be dismissed from these results, the higher numbers of observations of *C. septempunctata* on the abaxial leaf surface in the treated plot, together with the downward redistribution of the ladybirds in the crop canopy and the fact that all four ladybirds that flew out of the experimental plot during observation were in the treated plot may suggest that deltamethrin may have a short-term repellent effect on *C. septempunctata*. Redistribution may have important implications in terms of reducing the short-term risk posed by deltamethrin to ladybirds in cereals as spray residue levels will decline through the crop canopy (Chapter 6) and the risk posed by deltamethrin to *C. septempunctata* will be less on the soil than on foliage due to reduced bioavailability (Chapter 4).

CHAPTER 9

General discussion

9.1 Evaluating the risk posed by deltamethrin to beneficial invertebrates in temperate cereal crops

This study has taken a quantitative approach to aid the analysis of short-term pesticide side-effects on beneficial invertebrates. The series of laboratory and *in situ* bioassays that were carried out have provided an insight into the toxic risk posed by summer-applied deltamethrin sprays to a range of predator species and a parasitoid species that inhabit temperate cereal crops. The mechanistic experimental approach adopted throughout the study has achieved the goals that were set at the beginning of work and has enabled;

a) the determination of the susceptibility of predators to deltamethrin via the three main routes of exposure (topical, residual and dietary routes) and the establishment of susceptibility spectra;

b) the investigation of the mechanisms of toxicity and exposure that mediate the risk posed by deltamethrin residues to predators;

c) the determination and/or prediction of the likely levels of mortality that may result from exposure of predators to realistic concentrations of deltamethrin in cereal crops;

d) the investigation of the use of reduced-dose rates of deltamethrin to minimise mortality for a single predator species in a cereal crop.

The implications of these findings have been discussed in detail in each respective chapter and therefore the following sections only discuss the overall implications of the results and how they may be used for risk assessment, for interpreting the results of field trials and to provide a basis for the selection of organisms that may be suitable for pesticide registration testing. Finally suggestions for future work are made.

9.1.1 Qualitative and quantitative risk assessment procedures

Qualitative estimates of risk are often based on ecological criteria such as the diurnal activity cycle and position in the crop canopy of the invertebrate concerned (eg. Jepson, 1989; Jepson, 1993b). This approach provides a useful basis for deciding which species are likely to be most at risk from a spray application. For example in Table 9.1 a range of beneficial invertebrates that inhabit cereal crops were assigned arbitrary values of risk depending on their ecological characteristics. Species that were active in the crop canopy or active during the day were given scores of 2 for each

characteristic whereas species that were active on the ground or at night were given scores of 1 for each characteristic (Table 9.1). The scores were added together and the species with the highest scores were predicted to be most at risk based on the assumptions that sprays are applied to the crop during the day and that organisms present in the crop are likely to be at greater risk of exposure than those on the ground.

Table 9.1 A qualitative approach to the analysis of exposure of different taxonomic groups of beneficial arthropods in cereals to direct spraying and residual uptake of pesticides applied to cereal crops (adapted from Jepson, 1989). For direct contact, organisms active in the day (D) or on foliage (P) score 2 for each characteristic, those active at night (N) or on the ground (G) score 1. Species with the highest total score are likely to be at most risk from a spray application.

Order: Family *Species*	Score for activity and crop distribution				Total risk
	N	D	P	G	
Araneae: Linyphiidae					
E. atra	1			1	2
L. tenuis	1		2		3
Coleoptera: Staphylinidae					
T. hypnorum	1		2		3
Coleoptera: Coccinellidae					
C. septempunctata (A)		2	2		4
C. septempunctata (L)		2	2		4
Coleoptera: Carabidae					
B. obtusum		2		1	3
B. lampros		2		1	3
T. quadristriatus		2		1	3
D. atricapillus		2	2		4
A. dorsale	1			1	2
H. rufipes	1			1	2
N. brevicollis	1			1	2
P. melanarius	1			1	2
Hymenoptera: Braconidae					
A. rhopalosiphi		2	2		4
Diptera: Syrphidae					
E. balteatus (A)		2	2		4
E. balteatus (L)	1		2		3

The predictions suggested that species such as the coccinellid *C. septempunctata* (adults and larvae), the carabid beetle *D. atricapillus*, the parasitoid *A. rhopalosiphi* and the adult stages of the hoverfly *E. balteatus* may be most at risk from a spray application. The limitations of this approach however are that the predictions are crude and cannot distinguish between the risks posed by a spray to organisms with opposite habits i.e. nocturnal and plant-active species and diurnal and ground-active species. Therefore the main value of this approach is to structure the system under investigation and to provide a guide for the selection of appropriate substrate and

routes of exposure for further toxicological tests.

The usefulness of these predictions may be improved by incorporating toxicological criteria in the form of weighting factors to the rankings. These weighting factors may include measurements of the relative susceptibilities of the given species to the pesticide and the relative toxicity of the pesticide on the substrates that the organisms are likely to be exposed, i.e. crop foliage and the soil. In this study the twelve species of beneficial invertebrates tested in the laboratory topical bioassays were found to have a 300 fold range of susceptibilities to deltamethrin (Chapter 2). Also the toxicity of fresh residues of deltamethrin to given predator species on cereal flag leaves was estimated to be approximately 60 times greater than residues on a sandy loam soil (Chapter 4). The values of the relative susceptibilities of the predators to deltamethrin and the estimate of the relative toxicity of deltamethrin on flag leaves and soil were incorporated within the ecological exposure categories in Table 9.2. The risk posed by the direct contact route of exposure to the predators was not quantified for all species

Table 9.2 A quantitative approach to the analysis of exposure of different taxonomic groups of beneficial arthropods in cereals to direct spraying and residual uptake of deltamethrin. For direct contact, organisms active in the day (D) score 2 and those active at night (N) score 1. For residual exposure those active on the plant (P) score 60 whereas those that are active on the ground score 1. The susceptibility values were calculated relative to the susceptibility of the least susceptible predator species (*D. atricapillus*). The species with the highest multiplicative score are likely to be at most risk from a spray application.

Order: Family *Species*	Relative susceptibility Topical 72 h LD_{50} (ng AI arthropod^{-1})	Score for activity and crop distribution N	D	P	G	Total risk
Araneae: Linyphiidae						
E. atra	289.5	1			1	289.5
Coleoptera: Staphylinidae						
T. hypnorum	18.53	1		60		1111.8
Coleoptera: Coccinellidae						
C. septempunctata (A)	2.33		2	60		279.6
C. septempunctata (L)	7.42		2	60		890.4
Coleoptera: Carabidae						
B. obtusum	18.23		2		1	36.5
B. lampros	17.95		2		1	35.9
T. quadristriatus	15.04		2		1	30.1
D. atricapillus	1.00		2	60		120.0
A. dorsale	2.77	1			1	2.8
H. rufipes	1.57	1			1	1.6
N. brevicollis	1.05	1			1	1.0
P. melanarius	1.71	1			1	1.7

and therefore no changes were made to the arbitrary values used in the qualitative

analysis. However studies of direct contact exposure have shown that considerable differences in exposure levels will exist for predators distributed at different levels in the crop canopy (Chapter 6).

The predicted values of risk given in Table 9.2 differed from those produced in Table 9.1. Most noticeably the linyphiid spider *E. atra* was predicted to be at lower risk to a spray application than the diurnal Carabidae in Table 9.1 whereas this species was predicted to be at a higher risk than these species in the quantitative ranking in Table 9.2. This was attributable to its' high susceptibility to deltamethrin relative to the other predators tested. The predator predicted to be most at risk by this ranking was the staphylinid beetle *T. hypnorum*. This predator was also highly susceptible to deltamethrin and may be at a high risk from exposure to deltamethrin because it is plant-active. Both adults and larvae of the coccinellid *C. septempunctata* were predicted to be at a high risk from a deltamethrin spray relative to the other predators and even the least susceptible predator tested, *D. atricapillus*, was predicted to be at relatively high risk from exposure to deltamethrin than the more susceptible ground-active carabid beetles. These risk predictions have indicated that plant-active species are likely to be at greater risk of suffering effects from a deltamethrin spray than ground-active predators because of the large difference in toxic risk posed by the two substrates. The exception to this was the ground-active linyphiid spider *E. atra* which was one to two orders of magnitude more susceptible to deltamethrin than any of the other species and was therefore predicted to be at a relatively high risk even on the soil.

This simple approach to risk prediction has enabled the identification of species which may be most at risk from a deltamethrin spray. The predictions agreed with the reductions observed in field trial studies. For example Basedow *et al.* (1985) and Fischer and Chambon (1987) found reductions in linyphiid spiders, Basedow *et al.* (1985) and Vickerman *et al.* (1987b) found reductions in staphylinid beetles, *Tachyporus* spp., and Vickerman *et al.* (1987b) found reductions in *D. atricapillus* and *C. septempunctata* larvae. Therefore quantitative risk assessment procedures of this form may offer a relatively simple method for determining which species are likely to be at most risk from exposure to a given chemical by using a limited amount of data concerning the susceptibility of the test organisms to the pesticide and the relative toxicity of the compound on realistic substrates.

In order to improve the predictions further it may be necessary to take into account

other routes of exposure, such as dietary exposure and/or intrinsic species characteristics which may affect exposure to pesticide deposits, such as the contact area of a given species with treated substrates. Dietary exposure experiments with the large carabid beetle *N. brevicollis* in this study have indicated that this route of exposure may potentially be an important cause of mortality for some predators after a spray application (Chapter 5 and Appendix 4). Higher levels of mortality were observed in the laboratory from the consumption of deltamethrin contaminated prey by *N. brevicollis* than were predicted from direct contact with spray under a cereal crop and from contact with spray residues on the soil (Appendix 4). The effects of this route of exposure are still unquantified in the field however and require further investigation with a wider range of species to determine the importance of this route of exposure. Also studies undertaken to investigate species specific characteristics that may influence an organisms' exposure to pesticide residues have indicated that within a group of seven coleopteran predators differences in exposure may vary by approximately twenty-three fold based on an index of exposure from measurements of walking speed and contact area with the substrate (Chapter 3). Therefore this suggests that even within species with similar habits on the same substrate large differences in pesticide exposure and uptake may occur.

9.1.2 Susceptibility rankings for beneficial invertebrates in cereals to deltamethrin

Rankings of the relative susceptibilities and mortalities determined in the laboratory and *in situ* bioassays carried out in this study are summarised in Table 9.3. These rankings may provide a useful overall guide for the selection of species which may be at greatest risk from different routes of exposure or aid the selection of organisms for pesticide registration testing in cereals. The rankings may only be compared between species within each particular bioassay type, however overall trends may be found within species between the different bioassays. For example the linyphiid spiders were found to be the most susceptible predators tested topically in the laboratory and when exposed to deltamethrin residues on the soil. Therefore, this suggests that these predators are very sensitive to deltamethrin. This agrees with the findings of several other authors eg. Everts (1990), Mullié and Everts (1991) and Jagers op Akkerhuis and Hamers (1992). Other species that were also ranked highly in several different tests were *C. septempunctata* 4th instar larvae and the staphylinid *T. hypnorum*. These predators are both plant-active and are known to be relatively susceptible to deltamethrin (Chapters 2, 3 & 4) compared to the other species tested. The predators that were ranked at the lowest risk in the bioassays were the larger carabids (eg. *N.*

Table 9.3 Quantitative susceptibility rankings for aphid predators that inhabit temperate cereal crops to deltamethrin.

LD_{50} values, susceptibility index values and % mortality values for the species tested are ranked in order of susceptibility; Ranking value 1 = most susceptible, the highest ranking value = least susceptible species.

Order: Family *Species*	Laboratory topical bioassays LD_{50} (ng AI arthropod^{-1}) (Chapter 2)	Susceptibility index (v.w.a/LD_{50}) (Chapter 3)	Laboratory residual bioassays LD_{50} (g AI ha^{-1}) 2 h Flag leaf	72 h Soil (Chapter 4)	Direct exposure to spray in the (Predicted % mortality) Foliage	Soil* (Chapter 6)	Residual exposure in the field (% Mortality) Flag leaf	Soil (Chapter 7) (Appendix 3)
Araneae: Linyphiidae								
E. atra (♀)	1							
L. tenuis								1
Coleoptera: Staphylinidae								
T. hypnorum	2	1	2	1			1	
Coleoptera: Coccinellidae								
C. septempunctata (1)	7							
C. septempunctata (L)	6		1		1	1		
C. septempunctata (2)	9	6	3	3	2	3	2	2
Coleoptera: Carabidae								
B. obtusum	3							
B. lampros	4	3		2		5		3
T. quadristriatus	5							
D. atriacapillus	13	7	6	6	3	5	3	
A. dorsale	8	4						
N. brevicollis	12	5		6				
H. rufipes	11			5		4		
P. melanarius	10	2		4		2		4
Diptera: Syrphidae								
E. balteatus (A)			4					
Hymenoptera: Braconidae								
A. rhopalosiphi			5					

C. septempunctata (1) - Adults captured in May, *C. septempunctata* - 4th instar larvae captured in July, *C. septempunctata* (2) - Adults captured in July. *E. balteatus* (A) = Adults

* - Rankings of direct exposure on soil assume that all species were present on the soil during spray application. This may not be the case for some of the nocturnal species (see Table 9.1).

brevicollis and *H. rufipes*) which were of relatively low susceptibility to deltamethrin (Chapters 2 & 4) and may be at lower risk from deltamethrin because they are nocturnal and ground-active. The small carabid beetle *D. atricapillus* also had a low rank which may be explained by the fact that it appeared to be tolerant to deltamethrin (Chapter 2) and to have a low contact area with substrates and therefore may experience lower levels of contact with spray residues than the other predator species.

9.1.3 The development of methodologies to select optimum dose rates to preserve beneficial invertebrates and promote biological control

A simple framework for predicting optimum pesticide dose rates to minimise predator mortality was developed during the study. Predictions of mortality levels were made by integrating topical dose-response data and spray deposition data for a given species to predict the levels of mortality that may result from direct contact with spray. These were then combined with the levels of mortality of the organism that occured from exposure to pesticide residues using *in situ* bioassays.

The experiments carried out with reduced dose rates of deltamethrin in this study have suggested that dose rates of a quarter of the recommended field rate of deltamethrin (1.56 g AI ha^{-1}) may provide a degree of aphid control and was predicted to preserve approximately 50% of the adult coccinellids in the crop at the time of spraying and the following 10 days. Therefore this may suggest that there is scope for reducing dose rates of deltamethrin in cereals as an approach to promote biological control and reduce pesticide inputs and the cost to the farmer. To do this however the predicted effects of deltamethrin applied at a quarter of the recommended field rate needs validation for natural predator populations in the field. Also the reliability of the predators in providing control of residual pest populations needs to be determined.

9.1.4 Conclusions

Overall the predictive approach developed in this study may be applied to many other chemicals and cropping systems. The techniques used are based on relatively simple and flexible bioassay and spray quantification techniques set out in a basic experimental framework. This approach may provide a first step to improve our ability to manage the short-term side-effects of pesticides and to limit the impact of sprays on beneficial invertebrates. By defining dose rates which minimise effects on predators it may be possible to incorporate chemical pest control with integrated pest management strategies which maximise the contribution made by beneficial organisms to pest control.

9.2 Future work

In order to improve the accuracy of risk predictions for invertebrates advances in measuring pesticide uptake and body burdens received from the various routes of exposure are required. This may be achieved by the use of radio-labelled pesticides (eg. Mullié and Everts, 1991; Jagers op Akkerhuis and Hamers, 1992) or residue analysis techniques. Also detailed toxicological information is required concerning the toxic interactions of pesticide doses received by different routes of exposure in order that mortality derived from mechanistic studies may be used to predict overall effects on given invertebrate species.

Of the main routes of exposure, advances are most needed in quantifying the residual uptake of pesticides by organisms. *In situ* bioassays may be the most readily available and cost effective methods for determining uptake in a crop but they are limited as they confine invertebrates on relatively small and localised areas of the crop (see Appendix 3). Modelling uptake may be one of the most promising ways forward, however the existing models that seek to predict exposure, uptake and effects mediated by pharmacokinetics are complex and are over-parameterised to be of practical value (eg. Salt and Ford, 1984). An alternative may be dynamic models, which are based on differential equations for pesticide deposition and degradation, differential equations for rates of pesticide intake and clearance and hazard functions related to pesticide retention curves (eg. Schaalje, 1990). This approach however requires the use of parameter estimation techniques which provide little information to explain differences between species. Also neither of these approaches explicitly consider the heterogenous distribution of pesticides in the crop (eg. Chapter 6; Cilgi and Jepson, 1992) or the differing properties of plant foliage and soil with respect to bioavailability (eg. Chapter 4). Therefore there is a need to identify which parameters are most important in order to develop a more practical predictive approach. Once developed and validated these predictive models may improve our understanding and ability to manage pesticide side-effects and to select optimum dose rates for pest management purposes. However, they cannot be viewed as an endpoint in themselves and may eventually be incorporated within models of long-term effects, which are mediated by processes of recolonisation and dispersal, to predict effects on populations.

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APPENDIX 1

Topical dose-response data from definitive bioassays.

n = number of individuals tested per dose

M = numbers moving as normal

KD = number knocked down

D = number dead

Species : *P. melanarius*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	30	30	0	0
100	30	16	11	3
200	30	14	10	6
300	30	12	7	11
400	30	11	5	14
500	30	6	7	17
600	30	3	12	15

48 H

Control	30	30	0	0
100	30	21	5	4
200	30	19	2	9
300	30	16	2	12
400	30	12	4	14
500	30	6	4	20
600	30	3	3	24

72 H

Control	30	30	0	0
100	30	17	0	13
200	30	10	2	18
300	30	7	2	21
400	30	6	1	23
500	30	6	0	24
600	30	3	1	26

96 H

Control	30	30	0	0
100	30	17	0	13
200	30	12	0	18
300	30	9	0	21
400	30	7	0	23
500	30	5	1	24
600	30	4	0	26

Species : *N. brevicollis*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	30	30	0	0
100	30	12	12	6
200	30	9	13	8
400	30	6	12	12
600	30	2	8	20
800	30	0	9	21

48 H

Control	30	30	0	0
100	30	22	0	8
200	30	15	4	11
400	30	12	2	16
600	30	7	2	21
800	30	0	6	24

72 H

Control	30	30	0	0
100	30	20	0	10
200	30	17	0	13
400	30	9	3	18
600	30	4	3	23
800	30	0	4	26

96 H

Control	30	30	0	0
100	30	20	0	10
200	30	17	0	13
400	30	11	0	19
600	30	6	0	24
800	30	4	0	26

Species : *D. atricapillus*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	30	30	0	0
100	30	7	18	5
200	30	5	17	8
250	30	6	14	10
300	30	5	13	12
400	30	3	10	17
600	30	2	7	21

48 H

Control	30	30	0	0
100	30	10	14	6
200	30	6	13	11
250	30	6	11	13
300	30	6	9	15
400	30	4	8	18
600	30	3	4	23

72 H

Control	30	30	0	0
100	30	17	5	8
200	30	11	4	14
250	30	11	4	15
300	30	11	2	17
400	30	8	3	19
600	30	4	2	24

96 H

Control	30	30	0	0
100	30	17	4	9
200	30	13	3	14
250	30	12	3	15
300	30	8	2	20
400	30	6	3	21
600	30	4	0	26

Species : *H. rufipes*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	20	20	0	0
50	20	20	0	0
100	20	11	7	2
200	20	8	8	4
300	20	6	10	2
400	20	4	5	11
500	20	1	13	6

48 H

Control	20	20	0	0
50	20	20	0	0
100	20	14	2	4
200	20	8	1	11
300	20	4	3	13
400	20	4	2	14
500	20	1	1	18

72 H

Control	20	20	0	0
50	20	20	0	0
100	20	12	1	7
200	20	6	1	13
300	20	6	0	14
400	20	3	0	17
500	20	0	0	20

96 H

Control	20	20	0	0
50	20	20	0	0
100	20	12	0	8
200	20	7	1	12
300	20	6	0	14
400	20	2	0	18
500	20	0	0	20

Species : *A. dorsalis*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	30	30	0	0
50	30	8	18	4
70	30	10	13	7
100	30	2	18	10
150	30	6	12	12
200	30	2	14	14
300	30	1	9	20
400	30	0	3	27

48 H

Control	30	30	0	0
50	30	8	14	8
70	30	8	14	8
100	30	6	10	14
150	30	2	10	18
200	30	4	6	20
300	30	0	6	24
400	30	0	0	30

72 H

Control	30	30	0	0
50	30	12	8	10
70	30	8	8	14
100	30	8	6	16
150	30	2	8	20
200	30	3	3	24
300	30	0	0	30
400	30	0	0	30

96 H

Control	30	30	0	0
50	30	16	2	12
70	30	13	1	16
100	30	11	1	18
150	30	8	0	22
200	30	2	0	28
300	30	0	0	30
400	30	0	0	30

Species : *B. obtusum*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	20	20	0	0
5	20	8	10	2
10	20	5	10	5
20	20	2	10	8
50	20	0	6	14
100	20	0	2	18

48 H

Control	20	20	0	0
5	20	10	6	4
10	20	6	7	7
20	20	3	6	11
50	20	1	4	15
100	20	0	1	19

72 H

Control	20	20	0	0
5	20	13	2	5
10	20	11	0	9
20	20	5	2	13
50	20	3	1	16
100	20	0	0	20

96 H

Control	20	19	0	1
5	20	10	2	8
10	20	11	0	9
20	20	6	0	14
50	20	4	0	16
100	20	0	0	20

Species : *T. quadristriatus*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	40	40	0	0
10	40	25	10	5
20	40	8	20	12
30	40	7	22	11
40	40	2	10	28
50	40	0	10	30

48 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	40	40	0	0
10	40	29	1	10
20	40	11	9	20
30	40	9	2	29
40	40	4	4	32
50	40	0	4	36

72 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	40	40	0	0
10	40	27	0	13
20	40	17	1	22
30	40	7	1	32
40	40	4	0	36
50	40	0	0	40

96 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	40	39	0	1
10	40	27	0	13
20	40	16	0	24
30	40	7	0	33
40	40	2	0	38
50	40	0	0	40

Species : *T. hypnorum*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	30	30	0	0
5	30	22	8	0
10	30	10	14	6
20	30	8	8	14
30	30	3	7	20
40	30	2	7	21
60	30	0	4	26

48 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	30	30	0	0
5	30	21	4	5
10	30	14	6	10
20	30	8	6	16
30	30	4	3	23
40	30	1	3	26
60	30	0	1	29

72 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	30	30	0	0
5	30	19	6	5
10	30	18	2	10
20	30	8	1	21
30	30	4	0	26
40	30	0	0	30
60	30	0	1	29

96 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	30	30	0	0
5	30	17	8	5
10	30	17	1	12
20	30	7	0	23
30	30	2	0	28
40	30	0	0	30
60	30	0	0	30

Species : *E. atra* (Females)

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	10	10	0	0
0.5	10	5	2	3
1.0	10	4	3	3
1.5	10	3	1	6
2.0	10	2	3	5
2.5	10	2	0	8
3.0	10	2	0	8

48 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	10	10	0	0
0.5	10	5	1	4
1.0	10	3	1	6
1.5	10	3	1	6
2.0	10	1	3	6
2.5	10	1	0	9
3.0	10	0	0	10

72 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	10	10	0	0
0.5	10	5	1	4
1.0	10	3	1	6
1.5	10	2	1	7
2.0	10	0	0	10
2.5	10	0	0	10
3.0	10	0	0	10

96 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	10	7	0	3
0.5	10	6	0	4
1.0	10	2	0	8
1.5	10	1	0	9
2.0	10	0	0	10
2.5	10	0	0	10
3.0	10	0	0	10

Species : *B. lamprce*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	20	20	0	0
5	20	14	5	1
10	20	11	6	3
20	20	6	7	7
50	20	2	2	16
70	20	1	1	18
100	20	0	0	20

48 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	20	20	0	0
5	20	15	2	3
10	20	11	6	3
20	20	7	4	9
50	20	2	2	16
70	20	1	1	18
100	20	0	0	20

72 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	20	20	0	0
5	20	13	2	5
10	20	11	2	7
20	20	7	3	10
50	20	1	0	19
70	20	0	0	20
100	20	0	0	20

96 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	20	20	0	0
5	20	14	0	6
10	20	11	0	9
20	20	7	1	12
50	20	0	0	20
70	20	0	0	20
100	20	0	0	20

Species : *C. septempunctata* (4th instar larvae)

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	20	20	0	0
10	20	1	19	0
20	20	0	18	2
40	20	0	17	3
80	20	0	16	4
100	20	0	13	7
200	20	0	11	9

48 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	20	18	0	2
10	20	3	17	0
20	20	0	18	2
40	20	0	17	3
80	20	0	16	4
100	20	0	12	8
200	20	0	7	13

72 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	20	18	0	2
10	20	12	4	4
20	20	8	3	9
40	20	4	4	12
80	20	3	0	17
100	20	0	0	20
200	20	0	0	20

96 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	20	18	0	2
10	20	13	0	7
20	20	10	0	10
40	20	6	0	14
80	20	3	0	17
100	20	0	0	20
200	20	0	0	20

Species : *S. avenae (Adults)*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	30	30	0	0
10	30	13	6	11
25	30	5	9	16
50	30	10	5	15
100	30	2	4	24
200	30	0	4	26
500	30	0	0	30

48 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	30	30	0	0
10	30	11	4	15
25	30	5	4	21
50	30	7	3	20
100	30	4	0	26
200	30	0	2	28
500	30	0	0	30

72 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	30	30	0	0
10	30	12	0	18
25	30	8	0	22
50	30	6	0	24
100	30	1	0	29
200	30	0	0	30
500	30	0	0	30

96 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	30	28	0	2
10	30	12	0	18
25	30	8	0	22
50	30	5	0	25
100	30	0	0	30
200	30	0	0	30
500	30	0	0	30

Species : *C. septempunctata (May Gen. Adults)*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	30	30	0	0
10	30	13	17	0
25	30	5	25	0
50	30	3	27	0
100	30	5	23	2
150	30	6	18	6
200	30	5	18	7
300	30	5	12	13
400	30	1	21	18

48 H

Control	30	30	0	0
10	30	25	3	2
25	30	18	7	5
50	30	5	19	6
100	30	5	16	9
150	30	3	14	13
200	30	5	5	20
300	30	3	5	22
400	30	0	4	26

72 H

Control	30	30	0	0
10	30	27	0	3
25	30	20	4	6
50	30	17	3	10
100	30	12	4	14
150	30	8	2	20
200	30	4	2	24
300	30	2	0	28
400	30	0	0	30

96 H

Control	30	30	0	0
10	30	27	0	3
25	30	21	0	9
50	30	15	0	15
100	30	9	0	21
150	30	5	1	25
200	30	2	0	28
300	30	0	0	30
400	30	0	0	30

Species : *C. septempunctata (July Gen. Adults)*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (ng AI/ Arthropod)	n	M	KD	D
Control	30	30	0	0
50	30	18	11	1
100	30	4	20	6
200	30	0	20	10
300	30	0	14	16
400	30	0	9	21
500	30	0	4	26

48 H

Control	30	30	0	0
50	30	18	9	3
100	30	6	14	10
200	30	2	12	16
300	30	0	12	18
400	30	0	6	24
500	30	0	3	27

72 H

Control	30	30	0	0
50	30	21	0	9
100	30	15	0	15
200	30	8	0	21
300	30	5	1	24
400	30	4	1	26
500	30	0	0	30

96 H

Control	30	30	0	0
50	30	21	0	9
100	30	15	0	15
200	30	8	0	21
300	30	5	1	24
400	30	3	0	27
500	30	0	0	30

APPENDIX 2

Residual dose-response data from definitive bioassays.

Surfaces sprayed under a Potter Tower calibrated to deliver spray at a volume rate equivalent to 200 l/ha.

n = number of individuals tested per dose

M = number moving as normal

KD = number knocked down

D = number dead

Bioassay : 2 H Soil

Species : *T. hypnorum*

Chemical : Deltamethrin 2.5% E.C.

2 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
9.2	20	6	14	0
18.3	20	0	20	0
27.5	20	0	20	0
36.7	20	0	20	0
91.7	20	0	20	0
183.4	20	0	20	0

24 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
9.2	20	17	3	0
27.5	20	8	9	3
36.7	20	6	10	4
91.7	20	8	9	3
183.4	20	3	9	8

48 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
9.2	20	19	0	1
27.5	20	8	9	3
36.7	20	6	10	4
91.7	20	7	6	7
183.4	20	0	7	13

72 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
9.2	20	16	1	3
27.5	20	12	1	7
36.7	20	10	1	9
91.7	20	6	3	11
183.4	20	3	1	16

96 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
9.2	20	16	1	3
27.5	20	10	3	7
36.7	20	10	1	9
91.7	20	6	3	11
183.4	20	4	0	16

120 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
9.2	20	17	0	3
27.5	20	12	1	7
36.7	20	10	0	10
91.7	20	7	1	12
183.4	20	4	0	16

Bioassay : 2 H Soil

Species : *C. septempunctata (Adults)*

Chemical : Deltamethrin 2.5% E.C.

2 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
36.7	20	2	18	0
91.7	20	0	20	0
183.4	20	0	20	0
366.8	20	0	20	0

24 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
36.7	20	16	11	3
91.7	20	14	10	6
183.4	20	12	7	11
366.8	20	11	5	14

48 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
36.7	20	16	11	3
91.7	20	14	10	6
183.4	20	12	7	11
366.8	20	11	5	14

72 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
36.7	20	16	0	4
91.7	20	11	0	9
183.4	20	3	2	15
366.8	20	2	1	17

96 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
36.7	20	16	0	4
91.7	20	11	0	9
183.4	20	4	1	15
366.8	20	2	1	17

120 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
36.7	20	16	0	4
91.7	20	11	0	9
183.4	20	5	0	15
366.8	20	3	0	18

Bioassay : 2 H Soil

Species : *N. brevicollis*

Chemical : Deltamethrin 2.5% E.C.

2 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
43.1	20	8	12	0
91.2	20	3	17	0
121.2	20	0	20	0
169.7	20	0	20	0

24 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
43.1	20	18	2	0
91.2	20	14	6	0
121.2	20	13	5	0
169.7	20	12	6	2

48 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
43.1	20	19	1	0
91.2	20	17	3	0
121.2	20	17	3	0
169.7	20	14	4	2

72 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
43.1	20	18	2	0
91.2	20	19	0	1
121.2	20	19	1	0
169.7	20	16	1	3

96 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
43.1	20	18	2	0
91.2	20	19	0	1
121.2	20	19	1	0
169.7	20	16	1	3

120 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
43.1	20	20	0	0
91.2	20	19	0	1
121.2	20	20	0	0
169.7	20	16	0	4

Bioassay : 2 H Soil

Species : D. atricapillus

Chemical : Deltamethrin 2.5% E.C.

2 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
31.2	20	12	9	0
72.4	20	7	13	0
151.2	20	6	14	0
201.3	20	0	20	0
499.7	20	0	20	0

24 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
31.2	20	19	1	0
72.4	20	16	4	0
151.2	20	18	2	0
201.3	20	16	4	0
499.7	20	11	9	0

48 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
31.2	20	20	0	0
72.4	20	19	1	0
151.2	20	20	0	0
201.3	20	18	2	0
499.7	20	18	1	1

72 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
31.2	20	20	0	0
72.4	20	19	0	1
151.2	20	20	0	0
201.3	20	20	0	0
499.7	20	18	1	1

96 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
31.2	20	20	0	0
72.4	20	19	0	1
151.2	20	20	0	0
201.3	20	20	0	0
499.7	20	18	0	2

Bioassay : 24 H Soil

Species : C. septempunctata (Adults)

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
1.83	20	8	12	0
9.17	20	6	14	0
18.34	20	2	16	2
36.67	20	0	14	6
55.02	20	0	15	5

48 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
1.83	20	8	9	3
9.17	20	6	8	6
18.34	20	1	11	8
36.67	20	1	8	11
55.02	20	0	7	13

72 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
1.83	20	16	0	4
9.17	20	13	0	7
18.34	20	7	4	9
36.67	20	7	2	11
55.02	20	4	1	15

96 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
1.83	20	17	0	3
9.17	20	15	0	5
18.34	20	10	1	9
36.67	20	7	0	13
55.02	20	3	0	17

120 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
1.83	20	17	0	3
9.17	20	15	0	5
18.34	20	11	0	9
36.67	20	7	0	13
55.02	20	3	0	17

Bioassay : 2 H Glass

Species : *D. atricapillus*

Chemical : Deltamethrin 2.5% E.C.

2 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
1.7	30	9	21	0
8.5	30	0	30	0
16.9	30	0	30	0
25.4	30	0	30	0
36.7	30	0	30	0

24 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
1.7	30	22	8	0
8.5	30	21	8	1
16.9	30	15	15	0
25.4	30	12	18	0
36.7	30	5	25	0

48 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
1.7	30	26	4	0
8.5	30	22	7	1
16.9	30	19	8	3
25.4	30	21	6	3
36.7	30	18	7	5

72 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
1.7	30	28	0	2
8.5	30	24	2	4
16.9	30	23	1	6
25.4	30	18	4	8
36.7	30	19	2	9

96 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
1.7	30	28	0	2
8.5	30	25	1	4
16.9	30	23	0	7
25.4	30	20	2	8
36.7	30	19	1	10

Bioassay : 2 H Glass

Species : *T. hypnorum*

Chemical : Deltamethrin 2.5% E.C.

2 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.57	20	1	19	0
0.85	20	0	20	0
1.7	20	0	20	0
2.54	20	0	20	0

24 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.57	20	13	7	0
0.85	20	7	11	2
1.7	20	7	7	6
2.54	20	0	20	5

48 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.57	20	11	6	3
0.85	20	9	5	6
1.7	20	6	3	11
2.54	20	2	4	14

72 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.57	20	16	0	4
0.85	20	12	1	7
1.7	20	9	0	11
2.54	20	2	1	17

96 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.57	20	16	0	4
0.85	20	13	0	7
1.7	20	9	0	11
2.54	20	2	1	17

120 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.57	20	16	0	4
0.85	20	12	0	8
1.7	20	9	0	11
2.54	20	3	0	17

Bioassay : 2 H Glass

Species : *C. septempunctata* (Adults)

Chemical : Deltamethrin 2.5% E.C.

2 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.51	20	4	16	0
0.85	20	5	15	0
1.7	20	0	20	0
2.7	20	0	20	0
3.4	20	0	20	0

24 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.51	20	14	5	1
0.85	20	10	9	1
1.7	20	5	10	5
2.7	20	5	9	6
3.4	20	5	7	8

48 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.51	20	18	1	1
0.85	20	15	2	3
1.7	20	8	4	8
2.7	20	7	6	7
3.4	20	3	3	14

72 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.51	20	18	0	2
0.85	20	17	0	3
1.7	20	6	1	13
2.7	20	7	0	13
3.4	20	3	1	16

96 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.51	20	18	0	2
0.85	20	18	0	3
1.7	20	6	1	13
2.7	20	6	1	13
3.4	20	4	0	16

120 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.51	20	18	0	2
0.85	20	17	0	3
1.7	20	7	0	13
2.7	20	7	0	13
3.4	20	4	0	16

Bioassay : 2 H Glass

Species : *N. brevicollis*

Chemical : Deltamethrin 2.5% E.C.

2 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
1.83	20	3	17	0
9.17	20	0	20	0
18.34	20	0	20	0
36.67	20	0	20	0

24 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
1.83	20	15	5	0
9.17	20	13	7	0
18.34	20	10	10	0
36.67	20	12	7	1

48 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
1.83	20	18	2	0
9.17	20	15	5	0
18.34	20	10	8	2
36.67	20	10	5	5

72 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
1.83	20	20	0	0
9.17	20	20	0	0
18.34	20	17	0	3
36.67	20	11	1	8

96 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
1.83	20	20	0	0
9.17	20	20	0	0
18.34	20	15	0	5
36.67	20	11	0	9

120 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
1.83	20	20	0	0
9.17	20	20	0	0
18.34	20	15	0	5
36.67	20	11	0	9

Bioassay : 2 H Flag leaf

Species : *T. hypnorum*

Chemical : Deltamethrin 2.5% E.C.

2 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.51	20	0	20	0
1.02	20	0	20	0
1.35	20	0	20	0
2.03	20	0	20	0

24 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.51	20	14	3	3
1.02	20	10	6	4
1.35	20	7	9	4
2.03	20	1	7	12

48 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.34	20	14	2	4
0.85	20	11	2	7
1.2	20	7	3	10
1.7	20	5	3	12

72 H

Dose (g AI/ha)	n	M	KD	D
Control	20	19	0	1
0.51	20	14	0	6
1.02	20	12	1	7
1.35	20	7	1	12
2.03	20	4	0	16

96 H

Dose (g AI/ha)	n	M	KD	D
Control	20	19	0	1
0.51	20	14	0	6
1.02	20	13	0	7
1.35	20	7	0	13
2.03	20	4	0	16

120 H

Dose (g AI/ha)	n	M	KD	D
Control	20	19	0	1
0.51	20	14	0	6
1.02	20	13	0	7
1.35	20	7	0	13
2.03	20	4	0	16

Bioassay : 2 H Flag leaf

Species : *C. septempunctata* (Adults)

Chemical : Deltamethrin 2.5% E.C.

2 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.85	20	0	20	0
1.70	20	0	20	0
3.40	20	0	20	0
4.25	20	0	20	0

24 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.85	20	3	17	0
1.70	20	0	16	4
3.40	20	0	14	6
4.25	20	0	10	10

48 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.85	20	11	6	3
1.70	20	8	3	9
3.40	20	6	3	11
4.25	20	3	3	14

72 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.85	20	17	0	3
1.70	20	10	1	9
3.40	20	4	2	14
4.25	20	3	1	16

96 H

Dose (g AI/ha)	n	M	KD	D
Control	20	19	0	1
0.85	20	17	0	3
1.70	20	10	0	10
3.40	20	4	2	14
4.25	20	3	0	17

120 H

Dose (g AI/ha)	n	M	KD	D
Control	20	19	0	1
0.85	20	17	0	3
1.70	20	10	0	10
3.40	20	5	1	14
4.25	20	3	0	17

Bioassay : 2 H Flag leaf

Species : *C. septempunctata* (4th Instar)

Chemical : Deltamethrin 2.5% E.C.

2 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.10	20	0	20	0
0.21	20	0	20	0
1.01	20	0	20	0
2.03	20	0	20	0

24 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
0.10	20	14	17	0
0.21	20	9	11	0
1.01	20	6	10	4
2.03	20	0	13	7

48 H

Dose (g AI/ha)	n	M	KD	D
Control	20	19	0	1
0.10	20	11	5	4
0.21	20	10	4	6
1.01	20	5	6	9
2.03	20	3	3	14

72 H

Dose (g AI/ha)	n	M	KD	D
Control	20	19	0	1
0.10	20	17	0	5
0.21	20	10	1	8
1.01	20	4	3	13
2.03	20	1	1	18

96 H

Dose (g AI/ha)	n	M	KD	D
Control	20	17	0	3
0.10	20	17	0	5
0.21	20	11	0	8
1.01	20	4	1	14
2.03	20	1	0	19

120 H

Dose (g AI/ha)	n	M	KD	D
Control	20	16	0	4
0.10	20	17	0	3
0.21	20	10	0	10
1.01	20	2	0	18
2.03	20	0	0	20

Bioassay : 2 H Flag leaf

Species : *D. atricapillus*

Chemical : Deltamethrin 2.5% E.C.

2 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
16.9	20	1	19	0
25.4	20	0	20	0
33.9	20	0	20	0
50.8	20	0	20	0

24 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
16.9	20	20	0	0
25.4	20	16	4	0
33.9	20	16	4	0
50.8	20	14	6	0

48 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
16.9	20	20	0	0
25.4	20	20	0	0
33.9	20	16	3	1
50.8	20	17	2	1

72 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
16.9	20	20	0	0
25.4	20	20	0	0
33.9	20	19	0	1
50.8	20	17	2	1

96 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
16.9	20	20	0	0
25.4	20	20	0	0
33.9	20	19	0	1
50.8	20	17	1	2

120 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
16.9	20	20	0	0
25.4	20	20	0	0
33.9	20	19	0	1
50.8	20	17	1	2

Bioassay : 2 H Flag leaf

Species : *E. balteatus* (Adults)

Chemical : Deltamethrin 2.5% E.C.

2 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
3.39	20	0	20	0
6.78	20	0	20	0
10.16	20	0	20	0
13.54	20	0	20	0

24 H

Dose (g AI/ha)	n	M	KD	D
Control	20	20	0	0
3.39	20	12	2	6
6.78	20	8	4	8
10.16	20	3	5	12
13.54	20	2	3	15

48 H

Dose (g AI/ha)	n	M	KD	D
Control	20	19	0	1
3.39	20	10	2	8
6.78	20	10	1	9
10.16	20	4	3	13
13.54	20	2	3	15

72 H

Dose (g AI/ha)	n	M	KD	D
Control	20	19	0	1
3.39	20	10	0	10
6.78	20	8	1	11
10.16	20	4	1	15
13.54	20	1	0	19

96 H

Dose (g AI/ha)	n	M	KD	D
Control	20	18	0	2
3.39	20	10	0	10
6.78	20	8	0	12
10.16	20	4	1	15
13.54	20	1	0	19

120 H

Dose (g AI/ha)	n	M	KD	D
Control	20	17	0	3
3.39	20	10	0	10
6.78	20	8	0	12
10.16	20	4	0	16
13.54	20	1	0	19

Bioassay : 2 H Flag leaf

Species : *A. rhopalosiphi*

Chemical : Deltamethrin 2.5% E.C.

2 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
2.82	30	0	30	0
5.64	30	0	30	0
8.46	30	0	30	0
11.28	30	0	30	0

24 H

Dose (g AI/ha)	n	M	KD	D
Control	30	28	0	2
2.82	30	23	0	7
5.64	30	12	7	11
8.46	30	10	8	12
11.28	30	8	3	19

48 H

Dose (g AI/ha)	n	M	KD	D
Control	30	28	0	2
2.82	30	23	0	7
5.64	30	16	0	14
8.46	30	12	0	18
11.28	30	9	0	21

72 H

Dose (g AI/ha)	n	M	KD	D
Control	30	28	0	2
2.82	30	23	0	7
5.64	30	16	0	14
8.46	30	12	0	18
11.28	30	9	0	21

96 H

Dose (g AI/ha)	n	M	KD	D
Control	30	28	0	2
2.82	30	23	0	7
5.64	30	16	0	14
8.46	30	12	0	18
11.28	30	9	0	21

Bioassay : 72 H Continuous exposure on soil

Species : *T. hypnorum*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
1.6	30	26	4	0
3.1	30	21	8	1
7.8	30	7	17	6
10.9	30	6	11	9

48 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
1.6	30	23	7	0
3.1	30	23	5	2
7.8	30	7	16	7
10.9	30	6	11	13

72 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
1.6	30	15	15	0
3.1	30	20	6	4
7.8	30	9	11	11
10.9	30	3	9	18

96 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
1.6	30	16	12	2
3.1	30	7	16	7
7.8	30	9	6	15
10.9	30	4	6	20

120 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
1.6	30	16	0	4
3.1	30	7	0	13
7.8	30	9	0	21
10.9	30	4	2	24

144 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
1.6	30	16	0	4
3.1	30	7	0	13
7.8	30	9	0	21
10.9	30	4	0	26

Bioassay : 72 H Continuous exposure on soil

Species : *C. septempunctata* (Adults)

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
3.1	30	14	16	0
7.8	30	13	17	0
10.9	30	9	21	1
15.5	30	10	16	4
31.0	30	2	21	7
62.1	30	2	17	11

48 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
3.1	30	18	12	0
7.8	30	14	14	2
10.9	30	14	9	6
15.5	30	12	13	5
31.0	30	5	16	9
62.1	30	4	12	14

72 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
3.1	30	28	2	0
7.8	30	20	5	5
10.9	30	10	10	10
15.5	30	7	12	11
31.0	30	3	2	25
62.1	30	3	6	21

96 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
3.1	30	28	2	0
7.8	30	22	2	6
10.9	30	14	6	10
15.5	30	12	8	10
31.0	30	9	2	19
62.1	30	3	3	24

120 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
3.1	30	28	0	2
7.8	30	22	0	8
10.9	30	15	5	10
15.5	30	15	3	12
31.0	30	9	2	19
62.1	30	4	0	26

144 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
3.1	30	28	0	2
7.8	30	22	0	8
10.9	30	18	1	11
15.5	30	15	0	15
31.0	30	9	1	20
62.1	30	4	0	26

Bioassay : 72 H Continuous exposure on soil

Species : *N. brevicollis*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
15.5	30	29	1	0
31.0	30	25	5	0
62.1	30	16	14	0
93.1	30	19	9	2
124.1	30	15	11	4

48 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
15.5	30	26	4	0
31.0	30	23	7	0
62.1	30	17	12	1
93.1	30	7	19	4
124.1	30	10	13	7

72 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
15.5	30	26	4	0
31.0	30	21	6	3
62.1	30	21	4	5
93.1	30	11	9	10
124.1	30	6	12	12

96 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
15.5	30	21	6	3
31.0	30	19	4	7
62.1	30	12	5	13
93.1	30	9	8	13
124.1	30	4	9	17

120 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
15.5	30	22	3	5
31.0	30	20	2	8
62.1	30	15	1	14
93.1	30	9	2	19
124.1	30	6	2	22

144 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
15.5	30	25	0	5
31.0	30	20	0	10
62.1	30	16	0	14
93.1	30	10	0	20
124.1	30	6	0	24

Bioassay : 72 H Continuous exposure on soil

Species : *P. melanarius*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
15.5	30	25	5	0
31.0	30	22	8	0
62.1	30	14	14	2
93.1	30	19	9	2
124.1	30	14	11	5

48 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
15.5	30	24	6	0
31.0	30	20	10	0
62.1	30	14	13	3
93.1	30	8	17	5
124.1	30	7	14	9

72 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
15.5	30	27	3	0
31.0	30	25	4	1
62.1	30	18	7	5
93.1	30	6	16	8
124.1	30	6	11	13

96 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
15.5	30	19	6	4
31.0	30	18	6	5
62.1	30	13	4	13
93.1	30	12	7	11
124.1	30	5	6	19

120 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
15.5	30	26	0	4
31.0	30	20	0	10
62.1	30	13	3	14
93.1	30	9	1	20
124.1	30	6	2	22

144 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
15.5	30	26	0	4
31.0	30	20	0	10
62.1	30	13	1	16
93.1	30	9	0	21
124.1	30	6	0	24

Bioassay : 72 H Continuous exposure on soil

Species : *D. atricapillus*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
93.1	30	14	16	0
124.1	30	12	18	0
155.2	30	8	22	0
310.3	30	5	24	1
463.4	30	9	19	2

48 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
93.1	30	17	12	1
124.1	30	16	14	0
155.2	30	7	22	1
310.3	30	10	16	4
463.4	30	8	15	7

72 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
93.1	30	10	19	1
124.1	30	12	18	0
155.2	30	6	20	4
310.3	30	4	18	8
463.4	30	4	14	12

96 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
93.1	30	23	5	2
124.1	30	22	7	1
155.2	30	15	7	8
310.3	30	10	7	13
463.4	30	8	6	16

120 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
93.1	30	24	2	4
124.1	30	22	4	4
155.2	30	14	5	11
310.3	30	12	3	15
463.4	30	8	3	19

144 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
93.1	30	25	0	5
124.1	30	24	0	6
155.2	30	19	0	11
310.3	30	12	1	17
463.4	30	10	0	20

Bioassay : 72 H Continuous exposure on soil

Species : *B. obtusum*

Chemical : Deltamethrin 2.5% E.C.

24 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
3.1	30	21	8	1
7.8	30	13	16	1
10.9	30	15	13	2
15.5	30	18	9	3
31.0	30	10	12	8
62.1	30	8	12	10

48 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
3.1	30	22	7	1
7.8	30	16	12	2
10.9	30	19	9	2
15.5	30	15	11	4
31.0	30	12	8	10
62.1	30	8	9	13

72 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
3.1	30	17	12	1
7.8	30	14	14	2
10.9	30	14	10	6
15.5	30	11	13	6
31.0	30	5	14	11
62.1	30	5	10	15

96 H

Dose (g AI/ha)	n	M	KD	D
Control	30	30	0	0
1.6	30	27	1	2
3.1	30	25	1	4
7.8	30	17	4	9
10.9	30	13	8	9
15.5	30	7	3	20
18.6	30	3	5	22

120 H

Dose (g AI/ha)	n	M	KD	D
Control	30	29	0	1
1.6	30	26	0	4
3.1	30	24	0	6
7.8	30	17	0	13
10.9	30	13	2	15
15.5	30	8	0	22
18.6	30	2	1	27

144 H

Dose (g AI/ha)	n	M	KD	D
Control	30	29	0	1
1.6	30	26	0	4
3.1	30	24	0	6
7.8	30	17	0	13
10.9	30	13	0	17
15.5	30	8	0	22
18.6	30	2	0	28

APPENDIX 3

(In " Interpretation of Pesticide Effects on Beneficial Arthropods", Aspects of Applied Biology 31 (1992), 61-68. Eds. R.A. Brown, P.C. Jepson & N.W. Sotherton.)

In situ bioassay techniques to evaluate the toxicity of pesticides to beneficial invertebrates in cereals.

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SUMMARY

This paper describes two foliar and one soil *in situ* bioassay techniques, that have been developed to evaluate the toxicity of pesticide residues to beneficial invertebrates in cereals. These techniques were used to assess the toxicity of summer-applied synthetic pyrethroids to aphid predators such as Carabidae, Stapylinidae, Coccinellidae, and Linyphiidae that inhabit UK cereal crops. The methodologies are not intended to act as definitive test guidelines but may provide an approach to experimental design which could be adapted to suit the purpose of registration tests. The uses and limitations of *in situ* bioassays are discussed.

INTRODUCTION

In situ bioassays aim to confine and therefore expose the test organism(s) to realistic levels of a pesticide on localised parts of the crop (e.g. foliage or soil) under field conditions. These bioassays can provide detailed information concerning the toxicity of a compound or compounds to given species and aid understanding of the behaviour of the compounds, for example bioavailability and persistence, in the crop environment. The advantages of taking this approach are that it :-

a) is cost-effective, for example only relatively small areas of crop are required, which may be sprayed using conventional tractor mounted sprayers or small plot, hand-held sprayers; the bioassay cages are relatively inexpensive to construct and it is often cheaper and more practical to design replicated experiments, whilst being mindful of pseudo-replication, in small plots than on a larger field scale.

b) enables the design of novel methodologies to answer specific questions for different compounds or test species on different substrates.

c) enables comparison of species susceptibility on a given substrate, pesticide toxicity on different substrates (i.e foliage and soil), and comparison of toxicity of different compounds and different application rates to given species.
d) may aid the interpretation of field trials.

When developing bioassay techniques careful consideration must be given to a large number of biological and operational factors. It is important to take account of the ecology of the species being tested and expose the organism on surfaces it is likely to come into contact in the field, to provide suitable food for test invertebrates if the exposure period is relatively long and to provide sufficient ventilation to reduce or eliminate possible excessive vapour effects of the compound in the cage or chamber. The microclimate created within the chamber is also important as this may effect the behaviour of the pesticide and/or the organism. If the chamber is present during spray application factors such as side contamination and physical effects on spray deposition patterns need consideration. It is also important to ensure that the test organisms are confined on the treated surface and are not able to rest on the cage sides and that the length of exposure period and the size of the exposure area are suitable relative to the activity pattern of the organism.

The three *in situ* bioassay techniques described in this paper were developed specifically for use in cereal crops and are additional to those referred to in Jepson (1992) and Jepson and Mead-Briggs (1992a & b). The *in situ* bioassays were used to assess the toxicity of residues of summer-applied synthetic pyrethroids to a range of aphid predators such as Carabidae, Staphylinidae, Linyphiidae, and Coccinellidae that inhabit UK cereals. Two are foliar bioassays which enable confinement of test organisms on leaves and the third is a soil-based bioassay. The descriptions give details of the construction of the bioassay chambers and some advice concerning their practical use in the field. Details of plot size, plot layout, degree of replication or spray application and quantification techniques are not given. Control treatments should be made in all cases using identical methodology with either unsprayed or water sprayed plots. The mortality of test organisms in treated plots should then be adjusted for or compared with mortality in the control plots.

MATERIALS AND METHODS

i) Foliar Bioassay Techniques

a) Modified Petri dish chambers

These were used to confine coccinellids and carabid beetles and can

accommodate one or two beetles per chamber. The chambers were constructed as in Figure 1.

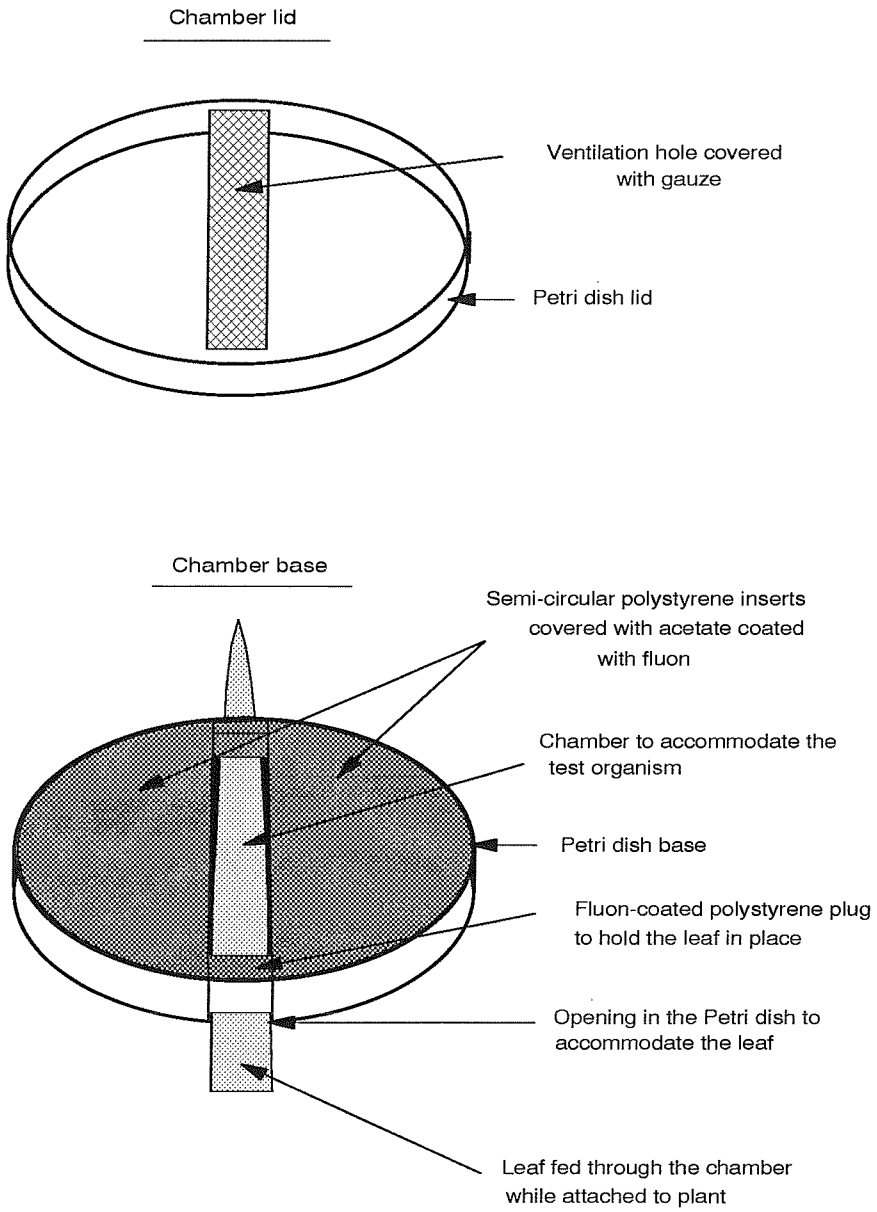

Figure 1. Modified Petri dish chamber

A 2cm long opening was cut into opposite walls in the bottom halves of disposable, perspex Petri dishes (9.5 cm diameter). Polystyrene semi-circles were then cut and two were placed in each Petri dish, leaving a central channel which was marginally narrower than the width of a single cereal leaf (approximately 1.6 - 1.9 cm). The polystyrene semi-circles were covered with acetate sheeting that had been coated with "fluon" (PTFE) to prevent test organisms from climbing the chamber sides. A large ventilation hole was cut into the lid of the Petri dishes and covered with gauze to

provide ventilation. Small polystyrene plugs (the width of the channel) were covered in fluon-coated acetate sheeting to seal the openings at the ends of the chamber once it was fitted over the leaf.

After spray application the deposits were allowed to dry. Individual leaves were chosen at random from the part of the crop being tested and were fed down the channel within the chamber, adaxial surface uppermost. The fluon-coated plugs were then inserted at the entry and exit holes to hold the leaf in position and to prevent test organisms from escaping. The test organisms were then placed on the leaf surface within the chambers and the lids were secured with adhesive tape. The chambers were supported either on surrounding foliage or with canes to keep them upright during the bioassay.

b) Multiple-leaf bioassay chambers

These chambers enable confinement of batches of up to five carabid, staphylinid or coccinellid beetles on plates covered with foliage from a series of plants. Variations in deposition rate between plants may be taken into account by this test method. The chambers were constructed as in Figure 2.

Three strips of double-sided adhesive tape were laid in parallel to glass plates (12cm x 12cm). Plastic tubs (9.5cm diameter and 6.5cm high) with ventilation holes cut into the bottom covered with gauze were coated on the inside walls with fluon.

After spraying, residues were allowed to dry and then leaves from the part of the plant being tested were removed at random from within the treated plots and attached carefully in parallel, base to tip, adaxial surface upwards, to the double-sided adhesive tape on each plate, ensuring that the glass was completely covered. The test organisms were then introduced onto the foliage and fluon-coated tubs were inverted over the plates and secured with adhesive tape. These chambers were then left in the crop to experience field conditions. New plates, with fresh foliage from the treatment plots were made each day because leaves excised from the plant tend to become desiccated after 24 h. The test organisms may be removed from the chambers and placed in freshly-prepared chambers or new batches of organisms may be used, depending on the required exposure period and the aims of the bioassay.

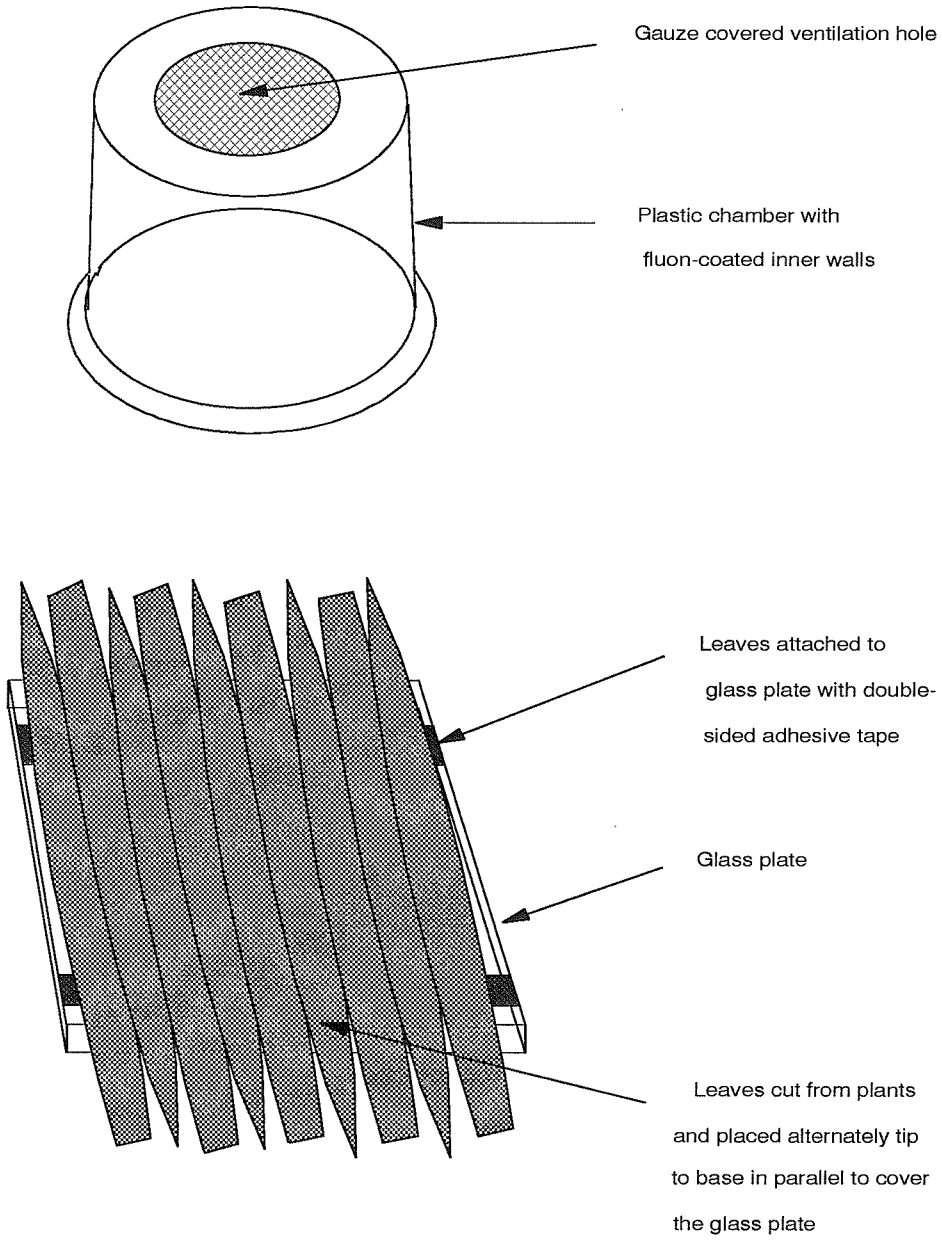

Figure 2. Multiple-leaf bioassay chamber

ii) Soil Bioassay

a) Soil Exposure Chambers

These chambers were used to confine carabid, staphylinid, and coccinellid beetles and linyphiid spiders on soil in batches of up to five individuals per chamber. The chambers were constructed as in Figure 3.

The inside surfaces of plastic tubs (9.5cm diameter x 6.5 cm high) were coated with fluon. Plastic inlays were made from tubs with their bottoms cut out. Ventilation holes were cut in the tub lids and covered with gauze.

The tubs were slightly sunk into the soil surface under the crop canopy. The chambers were then filled with a layer of soil, either from the experimental field or with a standard test soil, depending on experimental procedure. The soil was lightly compressed in the chambers to provide a flat surface and factors such as environmental conditions and soil moisture levels recorded. Plastic inlays were

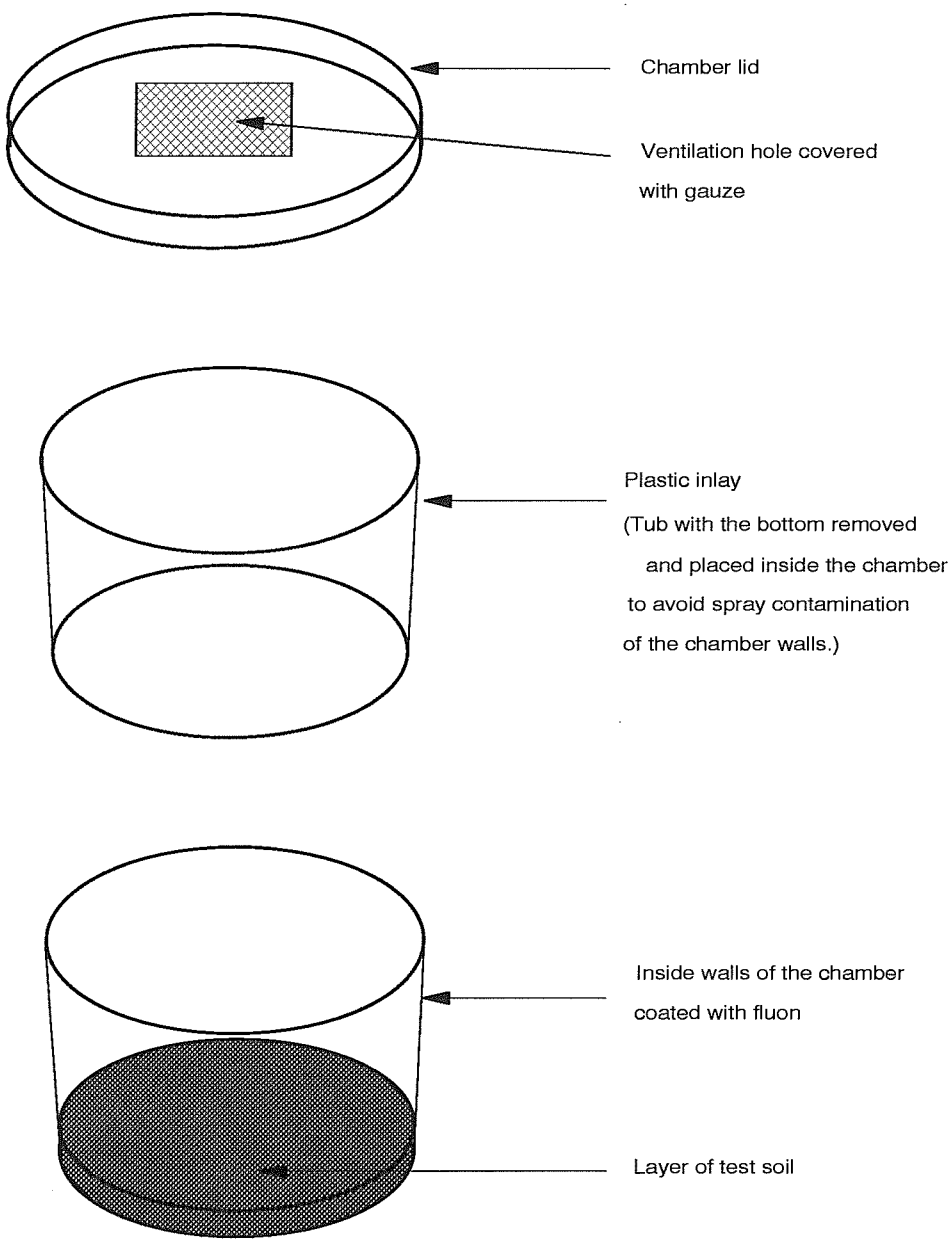

Figure 3. Soil Exposure Chamber

placed in each chamber prior to spraying to prevent spray contamination on the chamber walls above the soil surface. These were removed after spraying. The test organisms were introduced after the spray deposit had dried. Lids, with ventilation holes, were then placed over the chambers to prevent flying invertebrates from

escaping and to reduce the chance of predation. This method of soil bioassay enables complete assessment of response as the chambers can be collected and the contents may be thoroughly searched in the laboratory enabling complete recovery of the organisms released.

RESULTS

Examples of *in situ* bioassay data are given in Figures 4 and 5 for the aphid predators, *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae), *Bembidion lampros* (Herbst.) and *Pterostichus melanarius* (Illiger) (Coleoptera: Carabidae) and *Leptyphantes tenuis* (Blackwall) (Aranaea: Linyphiidae) in a cereal crop cv. Galahad G.S. 67 (Zadoks *et al.* 1974) sprayed with the synthetic pyrethroid insecticide deltamethrin (2.5% E.C.) at a rate of 6.25 g a.i./ha in 200 l water.

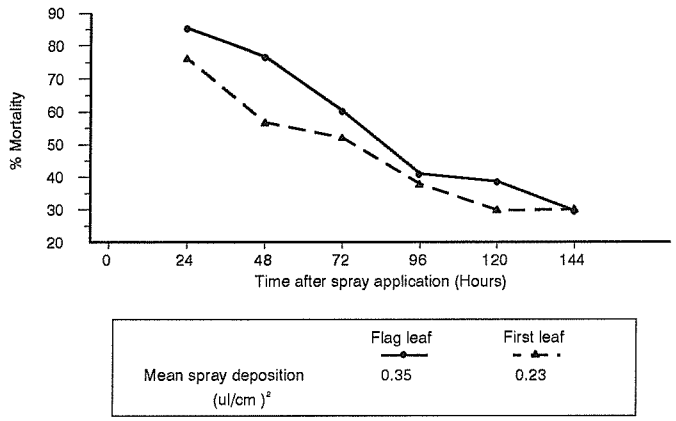

Figure 4. 24 h exposure foliar bioassays to assess the toxicity of deltamethrin residues to *C. septempunctata* at two different strata in a cereal crop.

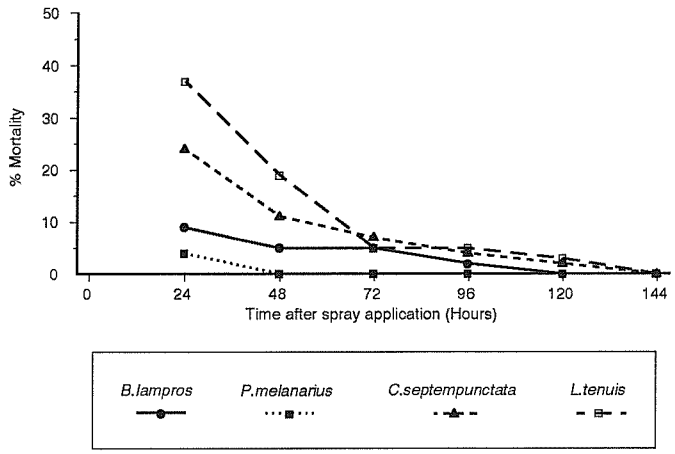

Figure 5. 24 h soil exposure bioassay to assess the soil toxicity of deltamethrin to four species of aphid predators.

Figure 4 shows the mortality, corrected for control mortality, of *C. septempunctata* adults during a 24 h exposure bioassay using modified Petri dish chambers. Individuals were exposed to deltamethrin deposits on flag leaves or first leaves for 24 h on the six days after spray application. Figure 5 gives the mortality of four species of aphid predators, *C. septempunctata*, *B. lampros*, *P. melanarius* and *L. tenuis* for a 24 h bioassay on soil under the crop canopy treated with deltamethrin on the six days after spray application. The results indicate that the toxicity of deltamethrin residues declined rapidly over the six days after spray application. Figure 4 shows that the toxicity of foliar residues of deltamethrin to *C. septempunctata* was higher on the flag leaf than the first leaf due to differences in spray deposition. Figure 5 indicates that of the four predator species tested, the linyphiid spider *L. tenuis* was the most susceptible to deltamethrin residues on the soil followed by *C. septempunctata* and the least susceptible species was the large carabid beetle *P. melanarius*.

DISCUSSION

In situ bioassays are cost-effective tools for the quantification of the toxicity of pesticides to a large range of species on relevant substrates under field conditions. They may be designed to answer specific questions concerning the relative toxicity of parts of the sprayed crop canopy, relative susceptibilities of species, the toxicity of different pesticides and the persistence of toxic effects. Great care must be taken however if the results of bioassays are to be extrapolated to the field. Bioassays may under-estimate mortality as they often only take into account one route of exposure. Conversely they may over-estimate mortality by exposing organisms to residues that they would not come into contact with in the field, for example the pyrethroid cypermethrin is intrinsically highly toxic to honeybees but in the field this toxicity is not realised because of repellency (Delabie *et al.* 1985). They may however aid the interpretation of field trials, for example Unal and Jepson (1991).

In situ bioassays of the type described here provide a link between laboratory and field experiments but are, at least at present, unlikely to replace either. They are not as reproducible as laboratory studies, mainly due to environmental variables and they cannot replace field trials as they, by their nature, cannot measure factors that determine ecological hazard such as colonisation rates and dispersal patterns. However by using laboratory reared test organisms (where possible) and recording factors such as weather conditions, crop type, density, growth stage, soil type, soil conditions and by quantifying spray deposition (see Cilgi and Jepson 1992)

detailed and useful information on compounds can be obtained and progress may be made towards predicting short-term effects on given species (Jepson *et al.* 1990) and developing integrated pest management strategies for example, determining optimum dose-rates.

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APPENDIX 4

(In *Science of the Total Environment*, in press.)

Predicting the short-term toxicity of deltamethrin to *Nebria brevicollis* (F.) (Coleoptera: Carabidae) in a temperate cereal crop.

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ABSTRACT

Laboratory bioassay and spray deposition data were used to predict the levels of mortality that may result from the exposure of the carabid beetle *Nebria brevicollis* to the synthetic pyrethroid deltamethrin sprayed at a rate of 6.25g AI/ha in a cereal crop in the 72 hours after spray application. Mortalities from direct contact with spray droplets, uptake from spray deposits and dietary intake of spray contaminated prey were considered. At decimal growth stage 73 the predicted direct contact mortality was 1 to 3% for *N. brevicollis*; mortality via uptake from deltamethrin residues on soil was 4 to 5%; and mortality from dietary intake was between 13 and 27%, depending on the state of beetle hunger. The integration of these findings to predict toxic effects in the field is discussed.

INTRODUCTION

Predatory invertebrates may be exposed to pesticides by direct and residual contact with spray droplets and by the consumption of pesticide contaminated prey (Jepson, 1989: Everts *et al.* 1991). This paper aims to quantify the short-term mortality that may occur via these routes for a predatory carabid beetle *Nebria brevicollis* (F.)(Coleoptera: Carabidae) following the application of a synthetic pyrethroid insecticide, deltamethrin, in a temperate cereal crop as a contribution to the estimation of short-term risks of pesticides to beneficial invertebrates (Jepson, 1993). Deltamethrin was provisionally approved for use as a summer-applied aphicide in UK cereals in 1990. Until then the use of synthetic pyrethroid insecticides in cereals was limited to the autumn only because of fears concerning their broad activity spectrum against non-target invertebrates.

Research over the last 10 years has shown that polyphagous predators, such as the carabid beetles, have considerable value as ground active aphid predators in cereals (Sunderland *et al.* 1987; Sopp, 1987; Winder, 1990; Wratten and Powell, 1991). *N. brevicollis* was chosen as the test species as it is representative of the relatively large carabid species found in cereal crops and is present in cereal crops in the early summer and the autumn when pyrethroids are applied. This species has also been shown to be susceptible to autumn-applied pyrethroid sprays (Pullen, Jepson and Sotherton, 1992).

The study took a mechanistic approach to answer the following questions concerning the possible mortality of large species of carabid beetles in the 72 hours following a deltamethrin spray application;

1) What are the toxic effects of direct contact with spray deposits under a mature cereal crop canopy ?
2) What are the toxic effects of exposure to soil residues of deltamethrin ?
3) What are the toxic effects of the consumption of deltamethrin contaminated prey ?

A similar study has recently been reported by Everts *et al.* (1991) with the linyphiid spider *Oedothorax apicatus* (Blackwall) (Erigonidae), to calculate the contribution that these different routes of exposure make to the body burden of insecticide when spiders were exposed to sub-lethal rates of deltamethrin. They found residual exposure to be of greatest importance, with direct contact exposure contributing a smaller proportion of total uptake and dietary intake being negligible. The rationale we present in this paper was designed to estimate uptake from each exposure route at field concentration and differs from the spider study in that we aimed to generate predictions to contribute to the calculation of optimal dose-rates (Jepson, 1993).

EXPERIMENTAL METHODS

Test invertebrates

Adult *N. brevicollis* beetles were captured between September and December in 1989 and 1990 in cereal fields and field-margins at Leckford, near Stockbridge, Hampshire, by dry pitfall trapping and surface searching (Southwood, 1987). The aphid species used as prey in the dietary experiment was the Rose-Grain aphid *Metapolophium dirhodum* (Walk.) (Homoptera: Aphididae).

All invertebrates were kept in a controlled environment room in an insectary, maintained at 19-22°C and 55-70% relative humidity with a 16:8 L:D photoperiod, prior to the experiments. The beetles were kept in plastic aquaria, containing a layer of moist soil and were fed on ground, moist, cat biscuits ("Delicat"- Quaker Latz). The aphids were cultured on barley seedlings.

Test Chemical

Formulated deltamethrin (2.5% E.C.) was used as the test chemical, with distilled water as the diluent and as the control treatment.

Determination of the level of mortality via direct contact with spray droplets.

The mortality likely to occur by direct contact with spray deposits was evaluated by quantifying the predator susceptibility to deltamethrin and the degree of spray impaction on the predators on the soil surface under a cereal crop canopy.

i) Predator susceptibility to deltamethrin.

The susceptibility of *N. brevicollis* to deltamethrin was determined by laboratory topical bioassay, in which beetles were treated with precise doses of deltamethrin using a microapplicator (Burkard Manufacturing Co. Ltd.)(Arnold, 1967). The response was recorded as unaffected (i.e. moving as normal) or affected, either "knocked down" (with moving antennae, mandibles or legs but unable to right themselves permanently) or dead (showing no response to stimulation). Response assessments were made at 24 hour intervals and the dose-response data for dead individuals were analysed by probit analysis (Finney, 1971) to obtain toxicological statistics. The end-point of toxicity was found to be approximately 72 (Wiles and Jepson, 1993a).

ii) Quantification of spray deposition on beetles under the cereal crop canopy.

Due to lack of availability of *N. brevicollis* at the time of testing the large carabid beetle *Pterostichus melanarius* L. (Coleoptera: Carabidae) was used to determine mean spray deposition under the crop canopy and these deposition rates were corrected for mean body surface area to predict a mean spray deposition for *N. brevicollis*. Forty-two freshly killed *P. melanarius* beetles were placed at random on the soil surface in plots of winter wheat cv. Galahad at growth stage 73 (Zadoks *et al.* 1974) and a crop density of 420 tillers/m^2. The plots were then sprayed with a water and 0.05% fluorescent dye (Fluorescein- Acid Yellow 73, Aldrich) spray mixture using a hand-held sprayer calibrated to deliver spray at a volume rate of 200 l/ha, which is the current recommended volume application rate for aphicides in UK cereal crops. The beetles

were collected individually after spraying and placed in a phosphate buffer solution. The mean spray depositions per beetle were calculated in the laboratory from spectrofluorimeter readings and calibration curves using the procedure described by Çilgi (1988) and Çilgi and Jepson (1992).

Determination of the level of mortality via contact with soil residues.

Laboratory bioassays of soil residues were carried out using small chambers (9.5 cm diameter) filled with a layer of lightly compacted, sieved, sandy loam soil with 22% moisture content. The chambers were sprayed with five definitive doses, selected from range-finding bioassays, ranging from 15 to 124 g AI/ha under a Potter Tower (Potter, 1952). Thirty *N. brevicollis* per dose were released, in batches of two, into the chambers exposed for a period of 72 hours. Ground, moist, cat biscuits were provided as food during this period. The beetles were removed after 72 hours and placed in clean, ventilated chambers were the mortality was recorded at 24 hour intervals until an end-point was reached. Mortality data were analysed by probit analysis to obtain dose-response statistics.

Determination of the level of mortality via consumption of contaminated prey.

Freeze-killed aphids were dosed topically with 30 ng AI deltamethrin using a microapplicator. This dose was chosen as it approximates to the aphids receiving a 1μl droplet of the recommended field rate concentration of deltamethrin (31.25 ng/μl, i.e. 6.25 g AI in 200l water) applied as a summer cereal aphicide in the U.K. Control aphids were dosed with water alone. Fifteen treated or control aphids were then placed in plastic tubs (9.5 cm diameter) and single *N. brevicollis* were then placed in each tub. The beetles were returned to the insectary and kept under constant conditions of 19-22°C and 55-70% relative humidity with a day length of 16:8 L:D during the experiment. Batches of beetles were last fed 24, 48, 72 and 120 hours before the experiment to test the effect of hunger level on dietary intake. The number of contaminated or control aphids eaten by each beetle over a 24 hour period was recorded. The beetles were then removed and placed in clean, ventilated, tubs with fifteen untreated freeze-killed aphids. The response of the beetles, i.e. moving as normal, knocked down or dead, and the numbers of aphids eaten were recorded at 24 hour intervals on the following days.

Outline methodologies are given here. Full details of procedures will be given in Wiles and Jepson (a, b & c).

RESULTS

Mortality via direct contact with spray droplets.

The predicted mean spray deposition and mortality of *N. brevicollis* on soil under a cereal crop canopy at growth stage 73 is given in Table 1. The mean beetle surface area measurements were taken from Çilgi (1988). The mean spray deposition on *P. melanarius* was 0.83 µl/beetle on a mean surface area of 1.44 cm^2 and was corrected for the surface area of *N. brevicollis*. The mean spray deposition value was multiplied by the recommended field rate concentration for deltamethrin (31.25 ng/µl, i.e. 6.25g AI in 200l water) to obtain an estimate of mean dose received per beetle. This dose was then substituted into the 72 h topical probit equation to obtain a predicted percent mortality. This was 1 to 3% for *N. brevicollis.*

Table 1. Predicted 72h direct contact mortality of *N. brevicollis* under a cereal crop canopy (GS 73) sprayed with 6.25 g AI/ha deltamethrin.

Species	Mean surface area (± s.e.) (cm^2)	Predicted mean spray deposition (± s.e)(µl/beetle)	Predicted mean dose received (± s.e) (ng/beetle)	72 h Probit equation	Predicted % mortaiity
N. brevicollis	0.84 (0.02)	0.48 (0.11)	15.1 (3.3)	y = 1.71x + 1.0	1-3
				χ^2 = 1.22 (df.3) ns*	

* ns = not significant, P>0.05.

Mortality via contact with soil residues.

The probit line from the 72 hour exposure of *N. brevicollis* to soil deposits of deltamethrin is plotted in Figure 1. The dose-response statistics indicate that at current recommended field rate (6.25 g AI/ha) 4 to 5% mortality would be expected for *N. brevicollis*.

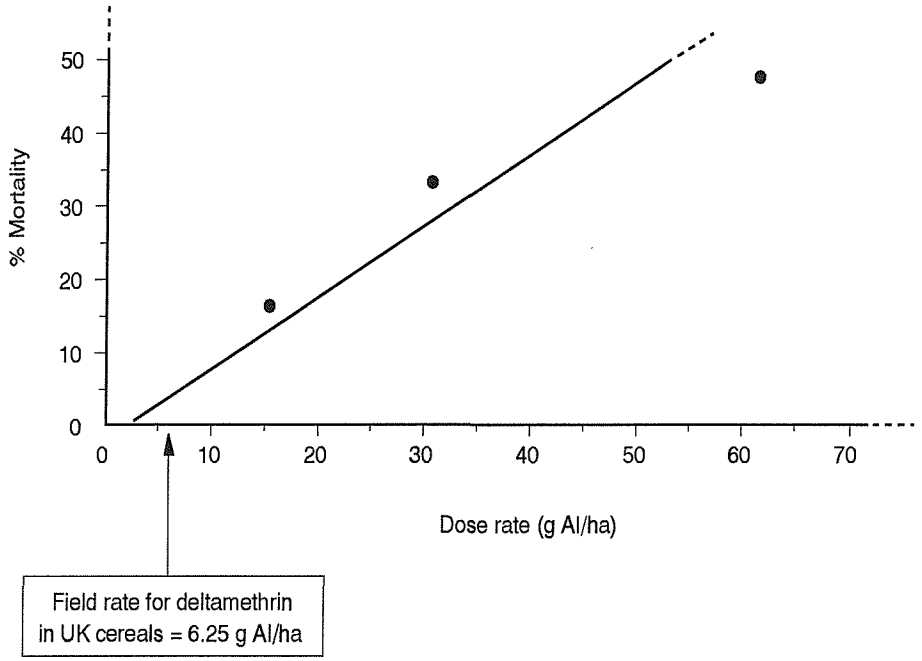

Fig. 1. 72 h probit line for *N. brevicollis* after 72 h exposure to soil treated with deltamethrin. Probit statistics for 72 h residual exposure probit line; $y = 1.89x + 1.73$, $\chi^2 = 1.2$ (d.f.3) $P>0.05$.

Mortality via consumption of contaminated prey.

The observed percentage mortalities of *N. brevicollis* over 72 hours due to the consumption of contaminated prey in the four experiments are given in Table 2. The mortality increased with beetle hunger and varied between 13%, for those beetles that had been fed 24 or 48 hours before exposure, to 27% for beetles that had been starved for 120 hours prior to exposure.

Table 2. 72 h mortality of *N. brevicollis* after consumption of deltamethrin contaminated aphids.

Beetle hunger level	% Mortality
Fed 24 hours prior to exposure	13
Fed 48 hours prior to exposure	13
Fed 72 hours prior to exposure	20
Fed 120 hours prior to exposure	27

Predicted combined mortalities from topical, residual and dietary exposure.

The predicted percentage mortalities of *N. brevicollis* for different combinations of the three routes of exposure are given in Table 3.

Table 3. Predicted 72 h mortalities from combined routes of exposure for *N. brevicollis* after deltamethrin application (6.25 g AI/ha) in a temperate cereal crop.

Routes of exposure	Predicted overall % mortality
Topical + Residual	5 - 8
Topical + Dietary*	14 - 30
Residual + Dietary*	17 - 32
Topical + Residual + Dietary*	18 - 35

* - Mortality dependent on level of beetle hunger.

DISCUSSION

These experiments have shown that, under the given conditions, mortality of large carabid beetles, such as *N. brevicollis,* may result in a cereal crop via direct and residual contact with spray droplets and by the consumption of contaminated prey. The mortalities via direct contact and residual uptake were lower than from dietary intake. The overall maximum predicted mortality of *N. brevicollis* from all three routes of exposure was 35%, based on the assumptions that all the beetles were on the soil surface at the time of spray application, that the beetles were in a high state of hunger and that doses received from the different routes are additive. This predicted mortality may be higher than field trial data would suggest for the Carabidae (i.e. Vickerman *et al.* 1987) however field assessment methods often assess changes in activity and abundance rather than actual mortality.

In order to make more accurate predictions of the short-term effects of a spray application on a given species, more information is required concerning the toxic interactions of doses received via different routes of exposure. Mullié and Everts (1991) have shown that the mortality from combined topical and residual exposure of the linyphiid spider *Oedothorax apicatus* (Blackwall) (Arachnida: Erigonidae) to [^{14}C] deltamethrin was lower than predicted by addition of topical and residual mortality. Toxicokinetic theory may predict that the toxicity of certain doses from different routes of exposure may be additive (Ford pers. comm.) but sub-lethal effects on arthropod behaviour via uptake from several routes may reduce activity and thus exposure and therefore reduce overall mortality. Further information is also needed concerning biological factors in the field, such as arthropod distribution in the crop, behaviour, activity and hunger levels and operational factors including pesticide application

parameters and environmental influences on pesticide bioavailability and toxicity (Critchley, 1972; Harris and Turnbull, 1978; Jepson, 1989; Croft, 1990; Jagers op Akkerhuis and Hamers, 1992).

The importance of the different routes of exposure is likely to vary with the ecology and behaviour of the given arthropod species. Dietary intake of spray contaminated prey may be an important short-term route of exposure for carabid beetles however it is likely that exposure to residues may be a more important route of exposure for active predators in the long-term. Many carabid beetles are nocturnal and therefore the importance of the direct contact route of exposure may be low because the beetles are likely to be hidden in soil refuges during the day. Spray deposition on the soil in the field will be less than in these experiments as the foliage will intercept a proportion of the spray. Also a windfall of contaminated aphid prey on the soil surface after a spray application may increase feeding as a result of a behavioural functional response, leading to enhanced effects: sub-lethal poisoning effects via dietary (Wiles and Jepson, 1993c) or other routes of exposure (Mullié and Everts, 1991) may however reduce feeding upon pyrethroid contaminated prey. Further exploration of the mechanisms of exposure via different routes in the field are required to make accurate predictions of the short-term effects of a spray application and to determine optimum dose-rates.

ACKNOWLEDGEMENTS

J.A.W. is in receipt of a MAFF Case Award with The Game Conservancy Trust. We would like to thank Dr N.W. Sotherton for his advice.

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Wiles, J.A. and P.C. Jepson, 1993a. The susceptibility of a cereal aphid pest and it's natural enemies to deltamethrin. *Pestic. Sci.* (In press)

Wiles, J.A. and P.C. Jepson, 1993b. Substrate-mediated toxicity of deltamethrin residues to aphid predators. (In prep.)

Wiles, J.A. and P.C. Jepson, 1993c. The dietary toxicity of deltamethrin to *Nebria brevicollis* (F.) (Coleoptera: Carabidae). *Pestic Sci.* (In press)

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APPENDIX 5

List of publications from this study: (In chronological order)

John A. Wiles (1990)
Assessing the effect of summer applied synthetic pyrethroids on aphid predators in cereals. *In "The Game Conservancy Annual Review 1990"*, pp 62-63.

P.C. Jepson, S.J. Duffield, J.R.M. Thacker, C.F.G. Thomas, & J.A. Wiles (1990)
Predicting the side-effects of pesticides on beneficial invertebrates. *Proceedings of the BCPC Conference, Pests and Diseases,* 1990. Vol.**3**. pp 957-962.

J.A. Wiles, P.C. Jepson, D.W. Salt, & M.G. Ford (1991)
Evaluating the Hazard of Pesticides To Target and Non-Target Terrestrial Invertebrates. (Poster presented at the 7th IUPAC Conference, Hamburg, July 1990)(Extended summary). *Pesticide Science* **31**, 98-99.

John A. Wiles (1992)
Assessing the short-term side-effects of a pyrethroid insecticide on aphid predators in cereals. (Poster presented at the Society of Chemical Industry Symposium,"Postgraduate Research In Pesticides." London, March 1991). (Extended summary) *Pesticide Science* **34**, 94-95.

John A. Wiles & Paul C. Jepson (1992)
In situ bioassay techniques to evaluate the toxicity of pesticides to beneficial invertebrates. *Aspects of Applied Biology* **31**, 61-68.

John A. Wiles & Paul C. Jepson (1993a)
The Susceptibility of a Cereal Aphid and its' Natural Enemies to Deltamethrin. *Pesticide Science* (In press).

John A. Wiles & Paul C. Jepson (1993b)
An Index of the Intrinsic Susceptibility of Non-Target Invertebrates to Residual Deposits of Pesticides. In *"Ecotoxicology of Soil Organisms"*, Eds. H. Eijsackers, F. Heimbach & M. Donker. Lewis Publishers, Chelsea, USA. (In press).

John A. Wiles & Paul C. Jepson (1993c)
Predicting the short-term toxicity of deltamethrin to *Nebria brevicollis* (F.) (Coleoptera: Carabidae) in a temperate cereal crop. *Science of the Total Environment* (In press).

John A. Wiles & Paul C. Jepson (1993d)
The Dietary Toxicity of Deltamethrin to *Nebria brevicollis* (F.) (Coleoptera: Carabidae). *Pesticide Science* (In press).