UNIVERSITY OF SOUTHAMPTON

THE EFFECTS OF PYRETHROID INSECTICIDES UPON PARASITIC HYMENOPTERA IN THE CEREAL ECOSYSTEM

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ABSTRACT

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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 parasitic Hymenoptera associated with aphids in temperate cereal crops. Such a study was required to aid short-term risk predictions for parasitoids in the field, derived from current laboratory and semi-field experiments.

Standard laboratory bioassays were used to investigate lethal and sublethal effects of deltamethrin upon parasitoid life-stages. Modifications were made to these tests to improve realism and enable field risk predictions. Results indicated that the toxicity of deltamethrin residues was dependent upon concentration, parasitoid exposure time and substrate. Immature parasitoid stages within aphid hosts were shown to be protected from deltamethrin. Estimates of actual exposure of mummies to pesticides in the field suggested that current laboratory bioassays overestimate exposure and therefore possibly the toxic effects of insecticides. A quantification of the contribution made by the different routes of exposure (topical, residual and dietary) of parasitoid life-stages to insecticides was obtained with radio-labelled and radioinert diazinon. Uptake by these routes was shown to be dependent upon insecticide concentration and time of exposure.

Laboratory behavioural studies with parasitoids were undertaken to investigate the degree of attraction/arrestment by honeydew deposits in the presence of deltamethrin, which has repellent properties. Insecticide residues altered foraging behaviour, with honeydew causing parasitoids to remain longer in insecticide-treated areas. The implications of this for foraging parasitoids in the field are discussed.

Field studies in winter wheat investigated the short-term effects of deltamethrin applied at a series of reduced dose-rates on aphid and parasitoid populations. Aphid colonies were found to survive on the lower crop strata and toxicity of deltamethrin residues to parasitoids declined over subsequent days after treatment. Results suggested that concentrations as low as 1/6th of the current recommended field rate may be suitable for uniting chemical and parasitic control in future integrated pest management programmes. Larger field studies investigating the longer-term effects of deltamethrin applications indicated that aphid primary parasitoid and hyperparasitoid populations rapidly recovered in 4ha treated plots of winter wheat through a progressive reinvasion of individuals from surrounding untreated areas.

Overall, the results from this study are discussed in terms of how toxicological and ecological data may be collected and used to improve risk predictions for parasitoid species exposed to insecticides. Recommendations are given for the design and analysis of future laboratory, semi-field and field studies of aphid parasitoids.

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CHAPTER ONE

GENERAL INTRODUCTION

Cereal aphids

The three most economically important cereal aphid species in Europe are the grain aphid, *Sitobion avenae* (F.), the rose-grain aphid, *Metopolophium dirhodum* (Wlk.), and the bird-cherry oat aphid, *Rhopalosiphum padi* (L.) (Homoptera: Aphididae) (Dixon, 1987). Their abundance varies from year to year (Rabbinge *et al.*, 1979; Carter *et al.*, 1980) and is variable between regions (George & Gair, 1979). In wheat, *S. avenae* feed on the upper leaves, but has a preference for the ears once these are available (Vickerman & Wratten, 1979). *M. dirhodum* is entirely leaf-feeding, showing a preference for the leaf under-sides, and *R. padi* is mainly a leaf-feeder with few reports of it feeding on the ear (Blackman & Eastop, 1984).

These species have been considered as pests in the U.K. since the 1960's when major outbreaks occurred (Fletcher & Bardner, 1969). Aphid infestation can result in a loss of crop yield (Wratten, 1975) and a reduction in grain quality (Lee *et al.* 1982), through the consumption of plant sap and through plant virus transmission. Barley Yellow Dwarf Virus (BYDV) is the major cereal virus transmitted by aphid feeding and results in stunted plants (Kendall *et al.*, 1984). Further secondary damage is caused by the deposition of honeydew, a sugary solution excreted from the aphid onto plant foliage, which acts as a substrate for growing fungi and mould, thus causing a reduction in light reaching leaf chloroplasts (Bardner & Fletcher, 1974; Vereijken, 1979).

Aphid outbreaks do not occur every year (Kröber & Carl, 1991). Therefore the economic and environmental consequences of prophylactic insecticide spraying are leading to an increased interest in the role of native natural enemies for controlling aphid outbreaks (Basedow *et al.*, 1990).

Natural enemies of cereal aphids

Cereal aphids are attacked by a wide spectrum of aphid-specific and polyphagous predators, entomopathogenic fungi and hymenopterous parasitoids (Kröber & Carl, 1991; Wratten & Powell, 1991).

Aphid-specific predators are found within the families Coccinellidae (Coleoptera) and Syrphidae (Diptera). Experiments involving the enclosure of coccinellids within field cages

over cereal crops have clearly demonstrated their role in reducing aphid abundance (Dixon, 1987). In the Syrphidae, only larvae are aphid predators, although they are very voracious feeders and have the potential to limit the rate of aphid population increase (Dixon, 1987; Chambers, 1988).

Polyphagous predators of aphids include arthropods from different groups such as Coleoptera, Heteroptera, Neuroptera, Opiliones, Acari and Araneae (Sunderland & Vickerman, 1980). The impact of these predators was first highlighted by one of the earliest studies of the cereal ecosystem which demonstrated negative associations of polyphagous predators and aphid population peaks (Potts & Vickerman, 1974). Also entomopathogenic fungi have been shown to make an important contribution to the suppression of aphid numbers in certain years (Dean & Wilding, 1971; Chambers *et al.*, 1986).

The primary parasitoids of cereal aphids belonging to two families of Hymenoptera, the Aphidiinae (Ichneumonoidae) and Aphelinidae (Chalcidoidea) have been reported to cause reductions in aphid population growth rate (e.g. Powell *et al.*, 1983; Carter & Sotherton, 1983; Vorley & Wratten, 1985).

Aphid parasitoids

The most abundant and important aphid primary parasitoids belong to the highly specialised family, Aphidiinae, members of which (more than 400 species known), parasitise only aphids (Starý, 1970). There is current taxonomic debate as to whether these aphid parasitoids should be regarded as a separate family (Aphidiidae), or as a sub-family (Aphidiinae) within the Braconidae family (Gärdenfors, 1986; O'Donnell, 1989). For the purposes of this thesis they shall hereafter be referred to as the Aphidiinae.

The three most economically important aphids infesting British cereal crops, *S. avenae*, *M. dirhodum* and *R. padi*, are attacked by at least seven different genera of primary parasitoids (Table 1.1), which are in turn attacked by at least five different genera of hyperparasitoids (Table 1.2) (Powell, 1982). Species of the genus *Aphidius* occur most frequently in the cereal ecosystem, although the species composition of primary parasitoids vary between years (Borgemeister & Poehling, 1988). Throughout the season, a female biased sex-ratio of primary parasitoids is maintained (Borgemeister & Poehling, 1988; Carter *et al.*, 1980).

The later appearance of hyperparasitoids in fields enhances the probability that they will coincide with suitable host stages, e.g. in the case of *Dendrocerus carpenteri* (Curtis), fully formed mummies are required. *D. carpenteri* has been recorded as being the most predominant hyperparasitoid species in cereal aphids (Starý, 1977; Leather *et al.*, 1984; Borgemeister & Poehling, 1988).

Frequently parasitoids appear to have a major effect at an early stage during the development of aphid populations, at densities as low as 0.1 aphids/shoot (Chambers *et al.*, 1986). Parasitoid abundance in cereal fields in early spring is influenced by a number of factors, including winter weather conditions and crop sowing date (Powell, 1983; Vorley & Wratten, 1987). By reducing potential peak populations of aphids they can increase the likelihood of other natural enemies keeping aphids below economic damage levels later in the season. Parasitoids themselves appear to be less effective during later stages of aphid population development because they have been shown to emigrate from the crop rather early, before the crop has matured (Vorley & Wratten, 1985).

Twenty three species of parasitoids in the family Aphidiinae have been exploited in classical biological control programmes targeted against aphids in the open field. The parasitoids became established in 32 out of 55 attempts (Greathead, 1989). In glasshouse environments, *Aphidius matricariae* Haliday is the most widely used species, with *Ephedrus cerasicola* Starý suggested as a suitable candidate for biological control of *Myzus persicae* (Sulzer) (Hågvar & Hofsvang, 1991).

Table 1.1. Aphid / parasitoid checklist from Powell (1982) . * indicates the relationship has been found in Britain; (*) indicates that the relationship has not yet been recorded in Britain, but has been recorded elsewhere in Europe.

Primary parasitoid species	Aphid species		
	Sitobion avenae	*Metopolophium dirhodum*	*Rhopalosiphum padi*
Aphelinus abdominalis	*	*	*
Aphidius ervi	*	*	*
A. frumentarius	*	*	*
A. matricariae			*
A. picipes	*	*	*
A. rhopalosiphi	*	*	*
A. uzbekistanicus	*	*	*
Diaeretiella rapae	(*)		(*)
Ephedrus plagiator	*	*	*
Lysiphlebus fabarum	(*)		
Monoctonus cerasi			(*)
Praon volucre	*	*	*
Toxares deltiger	*	*	

Table 1.2. Parasitoid / hyperparasitoid checklist from Powell (1982). * indicates the relationship has been found in Britain; (*) indicates that the relationship has not yet been recorded in Britain, but has been recorded elsewhere in Europe.

Hyperparasitoid species	Species of primary parasitoids				
	Aphelinus	*Aphidius*	*Ephedrus*	*Praon*	*Toxares*
Alloxysta circumscripta		*			
A. curvicornis		(*)			
A. macrophadna		*			
A. victrix		*			
Asaphes suspensus		*		*	
A. vulgaris		*	*	*	
Coruna clavata		*	*	*	
Dendrocerus aphidium		*	*	*	
D. carpenteri		*		*	
Phaenoglyphis villosa	*	*	*	*	*

Life history of aphid primary parasitoids

During the oviposition act, the female parasitoid bends her abdomen underneath her thorax and extends it forward, between her legs, to insert her ovipositor tip into an aphid host. One egg is laid, and only one parasitoid larva can complete development, classifying aphidiids as solitary endoparasitoids (Starý, 1970). After hatching from the egg, the legless larva passes through four instars before pupation (O'Donnell, 1987). Immediately prior to parasitoid pupation, the aphid dies and only the outer aphid cuticle is left. This becomes attached onto the surface of a plant by the larva cutting a small hole in the ventral side of the host and using a secretion from the silk glands. The parasitoid then spins a cocoon inside the aphid case (in most genera) or beneath it (e.g. *Praon* spp.) and the aphid cuticle becomes hardened and changes colour to form the characteristic mummified aphid ("mummy"). The parasitoid then passes through a prepupal stage into pupation, during which it has the ability to overwinter in temperate regions as a diapausing prepupa (Mackauer & Chow, 1986), or undergo seasonal diapause during hot periods or when the aphids migrate to summer host plants in another habitat (Starý, 1988). Once emerged from the pupa, the adult cuts a circular emergence hole in the dorsal surface of the mummy through which it exits. The whole developmental period of *Aphidius* species takes about 2 weeks (Table 1.3).

Male parasitoids have been shown to respond to the sex pheromones of females (Read *et al.*, 1970; Powell & Zhi-Li, 1983; Bouchard & Cloutier, 1985; Decker, 1988), with female aphidiids mating only once whilst males are capable of mating several times (Starý, 1988). Biparental reproduction occurs in the Aphidiinae, with unfertilised eggs giving rise to males, and females being produced from fertilised eggs (arrhenotoky).

Table 1.3. Developmental period in *Aphidius* species at 20-22°C (taken from Hågvar & Hofsvang, 1991)

	Egg stage (days)	Oviposition to mummification (days)	Mummification to emergence (days)	Oviposition to emergence (days)
Aphidius spp.	2.5 - 4.0	6.3 - 9.0	4.9 - 6.0	11.2 - 15.0

The aphidiids are synovigenic, with some ripe eggs present in the oviducts at emergence and further eggs maturing during the females' life time (Starý, 1988). Work by Shirota *et al.* (1983) found that *A. rhopalosiphi* females, maintained at 18°C, had a mean fecundity of 212 eggs and a mean longevity of 13.1 days. The maximum daily fecundity was reached a few days after emergence, then gradually decreased, though the aphidiids oviposited throughout most of their lives.

Most species of aphid parasitoid rarely exploit more than a small percentage of the host resources available in the field (Starý, 1970; Mackauer & Chow, 1986). The incidence of parasitism generally ranges between 1% and 10%, according to the growing season and aphid colony size, among other factors (Mackauer & Völkl, 1993).

Life history of aphid hyperparasitoids

Aphid hyperparasitoids are found within the following genera within five hymenopterous families; *Asaphes*, *Coruna* and *Pachyneuron* (Pteromalidae); *Aphidencyrtus* (Encyrtidae); *Tetrastichus* (Eulophidae); *Dendrocerus* (Megaspilidae) and *Alloxysta*, *Lytocysta* and *Phaenoglyphis* (Cynipidae). Of the major groups, *Alloxysta* and related genera are endoparasitic; the female oviposits directly into the embryonated egg or larva of a primary parasitoid inside a pre-mummified aphid. By contrast, species of *Asaphes*, *Pachyneuron* and *Dendrocerus* are ectoparasitoids; the female deposits her egg on the prepupa or pupa of a primary parasitoid inside a mummified aphid (Sullivan, 1987, 1988). Tertiary parasitism has also been reported, whereby a second hyperparasitoid develops on the larva of another hyperparasitoid which may belong to the same (Levine & Sullivan, 1983) or a different species (Matejko & Sullivan, 1984).

Hyperparasitoids generally have higher temperature requirements than primary parasitoids (Campbell *et al.*, 1974) and hence appear later in the season, frequently exceeding 50% of field samples (Sullivan, 1987; Horn, 1989; Völkl, 1992). The impact of hyperparasitoids on the effectiveness of primary parasitoids has not yet been clearly demonstrated. The reported high levels of hyperparasitism from field studies has led many authors to conclude this resulted in the inability of primary parasitoids to reduce their host populations (e.g. Luck *et al.*, 1981). However, other workers (e.g. Horn, 1989; Höller *et al.*, 1993; Mackauer & Völkl, 1993) have questioned whether hyperparasitoids necessarily reduce the potential impact of the primary parasitoids on their aphid hosts. Mathematical models suggest that the influence of hyperparasitoids in population dynamics varies with the initial model conditions (Hassell, 1978; Hassell & Waage, 1984). For example, in initially stable systems hyperparasitism may reduce host control by a primary parasitoid, whereas in initially unstable systems with excessive oscillations the addition of a hyperparasitoid can result in a stable equilibrium among the three consumer levels.

Host selection by primary parasitoids

In order for female parasitoids to locate their potential host populations, several behavioural steps are usually necessary in order to locate and parasitise their hosts (Vinson, 1981). These complex and interacting successive steps progressively decrease the area of search and hence increase the chances of finding hosts and successively parasitising them. The selection process was first divided by Doutt (1964) into the four steps (i-iv) below and a fifth step (v) was later added by Vinson (1975):

(i) Host habitat selection involves the female parasitoid searching for and reaching the habitat where host plants and hosts occur;

(ii) Host location involves the female searching for hosts, usually on or very close to the host plants, with the host being eventually encountered;

(iii) Host acceptance involves the examination of the host and a "decision" by the parasitoid as to whether it is suitable for oviposition;

(iv) Host suitability involves the fate of the parasitoid egg deposited into the host, being dependent on how suitable the host is for parasitoid development;

(v) Host regulation involves the way in which the developing parasitoid can affect the development, morphology, behaviour, physiology and biochemistry of the host.

Comprehensive reviews of host selection, consisting of host habitat location, host location and host acceptance (Vinson, 1976, 1977, 1981; Lewis *et al.*, 1976; Weseloh, 1981; Arthur, 1981), determination of host suitability (Vinson & Iwantsch, 1980a) and host regulation (Vinson & Iwantsch, 1980b) have been given covering most hymenopteran

parasitoids. For the context of this thesis, a brief review of primary parasitoid host selection, with particular emphasis on aphid parasitoids, is given below.

(i) Host habitat location

Upon emergence, female parasitoids may be in an environment containing few or no potential hosts. They have therefore evolved successful host location procedures to ensure the reproductive survival of individuals (Vinson, 1981).

Host habitat location involves the location of macrohabitats (e.g. forests, fields) and microhabitats (e.g. potential sources of insect host nutrition such as host plants). Female parasitoids use long-range cues including electromagnetic radiation (incorporating vision), sound and odour (Vinson, 1984). The role of vision in the host habitat location by *Diaeretiella rapae* M'Intosh (Vater, 1971) and *Aphidius ervi* Haliday (Goff & Nault, 1984) has been demonstrated, with both species responding to the colour green. This behavioural response may serve as a mechanism whereby a flying parasitoid locates and alights on vegetation where potential aphid hosts may be found. Olfactometer studies by various workers (e.g. Read *et al.*, 1970; Schuster & Starks, 1974; Akinlosotu, 1978; Singh & Sinha, 1982; Powell & Zhi-Li, 1983) have demonstrated positive attraction by various aphidiid species to host plant odours, whereas other studies involving aphid parasitoids of polyphagous hosts showed no attraction to odours from their host plants (Bouchard & Cloutier, 1985; Hågvar & Hofsvang, 1987, 1989).

Numerous studies involving wind tunnels, olfactometers and glasshouse experiments have involved determining the host habitat location cues for *D. rapae*, and conclude that the females use odours of the hosts food plant rather than from their hosts (Read *et al.*, 1970; Akinlosotu, 1978; Sheehan & Shelton, 1989a). It appears that *D. rapae* uses the odour of the mustard oil allylisothiocyanate, a secondary plant substance in crucifers, to locate the host plant of the cabbage aphid host. In addition, Sheehan & Shelton (1989b) found that the pre-emergent environment was a factor influencing the plant finding (orientation to plant odours) of female *D. rapae*, with females reared on collards flying significantly more often to collards in a wind tunnel experiment than those reared on potato, the less preferred plant.

(ii) Host location

Once in the host habitat, the female parasitoid searches for hosts, with different species responding to stimulii associated with their hosts. The stimulii may be either physical (e.g. vibrations, sound, vision, infrared radiation) or chemical, usually in the form of kairomones (Vinson, 1976; Weseloh, 1981). These kairomones can either be volatile and therefore detected by olfaction, or only detected after direct physical contact with them in

a solid or liquid form (Vinson, 1976). Once the kairomones are detected, parasitoids change their search behaviours which increases the probability of locating hosts. A reduction in walking speed, antennal examination of the substrate, and increased turning have been observed for various aphidiid species (e.g. Bouchard & Cloutier, 1984; van Alphen & Vet, 1986; Hood-Henderson & Forbes, 1988), and it has also been suggested that contact

Table 1.4. A list of published studies investigating the use of honeydew in host location by aphid parasitoids (modified from Hågvar & Hofsvang, 1991).

Species	Method	Behaviour of the parasitoids	Author
Aphidius nigripes Ashmead	Observations on plants and on filter paper	Increased searching time on plants contaminated with honeydew and on leaves and filter paper painted with water extracts of honeydew	Bouchard & Cloutier (1984)
Aphidius nigripes	Olfactometer	Females responded to honeydew from four aphid species: from preferred, less preferred and from a non-host aphid	Bouchard & Cloutier (1985)
Aphidius nigripes	Observations on plants	The residence and searching time were longest on aphid-infested plants, but almost twice as long on honeydew-contaminated plants compared with clean plants	Cloutier & Bauduin (1990)
Aphidius rhopalosiphi De Stefani-Perez	Observations on filter paper	The presence of honeydew increased the time spent searching during a visit on a leaf or on the ear of wheat	Gardner & Dixon (1985)
Aphidius rhopalosiphi	Observations on filter paper	Increased searching time on honeydew-treated areas	Budenberg (1990a)
Aphidius smithi Sharma & Subba Rao	Observation on leaves	Searching time during single visits significantly shorter on clean leaves vs. honeydew-covered leaves	McGregor & Mackauer (1989)
Diaeretiella rapae M'Intosh	Observations on plants	Honeydew is used as a kairomone shaping the foraging strategy on a plant	Ayal (1987)
Ephedrus cerasicola Starý	Observations on plants in cages and in glasshouses	Honeydew-contaminated plants had more parasitoids than fresh plants, aphid-infested plants had more parasitoids than honeydew-plants	Hågvar & Hofsvang (1989)
Praon pequodorum Viereck	Observations in Petri-dishes	The rate of travel slowed and the degree of turning increased upon contact with patches of fresh honeydew	Hood-Henderson & Forbes (1988)

kairomones can give information to female parasitoids about host densities and species before host encounter occurs (van Alphen & Vet, 1986; Ayal, 1987).

An important kairomone involved in the host location process by aphidiid parasitoids is the host-modified plant product honeydew, which has been shown in numerous studies to be a contact kairomone (Table 1.4).

(iii) Host acceptance

Host acceptance is the process whereby hosts are accepted or rejected for oviposition after antennal or ovipositor contact has been made by the female parasitoid (Vinson, 1976). Stimuli which can elicit host examination include shape, texture, size, chemicals, host movement, sound and sight (Arthur, 1981). Studies with aphidiids have shown the influence of host species, host size or age, aphid defences, chemicals (haemolymph), fungal infection, colour-form of one host species and previously parasitised hosts on host acceptance (Hågvar & Hofsvang, 1991).

(iv) Host suitability

After an egg has been deposited in the host, the successful development of the parasitoid depends upon the host suitability. Factors determining suitability include nutritional suitability, competition with other parasitoids inside the host, host immunity responses and host endocrine balance (Vinson, 1984; Vinson & Iwantsch, 1980a; Hågvar & Hofsvang, 1991).

(v) Host regulation

In order for a parasitoid to survive in a host, it may be necessary for the parasitoid to regulate the hosts development to meet its own needs. Some parasitoids have the ability to induce changes in host growth rate, food consumption, development, reproduction, morphology, behaviour, respiration, and biochemical and physiological activities (Vinson & Iwantsch, 1980b). These changes can occur as a result of the development of the parasitoid egg, through larval feeding, or as a result of chemical factors injected with the parasitoid egg.

Parasitism levels in relation to host density

Successful biological control is believed to be a result of low, stable equilibria between pest and enemy populations, brought about by density-dependent parasitism (Huffaker & Messenger, 1976). In accordance with optimal foraging theory (Hubbard &

Cook, 1978; Waage, 1979), effective parasitoids should concentrate their search within high-density patches of their hosts (spatial density-dependent parasitism), resulting in maximum pest depression and stability. However, records of density-dependent parasitism are not commonly reported in the literature (Stiling, 1987; Walde & Murdoch, 1988). Of the few studies combining observations of parasitoid foraging behaviour with resultant levels of parasitism, most have recorded a strong aggregative response of parasitoids to the host patches, but with resulting density-independent parasitism (e.g. Waage, 1983; Smith & Maelzer, 1986; Hirose *et al.*, 1990).

In the Aphidiinae, only the study by Hågvar & Hofsvang (1987) has investigated the relationship between foraging behaviour and parasitism levels. They concluded that *Ephedrus cerasicola* Starý females aggregated in areas of high aphid population density, but that the resultant parasitism was density-independent.

Conservation and manipulation of parasitoid populations on farmland

Various attempts have been made to enhance biological control by parasitoids, either released as part of a control programme, or 'wild' populations (Powell, 1986). These include the:

(1) provision of alternative hosts at times when the pest host is scarce;

(2) provision of food resources (pollen and nectar) for adult parasitoids;

(3) provision of refugia (e.g. for overwintering);

(4) maintenance of small populations of the pest hosts over extended periods to ensure the continued survival of the parasitoid population;

(5) release of behaviour-modifying chemicals.

Alternative hosts

Many of the parasitoid species which attack aphids on crops also attack other, non-pest aphids in semi-natural habitats. For example, *A. ervi* attacks the cereal aphids *S. avenae* and *M. dirhodum*, but also the pea aphid *Acyrthosiphon pisum* (Harris) and the nettle aphid *Microlophium carnosum* (Buckton) (Powell & Wright, 1988). The provision of these alternate hosts is important for maintaining local parasitoid populations when pest aphids are scarce and hence allows the build up of parasitoid numbers early in the year (Perrin, 1975; Starý, 1986).

Adult food resources

Many adult parasitoids require nectar and pollen to complete their life-cycles

(Zandstra & Motooka, 1978) and to ensure effective reproduction (van Emden, 1962). Most crop monocultures, especially annual crops, fail to provide adequate sources of these foods, and the manipulation, both quantitatively and qualitatively, of pollen and nectar sources within crops or in surrounding areas may have a role to play in enhancing parasitoid populations (Altieri & Whitcomb, 1979; Altieri & Letourneau, 1982).

Provision of refugia and the maintenance of small pest populations

Studies incorporating intercropping and mixed cropping of two or more plant species have been shown to result in higher levels of parasitism in pest populations, compared with monocultures of the same crops (Altieri *et al.*, 1978; Altieri & Todd, 1981). A system of strip cutting that leaves uncut strips of crop throughout the season can provide reservoirs of aphidiid parasitoids which can rapidly recolonise recently harvested strips (van den Bosch *et al.*, 1967).

Behaviour-modifying chemicals

Examples of the ways in which behaviour-controlling chemicals such as kairomones, synomones and pheromones (collectively called semiochemicals) might be used to manipulate parasitoids include: (i) to attract and retain parasitoids during the early stages of pest infestation when a favourable pest:parasitoid ratio is critical for efficient control (plant synomones; aphid kairomones), (ii) to increase the search times and attack rates of parasitoids (plant synomones; aphid kairomones), (iii) to disrupt the host finding and host acceptance behaviour of hyperparasitoids and so reduce their impact on primary parasitoid populations (parasitoid kairomones), (iv) to monitor parasitoid and hyperparasitoid abundance and activity as an aid to pest forecasting (parasitoid pheromones).

Chemical pest control

Modern agriculture has come to rely extensively on synthetic chemical pesticides for the control of a wide range of pests, including insects, mites, nematodes, rodents, weeds, and bacterial, viral and fungal pathogens. Although these compounds are targeted at the plant pests, many are broad-spectrum in activity causing mortality of non-target species within agricultural ecosystems.

After the advent of DDT in 1943, numerous other synthetic pesticides, including organochlorines, organophosphates and carbamates, were introduced onto the pesticide market (Elliott *et al.*, 1978). These provided good economic pest control, resulting in the production of high yields. However, it soon became evident that compounds such as the organochlorines had high chemical stability and were persistent in the environment. Accompanied with high lipid solubility, these compounds biomagnified resulting in indirect toxicity to non-target organisms, particularly those at the top of the food chain (Carson, 1962). The continued overuse of such compounds led to the development of resistance, resurgence and secondary pest outbreaks, necessitating the use of further pesticide applications (Metcalf, 1986; Leahey, 1985).

The integrated pest management (IPM) concept was developed essentially as a response of this incompatibility of pesticide and biological controls (Bartlett, 1964). Modern IPM is based on an understanding of the necessary interrelatedness of pesticides and natural enemies.

Synthetic pyrethroid insecticides

The pyrethroid insecticides are derivatives of the pyrethrins, a group of esters that occur naturally in the flowers of a number of *Chrysanthemum* species (Compositae) (Leahey, 1985). Synthetic pyrethroids were produced for the commercial market in the late 1970's (Jackson, 1989), and by 1989 they comprised approximately one-quarter of all foliar insecticides used in agriculture worldwide, over an estimated 100 million hectares (Cox, 1990). In Great Britain, the area of arable land used for cereal production has decreased over the period 1982-1992, but this has been accompanied by an increase in area treated with insecticides (Table 1.5). The percentage use of synthetic pyrethroids (mainly cypermethrin and deltamethrin) within this total has shown a dramatic increase over this period.

The success of pyrethroids as a class of insecticide can be attributed to their broad spectrum of activity resulting in high levels of control against a range of lepidopteran, coleopteran, homopteran, heteropteran, dipteran, orthopteran and thysanopteran pests. They exhibit low mammalian, avian and bee toxicity, rapid knockdown and antifeedant and

repellent effects in insects, accompanied by a lack of persistence (i.e. rapid biodegradability) in the environment. Commercial formulations are used at very low field dose rates, i.e. 5 to 25g A.I. ha^{-1}, therefore remaining cost-effective compared to other insecticide groups.

Table 1.5. Trends in pesticide usage and the area of cereals grown in Great Britain (data taken from Davis *et al.*, 1992).

Year	1982	1988	1990	1992
Area grown (million ha)	3.97	3.84	3.60	3.43
Total area treated with insecticides (million ha)	0.54	1.28	3.09	2.30
Percentage area treated with synthetic pyrethroids	14%	22%	69%	77%

The main routes of intoxication for organisms are through direct contact during spraying, contact with residual deposits and consumption of contaminated material (Hirano, 1989). The precise mode of action and toxicity of pyrethroids varies between different compounds and organisms. Differences in physiological resistance or tolerance to pyrethroids is governed by species-dependent differences in the site of primary lesion and also by the organisms ability to detoxify the insecticide by esterase activity or hydrolysis (Soderlund & Bloomquist, 1989). A major advantage of pyrethroids is their low mammalian toxicity, which results from their conversion, by hydrolytic or oxidative processes, to polar metabolites which are then eliminated in faeces and urine, unchanged or as conjugates, before sensitive sites are reached (Litchfield, 1985).

The sodium-ion channel is considered to be the primary site of pyrethroid action. A 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 (Gammon *et al.*, 1981; Ruigt, 1985; Soderlund & Bloomquist, 1989). The poisoning of houseflies, *Musca domestica* (L.), with pyrethroids has been extensively studied. Intoxication results in the uncoupling of their normal coordinated flight activity prior to the final inhibition of motor activity, supporting the idea of an initial bias towards peripheral sites of action (Adams & Miller, 1980).

The environmental fate of pyrethroids is determined by their high lipophilic tendencies and low volatility, resulting in contact rather than systemic action on plants (Graham-Bryce, 1987). Residues of pyrethroids on the soil are strongly adsorbed to colloids

(particularly organic matter) where they are metabolised by micro-organisms (Ruscoe, 1977; Elliott, 1989). The behaviour of residues on foliage and in soil is related to factors such as temperature, soil type and moisture content. An important feature of pyrethroids is their negative temperature coefficient (i.e. the toxicity increases as temperature decreases) (Sparks *et al.*, 1983; Ruigt, 1985; Heimbach & Baloch, 1994). For example, the half-life of deltamethrin in soil at 10°C is 10-11 weeks, but 5-6 weeks at 20°C (Hill & Schaalje, 1985). In the field, the photo-stable pyrethroids have been shown to persist on crops for 7-30 days (Elliott, 1989).

Insecticidal control of cereal aphids

Aphid populations in cereal crops are controlled either by prophylactic spraying with an aphicide or by monitoring aphid numbers in the crop and using threshold levels set by ADAS (Agricultural Development and Advisory Service) to decide when spraying is necessary to prevent economic damage. At present, farmers are advised to apply a summer aphicide when 66% of wheat stems are infested with one or more aphids between flowering and the milky ripe stage (decimal growth stages 61 to 75, Tottman & Broad (1987)).

Prophylactic spraying becomes uneconomic when aphids are not causing economic damage, and broad-spectrum insecticides may kill beneficial non-target invertebrates which may lead to later aphid outbreaks if favourable conditions exist (Powell *et al.*, 1985). To aid forecasting of damage caused by aphid populations, sophisticated computer models have been devised to account for pest numbers, crop growth stage and the economics of spraying (e.g. Mann & Wratten, 1986, 1991).

Before 1990, concerns about the broad-spectrum insecticidal nature of pyrethroids, and hence effects on non-target organisms, resulted in their restriction to autumn spray applications only. In 1990, two compounds, deltamethrin and alphacypermethrin, received provisional clearance for use as summer aphicides in cereals, to be applied between the onset of crop flowering and milky ripe stage.

Reduced pesticide inputs in cereals

Pesticide applications in cereals have been shown to reduce natural enemy population densities (Vickerman & Sunderland, 1977; Basedow *et al.*, 1985; Powell *et al.*, 1985; Fischer & Chambon, 1987; Vickerman *et al.*, 1987a, 1987b) and may even lead to local extinctions in some circumstances (Burn, 1992). Partly for this reason, the use of reduced dose-rates of aphicides in cereals have been advocated and has been shown to offer an economic means of aphid control with potential environmental benefits (Poehling, 1989, 1990; Mann *et al.*, 1991).

Registered pesticide dose rates normally lie beyond the upper asymptote of the dose response curves of pests, to ensure complete eradication (van Emden, 1989). In theory, it should be possible to achieve differences in the primary toxic effects on target and non-target organisms by reducing these dose rates below their maxima (Poehling, 1989). This hypothesis is based on the different type of dose responses shown by carnivores in comparison with many herbivores. The range of insecticide doses spanning low to high kills of herbivores is generally larger than that for carnivores (van Emden, 1989). One explanation for this is that it is connected with the wide range of enzymes, some of which can detoxify pesticides, which herbivores need to cope with the many secondary compounds they encounter in their host plants. The potential result is that the kill of natural enemies decreases faster than the kill for pests as insecticide dose decreases, thus resulting in insecticide selectivity at low doses (Poehling, 1989).

Based on economic and political pressures, rather than sound biological knowledge, the development of pesticide reduction programmes have been recently introduced in Western Europe (e.g. Sweden, Denmark, Netherlands), USA and Canada (Jansma *et al.*, 1993; Hill *et al.*, 1993). Before the advocation of such policies in UK cereal systems, an increased knowledge of optimum dose-rates and their effects upon pest and beneficial invertebrate populations is required.

Pesticide effects on non-target invertebrates

Non-target invertebrates, some of which are beneficial as predators and parasitoids, are killed along with the target pests because of the broad-spectrum activity of most insecticides. The destruction of these natural enemies may exacerbate pest problems (Vickerman & Sunderland, 1977; Vickerman *et al.*, 1987b). Five possible explanations for decrease in natural enemy effectiveness following pesticide application have been proposed (Waage, 1989):

(1) pesticides kill some natural enemies;

(2) pesticides have a sublethal effect on some natural enemies, reducing survival or foraging efficiency;

(3) pesticides enhance pest reproduction, allowing pests to escape control by natural enemies exhibiting inverse density-dependent functional responses;

(4) pesticides change local pest distribution, causing temporary emigration of natural enemies or reduced foraging efficiency;

(5) pesticides synchronise pest populations, causing the local extinction of natural enemy populations and pest increase as in (4).

Interest in natural enemy/pesticide research has grown rapidly since the mid 1970's (Croft & Brown, 1975; Croft, 1977). In order to catalogue the various research areas, a database (named SELCTV) incorporating pesticide effects on arthropod natural enemies of agricultural pests over the past 40 years was designed, including over 400 agricultural chemicals tested on 609 species of predators and parasitoids (Theiling, 1987; Theiling & Croft, 1988; Croft, 1990).

A review of the SELCTV database (Theiling & Croft, 1988) shows that parasitoids are generally more susceptible to pesticides than predatory natural enemies, and their prey or hosts (Croft & Morse, 1979; Plapp, 1981; Mullin & Croft, 1985). The life stages of a parasitoid show differences in their susceptibility to pesticides when assessed according to a toxicity rating scale where: 1 = 0% mortality; 2 = <10% mortality; 3 = 10-30% mortality; 4 = 30-90% mortality; and 5 = 90-100% mortality. The database shows that egg (mean toxicity rating=3.8) and adult (3.7) life stages show the most susceptibility, while larvae (3.2) and pupae (3.3) appear the most tolerant. Within all the insecticide classes present in the database, synthetic pyrethroids show the highest proportion (>40%) of the cited references appearing in the highest toxicity rating category (>90% mortality) for effects on natural arthropod enemies, and within this class deltamethrin appears to show the highest intrinsic toxicity (Theiling & Croft, 1988).

Modes of pesticide uptake by parasitoids

Direct mortality or sublethal effects of pesticides (Figure 1.1, solid lines) are caused by a combination of (i) direct contact resulting from direct interception of the spray droplets or vapour inhalation, (ii) residual contact involving external contact with the pesticide once it has landed, usually on a plant or soil surface, and (iii) food chain transfer which involves the parasitoid feeding on pesticide residues contained in or on an insect or plant host.

Uptake of the pesticide can kill the free-living adult stage of a parasitoid (Figure 1.1a) or an exposed ectoparasitoid (Figure 1.1c), however a developing endoparasitoid may normally be protected inside the host from immediate contact or residual uptake. Direct mortality (Figure 1.1c) can however occur through food chain transfer of the pesticide via feeding on the host (Figure 1.1e) or through the absorption of volatiles through the host tissues.

The pesticide can cause mortality or sublethal effects in the arthropod host (Figure 1.1b) and therefore indirectly affect any developing endo/ectoparasitoids (Figure 1.1d) and also the presence of the parasitoid may make the host more vulnerable to direct and residual pesticide uptake. Parasitism can also affect the susceptibility of the host to toxicants obtained in the food-chain from the host plant (Figure 1.1g), leading to mortality

of the insect host (Figure 1.1b) and indirectly affecting the parasitoid (Figure 1.1d).

The free-living adult stage of the parasitoid can come into contact with the pesticide

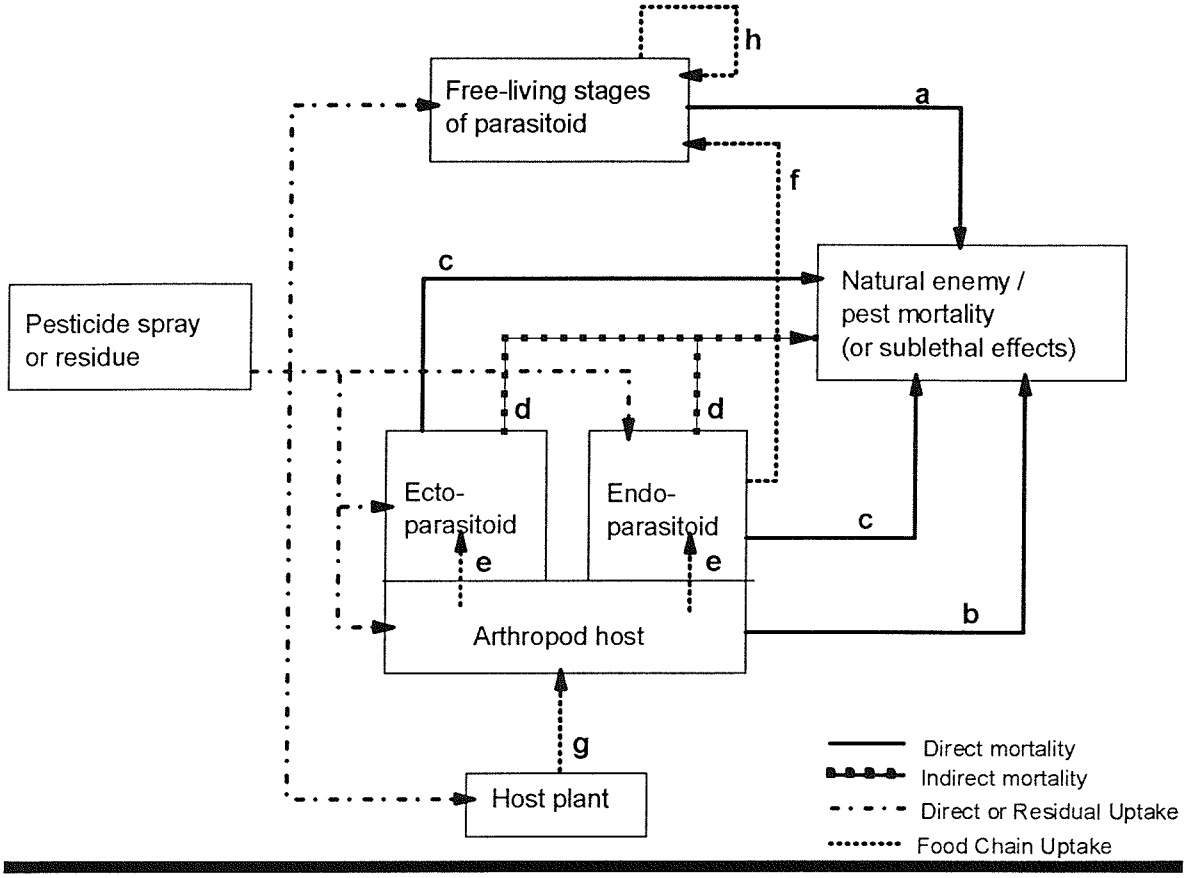


Figure 1.1. Modes of pesticide uptake by a parasitoid (taken from Croft, 1990).

via host feeding, cannibalism and nectar, honeydew or pollen feeding (Figure 1.1h). Latent effects of pesticides can also occur, resulting in the transfer of delayed mortality or sublethal effects from one life stage to another (Figure 1.1f).

The factors determining pesticide uptake by the different life stages of parasitoids are complex, interrelated and poorly documented in the literature (Croft, 1977). Therefore, work outlined in this thesis has concentrated on identifying the relevant importance of direct, residual and dietary exposure to adult stages, and protection in the mummy case, within laboratory, semi-field and field-based experiments.

Assessment of pesticide effects on non-target invertebrates

The development of testing methods and strategies for evaluating the effects of pesticides on non-target arthropods for regulatory and IPM purposes has been conducted by the IOBC/WPRS (International Organisation of Biological Control/West Palaeartic Regional Section), BART (Beneficial Arthropod Regulation Testing Group) and EPPO/CoE (European and Mediterranean Plant Protection Organisation with the Council of Europe). In order to quantify the effects of a pesticide on a beneficial non-target organism, a combination of tests that include laboratory, semi-field and field methods to be conducted in a set sequence is recommended (e.g. Hassan, 1989).

The methodologies cover the three temporal/spatial scales on which side-effects can be assessed (Figure 1.2). These are the "micro" (≡laboratory) scale, determining the level of initial uptake and toxicity of the pesticide by individuals in the crop during and after spray application, the "meso" (≡semi-field) scale, determining within-year effects on populations within treated plots, and the "macro" (≡field) scale, determining effects on populations in whole-field studies, between fields and seasons. The chronological sequence of the processes of pesticide contamination, including all the processes from initial exposure to final recovery of a non-target invertebrate is shown running from left to right in Figure 1.2. The three interlocking boxes indicate the three spatiotemporal scales, with a gradual increase in the environmental complexity and realism as scale increases, with a consequent decrease in the degree of experimental control and precision (Croft, 1990). The areas falling wholly within one box have independent methodologies associated with them and cannot be investigated using the techniques associated with a preceding spatial or temporal scale.

Within the IOBC/WPRS testing procedures, if a pesticide is found to be "harmless" to a particular beneficial organism in the initial laboratory toxicity test then no further testing is required. This indicates that it will remain "harmless" in the field situation. However, if a pesticide is shown to be "harmful" in laboratory screening, then further testing in semi-field experiments is needed to determine the duration of harmful activity, and/or the effect of a dry pesticide film on plant or soil. Further testing in field conditions may be warranted to show the effect of a direct spray of the pesticide on plants or soil inhabited by the beneficials.

Beneficial organisms chosen for testing should be relevant to the crops on which the pesticide is to be applied. The IOBC/WPRS group recommends the following for field crops, e.g. wheat: one general predator (e.g. Chrysopidae, Coccinellidae), one soil-inhabiting predator (e.g. Carabidae, Staphylinidae), and one aphid parasitoid (e.g. Aphidiinae).

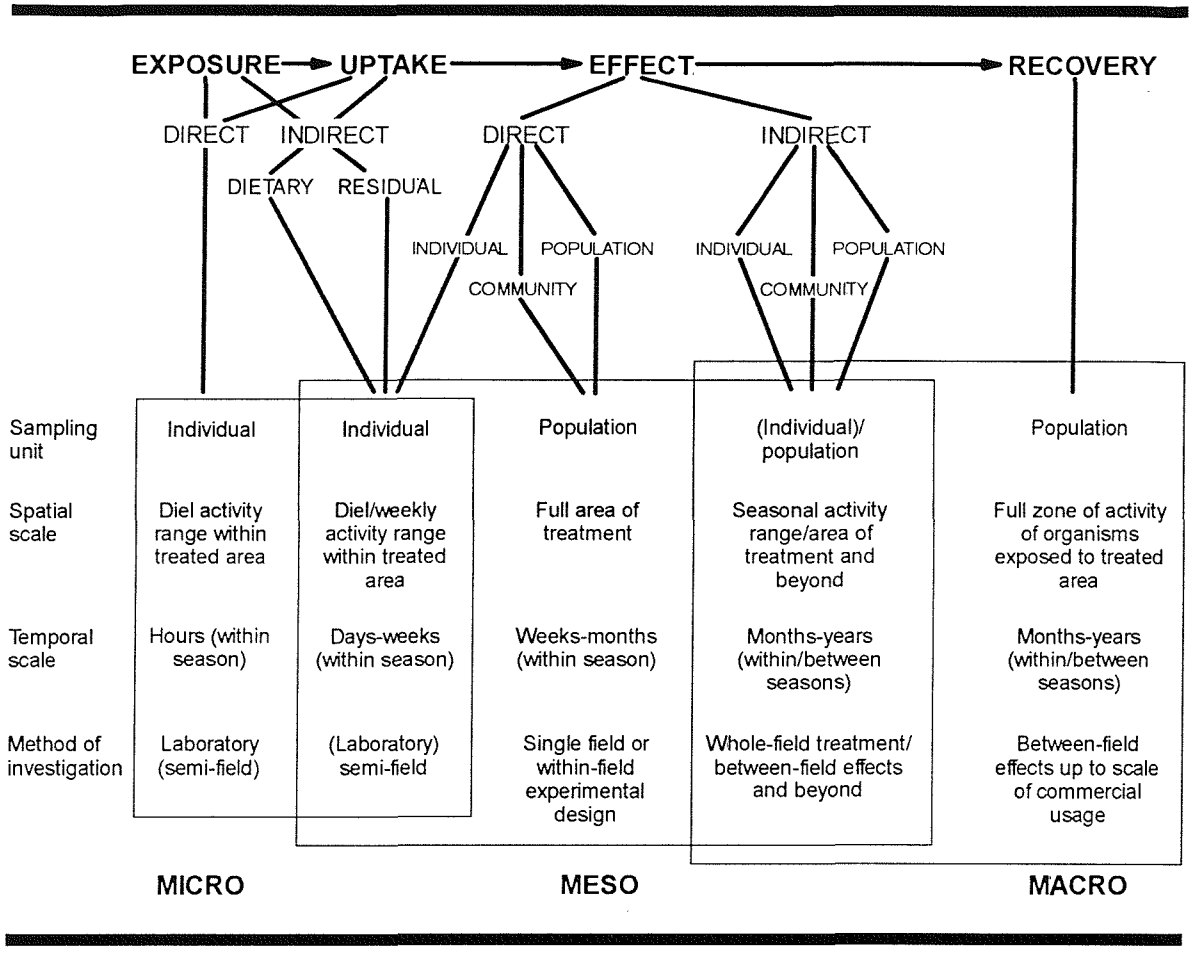


Figure 1.2. The temporal and spatial scales over which the processes of contamination and biological impact of pesticides act on non-target invertebrates in arable crops (from Jepson, 1989).

For the laboratory screening bioassays, the following methods are used (Hassan, 1989):

1. For the 'exposed' life stages (e.g. adults of parasitoids):

a) exposure to freshly dried pesticide deposits; b) recommended concentration of pesticide; c) application on glass plate, leaf or sand (soil); d) even film of pesticide, standard amount of 1-2 mg fluid/cm2 on glass or leaf and 6 mg fluid/cm2 on sand (soil); e) laboratory-reared organisms uniform in age; f) adequate exposure period before evaluation; g) adequate ventilation; h) water-treated controls; j) reduction in beneficial capacity/mortality; k) four evaluation categories: 1 = "harmless" (<50%), 2 = "slightly harmful" (50-79%), 3 = "moderately harmful" (80-99%), 4 = "harmful" (>99%). However, Hassan (1992) suggests that the "harmless" category should now be less than 30% and "slightly harmful" adjusted to 30-79%.

2. For the 'protected' or 'less exposed' life-stage (e.g. parasitoids within their hosts):

a) direct spray of organism; b) recommended concentration of pesticide; c) adequate ventilation; d) laboratory-reared organisms uniform in age; e) water-treated controls; f) reduction in beneficial capacity/mortality; g) four evaluation categories, as in test (1).

These laboratory studies are of limited value for predicting risk posed by insecticides to pest and beneficial populations in the field (Brown *et al.*, 1990). In addition to considering direct mortality in beneficial parasitoids, laboratory studies need to examine any sublethal effects which might ultimately result in reduced population numbers and effectiveness in suppressing host populations. However, to understand fully the impact of pesticides, realistic semi-field or field studies are necessary. These reflect the outcome of the complex interactions generated by parasitoid behaviour, development, and population dynamics, as well as pesticide residue dynamics and the ecological interactions of the parasitoid and host populations.

Aims of the study

This study was undertaken to investigate the side-effects of summer-applied synthetic pyrethroid insecticides upon cereal aphid parasitoids. This was to increase knowledge and understanding of pest-parasitoid-insecticide interactions, thereby improving risk predictions in the future.

Chapter 2 uses standard laboratory test methods to investigate the intrinsic toxicity of the pyrethroid insecticide, deltamethrin, at a range of concentrations, to parasitoids. The incorporation of further test methods, which provide greater realism to the field, are investigated to enable the calculation of "correction factors" for standard laboratory toxicological data.

Chapters 3 and 4 concern parasitoid foraging behaviours in the presence of honeydew and insecticide residues to enable improvement of current laboratory/semi-field tests and interpretation of field data.

Chapter 5 investigates the potential of using reduced dosage applications of deltamethrin to control cereal aphids. The study involves the measurement of within-plant spatial changes of the pest population after applications, accompanied by an assessment of residual toxicities of deltamethrin to the parasitoid.

Chapter 6 describes a laboratory investigation of the routes of exposure (topical, residual and oral) of parasitoid adults and pupae to insecticides.

Chapter 7 concerns a large field-scale experiment, involving measurements of insecticidal effects on aphid and hymenopteran populations. Recovery times and patterns

of reinvasion of the different invertebrate groups into treated areas are measured.

The experimental system

The cereal ecosystem was selected because it represents the largest arable land-use in Great Britain (Davis *et al.*, 1992), and is becoming something of a model for the development of more sophisticated ways of judging the risks that pesticides pose to natural enemies (Jepson 1993a, 1993b).

Within these crops, parasitoids are known to be important biological control agents of aphid pests (Powell, 1983). Aphid primary parasitoids within the genus *Aphidius* were chosen as the main study organisms because they represent the most abundant and important group within the cereal ecosystem (Powell, 1982; Borgemeister & Poehling, 1988). For the laboratory bioassays, *A. rhopalosiphi* was selected as the test organism because it is the commonest species in cereals in Western Europe (McLean, 1980), its biology is well documented (e.g. Shirota *et al.*, 1983) and it has been selected as the standard parasitoid species to be used in all future pesticide screening bioassays by the BART Group (Barrett *et al.*, 1994).

The pyrethroid insecticide, deltamethrin, was chosen as the experimental compound, because it represents one of the commonest insecticides applied to cereals in Great Britain for aphid control (Davies *et al.*, 1992), and is known to cause mortality within aphidiid populations (Krespi *et al.*, 1991).

CHAPTER TWO

LABORATORY TOXICITY ASSESSMENTS OF DELTAMETHRIN AGAINST DIFFERENT LIFE-STAGES OF *APHIDIUS RHOPALOSIPHI*

INTRODUCTION

Hymenopteran parasitoids are known to be extremely vulnerable to pesticides, with considerable research effort having been put into the quantification of the directly toxic effects of pesticides over a wide number of species (see Elzen (1989) for a review). To encourage the standardisation of current and future pesticide testing procedures, the International Organisation of Biological Control / West Palaearctic Regional Section (IOBC / WPRS) 'Pesticides and Beneficial Invertebrates' Working Group was established in 1974 (Hassan, 1985). Some testing procedures developed by this group are aimed at assessing the effect of pesticides on parasitoid species, by covering both the free-living adult stage and also the larval/pupal stage inside the host (Table 2.1).

The adult represents the most vulnerable stage of the parasitoid life-cycle to pesticides (Starý, 1970). Direct toxic effects occur as adult mortality, with additional sublethal effects including reduced survival, longevity and fecundity, which may remain detectable for up to two successive generations (e.g. Abo El-Ghar & El-Sayed, 1992).

Table 2.1. A list of published test methods for evaluating side-effects of pesticides against parasitic Hymenoptera.

Parasitoid species	Host species for cultures	Reference
Aphidius matricariae Haliday	*Myzus persicae* (Sulzer)	Polgar (1988)
Aphidius rhopalosiphi DeStefani-Perez	*Metopolophium dirhodum* (Walker)	Mead-Briggs (1992)
Coccygomimus turionellae (L.)	*Galleria mellonella* (L.)	Bogenschütz (1988)
Diaeretiella rapae McIntosh	*M. persicae* or *Brevicoryne brassicae* (L.)	Kühner *et al.* (1985a)
Encarsia formosa Gahan	*Trialeurodes vaporariorum* (Westwood)	Oomen (1985)
Phygadeuon trichops Thoms.	*Delia antiqua* (Meigen)	Naton (1988)
Trichogramma cacoeciae March	*Sitotroga cerealella* (Olivier)	Hassan (1988)

In aphid parasitoids, "less exposed" or "protected" life stages are also used in laboratory screening tests and include the late larval, prepupal and pupal stages within the mummified bodies of their aphid hosts. These have been shown to confer a high degree of protection to certain parasitoids against the influence of pesticides (Table 2.2). The level of protection provided by the mummified case against certain agrochemicals can be compared by measuring the degree of adult emergence and subsequent longevity and fecundity of surviving females. For each parasitoid species, these variables are determined by the type of insecticide, the dose applied, the method of application and the age of the mummy.

Within the cited studies, there are great differences in the range of chemicals tested and methodologies used, making comparisons between them difficult. The most variable aspect of the laboratory tests seems to be the method of pesticide application used (Table 2.2). This varies from dipping (immersing the whole mummy in a pesticide solution), micro-applicator procedures (placing a droplet of pesticide solution on the dorsal surface of the mummy), and laboratory and glasshouse sprayers (either spraying the pesticide solution to "run-off" or attempting to simulate the spray deposition of commercial field sprayers). Most of these do not reflect spray deposition found under field conditions and hence the true exposure of mummies to pesticides.

Current laboratory pesticide testing with parasitoids represent a worst-case scenario of exposure in the field (Brown *et al.*, 1990). To ensure maximum exposure and rates of chemical uptake, adults are forced to remain in contact with residual pesticide deposits, often on inert surfaces, for extended periods of time. The larvae/pupae are exposed through the direct application of the maximum recommended concentration of pesticide onto their hosts. These tests provide repeatable and predictable levels of exposure enabling the screening of a large number of different pesticides and ranking according to their toxicity. They are however, of limited use in interpreting and predicting the risks posed by pesticides to beneficial species in the field.

The bioavailability of pesticide residues to insects in the field is determined by species-dependent factors such as activity pattern, behaviour, and the degree of contact with the surface (Jepson *et al.*, 1990; Jepson, 1993a; Wiles & Jepson, 1993a). It is also dependent upon the properties of the chemical and the treated substrate. The interactions between chemical and substrate are governed by processes of adsorption, desorption, and volatilisation, which depend upon the nature of the plant cuticular wax layer (Adams *et al.*, 1987) and the organic matter and clay content of soil (Arnold & Briggs, 1990).

To aid risk predictions for adult parasitoids in the field from laboratory screening

Table 2.2. List of published studies concerning the degree of protection against pesticides of parasitoid larvae/pupae inside aphid mummy cases

Authors	Parasitoid species	Method of pesticide application	Pesticides and dose rates tested (% reduction in numbers emerging from treated mummies after correction for control mortality)
Lingappa *et al.* (1972)	*Lysiphlebus testaceipes*	Laboratory spray (no details given)	Label-recommended concentrations: disulfoton (11%), parathion (21%)
Süss (1982)	*Aphidius ervi*	Laboratory spray (no details given) on mummies positioned on wheat ears	Label-recommended concentrations: ethiocarb (21%), permethrin (28%), pirimicarb (33%), dimethoate (39%), mevinphos (63%)
Kühner *et al.* (1985)	*Diaeretiella rapae*	Sprayed to "run-off" using wash bottle	Highest label-recommended concentrations: glyphosate (0%), desmetryn (13%), propachlor (36%), cypermethrin (50%), pyrazophos (76%)
Hsieh & Allen (1986)	*Diaeretiella rapae*	Sprayed to "run-off" using laboratory pressurised atomiser	Full and half label-recommended concentrations: acephate (FR 8%; 1/2 FR 3%), permethrin (FR 11%, 1/2 FR 9%), methomyl (FR 10%, 1/2 FR 6%), malathion (FR 82%), diazinon (FR 100%)
El-Sayed & Abo El-Ghar (1989)	*Diaeretiella rapae*	Field spray on mummies positioned on plants, using knapsack sprayer	Minimum and maximum label-recommended concentrations: triazophos (7%-18%), malathion (7%-34%), profenofos (17%-44%)
Krespi *et al.* (1991)	*Aphidius uzbekistanicus*	Laboratory spray (no details given)	Label-recommended concentrations: lamda-cyhalothrin (7%), dimethoate (15%), deltamethrin (25%)
Shean & Cranshaw (1991)	*Aphelinus semiflavus/ Diaeretiella rapae*	Mummies dipped for 1sec in solution	Label-recommended concentrations: abamectin (*A. semiflavus* 40%; *D. rapae* 3%), endosulfan (*A. semiflavus* 0%; *D. rapae* 2%)
Abo El-Ghar & El-Sayed (1992)	*Diaeretiella rapae*	Sprayed to "run-off" using laboratory pressurised atomiser	Full (FR) and reduced label-recommended concentrations: cypermethrin (FR 68%; 1/2FR 55%), isoxathion (FR 93%; 1/2FR 73%; 1/4FR 50%; 1/10FR 38%), methomyl (FR 55%; 1/2FR 46%), prothiofos (FR 97%; 1/2FR 86%; 1/4FR 56%; 1/6FR 46%), tralomethrin (FR 73%; 1/1.3FR 48%; 1/2FR 58%)
Borgemeister *et al.* (1993)	*Aphidius rhopalosiphi*	Glasshouse sprayer calibrated for field application	Full (FR) and reduced label-recommended concentrations: demeton-s-methyl (FR 2%), fenvalerate (FR 0%; 1/2.5FR 2%); parathion (FR 20%); pirimicarb (FR 9%; 1/4FR 3%)

data, a comparison of deltamethrin toxicity on glass and foliage substrates was made in order to calculate a "correction factor". The quantification of latent toxic effects which result from parasitoids contacting insecticide residues for limited periods of time, before dispersing away from the treated area, was also made to incorporate more realism to predictions of risk in the field.

Applying a pesticide at the recommended field concentration directly onto aphid mummies ensures that they receive the maximum quantity of active ingredient that is possible from a field spray application. This quantity is likely to be unrealistically high, because mummies can be positioned at different sites throughout the crop strata and so may receive varying amounts of spray as a result of attenuation of spray deposition through the crop canopy (Çilgi & Jepson, 1992). Standard laboratory bioassays were conducted to assess the degree of protection against deltamethrin provided by the aphid mummy case to developing parasitoids. In addition, an experiment was conducted to quantify the direct spray exposure of aphid mummies positioned throughout a cereal crop canopy. This was to enable the calculation of "correction factors" for the laboratory-derived mortality data.

EXPERIMENTAL METHODS

Exposure of adult *Aphidius rhopalosiphi* to deltamethrin residues on glass and foliage

Test unit

The exposure chambers (modified from Mead-Briggs, 1992) consisted of two treated glass plates which were fitted to a section of plastic drainpipe (10.5cm diameter and 2.5cm high), with five holes (10mm diameter) drilled through the side walls for ventilation. The holes were covered on the inside with fine gauze, using a non-toxic glue (UHU). One hole was left uncovered for the introduction of the parasitoids and this was later sealed with a cotton wool plug, soaked in a 50:50 honey:water solution as a food source. The whole unit was held firmly together with rubber bands. In order to minimise the build-up of pesticide vapour, the exposure chambers were ventilated with humidified air using a small aquarium pump which was connected to one of the holes in the plastic drainpipe by rubber tubing.

For the glass bioassay, two glass plates (12cm x 12cm) that were thoroughly cleaned with the detergent ("Decon-90" , Decon Manufacturing Ltd.) and rinsed with distilled water were used as the substrate for exposure. In the flag leaf bioassay, clean glass plates (12cm x 12cm) were covered with freshly excised flag leaves from glasshouse-grown, untreated winter wheat plants, c.v. Galahad, at decimal growth stages 61-62 (Tottman & Broad, 1987). Leaves were attached to the glass using double-sided adhesive tape, in parallel, base to tip, with their adaxial surfaces exposed covering the whole glass plate.

Test insects

For each treatment, three replicate exposure chambers were set up, each containing 10 adult *A. rhopalosiphi* (5 male and 5 female) of uniform age (i.e. those arising from a discrete cohort and emerging over a single 24 hour period, and used within 48h of emergence). Parasitoids were cultured in an environment-controlled growth room on a population of cereal aphids, *Sitobion avenae* (F.), on barley seedlings (as described in Mead-Briggs, 1992).

Application of insecticide

A stock solution of the recommended field concentration of deltamethrin (Decis, 2.5% E.C.; 6.25g AI/ha in 200l of water) was prepared and further serial dilutions made to give concentrations of deltamethrin representing 1/2, 1/4, 1/8, 1/16,, 1/20, 1/32 and 1/64th of the recommended field concentration. Distilled water was used as the diluent and for the

control treatment. The test substrates were treated using a calibrated laboratory Potter tower (Potter, 1952) to give a volume application rate equivalent to 200l/ha (2mg solution/cm^2). The spray tower was thoroughly cleaned and flushed with acetone and water between treatments, and the control and chemical dose rates were applied in increasing order of concentration.

After treatment the plates were left to dry for approximately 1h and the chambers were assembled with the sprayed surfaces facing inwards. Parasitoids were carefully introduced to each chamber using an aspirator. The exposure chambers were then connected to the humidified air supply and kept in a controlled environment room maintained at 18-21°C with a RH of 50-80%. Continuous, low intensity illumination was provided above the chambers and the wasps were kept away from the untreated walls of the exposure chamber by placing a 2cm-wide strip of masking tape around the glass plate edge, thus ensuring that the wasps remained active mainly in the illuminated area.

Assessment of toxic effects

The parasitoids were confined in the exposure chambers for 24h and their condition assessed after 1, 2, 4, 6, 8, 14, 20 and 24 hours, without opening the chambers. The condition of the wasps were recorded as:

alive - apparently unaffected;

affected/dead - showing signs of uncoordinated movement/not moving.

No further tests for sublethal effects such as those acting on fecundity were carried out, because of the high mortality obtained in the treatments.

Statistical analysis

Probit analysis was performed on the mortality data to obtain dose-response statistics (Finney, 1971), using Abbott's correction for control effects (Abbott, 1925).

Assessment of longevity of adult *Aphidius rhopalosiphi* exposed for different lengths of time on deltamethrin residues

Test units

In order to provide an environment which ensured the maximum possible exposure of the parasitoids to the pesticide residues, a glass plate (12cm x 12cm) and a glass dish (6cm diameter) were sprayed as above at the recommended field concentration of deltamethrin. The two components were then placed together to form a sealed unit,

separated by a cork seal, through which hypodermic needles carrying the humidified air could be introduced. Individual female *A. rhopalosiphi* were placed inside the exposure chambers for either 2, 3, 5, 10, 15, 30 and 45 mins. Control parasitoids were exposed for 45 mins to chambers sprayed with distilled water. Fifteen parasitoids were tested for each exposure time. After exposure, parasitoids were transferred, using an aspirator, to a clean 9-cm Petri dish and provided with a 50:50 honey and water solution on cotton wool.

Assessment of toxic effects

An assessment of the wasps' condition was made immediately after exposure, 1h after exposure, and then at 24h intervals over the following 4 days.
Their condition was assessed as either:
alive - apparently unaffected;
affected/dead - showing signs of uncoordinated movement/not moving.

Statistical analysis

Percentage mortalities of parasitoids over time were plotted for each exposure period.

Assessment of *Aphidius rhopalosiphi* pupal exposure to topical insecticide applications

Test insects

In order to obtain a uniform generation of 4 day-old mummies, second-instar aphids (*S. avenae*) were exposed to newly-emerged mated female *A. rhopalosiphi* for a 24h period. After rearing the parasitised aphids on barley seedlings for a further 10 days in the controlled environment room, mummification occurred and sections of leaf containing mummified aphids were cut from the plants and attached (mummy upwards) onto a glass plate (7.5cm x 7.5cm) using double-sided adhesive tape. Eighty mummies were used for each treatment, divided equally amongst eight replicate plates.

An estimate of the surface area of the mummies used in this study was made by measuring the area of a flattened mummy case (excluding the under surface which would be attached to the plant substrate).

Application of insecticide

The glass plates containing the mummies were sprayed as described above, with the recommended field concentration of deltamethrin, a concentration representing 1/2 the

field rate, and a water control. After treatment, the plates were left to dry for 1h. Fine grain sand was sprinkled over the glass plates to minimise contact of the newly-emerged wasps with pesticide residues and also to prevent wasps becoming stuck to the double-sided sticky tape. A 9cm diameter Petri dish (with the base removed and replaced with a fine-mesh gauze) was placed over the plates, which were then kept in the controlled environment room. A 50:50 honey and water solution on cotton wool was provided as food for emerging parasitoids.

Assessment of toxic effects

Counts were made of the number of wasps successfully emerging from each treatment. These were removed within 12h of emergence for assessments of their reproductive viability. The longevity of emerged wasps was also recorded over the following 2 weeks, with parasitoids kept in ventilated 9cm Petri dishes with access to honey and water for food.

For fecundity assessments, 10 surviving females from each treatment were individually transferred, using an aspirator, to pots of barley seedlings (3 plants, 10-15 cm tall). The plants were previously infested with 40 2nd/3rd-instar *S. avenae* and enclosed within cylinders of clear acetate sheeting (9cm diameter, 25cm tall), covered with fine-mesh nylon netting. The plant chambers were kept in the controlled environment room with constant illumination for 24h, after which the female wasps were removed. The number of mummified aphids on the plants was then recorded after a further 12 days.

Statistical analysis

Differences in percentage emergence from mummies in the three treatments were arcsine-transformed to normalise the data and equalise variances prior to analysis. Homogeneity of variance was tested using the Levene test (Day & Quinn, 1989). Tukeys HSD multiple comparisons were calculated following one-way ANOVA in order to identify which means differed significantly. The fecundity results were examined using a two sample t-test. The longevity of parasitoids was determined by plotting percentage mortality over time for each treatment. The "total effect" of the deltamethrin was assessed using the formula proposed by Overmeer & van Zon (1982) which takes into account both initial toxicity (i.e. mortality of adults not emerging from mummies) and also sub-lethal effects (i.e. reduced fecundity of emerged females).

Spray deposition on aphid mummies in the field

The experiment was carried out in a mature winter wheat crop (cv. Brigadier, G.S. 75 (Tottman & Broad, 1987), with a tiller density of 470/m^2) on the Manydown Estate near Basingstoke, during July 1994.

Positioning of aphid mummies

Prior to spray application, 20 wheat ears containing aphid mummies (*S. avenae*) located between the individual wheat grains, and 20 ears with mummies attached on the awns were collected from the surrounding crop and attached to existing wheat ears using adhesive tape. Other field-collected mummies were carefully detached from their plant material and temporarily attached individually, using UHU spray adhesive, onto 2cm x 1cm pieces of paper. These were stapled horizontally on the central areas of the upper and lower surfaces of 20 flag leaves and 20 first leaves. An additional 20 mummies on paper were stapled vertically on the wheat stems between the flag leaf and ear. Different plants were selected at random for each mummy/position combination.

From initial observations it was concluded that neither the paper or spray adhesive interfered with spray droplet deposition or retention on the mummy.

Spray application of the fluorescein tracer

A fluorescein tracer formulation (Acid yellow 73, Aldrich) was applied as a 5% (w/v) solution in distilled water, with 0.05% (v/v) Farmon Blue as a wetting agent. The spray application was made using an Oxford Precision Sprayer fitted with four 110° flat-fan nozzles, operating at a pressure of 2 bar, and giving a swath width of 2m. Walking speed was adjusted to give a precise volume application rate of 200 l/ha following individual calibration of the nozzles.

Measurement of the fluorescein deposition

After the spray solution had dried (approximately 30 mins) the mummies were individually removed from the pieces of paper or ear/awn using fine forceps and placed into labelled glass tubes containing exactly 3ml of phosphate buffer solution (pH 6.8, 0.1M Na_2HPO_4 + $NaH_2PO_4H_2O$ in de-ionised water). After each mummy collection, the tip of the forceps was washed in the buffer solution to ensure no accidental cross-contamination of fluorescein occurred. Sample tubes were immediately transferred to a dark container and then kept in cold (6°C), dark conditions until analysis. A sample of the spray formulation in the tank mix was taken immediately after spraying and stored in identical conditions to the

sample tubes.

For the analysis, the samples were shaken for 1 min to ensure all the fluorescein residues were washed off into the buffer solution. The volume of tracer in solution in each sample was determined by analysis in a Perkin-Elmer LS-3B spectrofluorimeter operating at 490nm excitation and 515nm emission wavelengths. The fluorescence of each sample was corrected by using a sample of the buffer solution as a blank comparison.

A calibration curve was constructed by micropipetting a wide range of volumes of the original tank mix into 3ml of buffer to be analysed in the spectrofluorimeter. A linear regression analysis of log µl fluorescein tank mix and log fluorescence was undertaken to obtain the calibration curve from which the amount of fluorescein contained in the samples could be expressed as microlitres of the original spray formulation.

Statistical analysis

The volumetric data (representing µl/mummy) for each position in the crop was $\log_{10}$-transformed to normalise the data and equalise variances prior to analysis. Homogeneity of variance was tested using the Levene test (Day & Quinn, 1989). Tukeys HSD multiple comparisons were calculated following one-way ANOVA in order to identify which means differed significantly.

RESULTS

Susceptibility of *Aphidius rhopalosiphi* to deltamethrin residues on glass and foliage substrates

Parasitoids tested on different residue concentrations on the two substrates exhibited a range of responses over the 24h exposure period (Figure 2.1). No mortalities were recorded in the control group on either substrate, and therefore any observed mortalities in the treatment groups were considered to result from the toxic effects of the insecticide. The highest dose rates caused rapid mortality, with 100% recorded after 6h in the full field rate treatment on both substrates. With the reduced dose-rate applications, the mortality trends over time had shallower slopes as concentration declined. The lowest dose rate, representing 1/64 of the field concentration caused only 25% and 40% mortality of wasps after 24h exposure on glass and foliage substrates respectively.

Estimation of "toxicity factors"

The toxicity of the deltamethrin residues to *A. rhopalosiphi* adults on the two test substrates were compared by iterating a sequence of lethal time (LT) ratios calculated from probit mortality regression equations (Table 2.3) for each substrate, and thus calculating a mean ratio. The sequence of times selected represented responses between LT_{10} and LT_{90} to allow for differences between probit slopes. Mean values obtained for each concentration of deltamethrin (Table 2.4) were termed "toxicity factors" (following Wiles & Jepson, 1994a). These ratios give an estimate of the relative toxicity of deltamethrin residues to a given organism on the two substrates that are compared.

All Chi-squared values, except one, for the probit regression equations (Table 2.3) satisfied the statistical requirement for homogeneity ($P>0.05$). Probit analysis could not be performed on data from deltamethrin residues at 1/64 x field rate because wasp mortality remained low throughout the 24h bioassays (Figure 2.1).

The toxicity factors (Tf) varied throughout the range of dose-rates of deltamethrin applied. The values indicated that for 5 of the 7 dose rates tested, the toxicity of deltamethrin residues was greater on the glass substrates than on the foliage (suggested by Tf values > 1). The highest Tf value of 2.85, and hence the greatest difference in toxicity between the two substrates, was calculated for the full field concentration of deltamethrin. However, the probit regression equation for the full field rate application on the foliage indicated a significant heterogeneity with the χ^2 analysis (Table 2.3), and therefore this

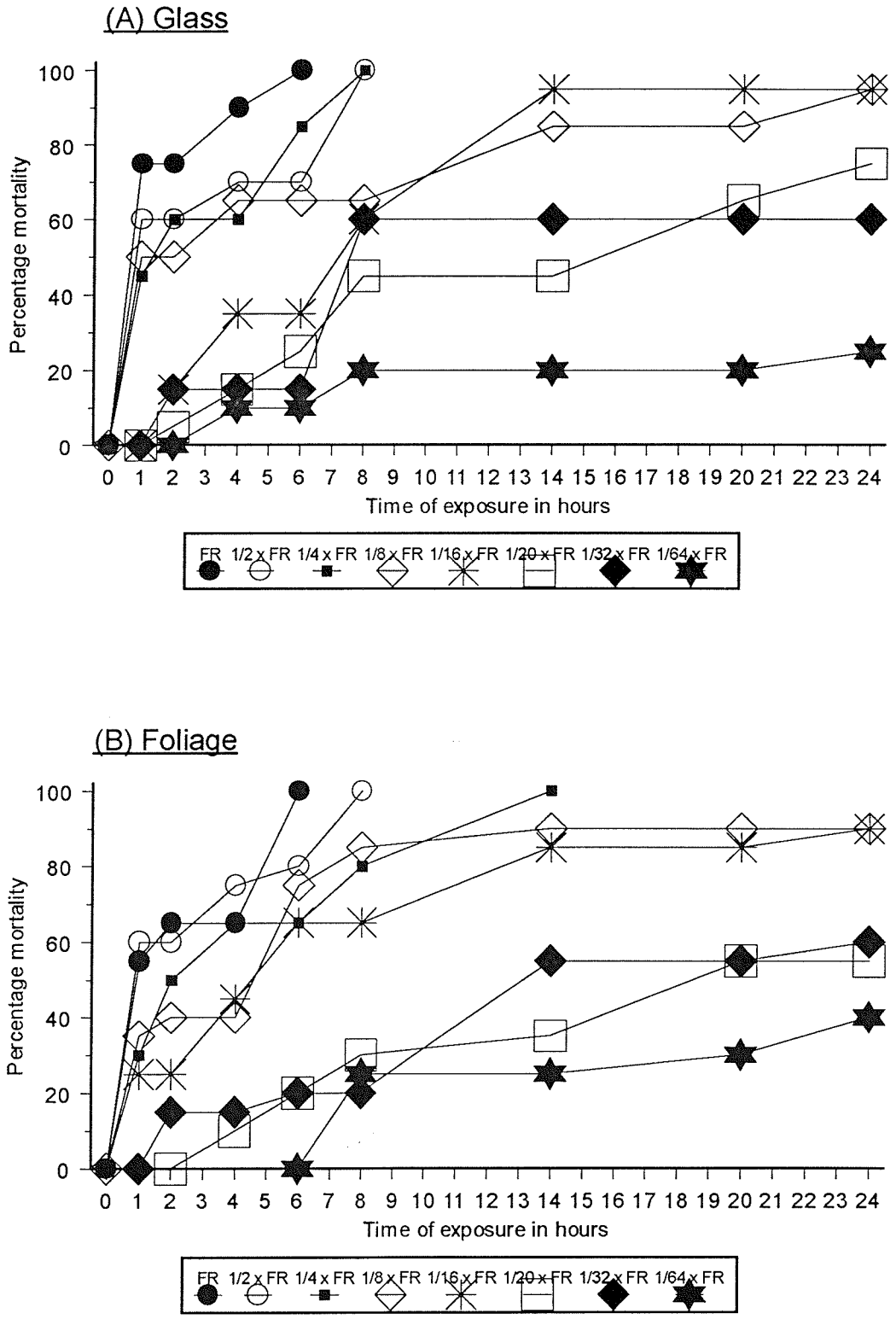


Figure 2.1. Percentage mortality trends of *Aphidius rhopalosiphi* exposed for 24h to different concentrations of deltamethrin (FR = field rate) on (A) glass and (B) foliage substrates.

Table 2.3. Summary statistics from the probit analyses of *Aphidius rhopalosiphi* tested on the residues of different dose rates of deltamethrin on glass and foliage substrates.

Dose of deltamethrin applied[a]	Probit mortality regression equation[b] $Y = a + bX$	LT50 (± S.E.) (hours)	Heterogeneity χ^2 (d.f.=5) significance[c]
Glass bioassay			
Field rate (FR)	5.58 + 1.47X	0.39 (0.42)	3.96 ns
1/2 x FR	4.41 + 2.29X	1.79 (0.47)	9.48 ns
1/4 x FR	4.52 + 2.26X	1.61 (0.29)	10.24 ns
1/8 x FR	4.58 + 1.28X	2.11 (0.55)	4.79 ns
1/16 x FR	2.88 + 2.82X	5.57 (0.61)	5.44 ns
1/20 x FR	2.77 + 2.03X	12.44 (1.74)	1.86 ns
1/32 x FR	3.32 + 1.50X	13.09 (2.48)	8.29 ns
1/64 x FR	3.17 + 0.84X	> 24.00	----
Foliage bioassay			
Field rate	4.96 + 1.97X	1.03 (0.48)	13.87 *
1/2 x FR	4.57 + 2.19X	1.56 (0.47)	5.04 ns
1/4 x FR	3.89 + 2.54X	2.71 (0.46)	7.20 ns
1/8 x FR	4.20 + 1.65X	3.01 (0.71)	4.66 ns
1/16 x FR	3.84 + 1.78X	4.44 (0.78)	0.82 ns
1/20 x FR	2.81 + 1.69X	19.34 (4.10)	1.19 ns
1/32 x FR	3.24 + 1.41X	17.56 (4.17)	3.00 ns
1/64 x FR	2.70 + 1.40X	> 24.00	----

[a] Field rate (FR) of deltamethrin: 6.25g AI/ha in 200l water.

[b] Probit mortality regression equation: Y is the probit mortality; a is the intercept value; b is the slope of the probit line; X is time (hours).

[c] Significance level: ns = not significant; * = $P < 0.05$.

value must be interpreted with caution. The two Tf values calculated as being 0.86 and 0.87 were for the 1/2 x field rate and 1/16 x field rate applications respectively. These were anomalous results compared to other dose-rates, however their small deviations from the value of 1, indicated the toxicity of deltamethrin residues on glass and foliage to be similar. The Tf values for the other dose rates tested (excluding full field rate) suggest that the insecticide residues are between 1.34 and 1.82 times more toxic to *A. rhopalosiphi* adults on the glass substrate compared with the foliage.

A plot of LT50 values against concentrations of deltamethrin indicated a direct correlation, with LT50 values decreasing proportionally with increasing concentration (Figure 2.2).

Table 2.4. Toxicity factors comparing the relative bioavailability of deltamethrin residues of different dose rates to *Aphidius rhopalosiphi* adults on glass and foliage substrates. The values were obtained from a mean of the ratios of LT10, LT30, LT50, LT70 and LT90 for the pair of substrates. (Foliage values divided by the corresponding glass substrate values).

	Field rate (FR)	1/2 FR	1/4 FR	1/8 FR	1/16 FR	1/20 FR	1/32 FR
Mean (±S.E.)	2.85 (±0.52)	0.86 (±0.02)	1.82 (±0.21)	1.51 (±0.26)	0.87 (±0.18)	1.58 (±0.16)	1.34 (±0.06)

(Field rate deltamethrin: 6.25g AI/ha in 200l water)

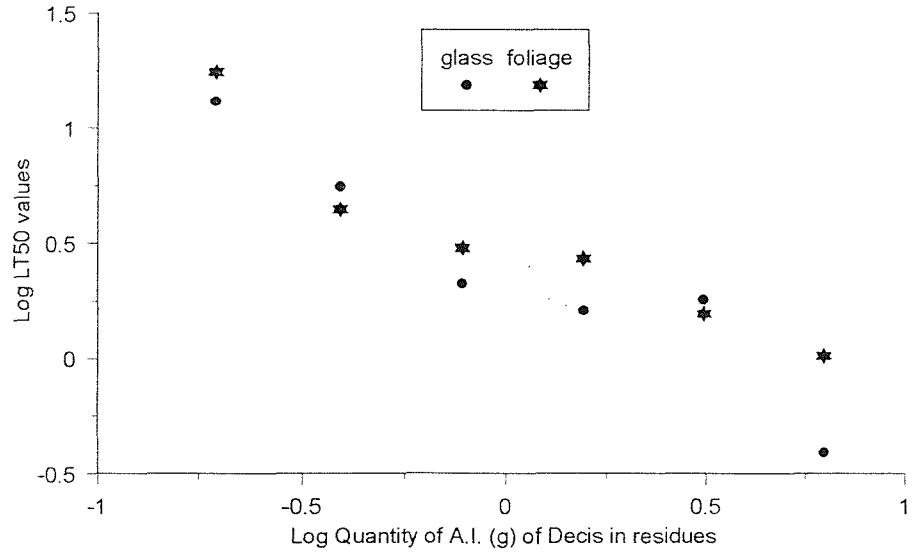


Figure 2.2. LT50 values obtained from the probit regression analysis of *Aphidius rhopalosiphi* exposed to different deltamethrin concentrations on glass and foliage substrates.

Effect of different exposure times on the susceptibility of *Aphidius rhopalosiphi* to deltamethrin residues on glass

The majority of parasitoids exposed to deltamethrin residues of field concentration for periods of time greater than 2 mins showed visible signs of intoxication in the form of uncoordinated behaviours, such as staggering (Table 2.5). The percentage of adults showing signs of intoxication decreased over 24h after each exposure period. Full recovery occurred with wasps exposed for less than 5 mins. Parasitoids exposed for longer periods showed lower rates of recovery over 24h, with higher mortality occurring as exposure period increased.

Survival of wasps over the following 4 days varied with the exposure time to deltamethrin residues (Figure 2.3). Greatest survival occurred for wasps exposed for the shortest time periods. Groups of parasitoids surviving from the 30 and 45 min exposure bioassays showed 100% mortality after 96 and 72 hours respectively.

Table 2.5. The number of parasitoids alive at intervals after the different exposure periods on residues of full field rate deltamethrin. Values in brackets indicate the percentage of insects showing signs of intoxication.

Exposure time (minutes)	Initial number of parasitoids tested	Immediately after exposure (%)	1 hour after exposure (%)	24 hour after exposure (%)
0 (control)	15	15 (0)	15 (0)	15 (0)
2	15	15 (0)	15 (0)	15 (0)
3	15	15 (27)	15 (13)	15 (0)
5	15	15 (80)	12 (17)	11 (18)
10	15	15 (100)	13 (100)	9 (0)
15	15	15 (100)	15 (100)	11 (0)
30	15	15 (100)	15 (100)	9 (0)
45	15	9 (100)	9 (100)	4 (0)

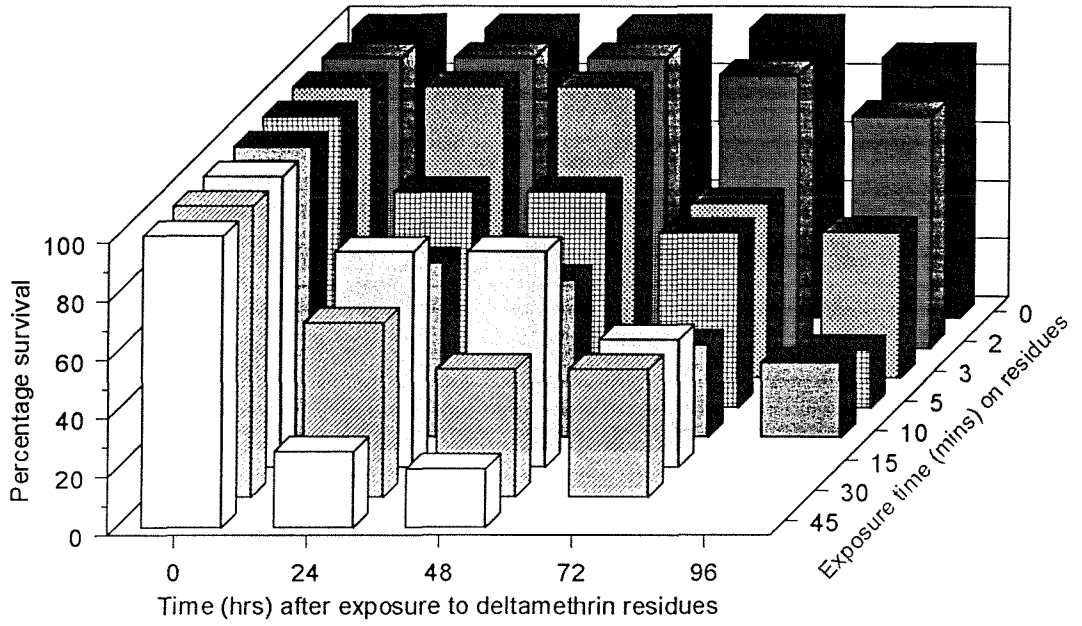


Figure 2.3. Percentage survival of *Aphidius rhopalosiphi* adults, over a 4 day period, following exposure to residues of field concentration deltamethrin (6.25g AI/ha in 200l water) on a glass substrate for different lengths of time.

Susceptibility of larval/pupal stages of *Aphidius rhopalosiphi* to topical applications of deltamethrin

There was a significant reduction in the population of adults emerging from the full field rate-treated mummies compared to that from the control and 1/2 x field rate treated mummies (Table 2.6). The reduced dose of deltamethrin (1/2 x field rate) had no significant effect on emergence. A total of 21.2%, 2.5% and 0% of the mummies contained wasps which had died during the emergence process, in the field rate, 1/2 x field rate and control treatments respectively.

The field-rate dose of deltamethrin caused a decrease, but not a statistically significant difference ($P>0.05$), in the fecundity of emerged females, compared to the control wasps (Table 2.6).

Survival of the post-emergent adults was shown to be affected by the insecticide treatments, with higher rates of mortality, compared to the control, occurring in the population of wasps emerged from the field rate-treated mummies (Figure 2.4). The 1/2 x field rate dose had minimal effect on the longevity of adults compared to the control.

Table 2.6. The effects of deltamethrin, applied at two different concentrations to mummies, on the emergence rate and fertility of *Aphidius rhopalosiphi* females. (n_1 = total number of mummies; n_2 = total number of female wasps tested).

	Percentage emergence	n_1	Mean (± S.E.) fecundity per female	n_2
Control	94% a	80	6.7 (± 1.11) a	10
1/2 x field rate	91% a	80	----	----
Field rate	70% b	80	5.2 (± 0.96) a	10

Emergence: ANOVA with additional Tukey HSD test ($F = 9.39$, d.f. = 23, $P < 0.01$). Fecundity: two sample t-test ($t = 1.01$, d.f. = 18, $P > 0.05$). Different letters in a column indicate statistical differences ($P < 0.05$).

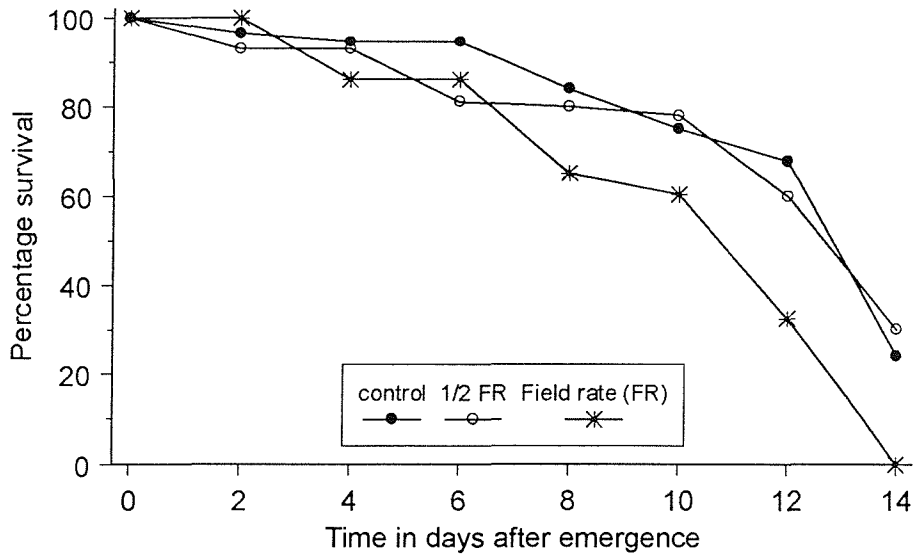


Figure 2.4. Percentage survival over 14 days of adult *Aphidius rhopalosiphi* emerged from control (n=41), field rate (FR) (n=43) and 1/2 FR (n=47) deltamethrin-treated mummies.

Interpretation of the toxic effects of deltamethrin

The "total effect" (E) of the deltamethrin treatment applied at the full recommended field rate can be calculated using the formula of Overmeer & van Zon (1982), which takes into account both initial toxicity (i.e. mortality of adults not emerging from mummies) and

also sub-lethal effects (i.e. reduced fecundity of emerged females).

A value for corrected mortality (M) was derived using Abbott's (Abbott, 1925) formula (Equation 1):

$$M = \frac{Mt - Mc}{100 - Mc} \times 100 \qquad \text{(Equation 1)}$$

where Mt is the percentage mortality of the treated group and Mc is the percentage mortality of the control group. In this study the value of M was 25.53.

A value for the treatment effect on reproductive capacity (R) of the test organism was derived from equation 2:

$$R = \frac{Rt}{Rc} \qquad \text{(Equation 2)}$$

where Rt is the mean number of mummies per female surviving at the end of the test in the treated group and Rc is the mean number of mummies per female surviving at the end of the test in the control group. In this study the value of R was 0.78.

The overall treatment effect (i.e. the reduction in beneficial capacity) was then calculated from equation 3:

$$E = 100 - [(100 - M) \times R] \qquad \text{(Equation 3)}$$

Therefore in this study, the treatment of mummies with the full field concentration of deltamethrin gave a value for the overall treatment effect (E) of 41.9. This falls into the IOBC/WPRS category of "slightly harmful" (category range: 30%-79%; Hassan, 1992; Chapter 1).

Spray deposition on mummies within a cereal crop

There was significant heterogeneity in deposition rates of spray between the different positions in the crop (Table 2.7). Deposition was highest on the mummies positioned on the awns and upper surfaces of flag leaves, with significantly less ($P<0.05$) spray depositing on mummies located on wheat ears and upper surfaces of the first leaves. Very low spray deposition levels were found on mummies on the lower surfaces of leaves and the stem.

Table 2.7. Volume of spray (mean from 20 mummies) deposited on mummies at different positions in the winter wheat crop.

Position of mummy in crop	Volume (µl/mummy) (± S.E.)*	Volume (µl/cm^2) (± S.E.)**
Awn	0.035 (± 0.0014) **a**	0.591 (± 0.023)
Ear	0.016 (± 0.0013) **b**	0.273 (± 0.022)
Flag leaf (upper surface)	0.034 (± 0.0021) **a**	0.573 (± 0.034)
Flag leaf (lower surface)	0.00036 (± 0.00007) **c**	0.0075 (± 0.001)
First leaf (upper surface)	0.019 (± 0.00095) **b**	0.328 (± 0.015)
First leaf (lower surface)	0.0009 (± 0.00023) **c**	0.023 (± 0.0045)
Stem	0.0012 (± 0.00013) **c**	0.025 (± 0.0018)

* ANOVA with an additional Tukey HSD test ($F = 174.4$, d.f. = 69, $P < 0.0001$). Different letters in the column indicate statistical differences ($P < 0.05$).
** Calculated using the mean surface area of the mummies (=6mm^2).

DISCUSSION

The high mortality trends shown by *A. rhopalosiphi* adults to the insecticide residues on both glass and foliage substrates indicate their extreme vulnerability to deltamethrin residues over extended periods. According to the classification of toxicity of pesticides devised by the IOBC/WPRS (Hassan, 1992), deltamethrin at the recommended field concentration in this study is regarded as "harmful" (category 4, >99% effect) for *A. rhopalosiphi*. This risk level is, however, reduced if lower initial dose-rates are used.

There was a trend for deltamethrin residues to exhibit greater toxicity (in the range of 1.34 and 2.85 times higher) towards parasitoids on the glass, compared to the foliage substrate, under the given conditions. This could indicate a change in bioavailability of the pesticide which can become bound up in the leaf surface. The rates of uptake of pesticides by plant material are known to differ depending on plant species, type of pesticide, adjuvants and environmental conditions (Hartley & Graham-Bryce, 1980). The epicuticular waxes in cereal crops are important in the uptake process and significant amounts of solute can be sorbed to epicuticular waxes and the cuticle underneath (Shreiber & Schönherr, 1992). This makes the pesticide less available for residual uptake by insects, compared to residues on inert, non-porous surfaces, such as the glass plate.

Toxicity factors, such as those calculated in this study, may be used to provide corrections to mortality data from glass bioassays. They can allow extrapolation of results to more natural substrates and help in the refinement of risk assessments (Wiles & Jepson, 1994a). For use in other risk assessment studies, toxicity factors need to be calculated for the particular crop, insect and chemical in question. Toxicity factors will vary with: (i) different substrates (i.e. leaves with different wax properties or thickness, and soil with different mineral or organic matter compositions); (ii) different bioassay conditions; (iii) durations of exposure; and (iv) between test organisms with different habits. Standardised test conditions and exposure methods, such as those employed in the current study, are therefore needed to ensure reliable comparisons between different chemicals and insects.

The bioassays conducted to investigate the effects of different exposure times of *A. rhopalosiphi* adults on deltamethrin residues represent a more realistic situation of parasitoid exposure in the field. Instead of the confinement of a parasitoid for 24h on insecticide residues, they simulate the scenario of a parasitoid entering an insecticide-contaminated area of crop, becoming exposed to insecticide residues, and then leaving, or remaining in intermittent rather than continuous contact with the treated foliage. In

conjunction with the standard 24h dose-mortality bioassays, knowledge of the long-term sublethal effects, such as survival, as a consequence of a parasitoid contacting insecticide residues over a range of exposure times, can be used to help in risk assessments for parasitoids entering newly sprayed crops. The degree of impact caused by a pesticide will depend on the nature of the compound, the concentrations used, and also the area of crop treated. The latter not only determines the extent of reinvasion of new parasitoid recruits into the treated area, but also the ability of a parasitoid to escape once it has contacted insecticide residues.

A. rhopalosiphi exposed to residues of deltamethrin, at field concentration, for greater than 2 mins showed signs of intoxication, characterised by uncoordinated behaviour in the form of staggering. Recovery to "normal" behaviour patterns occurred in the majority of wasps, over the following 24h, whilst in uncontaminated chambers. These observations of the symptoms of poisoning agree with neurophysiological investigations indicating that pyrethroids act on the nervous system of insects (Graham-Bryce, 1987), resulting in the characteristic effect of rapid knockdown which can be reversible if a toxic dose has not been received. Despite the observed recovery in the majority of insecticide-exposed wasps, their longevity was affected, with highest mortalities occurring in the parasitoid groups exposed for the longest periods on residues. These findings are in agreement with Krespi *et al.* (1991) who exposed *A. uzbekistanicus* Luzhetzki to deltamethrin residues for 10 min and 1h time intervals. They found no change in longevity of wasps after a 10 min exposure, but a significant reduction after a 1h exposure, compared to controls.

The direct association found between LT50 values and the quantity of insecticide in the 24h bioassays on both test substrates, suggests that the uptake of active ingredient, and therefore corresponding toxicity, of deltamethrin by *A. rhopalosiphi* is directly correlated with their time of exposure. There is therefore a need for the quantification of insecticide uptake with regards to exposure time (Chapter 6), combined with detailed behavioural studies of parasitoids foraging on insecticide-contaminated substrates (Chapters 3 & 6) to aid the interpretation of laboratory toxicity data, thus improving risk assessments for parasitoids in the field.

The toxicity of deltamethrin to life stages of *A. rhopalosiphi* inside the mummified aphid was assessed by the extent of successful adult emergence. The number of adults emerging was significantly lower from the mummies treated with the field concentration of insecticide compared to that recorded from the 1/2 x field rate and control mummies. Hsieh & Allen (1986) noted that an insecticide can inhibit the emergence of the parasitoid from a treated mummy in one or more of the following ways: 1) parasitoids are killed in the late

larval, prepupal and pupal stages inside the mummified aphids; 2) parasitoids are killed as adults without fully developing wings within the mummified aphids; and 3) parasitoids are killed as adults with fully developed wings as they cut holes in the dorsal portions of the mummified aphids with their mandibles and start to emerge. The latter was evident in this current study, with 21.2% of the *A. rhopalosiphi* adults dying whilst partially emerged from mummies treated with the full field concentration of deltamethrin. Mortality at the time of emergence has been reported for other parasitoid species (e.g. Bartlett, 1964; Lingren *et al.*, 1972; Hsieh & Allen, 1986; Krespi *et al.*, 1991) and has been linked to the ingestion of toxic residues of the chemical from the external surface of the cocoon or mummy (Polgar & Sagi, 1983). A newly emerging parasitoid is also susceptible to pesticides before its cuticle hardens (Croft & Brown, 1975), with the head and thorax considered to be the site of most rapid toxic action through the proximity to the central nervous system (Matsumura, 1975).

It has been demonstrated that organophosphate insecticides such as diazinon (Hsieh & Allen, 1986; Chapter 6), prothiofos and isoxathion (Abo El-Ghar & El-Sayed, 1992) possess the ability to penetrate the mummified aphid cuticle more effectively than pyrethroid insecticides (e.g. cypermethrin, permethrin and tralomethrin), killing the developing parasitoids before adulthood. The results from this study suggest that the mummy case did provide substantial protection against deltamethrin for the parasitoids, allowing them to complete their development and at least attempt emergence from the mummy. The observed differences between the two classes of insecticides could result from their chemical properties. For example, organophosphates generally have lower octanol/water partition coefficients and higher vapour pressures, compared to pyrethroids (Graham-Bryce, 1987; Worthing & Hance, 1991), thereby increasing their speed of uptake by insects.

The application of a reduced dose rate of deltamethrin (1/2 x field concentration) caused a similar number of *A. rhopalosiphi* adults to emerge successfully compared with controls (only 2.5% were recorded as dying during emergence). This type of insecticide selectivity, through reduced dose rates, has been demonstrated for other aphid parasitoids within mummy cases (e.g. Hsieh & Allen, 1986; Abo El-Ghar & El-Sayed, 1992; Borgemeister *et al.*, 1993) and could be utilised in future integrated pest management programs.

The fecundity of emergent females from the field rate deltamethrin-treated mummies was slightly lower, though not significantly different ($P>0.05$) from that in the controls. This is in agreement with other studies that reveal no effect on fecundity with pyrethroid insecticides (e.g. Hsieh & Allen, 1986; Krespi *et al.*, 1991; White *et al.*, 1991; Borgemeister *et al.*, 1993). An effect on the longevity of the emergent *A. rhopalosiphi* adults from the full

field rate deltamethrin-treated mummies was also shown, with higher mortality occurring compared to controls over the following 14 days. *A. uzbekistanicus* showed a reduction in longevity after emerging from deltamethrin-treated mummies (Krespi *et al.*, 1991), but no detrimental effects on survival were recorded for *A. rhopalosiphi* and *Diaeretiella rapae* M'Intosh with the pyrethroids, fenvalerate and permethrin (Hsieh & Allen, 1986; Borgemeister *et al.*, 1993). If, as in this current study, it is shown that a pesticide application causes no significant reductions in female fecundity, then any changes in the longevity of female parasitoids over the following days may not be of great importance. This is because fecundity of Aphidiinae females is greatest in their first few days after emerging (Starý, 1970).

Despite this, the IOBC/WPRS toxicity category for deltamethrin applied at field concentration in this study, taking into account the effects on emergence and fecundity, is "slightly harmful" (category 2, <79%, Hassan, 1992), again indicating the potential vulnerability of *A. rhopalosiphi* to current field rate applications of deltamethrin in cereals.

To determine the actual volume of spray impinging upon mummies in the field, they were positioned at different levels within the wheat crop to cover the normal distribution range found. For example, the location of 123 mummies counted on a wheat crop (described in Chapter 5), expressed as percentages, were: awn (12%), ear (43%), flag leaf upper surface (9%), flag leaf lower surface (18%), 1st leaf upper surface (9%), and 1st leaf lower surface (9%). The spatial distributions of aphid mummies seen over crop plants may differ depending on crop type and parasitoid species. For example, mummies of *A. nigripes* were found at all levels within a potato crop, but with more located on the upper compared to lower leaf surfaces (Brodeur & McNeil, 1992). The distributions are partly determined by the tendency of various aphidiid species to undergo mummification away from the host feeding site (e.g. Frazer & Gilbert, 1976; Powell, 1980; Lykouressis & van Emden, 1983; Höller, 1991). This phenomenon has been linked to an avoidance of their two major groups of natural enemies: hyperparasitoids and predators (Brodeur & McNeil, 1992).

The quantity of spray deposited on the mummies on different parts of the wheat plant was dependent on the vertical position of the plant part within the crop and its orientation toward the direction of spray. Therefore, mummies positioned on the awns (being closest to the spray nozzles), and the upper surface of flag leaves (being horizontal to the spray cloud), received the highest volumes of spray solution. The mummies concealed tightly within the perpendicular wheat ears received considerably less spray, with also very little reaching the mummies located on the upper surface of the first leaves and on the stem. This spray deposition trend showing an attenuation down through the crop

canopy has been recorded in other studies (e.g. Bryant & Courshee, 1985; Bryant *et al.*, 1985; Jepson *et al.*, 1987; Çilgi & Jepson, 1992). The highest and lowest mean deposition rates of 0.591µl/cm^2 and 0.0075µl/cm^2 were recorded on the mummies located on the awns and lower surfaces of flag leaves, respectively. In comparison, the current laboratory testing method for pesticides on aphid mummies, proposed by Mead-Briggs (1992), uses a calibrated laboratory spray tower delivering a deposit of 2µl/cm^2 (equivalent to a field application rate for deltamethrin of 200l/ha). Therefore, for this particular study, laboratory bioassays would overestimate the degree of exposure of mummies to pesticides in the field situation study by between 3.4 and 266 times.

Significant differences were found in emergence rate and longevity of emerged *A. rhopalosiphi* from mummies treated with water (control) and a field concentration of deltamethrin in the laboratory bioassays. However, fecundity of emerged females was not significantly affected by the insecticide application. These differences were lost with the application of the 1/2 x field concentration of deltamethrin. Therefore, with reference to the spray deposition levels on mummies in the field, and extrapolation of toxicity data from laboratory bioassays, it can be predicted that full field concentration of deltamethrin sprayed under the given conditions in a winter wheat crop poses no risk to developing *A. rhopalosiphi*, even in the most exposed mummies on the wheat awns.

It is recognised that the aim of standard laboratory testing procedures is to screen a large number of pesticides efficiently and economically. However, by acquiring further knowledge of mummy distributions on crops and subsequent exposure to spray deposition, risk predictions can be improved by incorporating a "correction factor" for laboratory-derived toxicity data.

Spray deposition on a crop is known to be affected by a series of factors including crop density, growth stage, cross wind speed and turbulence, droplet size and application rate (Gohlich, 1985). Therefore, the measurement of deposition on mummies should be conducted at different growth stages of a crop, to reflect different application timings.

A further extension of these field-based tests could be to attach leaves containing laboratory-reared mummies of equal age onto different positions of a plant. Insecticide could then be applied at the recommended dose rates using conventional crop sprayers, giving realistic spray deposition trends through the crop. After treatment, clip-cages (to prevent parasitism/predation) could be placed over the mummies left 'in situ' on the plant, and subsequent emergence of parasitoids assessed over the following days. This method would therefore incorporate weathering effects of the insecticide residues on the mummy case, which have shown to be important in determining toxicity (Obrtel, 1961).

Overall, this chapter has investigated the intrinsic toxicities of deltamethrin, at a range of concentrations, to *A. rhopalosiphi* in laboratory bioassays. Suggestions for future laboratory tests have been given to provide greater realism for risk predictions in the field. In continuation, the following chapter concerns the effects of deltamethrin residues on the foraging behaviour of parasitoids, again to help in the design and interpretation of future laboratory and semi-field tests.

CHAPTER THREE

THE INFLUENCE OF INSECTICIDE RESIDUES ON PRIMARY PARASITOID AND HYPERPARASITOID FORAGING BEHAVIOUR IN THE LABORATORY

INTRODUCTION

In comparison to direct mortality assessments, relatively little research has been undertaken into the sublethal effects of pesticides on parasitoids. Reviews by Moriarty (1969), Haynes (1988), Elzen (1989) and Croft (1990) have summarised the literature which covers a broad range of sublethal effects of insecticides. These effects can be manifested physiologically through altered reproduction, reduced longevity, or changes in egg viability and fitness, or behaviourally through altered host recognition, disruption of sexual communication, or changes in foraging patterns. Sublethal physiological effects of deltamethrin residues upon *Aphidius rhopalosiphi* DeStefani-Perez were demonstrated in Chapter 2, with reductions in longevity of adults exposed to field concentration residues in laboratory bioassays. In addition, both the fecundity and longevity of adults emerging from mummies, topically treated with the insecticide, were reduced. To continue the investigation of insecticide effects upon aphid parasitoids, this chapter concerns the behavioural changes that occur as a consequence of exposure to sublethal quantities of deltamethrin.

Most insecticides attack specific sites within the insect's nervous system (Matsumura, 1975) and sublethal concentrations have the potential to influence insect behaviour (Haynes, 1988). In addition, the peripheral sensory perception of insecticides by certain insects can also lead to alterations of their behaviour patterns. Various authors have described the repellency/dispersal of insects in response to insecticide residues (e.g. the horn fly (*Haematobia irritans*), Quisenberry *et al.*, 1984; the German cockroach (*Blattella germanica*), Bret & Ross, 1986; the pink bollworm moth (*Pectinophora gossypiella*), Haynes *et al.*, 1986; parasitic Hymenoptera, Bartlett, 1965, 1966).

Sublethal effects on insect behaviour are commonly reported with the pyrethroid insecticides. For example, "repellent/irritant" responses are shown by aphids (Rice *et al.*, 1983; Lowery & Boiteau, 1988), mites (Iftner & Hall, 1983; Berry *et al.*, 1990), parasitoids (Irving & Wyatt, 1973; Perera, 1982; Hoy & Dahlsten, 1984), coccinellids (Wiles & Jepson, 1994b) and honeybees (Delabie *et al.*, 1985; Pike *et al.*, 1982).

An understanding of the behavioural avoidance of insecticides by both pest and natural enemy species represents an important area for research because it could contribute to reductions in toxicological risks and to the evolution of behavioural resistance to insecticides (Lockwood *et al.*, 1984; Pluthero & Singh, 1984). It also has the added implication that standard laboratory bioassays may potentially overestimate the lethal effects of an insecticide on an insect population if they do not take into account these pesticide-induced behavioural effects.

Insecticides can: (i) affect the density and distribution of pests within and between plants (Trumble, 1985), (ii) vary in their distribution throughout the crop canopy (Çilgi & Jepson, 1992), and (iii) affect insect behaviour (Trumble, 1985), therefore they exhibit a large potential to disrupt parasitoid foraging. A limited number of studies have investigated the foraging of parasitoids on insecticide-sprayed plants (e.g. McGregor & Mackauer, 1989; Jiu & Waage, 1990; Borgemeister *et al.*, 1993). These conclude that for the particular aphid parasitoids in question, certain insecticides cause a reduced "attractiveness" of plants compared to control (unsprayed) plants, and an alteration of stereotypic search patterns on the plant. Before the effects of insecticide residues on parasitoid foraging can be determined, comprehensive documentation and understanding of their behaviours under "normal conditions" are needed. For example, previous behavioural studies have shown that female *A. rhopalosiphi* spend longer time periods searching on honeydew-treated filter papers (Budenberg, 1990a) and wheat plants (Gardner & Dixon, 1985) than on equivalent untreated substrates. The searching by female *Diaeretiella rapae* McIntosh on an experimental host plant can be divided into three basic responses: (i) a klinotactic response to a honeydew-contaminated horizontal surface followed by an upward flight if no host is encountered, alighting on the upper parts of the plant; (ii) an upward movement (negative geotaxis) to a honeydew-contaminated section encountered on a vertical surface (i.e. a stem); (iii) a downward movement (positive geotaxis) on an uncontaminated vertical surface encountered after visiting a honeydew contaminated surface (Ayal, 1987). It is believed that by exploiting this elaborate foraging behaviour, females can maximise the chance of locating aphid hosts. This complex behaviour pattern also, however, determines the extent of parasitoid exposure to insecticide residues on sprayed plants.

Incorporation of behavioural observations within the design of "extended-laboratory" bioassays (Hassan, 1992) could provide an important modification of the initial laboratory toxicity tests (Chapter 2). By mimicking the field situation where beneficial organisms are not forced to remain in contact with the treated plants, the avoidance of toxic chemicals by sub-lethally induced behavioural changes could be incorporated into risk assessment.

Testing procedures can incorporate aspects of the biology and behaviour of the test insect, the route by which the insects are exposed to the pesticides under field conditions, and approach more realistically the amounts of pesticide that they will be exposed to.

This chapter describes two studies conducted to investigate whether residues of the pyrethroid insecticide, deltamethrin, demonstrated any repellent properties or caused sub-lethal effects, leading to changes in the foraging behaviour of the primary parasitoid, *A. rhopalosiphi*, and one of its associated hyperparasitoids, *Dendrocerus carpenteri* (Curtis). In the first study, *A. rhopalosiphi* were exposed to insecticide residues prior to, and during visits to honeydew patches in a Petri-dish bioassay. The second study involved exposing *A. rhopalosiphi* and *D. carpenteri* to deltamethrin residues on mature wheat plants.

The specific questions to be answered were:

(1) Does exposure to deltamethrin residues of different concentrations affect the foraging behaviour of female *A. rhopalosiphi* on a honeydew patch?

(2) Does a prior exposure of female *A. rhopalosiphi* to deltamethrin residues affect the foraging time on a honeydew patch, and if so, does recovery to "normal" search patterns occur?

(3) Does exposure to deltamethrin residues affect the foraging behaviour and distribution of female *A. rhopalosiphi* and *D. carpenteri* on honeydew-contaminated mature wheat plants? Fundamental differences in the behaviour of these two parasitoid groups could lead to an enhancement or reduction in potential biocontrol.

By understanding these associations between parasitoid foraging behaviours and insecticide residues, improvements to risk prediction for the field could be achieved.

EXPERIMENTAL METHODS

Petri dish bioassays

Honeydew was collected by placing strips of Parafilm around colonies of *Sitobion avenae* (F.) on ears of mature wheat plants (cv. Mercia), maintained in plant growth rooms at 20 ± 2°C. After 2-3 days the Parafilm was removed and placed in an oven at 25°C for 30 mins. The dry honeydew was then scraped off with a scalpel blade and weighed. It was then dissolved in distilled water to a concentration of 0.25 mg/µl. This is a standard solution that elicits strong responses by *A. rhopalosiphi* to honeydew from *Metopolophium dirhodum* (Wlk.) (Budenberg, 1990a).

A 4 cm-diameter disc was cut from the centre of a 15 cm-diameter filter paper, and treated with 100µl of honeydew and/or deltamethrin solution. Control papers were treated with 100µl of distilled water. After drying, the discs were placed into the centres of the original filter paper which was then inserted within a 15 cm-diameter Petri dish.

Newly emerged parasitoids (less than 24h old), maintained in a perspex culture box, were allowed to mate, and provided with water and honey solution. Prior to experiments, females were placed individually into gelatin capsules and left for 30 mins before use.

Experiment 1. Foraging by female *A. rhopalosiphi* on a honeydew-treated patch in the presence and absence of deltamethrin residues

Bioassays were a modified version of those used by Budenberg (1990a). The visit times of 20 female *A. rhopalosiphi* were measured by placing them individually onto the centre of a honeydew-treated circle and recording the time taken until the wasp left the 4 cm-diameter disc for a period of greater than 5 secs. This was repeated with the addition of deltamethrin (Decis, 2.5% E.C.), at the recommended field rate (equivalent to 6.25g AI/ha in 200l of water), and serial dilutions representing concentrations of 1/2, 1/4, 1/8 and 1/16 of field rate. The 4 cm-diameter filter paper central disc was immersed in the appropriate solution and left to dry in a fume cupboard for 30 mins. The 100µl of honeydew solution was then applied to the insecticide-treated disc which was again dried. Tests on parasitoids were carried out as above.

Experiment 2. Behaviour of insecticide-exposed female *A. rhopalosiphi* on a honeydew-treated patch

Filter paper discs were immersed in a field-strength solution of deltamethrin and dried for 30 mins in a fume cupboard. The discs were then placed within 4.5cm-diameter

Petri dishes to completely cover the inside. Individual female *A. rhopalosiphi* were placed inside the dish, which was sealed. In order to minimise build up of insecticide vapour, and thereby confine wasp exposure only to direct contact with the dry deposit, the dishes were ventilated with humidified air using a small aquarium pump, via a micro-syringe inserted through the side wall of the dish.

Wasps were exposed for 1, 5, 10, 20 and 30 mins. Immediately after removal from the chamber, wasps were placed individually on a honeydew patch (as described in experiment 1) and the time before leaving was again recorded. The time spent walking, resting and grooming whilst on the patch were recorded using the "Observer" behavioural program (Noldus, 1990) on a micro-computer. After completion of these tests, the wasps were carefully removed from the honeydew patch using an aspirator and placed in a clean container with access to honey solution. After 2, 4 and 24h, the parasitoids were retested, as above, on a fresh honeydew patch. Twenty individual females were tested for each insecticide exposure period.

Mature wheat plant bioassays

Experiment 3. Foraging by *A. rhopalosiphi* and *D. carpenteri* on mature wheat plants

Individual wheat plants (cv. Mercia) were grown in a glasshouse to a mature stage (G.S. 73, Tottman & Broad (1987)) in 15 cm-diameter pots containing a soil mixture of 1/3 sharp sand and 2/3 John Innes No.2 peat. For each experiment, a single plant of standard size and structure was placed inside a tall clear perspex cylindrical cage (height 90cm, diameter 30cm) with mesh-covered openings around the base and on the top. A small extraction fan was positioned on the mesh top to continually draw a gentle stream of air through the cage. This minimised the build-up of pesticide vapours.

Female *A. rhopalosiphi* and *D. carpenteri* were reared together on a culture of *S. avenae* on barley seedlings. Mummies were collected and emerged females, of between 24-48h old, of both species were used. To standardise the condition of female wasps prior to experiments, individuals were kept in 8mm-diameter gelatin capsules for 30 mins.

Honeydew application

To increase the time spent searching on the plants, 0.8ml of an artificial honeydew solution was applied to all the test plants using a hand-held garden atomiser held 30cm above the plant. This provided a fine, even coverage of honeydew over the ear, stem and upper surfaces of leaves, representing the deposits from typical aphid infestations in the

field (pers. obs.). The composition of the artificial honeydew was based on the artificial aphid diet "a" of Kunkel (1977), with the amount of sucrose increased from 15% to 30% (Table 3.1). This has previously been shown to elicit similar search patterns in *A. rhopalosiphi* as a solution of honeydew collected from *M. dirhodum* (Budenberg, 1990b).

Table 3.1. Quantities (in mg) of chemicals in 100ml of artificial diet (from Kunkel, 1977)

Amino acids		**Vitamins**	
Alanine	100	Biotin	0.1
Arginine	270	Calcium pantothenate	5.0
Asparagine	550	Folic acid	2.0
Aspartic acid	140	Nicotinic acid	10.0
Cysteine	40	Pyridoxine-HCl	2.5
Glutamic acid	140	Thiamine-HCl	2.5
Glutamine	150		
Glycine	80	**Organics**	
Histidine	80	L-ascorbic acid	100
Isoleucine	80	Choline chloride	50
Leucine	80	Citric acid	10
Lysine	120	Myo-Inisitol	50
Methionine	80	Sucrose	30000
Phenylalanine	40		
Proline	80	**Inorganics**	
Serine	80	$FeCl_3$-$6H_2O$	1.1
Threonine	140	$CuCl_2$-$4H_2O$	0.2
Tryptophan	80	$MnCl_2$-$4H_2O$	0.4
Tyrosine	40	$ZnSO_4$	1.7
Valine	40	$MgSO_4$-$7H_2O$	123.0
		K_2HPO_4-$3H_2O$	1500.0

Insecticide application

To enable comparisons between the treatments, a technique for applying standard quantities of pesticide to large numbers of replicate plants was devised. By using one stroke of a 1.5" fine-bristled paint brush dipped in a solution of deltamethrin, a deposition on the plant foliage of 1.115µl ± 0.135µl/cm^2 (n=20) could be achieved. This deposition rate was measured by adding a fluorescent tracer to the insecticide solution, and measuring the

quantity washed off the plant material, using the technique described in Chapter 2. Concentrations of deltamethrin were then adjusted to give mass/unit area on different parts of the wheat plants, based on the volume/unit area of spray deposition on plant surfaces reported by Çilgi & Jepson (1992) (Table 3.2).

Table 3.2. Deposition rate of spray tracer solution (µl/cm^2) on winter wheat (crop growth stage, 62; tiller density, 503/m^2; crop variety, Rendezvous; application rate, 220 l/ha via tractor hydraulic spray) taken from Çilgi & Jepson (1992).

Position	Spray tracer solution (µl/cm^2)
Ear	0.309 ± 0.012
Flag leaf	0.317 ± 0.023
First leaf	0.208 ± 0.013
Stem	0.089 ± 0.008

The lower surfaces of the flag and first leaves were left untreated because negligible volumes of spray deposition are found in these areas in the field (Çilgi & Jepson, 1992; Chapter 5). An upward stroke with the brush on both sides of a wheat ear and stem gave an even coverage of the solution. After all the plant structures had been treated with their respective concentrations of insecticide, the plants were left to dry for 30 mins in a fume cupboard before use in the experiments. All plants were used within 2h of being treated with insecticide.

Behavioural observations

Searching by individual parasitoids was monitored by continuous observation for 10 mins. A mirror was placed at the back of the perspex cage at an angle of 45° to monitor parasitoid movements on leaf undersides and behind the plant. A visit for behavioural monitoring purposes was defined to have started when a female was allowed to walk from a gelatin capsule onto the upper surface of the flag leaf, and it was considered to have terminated when the parasitoid flew or walked off the plant, without returning, for more than 5 secs. During visits, an event recording computer software package ("Observer"; Noldus, 1990) was used to record the total time spent by the parasitoid on the plant and its behaviour (searching, resting or grooming), location (ear, flag leaf, first leaf or stem), and position on the leaf surface (upper or lower). A parasitoid was considered to be searching when it walked on the plant, making frequent brief stops to probe the plant surface with antennae or mouthparts. Resting was a motionless state with no antennal or mouthpart

contact with the plant surface. Grooming described the cleaning of any body part using the front or back legs.

Preliminary observations were made to determine whether search times on plants were affected by previous visits by parasitoids. Five wheat plants were searched separately by five individual female *A. rhopalosiphi*. The mean searching times of the first parasitoids to visit the 5 plants (702.4 secs, S.E.= 38.8 secs) did not differ significantly from that of the last parasitoids to search the plants (694.2 secs, S.E.= 46.3 secs) (t-test: t=0.14, d.f.=9, P>0.05). Therefore, for the experiments, 5 female wasps, each used once only, were tested individually on one of 4 replicate plants, giving a total of 20 wasps per treatment.

Statistical analysis

In experiments 1 and 2, the visit times of female *A. rhopalosiphi* in each treatment and at each interval after exposure, were $\log_{10}$-transformed to normalise the data and equalise variances prior to analysis. Homogeneity of variance was tested using the Levene test (Day & Quinn, 1989). Tukeys HSD multiple comparisons were calculated following ANOVA in order to identify which means differed significantly. For experiment 3, the mean visit times of parasitoids on control and deltamethrin-treated plants were compared using a t-test. Behaviours and locations of the wasps were expressed as percentages of the total time spent on the plant.

The behaviour patterns of parasitoids in experiments 2 and 3 were expressed as behavioural transition probabilities (BTPs) between pairs of behaviours. These were estimated from records of the specific behaviours at 10 sec intervals through the period of observation. BTPs were calculated using a purpose-written computer programme produced by D.W. Salt (University of Portsmouth), assuming the behavioural sequences to be a first order Markov chain process. Thus, BTPs described the likelihood of parasitoids changing from one behaviour to another over each 10 sec time interval, based solely on the behaviour during the previous interval.

Petri dish bioassays

Experiment 1. *Aphidius rhopalosiphi* on a honeydew patch

On initial contact with the honeydew-treated area, females stopped and examined the substrate with their antennae and mouthparts. They then walked over the area slowly with increased klinotaxis accompanied with continuous "drumming" of antennae on the filter paper. A parasitoid tended to follow the edge of the treated area, and executed turn angles exceeding 90° relative to its track direction each time it lost contact with the honeydew patch. This resulted in the parasitoid returning to the treated area. Brief abdominal protractions occurred within a few minutes of the female contacting the honeydew patch, with the abdomen positioned forward between the front legs in a typical ovipositional stance. Occasional oviposition stabs were made at the honeydew patch. In the presence of honeydew, all visit times by wasps were significantly greater than when honeydew was absent (Table 3.3).

In the absence of honeydew, females walked in a random pattern across the filter paper with antennae waving in the air, resulting in a departure from the 4cm-diameter circle within seconds (Table 3.3). Significantly shorter visit times were found at the two highest

Table 3.3. Mean visit times for female *A. rhopalosiphi* (n=20/treatment) on patches of honeydew and different concentrations of deltamethrin (field rate (FR) Decis, 2.5% E.C., equivalent of 6.25g AI/ha in 200l water). Values followed by different letters are significantly different (one-way ANOVA, P<0.05, Tukey HSD test).

Treatment	Visit time (secs ± S.E.)	Statistical separation (P<0.05)
Honeydew only	352.9 (± 27.7)	a
Honeydew + 1/16 FR	299.2 (± 17.8)	a b
Honeydew + 1/8 FR	276.0 (± 16.2)	b
Honeydew + 1/4 FR	121.4 (± 7.1)	c
Honeydew + 1/2 FR	112.1 (± 5.5)	c
Honeydew + FR	85.7 (± 4.8)	d
Distilled water only	4.4 (± 0.5)	e f g
1/16 FR only	4.7 (± 0.3)	e
1/8 FR only	4.4 (± 0.3)	e f
1/4 FR only	3.9 (± 0.2)	f g
1/2 FR only	3.7 (± 0.3)	g
FR only	2.3 (± 0.2)	h

dose-rates of deltamethrin, with faster walking and/or erratic movements causing the female to leave the area more quickly than when exposed to insecticide residues of lower concentration or distilled water.

Visit times to honeydew-treated patches were affected by the presence of deltamethrin residues, with mean times decreasing with increasing dose of insecticide (Table 3.3). The times spent by parasitoids on patches of honeydew containing residues of all deltamethrin dose rates, except 1/16 FR, were significantly lower than on patches with honeydew alone.

For comparison, the mean visit time of an *A. rhopalosiphi* female on a honeydew-only patch was 5.8 mins. This was shorten to 1.4 mins in the presence of deltamethrin residues of recommended field concentration.

Experiment 2: Insecticide-exposed *Aphidius rhopalosiphi* on a honeydew patch

Total visit times

The control parasitoids (previously exposed to filter paper treated with distilled water) showed the stereotypic searching pattern on the honeydew patch, described above, with visit times ranging from 6.4 to 6.9 mins (Table 3.4).

Examination of the residence times on a honeydew patch of parasitoids previously exposed to deltamethrin residues, revealed changes that were dependent upon the length of prior exposure to the insecticide and the time after treatment when observations were made. Immediately after exposure, visit times on the honeydew patch significantly decreased as duration of exposure increased (Figure 3.1a). After 20 mins of insecticide exposure, parasitoids showed knockdown symptoms and were therefore not tested until after a 2h recovery period. The wasps exposed for 30 mins all showed knockdown, with no subsequent recovery occurring.

The visit times of parasitoids after a 2h recovery period showed a similar trend as before, with wasps exposed for 10 mins and longer showing significantly shorter residence times on the honeydew patch than other treatment groups (Figure 3.1b).

This trend in visit times continued after a further 2h recovery period (i.e. 4h after insecticide exposure), with control wasps and those exposed for 1 min showing significantly longer patch residence times than the other treatment groups (Figure 3.1c).

After a 12h recovery period, the trend in visit times although similar to those found previously was not so distinct (Figure 3.1d). Again, the 5 and 10 minute-exposed wasps showed significantly lower residence times on the honeydew-treated patch compared to the

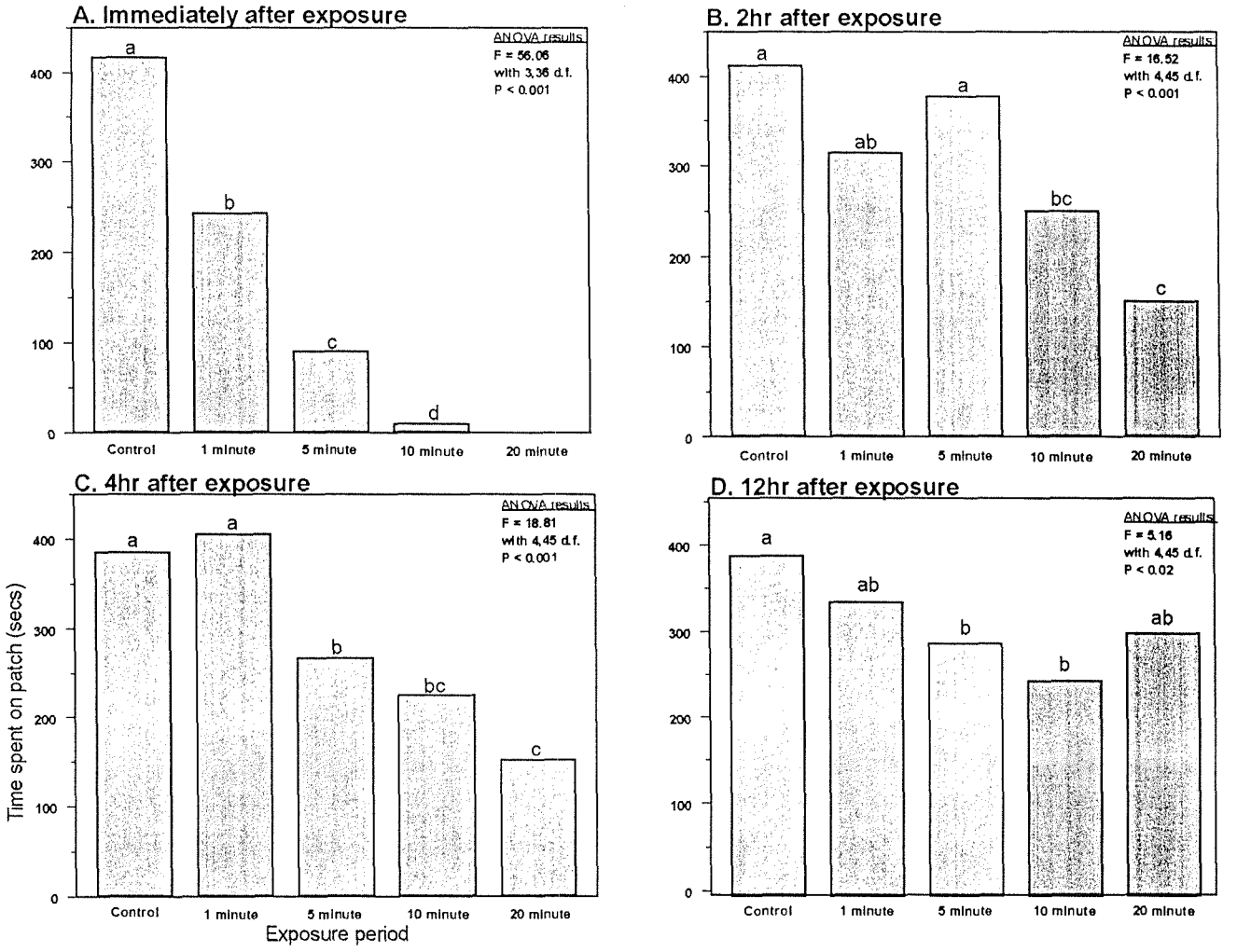


Figure 3.1. Time (seconds) spent by female *A. rhopalosiphi* on honeydew patch after different exposure times to residues of field concentration deltamethrin (equivalent of 6.25g AI/ha in 200l water), and after various "recovery times" after exposure. Values followed by different letters are significantly different (one-way ANOVA, Tukey HSD test).

control group. However, the 20 minute-exposed parasitoids, though showing shorter visit times, did not differ significantly from the controls.

Behaviours

For the control parasitoids tested at each interval after exposure, walking was the predominant behaviour (ranging from 79% to 85% of total time spent on the honeydew patch) with grooming and resting accounting for similar percentages of time (Table 3.4).

All parasitoids tested immediately after exposure to deltamethrin residues showed a higher percentage of time spent walking, compared to the control group. This trend was still evident after a 2h recovery period, but became undetectable during the 4h post-exposure observation (Table 3.4).

Table 3.4. Percentage of time spent walking, grooming and resting by female *A. rhopalosiphi* on honeydew patches, after different recovery intervals, in the different exposure groups.

			Percentage of total visit spent in each behaviour		
Length of exposure	Time after exposure	Visit time, secs (± S.E.)	Walking	Grooming	Resting
CONTROL	Immediate	417.55 (35.27)	82.65	8.83	8.52
	2h	412.37 (29.25)	79.11	11.64	9.25
	4h	385.29 (33.53)	85.87	8.83	5.30
	12h	394.54 (27.14)	85.29	6.55	8.16
1 MINUTE	Immediate	243.32 (22.18)	95.37	1.94	2.69
	2h	314.56 (17.89)	91.08	7.02	1.90
	4h	405.75 (31.31)	82.02	13.14	4.84
	12h	340.88 (29.50)	89.25	4.72	6.03
5 MINUTES	Immediate	90.55 (15.92)	87.10	12.51	0.39
	2h	377.57 (41.96)	84.67	13.68	1.65
	4h	266.95 (17.73)	96.13	1.20	2.67
	12h	293.18 (21.97)	75.08	13.32	11.60
10 MINUTES	Immediate	9.98 (2.03)	98.29	1.71	0.00
	2h	250.82 (20.69)	82.28	17.72	0.00
	4h	224.77 (21.09)	82.85	13.71	3.44
	12h	249.75 (20.77)	83.55	9.55	6.90
20 MINUTES	Immediate	---	---	---	---
	2h	151.08 (11.98)	98.07	1.89	0.04
	4h	151.65 (10.39)	96.68	2.42	0.9
	12h	304.72 (18.52)	95.44	3.01	1.55

These differences in walking behaviour were also indicated in the behavioural transition probabilities given in Table 3.5. Immediately after, and 2h after insecticide exposure, wasps showed a higher probability of walking during consecutive observations in all exposure groups, compared to the control group.

Table 3.5. Behavioural transition probabilities for female *A. rhopalosiphi* in control and deltamethrin-exposed groups during assessments immediately after, and 2h after exposure. Behaviour categories; w=walking, g=grooming, r=resting. Probabilities describe the likelihood of parasitoids changing from one behaviour to another after each 10 sec period.

Length of insecticide exposure	**Immediately after exposure**				**After 2h recovery period**			
CONTROL	From/To	w	g	r	From/To	w	g	r
	w	0.849	0.075	0.075	w	0.831	0.046	0.123
	g	0.500	0.458	0.042	g	0.589	0.386	0.025
	r	0.813	0.063	0.125	r	0.842	0.074	0.084
1 MINUTE	From/To	w	g	r	From/To	w	g	r
	w	0.943	0.026	0.031	w	0.928	0.049	0.022
	g	0.800	0.000	0.200	g	0.750	0.250	0.000
	r	1.000	0.000	0.000	r	0.800	0.200	0.000
5 MINUTE	From/To	w	g	r	From/To	w	g	r
	w	0.932	0.068	0.000	w	0.854	0.109	0.036
	g	0.455	0.545	0.000	g	0.311	0.689	0.000
	r	0.000	0.000	0.000	r	0.429	0.286	0.286
10 MINUTE	From/To	w	g	r	From/To	w	g	r
	w	1.000	0.000	0.000	w	0.884	0.116	0.000
	g	0.000	0.000	0.000	g	0.656	0.344	0.000
	r	0.000	0.000	0.000	r	0.000	0.000	0.000
20 MINUTE	From/To	w	g	r	From/To	w	g	r
	w	---	---	---	w	0.986	0.007	0.007
	g	---	---	---	g	1.000	0.000	0.000
	r	---	---	---	r	1.000	0.000	0.000

Mature plant bioassays

Experiment 3. Foraging of *Aphidius rhopalosiphi* and *Dendrocerus carpenteri*

Total visit times

Preliminary observations showed that female *A. rhopalosiphi* and *D. carpenteri* remained on uncontaminated wheat plants for up to 45 and 24 mins respectively. Walking/searching behaviour occurred immediately the parasitoid contacted the plant, followed later by long bouts of resting and grooming whilst remaining stationary on a particular plant structure. The maximum 10 min observation period per individual was therefore considered sufficient to quantify the main foraging patterns of the parasitoids.

None of the 20 *A. rhopalosiphi* left the untreated experimental plants during the 10 min observations, whereas *D. carpenteri* spent less time (a mean of 9 mins) on the equivalent plants. The presence of deltamethrin residues caused a significant decrease in mean visit times of *A. rhopalosiphi* (t-test: $t=3.73$, $P<0.01$) and *D. carpenteri* (t-test: $t=5.07$, $P<0.001$) (Table 3.6).

Table 3.6. Mean visit times for female *A. rhopalosiphi* and *D. carpenteri* (n=20/treatment) on untreated and deltamethrin-treated wheat plants.

Parasitoid species	Treatment	Visit time, secs (±S.E.)
Aphidius rhopalosiphi	untreated	600.0 (± 0.0)
	treated	455.0 (± 38.8)
Dendrocerus carpenteri	untreated	539.7 (± 16.0)
	treated	390.4 (± 24.7)

Behavioural activities

There were differences in the time allocated to the different behaviour categories between the two species and between plant treatments. *A. rhopalosiphi* spent an average of 74% of their total time on untreated plants searching. In the presence of deltamethrin residues, this behaviour decreased to 27% of total time, accompanied with an increase in grooming (Figure 3.2). Searching behaviour by *D. carpenteri* accounted for 84% of their total time spent on untreated plants. Behavioural patterns changed very little when *D. carpenteri* were exposed to deltamethrin-treated plants (Figure 3.2).

Behavioural transition probability tables (Table 3.7) emphasise the changes in

behaviour of *A. rhopalosiphi*, shown through the higher probability of searching during consecutive observations on the untreated plants than on the deltamethrin-treated plants. Higher probabilities of grooming during consecutive observations also resulted on the deltamethrin-treated, compared to untreated plants.

Probabilities of behaviour changes by *D.carpenteri* showed very little difference when compared for both untreated and treated plants (Table 3.7).

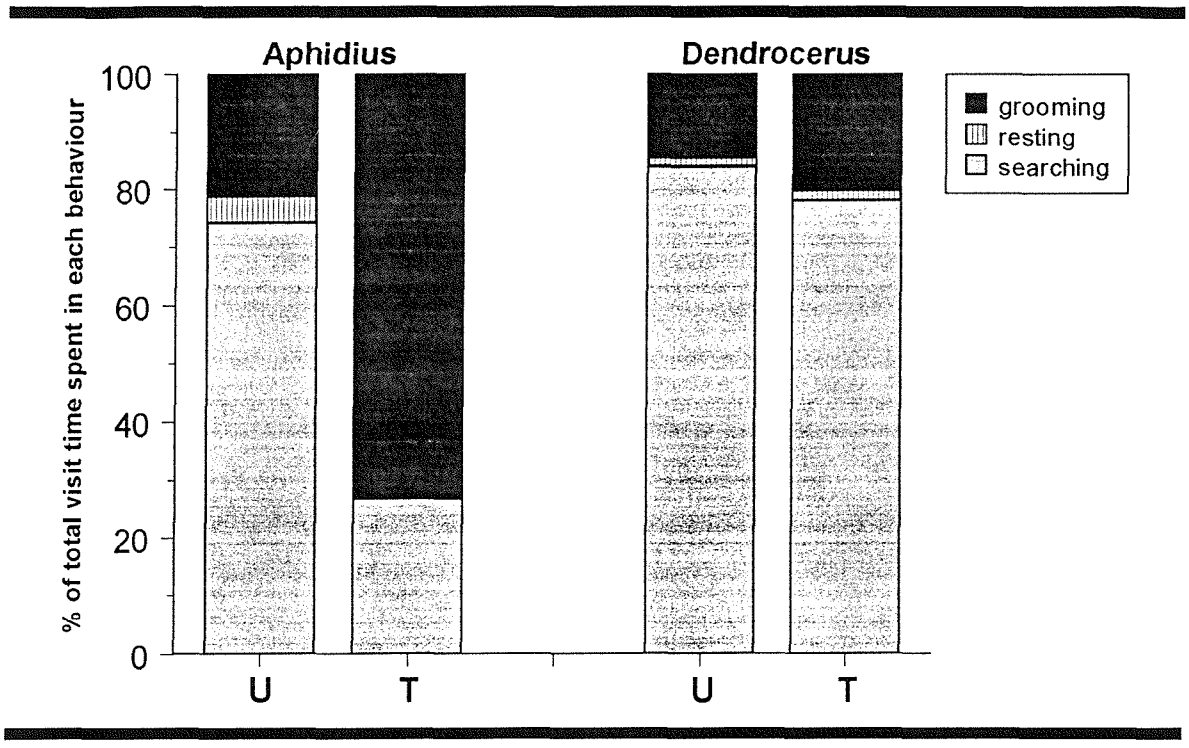


Figure 3.2. Percentage of total time spent in each behaviour category by female *A. rhopalosiphi* and *D. carpenteri* on the untreated (U) and deltamethrin-treated (T) wheat plants.

Location on plant

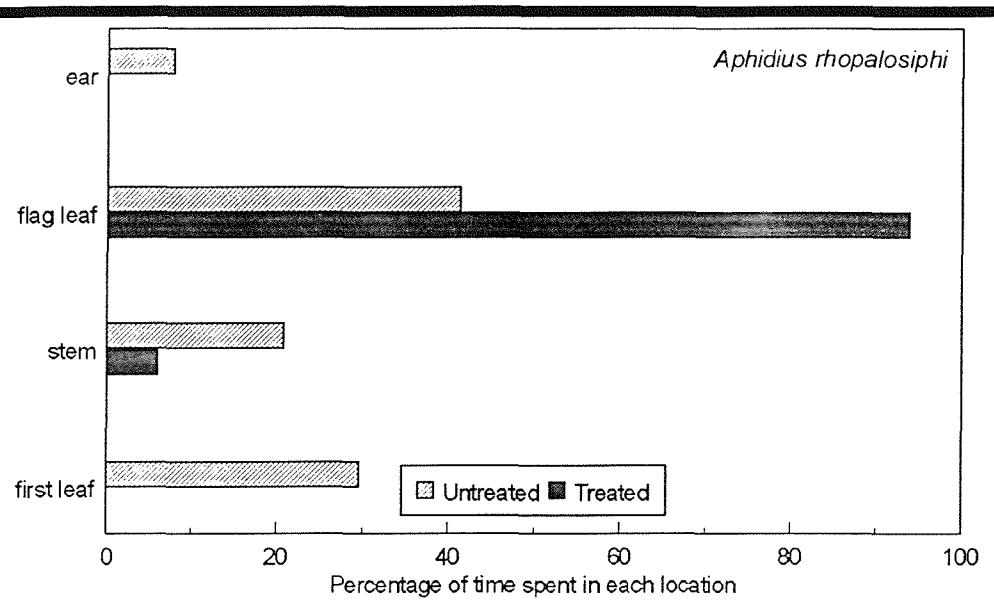
Once released onto the upper surface of the flag leaf, both *A. rhopalosiphi* and *D. carpenteri* undertook a thorough search of both leaf surfaces, with antennal drumming and mouthpart contact mostly on the upper leaf surface in the presence of honeydew deposits. When moving between different parts of the plant, parasitoids always walked along the stem of the plant, with flight only occurring when wasps left the plant without return.

Table 3.7. Behavioural transition probabilities for female *A. rhopalosiphi* and *D. carpenteri* during visits on untreated and deltamethrin-treated wheat plants. Behaviour categories; s = searching, g = grooming, r = resting. Probabilities describe the likelihood of parasitoids changing from one behaviour to another after each 10 sec period.

Untreated plants				**deltamethrin-treated plants**			
				Aphidius rhopalosiphi			
From/To	s	g	r	From/To	s	g	r
s	0.774	0.179	0.047	s	0.319	0.681	0.000
g	0.645	0.290	0.065	g	0.325	0.675	0.000
r	0.231	0.231	0.538	r	0.000	0.000	0.000
				Dendrocerus carpenteri			
From/To	s	g	r	From/To	s	g	r
s	0.741	0.213	0.046	s	0.683	0.278	0.039
g	0.713	0.189	0.098	g	0.566	0.420	0.014
r	0.384	0.199	0.417	r	0.732	0.211	0.057

On the untreated plants, both *A. rhopalosiphi* and *D. carpenteri* made frequent movements up and down the plant visiting the ear, flag leaf, stem and first leaf during the 10 mins of observations (Figure 3.3). There was more "reluctance" of *A. rhopalosiphi*, compared to *D. carpenteri*, to move onto the ear, with turning around occurring as soon as the ear was reached. *A. rhopalosiphi* spent a mean of 41% of their total time on the plant visiting the flag leaf, with the rest of the time allocated between the other plant structures. In the presence of deltamethrin residues, *A. rhopalosiphi* increased their time on the flag leaf (accounting for 94% of total time on plant), with no movement to other plant structures, except onto the adjacent stem for short time periods before returning back to the flag leaf. *D. carpenteri* exposed to deltamethrin residues on the plant also increased their time positioned on the flag leaf, but still exhibited downward movement onto the first leaf, as shown on the untreated plant. No upward movement onto the ear was recorded.

The percentage times spent on each plant structure during these experiments are



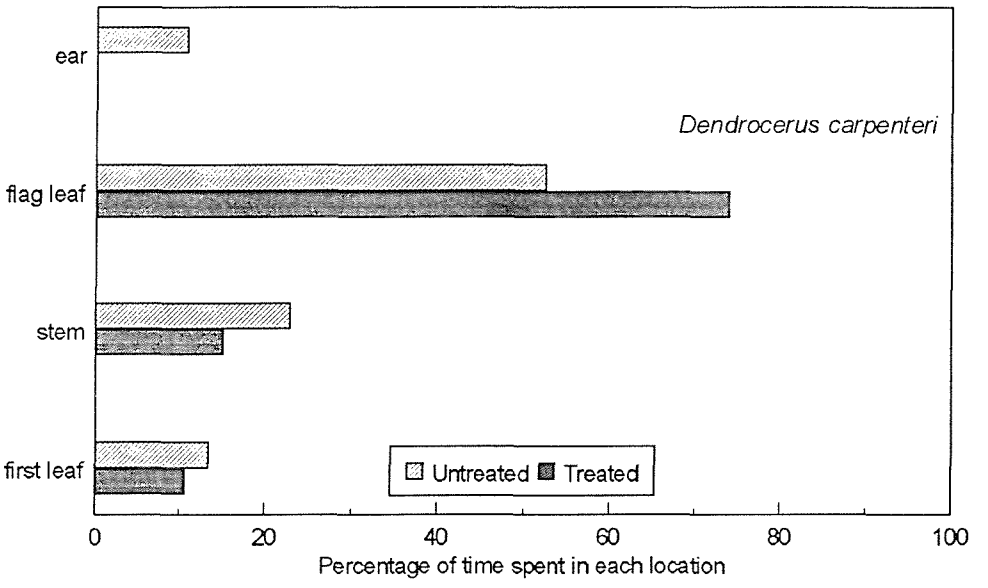


Figure 3.3. Percentage of time spent by female *A. rhopalosiphi* and *D. carpenteri* on different plant structures on the untreated and deltamethrin-treated wheat plants.

heavily biased towards the flag leaf, because this was the starting position for the parasitoids. Time spent on the stem represented the travelling of parasitoids between plant structures, with negligible time spent resting or grooming. Total visit times recorded on the first leaf and ear were probably underestimated because the end of the 10 min observation period often interrupted the search of these areas.

The percentage of *A. rhopalosiphi* moving off the flag leaf and then returning immediately was 46% on untreated plants, with the remainder showing a higher tendency to move downwards onto the first leaf, as opposed to the ear (Table 3.8). In the presence of deltamethrin residues, no upward or downward movement on the plant occurred.

On leaving the flag leaf, the percentage of *D. carpenteri* returning immediately was 54%, with similar numbers moving upwards onto the ear and downwards onto the first leaf (Table 3.8). The presence of deltamethrin residues resulted in no hyperparasitoids moving onto the ear, and an increase in numbers moving down the plant.

Table 3.8. Percentage of parasitoids moving to different parts of the plant, via the stem, after visiting the flag leaf. ('n' represents the number of parasitoid movements from the flag leaf that are used for the percentage calculations).

	From / To	flag leaf	ear	first leaf
APHIDIUS				
Untreated	flag leaf (n=35)	45.7	20.0	34.3
Treated	flag leaf (n=24)	100.0	0.0	0.0
DENDROCERUS				
Untreated	flag leaf (n=42)	53.9	25.7	20.4
Treated	flag leaf (n=28)	61.4	0.0	38.6

Leaf surface

A. rhopalosiphi and *D. carpenteri* spent equal amounts of time on the upper and lower surfaces of the flag and first leaves, on the untreated plants (Figure 3.4). On the insecticide-treated plants, *A. rhopalosiphi* altered their time spent on the leaf surfaces, giving a ratio of 79% : 21% for upper and lower respectively. *D. carpenteri*, in the presence of deltamethrin residues, showed an opposite trend, with more time on the lower leaf surfaces (60%).

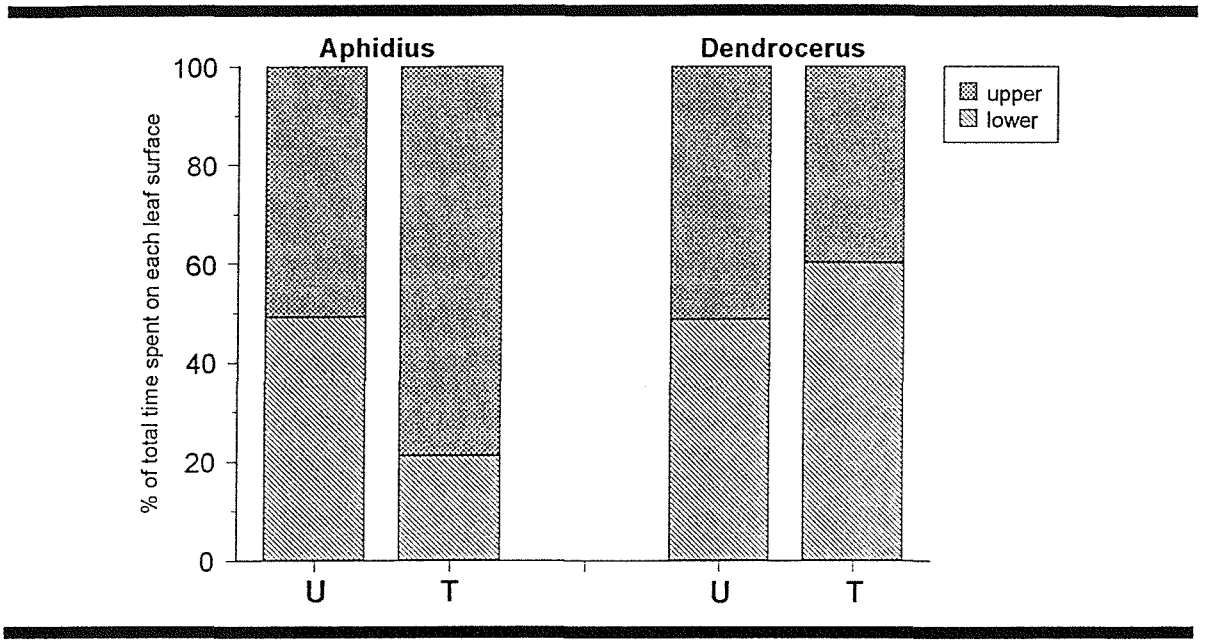


Figure 3.4. Proportion of time spent by female *A. rhopalosiphi* and *D. carpenteri* on the upper and lower leaf surfaces (flag and first leaf combined) on the untreated (U) and deltamethrin-treated (T) wheat plants.

DISCUSSION

Foraging on the honeydew patch

Results from the filter paper and mature plant bioassays confirm previous findings by Budenberg (1990a) and Hood-Henderson & Forbes (1988) that natural and artificial aphid honeydew are a source of kairomones and synomones used in host finding by female *A. rhopalosiphi* and *D. carpenteri*. The behavioural responses of *A. rhopalosiphi* to the honeydew-treated filter paper patches were similar to those reported by other authors working with various insect parasitoids in the presence of contact kairomones (e.g. Vinson *et al.*, 1978; Waage, 1978; Chiri & Legner, 1982; Bouchard & Cloutier, 1984; Budenberg, 1990a). The retention of females in honeydew-contaminated patches is evolutionarily advantageous because such areas are likely to contain aphid hosts. The arrestment, antennal examination, abdominal protraction, reduced walking speed, and increased turning observed in *A. rhopalosiphi* maximises the area searched and hence the number of potential hosts located. The reduced walking speed plays an important role in host location, as it increases the probability of finding cryptic aphid hosts (Gendron & Staddon, 1983), and reduces the likelihood of triggering alarm and escape reactions in aphids. The locomotory behaviours of parasitoids in contact with honeydew are similar to those found in aphid predators following prey encounter (Chandler, 1969; Carter & Dixon, 1982).

Habituation to the kairomones from honeydew presumably occurred in *A. rhopalosiphi* females causing them to leave the contaminated area after an average of 5.9 mins in the honeydew-only Petri dish bioassays. This habituation to contact kairomones has also been demonstrated for other insect parasitoids (Waage, 1978; Weseloh, 1980; Strand & Vinson, 1982; Bouchard & Cloutier, 1984; Budenberg, 1990a), and results in the parasitoid limiting the amount of time wasted in searching non-productive, honeydew-contaminated areas. Budenberg (1990a) has demonstrated that female *A. rhopalosiphi*, habituated to honeydew, showed a full recovery of their searching response after a 1h period away from the kairomones, although significant recovery was evident after only 10 mins.

Behavioural changes after exposure to insecticide residues

The inability of insecticide-exposed wasps to perform "normal" behaviours was demonstrated in this study, with decreased visit times to honeydew patches dependent on the concentration of insecticide residues present. During intervals after exposure, parasitoids resumed more natural behaviour patterns. This type of "recovery" by

invertebrates exposed to pyrethroid insecticides has been reported in other studies (e.g. Linn & Roelofs, 1984; Haynes & Baker, 1985). The potential for recovery depends on the mode of action of the neurotoxicant, how the insect metabolises it, and whether or not the insect has repeated contact with the insecticide (Haynes, 1988). In the current study, recovery occurred when the parasitoids were removed from the toxic chamber and placed in an uncontaminated area with free access to food and water. This may be highly unrealistic compared to a field situation where whole fields are sprayed and the parasitoid may lack the ability to disperse from the area once it has received a sublethal dose of insecticide. It is also important to note that an observed recovery of a specific behaviour pattern does not necessarily indicate that the insect has completely recovered from the insecticide exposure. It is therefore necessary to examine a whole range of responses to certain stimulii to determine whether "complete" recovery has occurred.

Wasps exposed to insecticide residues on wheat plants spent a significantly greater time grooming compared to those on untreated foliage. This has also been demonstrated for bees treated with permethrin, which spend significantly more time cleaning themselves and doing a trembling dance, and less time walking and foraging than untreated bees (Cox & Wilson, 1984). Grooming behaviour has been documented in many orders of insect including Diptera (Barber & Starnes, 1949; Hlavac, 1975; Golenda & Forgash, 1986), Hemiptera (Hlavac, 1975) and Hymenoptera (Jander, 1976; Jander & Jander, 1978). The behaviour is thought to be a reflex action initiated by an irritant on chemoreceptors or mechanoreceptors located on the insect body surface (Reingold & Camhi, 1978). The importance of insect cleaning behaviours in the process of insecticide uptake and elimination has been demonstrated by Gratwick (1957) and Kühner *et al.* (1985b) who recorded the spread of particles, picked up on the tarsi, around the body, and also the subsequent removal of the insecticide which they had acquired. The importance of this is explored further in Chapter 6.

Foraging on wheat plants

The pattern of parasitoid movement was a combination of horizontal and vertical searches bringing both species of wasp in contact with most of the available plant structures. The "reluctance" of some *A. rhopalosiphi* to move onto wheat ears on untreated plants was also noted by Gardner & Dixon (1985) who suggested it was due to the change in physical structure of the plant making it difficult for a parasitoid to move about between the grains. It was found that this structural complexity of the ear affected the aphid-parasitoid relationship by providing a refuge for aphids, which remained relatively protected

from parasitisation (Gardner & Dixon, 1985).

On untreated plants, the time spent searching by both species was equally divided between the upper and lower surfaces of the leaves. It has been suggested that aphid parasitoids can use honeydew on the upper surfaces of leaves as a cue for evaluating the numbers of aphids on the plant and therefore the amount of effort worth investing in further search (Bouchard & Cloutier, 1984; Ayal, 1987; Cloutier & Bauduin, 1990). Honeydew quickly builds up on leaf surfaces when an aphid colony infests a plant structure above, and the kairomonal effect of the honeydew soon wears off in the absence of aphids due to washing off and chemical degradation (Bouchard & Cloutier, 1984), therefore a minimal level of sampling of the available foliage is probably sufficient to detect the presence of hosts with accuracy (Cloutier & Bauduin, 1990). The lower leaf surfaces are searched by aphid parasitoids as they are the most preferred feeding sites for aphids due to easier access to the phloem and a sheltered environment (Dixon, 1987). In addition, parasitoids with limited vision may use leaf morphology to direct them off the leaf and onto the plant stem. This may be achieved by either walking along leaf edges or following veins on the lower leaf surface, from the fine peripheral ones to the large central vein in order to locate the petiole and hence the stem (Ayal, 1987).

Both female *A. rhopalosiphi* and *D .carpenteri* showed decreased visit times on the insecticide-treated plants, compared to the untreated plants. They also exhibited changes in their distribution patterns whilst on the treated plants, with no upward movement onto the wheat ear. Similar results were recorded for *D. rapae* (Jiu & Waage, 1990) and *A. rhopalosiphi* (Borgemeister *et al.*, 1993) which showed a decrease in visit times on insecticide-treated plants versus control plants. They also concentrated their activities on unsprayed surfaces, with *D. rapae* showing a reversal of their stereotypic upward foraging pattern on the plant (Jiu & Waage, 1990).

With the combined effects of shorter visit times to the insecticide-treated plants, and a tendency to move down the plant and stay on the lower surfaces of the leaves, the *D. carpenteri* would appear, by their behaviour, to be less vulnerable to insecticide residues than female *A. rhopalosiphi.* However, due to their smaller body size and their tendency to spend a higher proportion of their visit times actively searching, and therefore potentially contacting greater areas of insecticide contamination, this may result in higher mortality compared to *A. rhopalosiphi.* Insufficient numbers of *D. carpenteri* were available to test these theories in toxicity bioassays.

The flight of a wasp, in this study, onto the sides of the cage could have represented an attempt to make a short flight to a nearby plant onto which to continue searching or a flight upwards away from the vegetation to disperse over a greater distance. Logically one

could assume that if honeydew and/or hosts are located on a plant then a departing parasitoid would continue to search on nearby plants. Experiments conducted by Budenberg *et al.* (1992) monitored the direction of dispersal of *A. rhopalosiphi* from aphid-infested wheat plants in cages. The majority of female parasitoids leaving the plants were caught on the lower sides of the cage, coloured green to simulate the appearance of the crop, suggesting dispersal to nearby plants. The destination of dispersing wasps in this current study could not be determined, but it has important implications for the dispersal away from insecticide-treated plants in the field, i.e. are they moving to an adjacent treated plant or dispersing away to potentially reach reservoirs of untreated crop?

Although studies on the searching efficiency of aphid primary parasitoids are commonly reported (e.g. Gardner & Dixon, 1985; Ayal, 1987; Cloutier & Bauduin, 1990; Stadler & Völkl, 1991; Völkl, 1994) there are very few concerning the hyperparasitoids. However, one example reported by Chua (1979) indicates that the hyperparasitoid *Alloxysta brassica* (Ash.) has a more efficient searching pattern than the primary parasitoid *D. rapae*. It was suggested that these hyperparasitoids have to be more efficient because they have to perform an additional step in host finding. In other words, they have to seek not only the hosts of the primary parasitoids but also those containing primary parasitoids suitable for parasitising. This difference in behaviour has important implications for the design and interpretation of insecticide toxicity tests comparing different species. By conducting research to investigate these differences in behaviour, a comparison of the vulnerability of parasitoids with their hosts, predators and hyperparasitoids to insecticide residues can be made in order to aid risk prediction and possibly selectively control certain undesirable species in control programmes. A few studies concerning this question have provided promising results. For example, it was observed by Horn (1983) that hyperparasitoids spent more time walking over treated leaf surfaces searching for hosts than did primary parasitoids, thus potentially contacting more insecticide. This therefore raises the possibility of utilizing this behavioural difference to achieve insecticide selectivity, with at least five aphicides shown to be more toxic to the adult hyperparasitoid *Alloxysta curvicornis* (Cameron) than to the adult primary parasitoid *D. rapae* (Wiackowski & Herman, 1968). In addition, the carbamate Zectran, applied for California oakworm *Phrygaridia californica* (Packard) control was found to be more toxic to the hyperparasitoid *Dibrachys cavus* (Walker) than it was to the primary parasitoid *Itoplectis behrumsii* (Cresson) (Robertson, 1972).

With knowledge of the impact of agrochemicals on the foraging ability of hymenopteran parasitoids and the desirability for future integration of these chemicals and

beneficial invertebrates, it is paramount that these effects are investigated. *A. rhopalosiphi* have been shown, in this chapter, to undergo alterations in their behaviour as a result of exposure to insecticides. The extent of these changes on their potential risk in the field are still poorly understood and so therefore investigated further in subsequent chapters. The area of research of pesticide-induced behaviours is clearly very complex, but methods within this chapter have shown ways of measuring changes in behaviour to enable possible incorporation into models of insecticide encounter, uptake and intoxication, similar to that of Salt & Ford (1984).

CHAPTER FOUR

EFFECTS OF HONEYDEW AND INSECTICIDE RESIDUES ON THE DISTRIBUTION OF FORAGING APHID PARASITOIDS IN GLASSHOUSE AND FIELD CONDITIONS

INTRODUCTION

The opposing attractant/arrestment properties of aphid honeydew and the repellent properties of the pyrethroid insecticide, deltamethrin, to foraging aphid parasitoids were explored in Chapter 3. The relative strength of these responses will pose important constraints upon the foraging success of parasitoids searching in insecticide-treated crops.

Observations of parasitoid aggregation within host colonies and resulting parasitism levels under field or glasshouse conditions have been reported by only a few workers (e.g. Waage, 1983; Hågvar & Hofsvang, 1987). Such knowledge of parasitoid behaviour is important for the development of effective biological control programmes. It is desirable, from the point of view of an integrated pest management programme, that parasitoids are still attracted and arrested by the kairomonal properties of honeydew deposits on insecticide-treated crops, in order to locate surviving residual populations of aphids and provide biological control. However, by searching areas of honeydew-contamination, parasitoids will become exposed to fresh insecticide residues, increasing their toxicological risks through both lethal and sublethal effects (Chapter 2).

The results obtained from the laboratory-based experiments (Chapter 3) may not provide a true prediction of the interactions between honeydew and insecticide residues on parasitoid behaviour in a field situation. The lack of realism stems from the fact that they were conducted in enclosed ventilated chambers, using fresh insecticide residues, homogenous honeydew deposits, with no aphid hosts and with the parasitoids placed onto the treated surfaces, rather than engaging in their normal foraging behaviours. In order to understand the importance of honeydew and insecticide residues in shaping parasitoid foraging patterns, leading to improved risk predictions in the field, this chapter describes experiments conducted under both glasshouse and field conditions. These permitted the incorporation of important components such as spatially distributed aphid colonies and honeydew deposits, and the weathering of honeydew and insecticide residues.

The principal aims of this chapter were to:

(i) record the colonisation patterns of female aphid parasitoids, of the genus *Aphidius*, on wheat plants containing spatially distributed aphid colonies;

(ii) investigate the impact of the pyrethroid insecticide, deltamethrin, on these colonisation patterns;

(iii) determine the overall interaction of honeydew and insecticide residues on the behaviour of *Aphidius* parasitoids in a field situation.

Plant colonisation of female *Aphidius* parasitoids in an experimental glasshouse

Treatments

Experimental plants were separated into four groups to investigate the distribution patterns of aphids and parasitoids and the effects of deltamethrin upon this, under glasshouse conditions. Studies will hereafter be referred to as experiments 1, 2, 3 and 4, according to Table 4.1.

Table 4.1. Summary of the combination of aphids, parasitoids and insecticide used in the four experiments

	Aphid-infested plants present?	Parasitoids present?	Insecticide present?
Experiment 1	X	✓	X
Experiment 2	✓	X	X
Experiment 3	✓	✓	X
Experiment 4	✓	✓	✓

Plant material

Groups of mature wheat plants were removed from a commercial crop of winter wheat (cv. Tonic, G.S. 74, Tottman & Broad (1987)) and re-potted into 20cm-diameter pots. The plants in each pot were thinned out to leave 6 tillers of equal size, each gently washed with distilled water to remove any aphids or honeydew deposits.

Experimental layout

The studies were carried out in a small glasshouse (length: 4m, width: 3m) at Chilworth, Hampshire, with white-wash shading applied to the exterior of the glass. During all the experiments the roof vents, covered with fine mesh, were left fully open. Daytime temperatures were recorded during the experimental periods.

For each experiment, 48 pots of plants were placed in a 8 x 6 grid with 30cm spacings between each pot.

Aphid infestation

Aphids used for infestation of wheat plants were *Sitobion avenae* (F.), previously cultured on barley seedlings in an environmentally-controlled insectary.

For experiments 2, 3 and 4, eight pots of plants each received 150 aphids (3rd and 4th instars) placed at the base of the plants. These were left for 4-5 days prior to the experiments to permit aphid colonies to become established. The position of these aphid-infested pots of plants within the grid of plants was initially chosen at random (Figure 4.4a). This pattern then remained constant for subsequent experiments.

A sheet of white paper was positioned at the base of the aphid-infested plants, cut around the stems and covering the soil. This was designed to catch any aphids falling off the plants, which were subsequently recorded and removed.

Release of parasitoids

In order to collect sufficient parasitoid numbers, Dietrick vacuum-suction sampling (Dietrick, 1961) was carried out in a mature winter crop (cv. Tonic) and live female parasitoids from the genus *Aphidius* (identified by their characteristic wing venation, see Powell (1982)) were collected and kept in a culture box under environmentally-controlled conditions (20°C; 8h dark:16h light) for a maximum of 24h prior to the experiments. They were fed on a 50:50 honey:water solution placed on cotton wool pads. To standardise the condition of the wasps, they were kept in gelatin capsules and denied food/water for 2h prior to release.

For experiments 1, 3 and 4, six female *Aphidius* wasps were released at the base of each plant, giving a total of 288 wasps released in the glasshouse for each experiment. The release was at 09.00 British Summer Time (BST) and the number of wasps on each pot of plants was recorded during inspections at 30 and 60 mins after release, and then at one hourly intervals up to 8h after release. One inspection lasted for about 15 mins with equal time allocated to each group of plants. The pots of plants were searched in a fixed sequence around the glasshouse each time. All plant structures were carefully inspected, with parasitoids seemingly undisturbed by the examinations. A brief search for parasitoids on the glasshouse walls and a record of aphids on the white paper were also made.

At the completion of an experiment, the aphid-infested plants were transferred to an environment-controlled growth room and parasitised aphids, showing the characteristic mummy form, on the plants were counted after 14 days.

Insecticide application

Prior to experiment 4, the 40 aphid-uninfested pots of plants were sprayed outside

the glasshouse in a tightly packed block with a field recommended dose rate of deltamethrin (Decis, 2.5% E.C., equivalent of 6.25g AI/ha in 200 litres of water). The spray was applied using an Oxford Precision Sprayer fitted with four 110° flat-fan nozzles, operating at a pressure of 2 bar, and giving a swath width of 2m. Walking speed was adjusted to give a precise volume application rate equivalent of 200l/ha. After drying (approximately 1h), plants were returned to their allotted positions within the grid of pots and the parasitoid release and subsequent counting was carried out as described above.

Statistical analysis

The distribution patterns of parasitoids on plants were mapped throughout each experimental day, using a modified form of the visual representation method used by Hågvar & Hofsvang (1987). The percentage of initial number of parasitoids released, recorded on the plants and glasshouse walls during each assessment period are given. Total numbers of aphids recorded as "off" the aphid-infested plants in experiments 2, 3 and 4 were tested for homogeneity of variance using the Levene test (Day & Quinn, 1989). Tukeys HSD multiple comparisons were calculated following one-way ANOVA in order to identify which means differed significantly.

Two-sample t-tests were used to compare the cumulative numbers of parasitoids visiting the aphid-infested plants, and the resulting parasitism, in experiments 3 and 4.

Distribution of *Aphidius* parasitoids in the field

A field experiment was carried out in a mature winter wheat crop (cv. Brigadier; G.S. 74 (Tottman & Broad, 1987); with a tiller density of 470/m^2) on the Manydown Estate near Basingstoke. Twenty 2m^2 plots of wheat, with very low natural aphid infestations (a mean of <0.1 aphids per plant, n=100), were marked out in a 4 x 5 grid with a 5m area of crop left between them to avoid cross-contamination of treatments during spray application. Four treatments, each with five replicates were randomly assigned to the plots (Figure 4.1). The treatments applied were: (i) water, to act as a control, (ii) a fructose solution, (iii) the insecticide, deltamethrin, and (iv) a fructose solution and an additional application of deltamethrin.

The spray applications of the fructose solution (fructose dissolved in water, 25% w/v) and a field concentration of deltamethrin were applied to the respective plots using the Oxford Precision Sprayer, as described above. Walking speed was adjusted to give a precise volume application rate of 200l/ha.

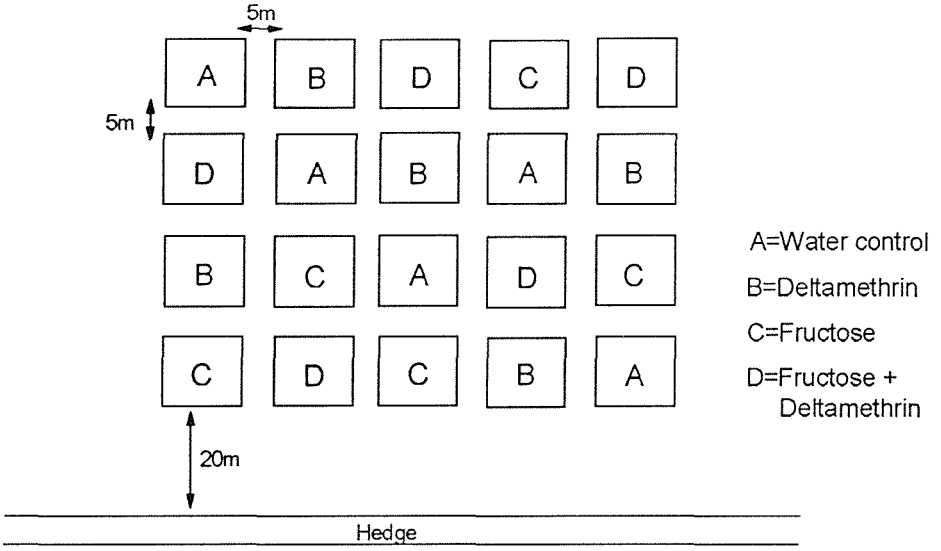


Figure 4.1. Plot layout for the four treatments applied

For the combined fructose and deltamethrin plots, the fructose solution was sprayed first and once dry, the insecticide was applied. After all the spray solutions had dried (approximately 30 mins), two flat, transparent sticky traps with a sticky area of 18cm x 18cm on both sides were attached to a cane in the centre of each plot at flag leaf height. Traps were left exposed for a 24h period after which they were collected and replaced by new ones, on each of the four days following spraying. Only cereal aphid parasitoids in the genus *Aphidius* were identified under a binocular microscope. This group was abundant and easy to identify due to their characteristic wing venation patterns (see Powell, 1982).

Statistical analysis

A mean number of parasitoids was computed from the two sticky traps in each of the five replicate plots for each treatment. G-tests were then used to test for homogeneity of means within treatments before pooling the data for subsequent analysis. On each day after treatment, the numbers of parasitoids trapped in each treatment plot were $\log_{10}$-transformed to normalise the data and equalise variances prior to analysis. Homogeneity of variance was tested using the Levene test (Day & Quinn, 1989). Tukeys HSD multiple comparisons were calculated following one-way ANOVA in order to identify which means differed significantly.

Distribution of parasitoids in an experimental glasshouse

Meteorological readings

The temperature profiles throughout the experimental days in the glasshouse showed very similar trends on each occasion (Figure 4.2). Temperatures varied between 18°C to 29°C, with the highest temperatures recorded during 13.00-15.00h (BST).The days were climatically similar, with slight cloud cover contributing to shaded conditions within the white-washed glasshouse.

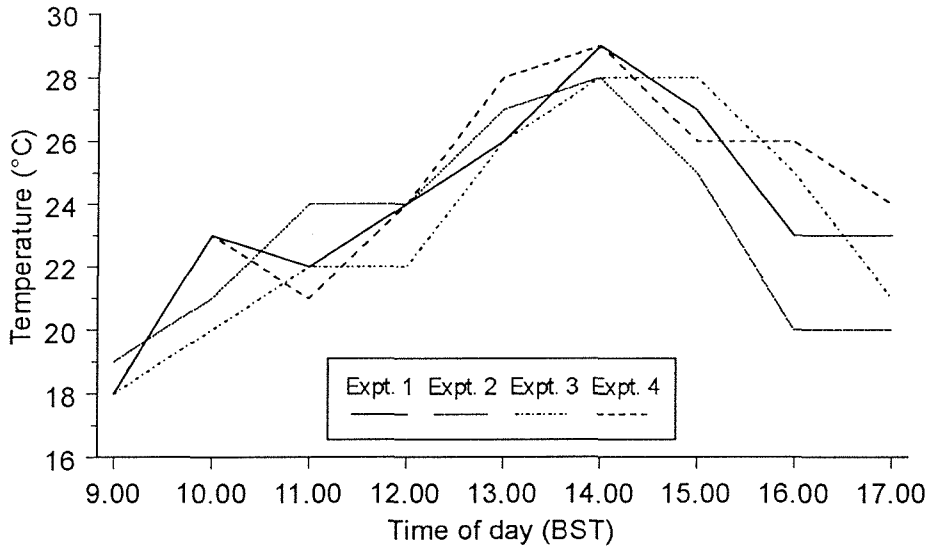


Figure 4.2. Temperature profiles in the glasshouse during the four experimental days

Parasitoid distribution on single plants

In experiment 1 (no aphid-infestation/no insecticide) the parasitoid colonisation pattern of plants, though low, was distributed randomly with no evidence for aggregation on particular plants or regions of the glasshouse (Figure 4.3a). A maximum of three wasps were found on a single plant at any time.

During the first observation period in experiment 3 (no insecticide present), a random pattern of plant colonisation by parasitoids was present, with wasps showing no preference for particular plants (Figure 4.4a). However, 1h after release, parasitoids showed strong aggregation patterns to the aphid-infested plants. No particular aphid-infested plant

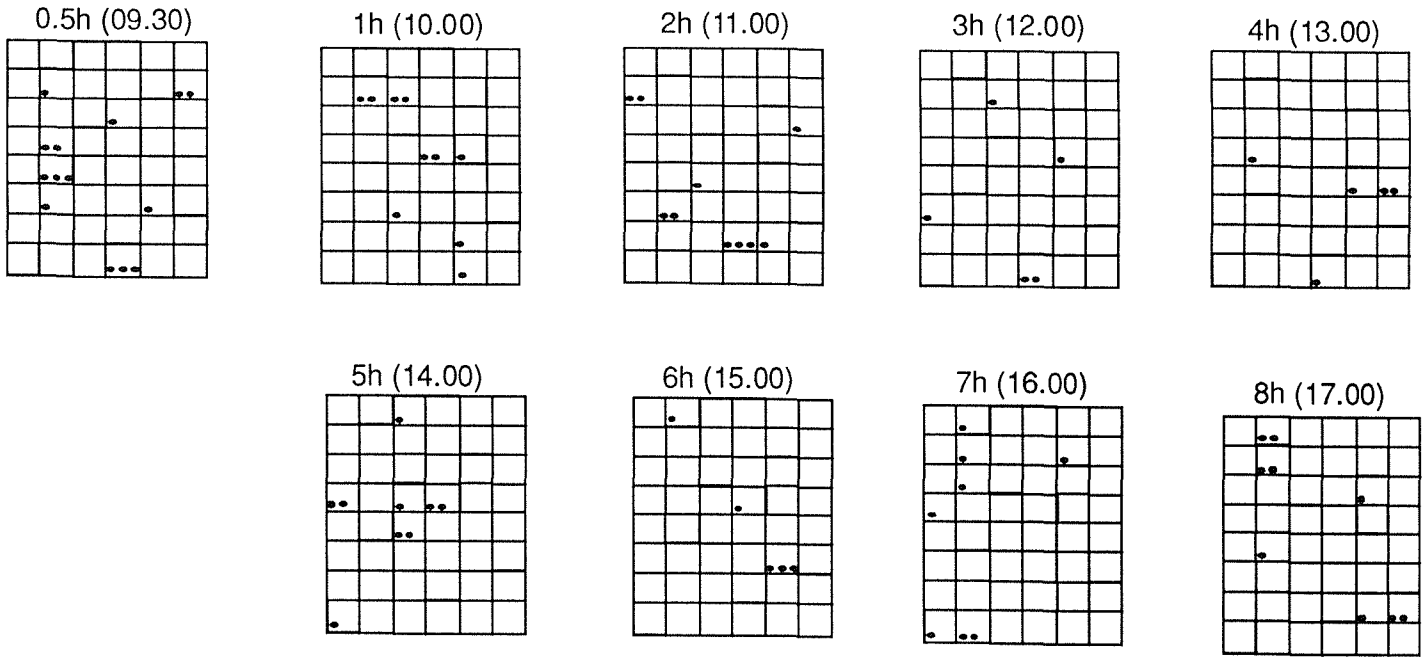


Figure 4.3a. Distribution patterns of individual *Aphidius* (dots) in experiment 1 on single plants (small squares) not infested with aphids, registered at various time periods (hours after release, and hour of day) after parasitoid release.

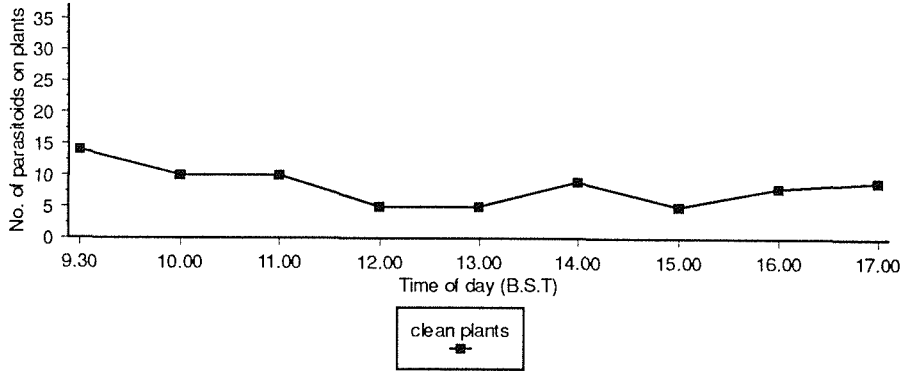


Figure 4.3b. Summary of the numbers of *Aphidius* observed on the clean plants (n=48) over the experimental period of experiment 1.

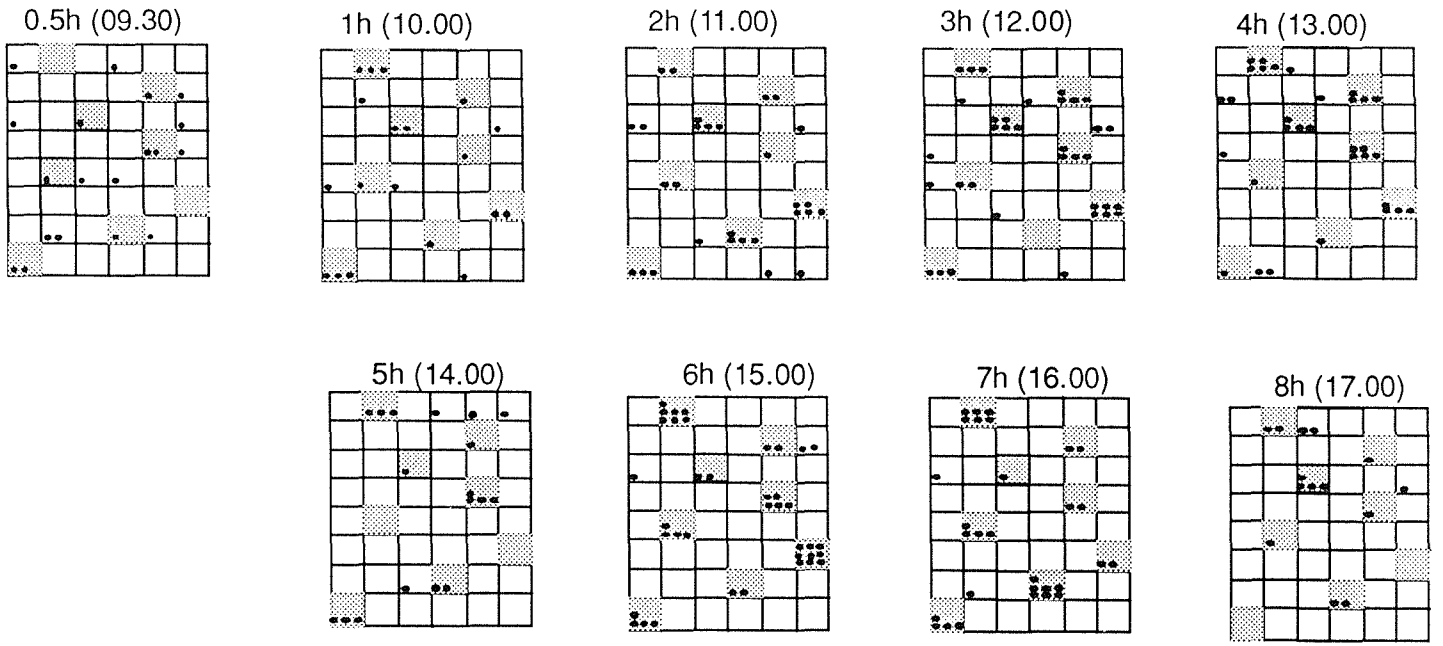


Figure 4.4a. Distribution patterns of individual *Aphidius* (dots) in experiment 3 on single plants (small squares) either aphid-infested (shaded) or uninfested (unshaded), registered at various time periods (hours after release, and hour of day) after parasitoid release.

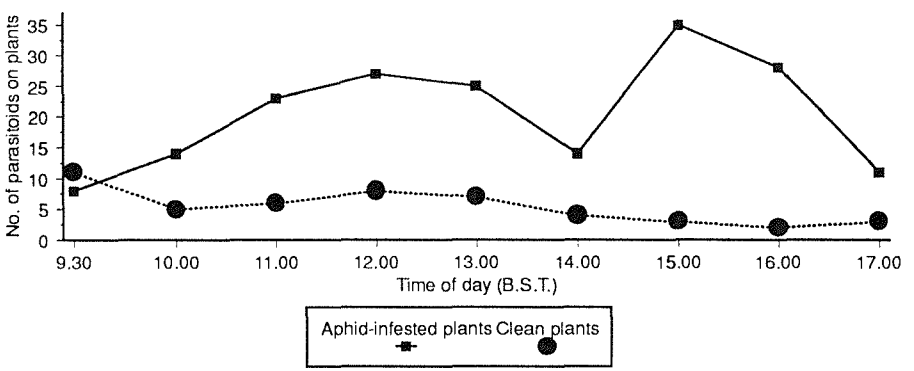


Figure 4.4b. Summary of the numbers of *Aphidius* observed on the aphid-infested plants (n=8) and the uninfested "clean" plants (n=40) over the experimental period of experiment 3.

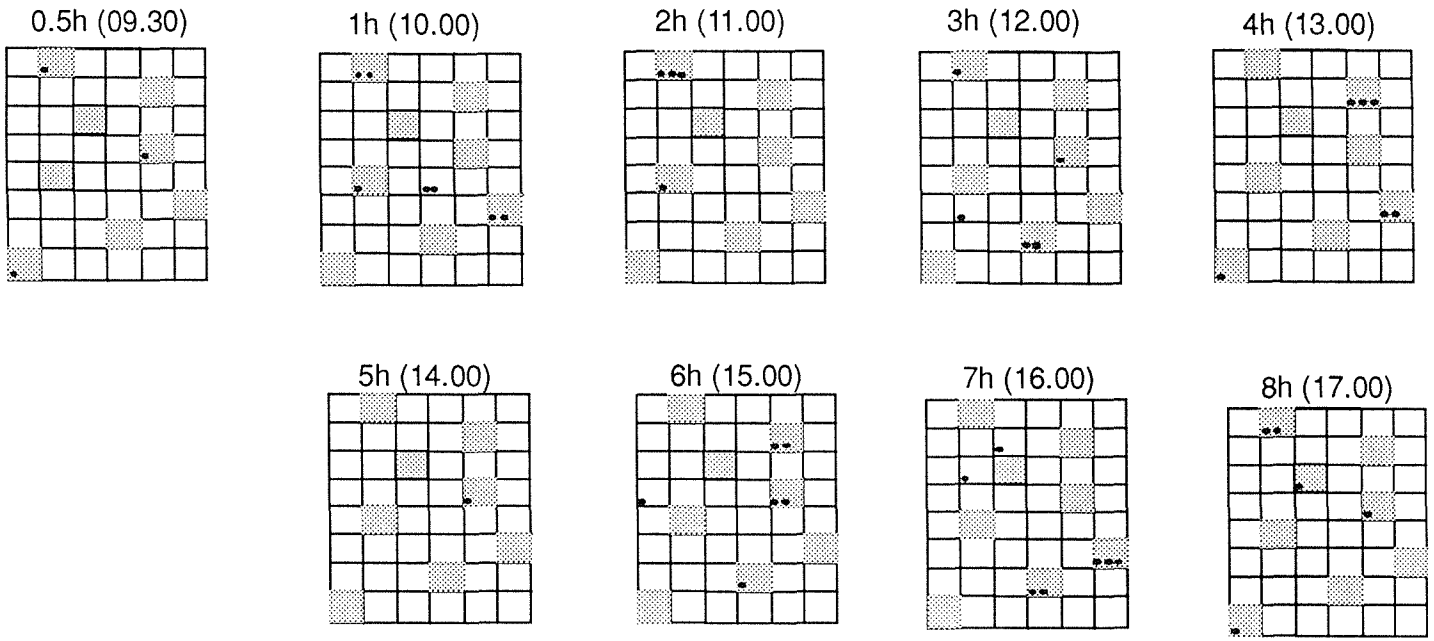


Figure 4.5a. Distribution patterns of individual *Aphidius* (dots) in experiment 4 on single plants (small squares) either aphid-infested (shaded) or uninfested (unshaded), registered at various time periods (hours after release, and hour of day) after parasitoid release.

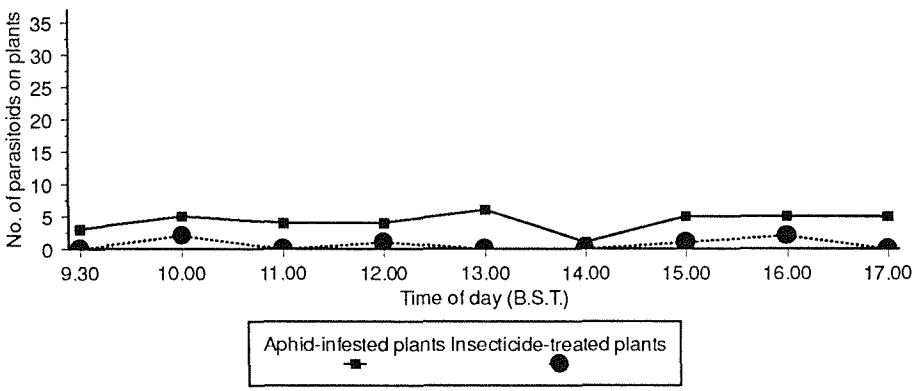


Figure 4.5b. Summary of the numbers of *Aphidius* observed on aphid-infested plants (n=8) and the insecticide-treated plants (n=40) over the experimental period of experiment 4.

appeared to be the most favoured by parasitoids, with a uniform spread of wasps over them throughout the day. The highest total of nine parasitoids was observed on a single plant at 15.00h (6h after release).

Throughout experiment 4 (insecticide present), observed parasitoid colonisation levels on all plants were very low (Figure 4.5a). A preference was shown by parasitoids for the aphid-infested plants.

Parasitoid colonisation on plants and walls

In all four experiments, parasitoids were frequently observed on the walls of the glasshouse (Table 4.2). High numbers were recorded in all experiments during the first observations (0.5h after release), suggesting that once released, parasitoids showed a strong tendency to disperse away from their immediate surroundings. Less were observed on walls during subsequent assessments, with a second highest peak in numbers occurring during 13.00-15.00h, coinciding with the highest recorded temperatures in the glasshouse (Figure 4.2).

Parasitoid numbers observed on uninfested plants in experiments 1 and 3 remained low during each assessment, with a generally decreasing trend in numbers throughout the day (Figures 4.3b & 4.4b). This suggests that uninfested plants became increasingly less favoured and were soon abandoned or avoided by the parasitoids.

During experiment 3, numbers of parasitoids observed on the aphid-infested plants increased during successive observations up to 12.00h, followed by a decrease between 13.00-14.00h assessment periods. A peak in numbers occurred again at 15.00h followed by a decline during the following two observation periods (Figure 4.4b). This observed trend of plant colonisation may be related to the temperature profile (Figure 4.2.), indicating the decline in parasitoid numbers occurred during the period of highest temperatures. There was also an observed increase in parasitoid numbers recorded on the glasshouse walls (Table 4.2).

During experiment 4, parasitoid numbers observed on aphid-infested plants remained low (Figure 4.5b). Again, lowest numbers were recorded at 14.00h when the hottest temperatures were recorded, accompanied by an increase in wasp numbers on the walls (Table 4.2). Only small numbers of parasitoids were recorded on insecticide-treated plants throughout the experimental period.

The total numbers of parasitoids observed on both plants and glasshouse walls varied throughout the day in each experiment (Table 4.2). The highest percentages of initial parasitoids released were recorded during the first observations (0.5h after release) and during 13.00-15.00h. The maximum number re-counted during any experiment occurred at

Table 4.2. The total number of *Aphidius* recorded on plants and glasshouse walls, with the combined percentage of the initial number released (n=288), registered at time intervals after release in experiments 1 (no aphids), 3 (aphids present) and 4 (aphids and insecticide present). [1]data shown in figure 4.3b; [2]data shown in figure 4.4b; [3]data shown in figure 4.5b.

	Hours after release	0.5	1	2	3	4	5	6	7	8
	Time of day (BST)	(9.30)	(10.00)	(11.00)	(12.00)	(13.00)	(14.00)	(15.00)	(16.00)	(17.00)
Experiment 1	Plants[1]	14	10	10	5	5	9	5	8	9
	Walls	36	29	24	27	39	34	41	22	18
	Total % of released wasps	17.4%	13.5%	11.8%	11.1%	15.3%	14.9%	15.9%	10.4%	9.3%
Experiment 3	Plants[2]	19	19	29	35	32	18	38	30	14
	Walls	32	14	19	24	17	28	26	22	21
	Total % of released wasps	17.7%	11.5%	16.7%	20.5%	17.0%	15.9%	22.2%	18.1%	12.2%
Experiment 4	Plants[3]	3	7	4	5	6	1	6	7	5
	Walls	45	32	22	28	39	53	44	27	31
	Total % of released wasps	16.7%	13.6%	9.0%	11.4%	15.6%	18.8%	17.4%	11.8%	12.5%

15.00h (6h after release) in experiment 3, with 64 (= 22.2%) found on both plants and walls. The generally low numbers observed during the experiments suggest that a large proportion of the parasitoids either escaped from the glasshouse or remained hidden.

Relationship between aphid fall-off and parasitoid activity

The mean number of aphids falling/walking off the plants in experiment 2 (no parasitoids present), remained at a very low level throughout the day (Figure 4.6). During experiment 3 (aphids+parasitoids present), high numbers of aphids were recorded on the white paper, coinciding with times of high parasitoid numbers on the plants and high temperatures. Significantly higher aphid numbers on the white paper (ANOVA, $P<0.05$; Tukeys HSD test; Table 4.3) were observed during the experiments in the following order: experiment 3 (parasitoids + aphids) > experiment 4 (parasitoids + aphids + insecticide) ≡ experiment 1 (aphids only). This trend correlates with the observed total numbers of parasitoids on plants in these experiments (Figures 4.4b & 4.5b).

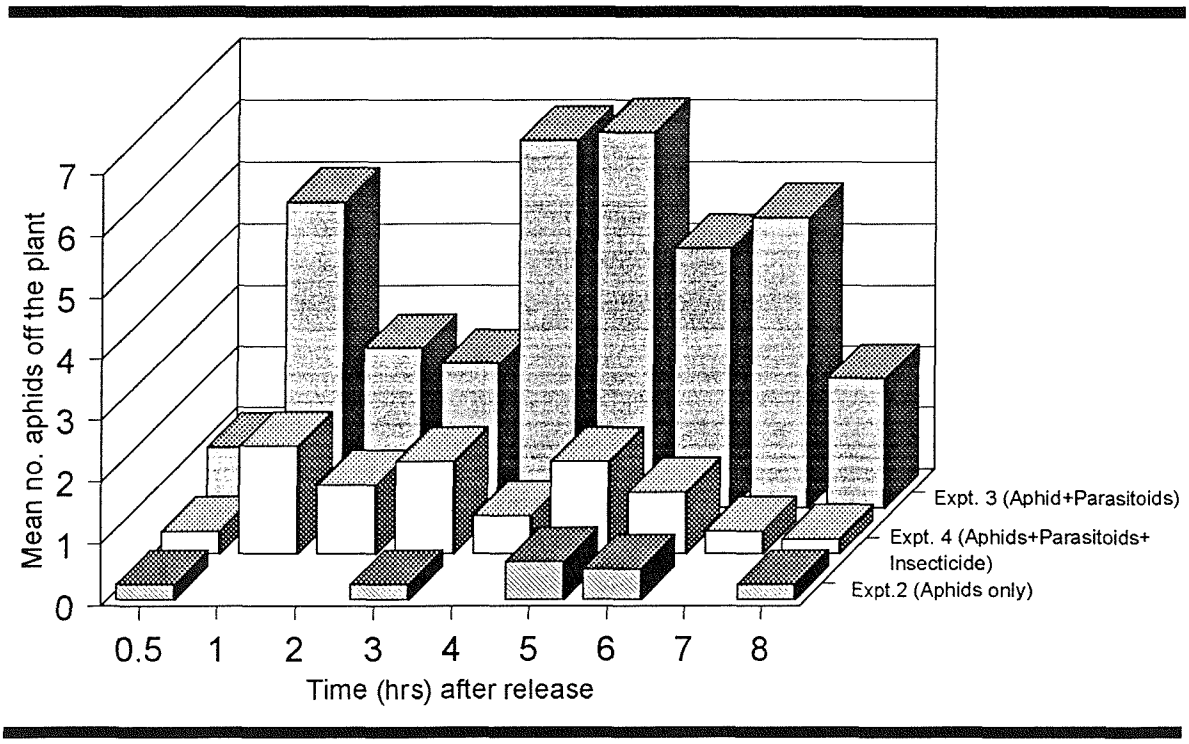


Figure 4.6. Number of aphids recorded as "off" the aphid-infested plants at time intervals after parasitoid release during experiments 2, 3 and 4.

Table 4.3. Summary statistics of one-way ANOVA conducted on aphid "fall-off" data, represented in Figure 4.6.

Analysis of variance					
Source	D.F.	Sum of squares	Mean squares	F ratio	F probability
Between groups	2	520.03	260.01	62.16	0.0000
Within groups	213	890.93	4.18		
Total	215	1410.96			

Parasitism in relation to parasitoid numbers

In experiment 3, the mean cumulative number (±S.E.) of parasitoids observed visiting a single aphid-infested plant during the 8h period was 23.1 (±1.7), which was significantly greater (two sample t-test, t=9.14, d.f.=15, P<0.001) than the cumulative number of parasitoids visiting the equivalent plants in experiment 4, (4.8±0.9). The subsequent formation of mummies revealed a mean of 19.5 (±1.7) per plant in experiment 3 which was significantly different from the mean of 3.8 (± 0.7) per plant in experiment 4 (two sample t-test, t=8.55, d.f.=15, P<0.001). This suggests that the recorded observations of parasitoids on the plants represented a realistic measure of the parasitic activity of the parasitoids.

Distribution of *Aphidius* parasitoids in the field

During the 24h immediately following spraying, the numbers of *Aphidius* caught on traps in the honeydew-only treatment were higher (though not statistically significant, P>0.05) than those in the honeydew+insecticide plots (Figure 4.7a). However, both these treatments showed significantly higher numbers (P<0.05) of trapped parasitoids than in the water and insecticide-only plots.

During day 2 after treatment, the same trend in parasitoid numbers caught in each treatment was shown (Figure 4.7b). No significant differences were found between the treatments, except for the insecticide-only treatment which still showed a significantly lower number of parasitoids caught than the other treatments. No significant differences, or biological trends, in the number of trapped parasitoids were detected between treatments during days 3 and 4 after spraying (Figures 4.7c & 4.7d).

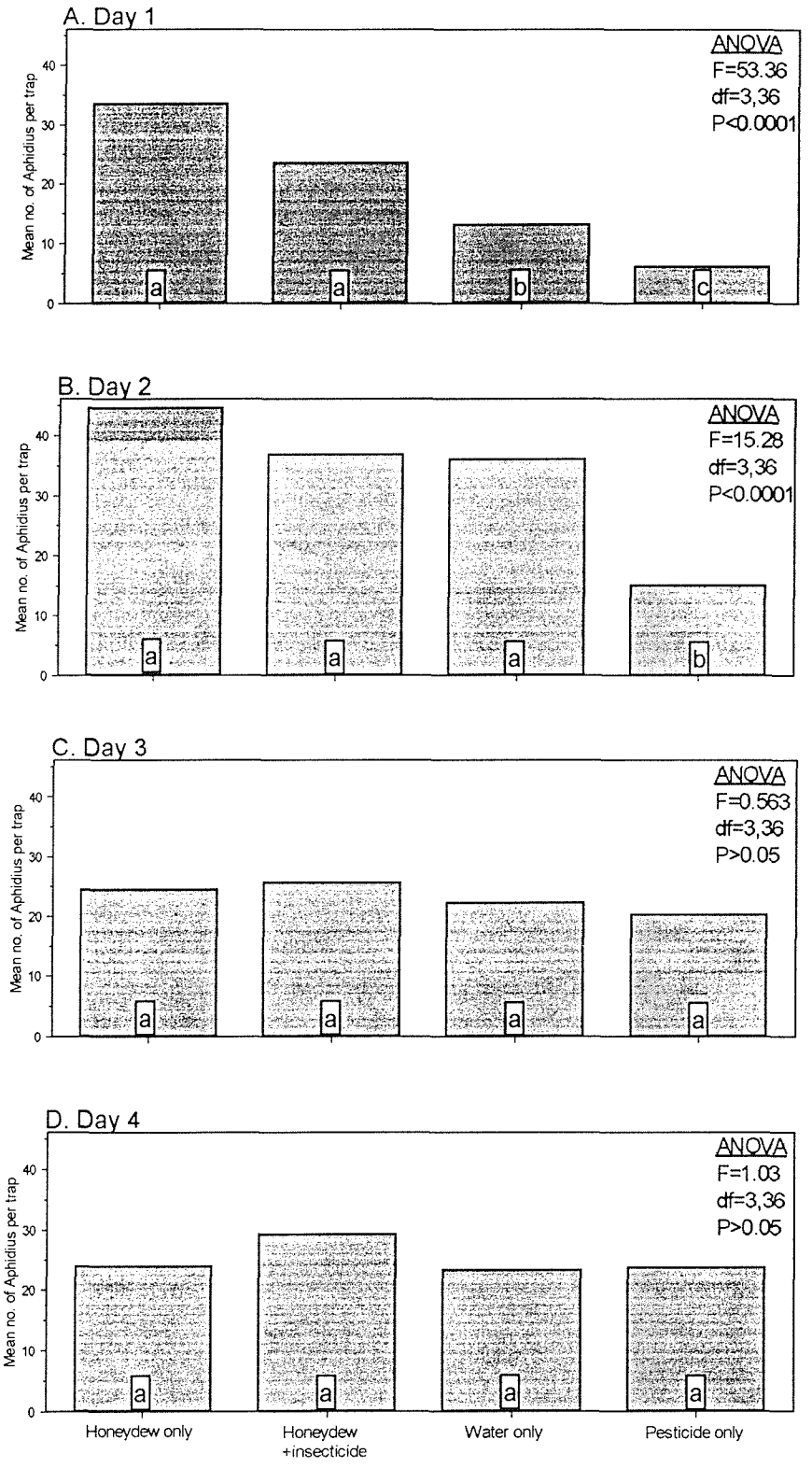


Figure 4.7. Mean number of *Aphidius* parasitoids caught per trap (n=10) in the four treatments on (A) day 1, (B) day 2, (C) day 3 and (D) day 4 after spray application. ANOVA conducted on $\log_{10}$-transformed data, columns showing different letters differ significantly (P<0.05) (Tukeys HSD test).

DISCUSSION

Parasitoid foraging in the experimental glasshouse

The results indicate that females of *Aphidius* spp. have a searching behaviour that rapidly leads them to aphid-infested plants. Initial plant colonisation appeared to be random, with no evident bias towards any particular plant, presumably a result of the release of equal numbers of wasps at the base of each plant. In the absence of aphids (experiment 1), a small number of parasitoids were observed to visit the wheat plants throughout the 8h experimental period. Similarly, Akinlosotu (1978) found that *Diaeretiella rapae* M'Intosh distributed themselves randomly on aphid-free brussels sprout plants in a $25m^2$ field cage immediately after release. The attraction of aphid parasitoids to host-free plants may be explained by the fact that in olfactometer experiments, certain species have been shown to be attracted to odours from the food plant of their aphid hosts (Read *et al.*, 1970; Schuster & Starks, 1974; Akinlosotu, 1978; Powell & Zhi-Li, 1983). In addition, colour has also been implicated with host habitat location, with both *A. ervi* Haliday and *D. rapae* responding strongly to the colour green (Vater, 1971; Goff & Nault, 1984).

Parasitoids initially colonising plants at random left aphid-uninfested plants presumably in response to a lack of kairomones or hosts, as was demonstrated for *A. rhopalosiphi* in laboratory bioassays (Chapter 3). A continuous localised dispersal away from "unproductive" plants could potentially bring the wasp into contact with an aphid-infested plant. Alternatively, parasitoids could have been attracted to plants containing hosts solely by exploiting honeydew kairomones (Bouchard & Cloutier, 1985; Hågvar & Hofsvang, 1987; Wickremasinghe, 1989). For example, *Ephedrus cerisicola* Starý have been shown to use odours from colonies of their host aphids, with plants containing a high density of aphids (approximately 500 to 1000) more readily detected than those with low aphid densities (50 to 200) (Hågvar & Hofsvang, 1987).

The observed higher numbers of parasitoids on the aphid-infested, compared to the uninfested, plants in the current study could have resulted from a combination of an increased attraction of flying parasitoids to plants with aphids and fresh honeydew deposits, and to an increase in time spent searching and undertaking resting and grooming behaviours after successful host encounters. Increased search times have been shown for *A. rhopalosiphi* on honeydew-contaminated substrates (Chapter 3), and following oviposition in aphid hosts (Gardner, 1982; Budenberg *et al.*, 1992). In addition, the congregation of parasitoids on an aphid-infested plant over time may be as an indirect result of parasitoids already present, as it is believed that aphid alarm pheromones emitted from parasitoid-

disturbed aphid colonies can attract new parasitoids to the area (Hågvar & Hofsvang, 1989).

Throughout the experimental days, only small numbers of parasitoids were observed during any one observation period. It is possible that the actual numbers of parasitoids visiting the plants were underestimated as a result of the long time intervals between observations. If practical for further experiments, continuous recording of parasitoid visits to plants would allow better predictions of host location success.

Aphid numbers recorded "off" the plant in the absence of parasitoids (experiment 1) were significantly lower than when parasitoids were present (experiment 3). This disturbance of aphids by parasitoids has been previously documented (Tamaki *et al.*, 1970; Hight *et al.*, 1972; Ruth *et al.*, 1975; Gowling & van Emden, 1994). It may result from the disturbance by foraging parasitoids, accompanied by aphid defence behaviours such as kicking and emission of alarm pheromones which frequently result in the escape of aphids (Gowling & van Emden, 1994). This factor has important consequences for the assessment of parasitoid impact, because fallen aphids may be caught in spider webs between stems, consumed by ground-dwelling predators (Winder, 1990) or debilitated through the action of stabbing by the parasitoid. In the current study, the number of aphids recorded on the white paper presumably represented only a small proportion of the aphids that fell off or left the plant during the 8h experimental period. Future experiments could use water traps or double-sided adhesive tape placed at the base of plants to provide accurate estimates of falling aphids.

The recorded parasitism in experiments 3 and 4 were surprisingly low, and could have been a result of various unidentified factors. The low numbers of parasitoids actually foraging on the plants, as well as defence and escape behaviours could have been important in limiting the number of aphids parasitised. Interference between foraging parasitoids has also been attributed to a reduction in potential parasitism by causing increased dispersal, as was shown in cage experiments with *D. rapae* (Akinlosotu, 1978). However, it is unlikely that interference between foraging female parasitoids was a major factor influencing parasitism rate in these experiments because only a maximum of 9 parasitoids were seen searching the same plant at any one time.

Parasitoid foraging in the field

The significantly higher numbers of parasitoids caught on day 1 after treatment in plots sprayed with fructose solution, compared to water, highlights the potential of using artificial honeydews in pest management programs. The fructose solution applied in the current study was shown to encourage longer arrestment times by *A. rhopalosiphi* compared to a sucrose solution (Budenberg, 1990b), and may have acted as a combined

attractant/arrestment and food source for the parasitoids in the field. However, the effects were short-term as no significant differences between treatments were detected on day 2 after treatment.

There has been considerable interest in the possibility of utilising semiochemicals of various types to influence beneficial insects in biological control programs (e.g. Hagen *et al.*, 1971, 1976; Ben Saad & Bishop, 1976; Finch, 1978, 1980; Lewis *et al.*, 1979; Altieri *et al.*, 1981, 1982; Titayavan & Altieri, 1990). By applying these artificial solutions to crops, a reinforcement of the host location behaviour of predators and parasitoids can be achieved by increasing their activity and retaining them in the environment. The identification of the kairomone(s) in aphid honeydew could lead to their increased use for the enhancement of aphid parasitism (Powell, 1986), however an attempt at the chemical analysis by Budenberg (1990b) proved relatively unsuccessful. For future work on artificial honeydews, further knowledge of the proper active compounds in honeydew is needed, along with their effects on the behaviour of released parasitoids, wild parasitoids, hyperparasitoids and aphidophagous predators, to ensure they are not detrimental to the control of aphids. The presence of artificial honeydew could reduce both rates of parasitism and predation because the distribution of natural honeydew, normally used to locate aphids, would be obscured. The high sugar contents could also encourage the growth of sooty moulds and other fungi.

Consequences of the repellency phenomenon

In the glasshouse and field studies, the significantly lower numbers of parasitoids recorded on, or in the vicinity of, insecticide-treated plants could have resulted from a combination of reduced residence times of the wasps on the plants or a repellent effect by the insecticide, deterring wasps from alighting on the treated foliage. The residence times of parasitoids upon insecticide-treated plants have been shown to be significantly reduced compared to untreated plants (Chapter 3; Jiu & Waage, 1990; Borgemeister *et al.*, 1993). Reduced flight activity by parasitoids towards insecticide-treated plants (Elzen *et al.*, 1989), and reduced foraging by bees in a treated fields (Pike *et al.*, 1982) have been reported.

The repellent properties of pyrethroid insecticides to an insect can occur through the masking of attractive plant odours and/or an interference with the receptors for detecting the odours (Haynes, 1988). A disruption of the foraging activities of parasitoids through repellency may prove beneficial from the point of view of integrated pest management programmes, because it can minimise their exposure to toxic residues. However, as was shown in the glasshouse study, it could also prove detrimental to parasitoids which must locate patchily distributed hosts. For example, the presence of deltamethrin residues on

wheat plants surrounding those housing aphids resulted in a decrease in observed parasitoid foraging, with subsequent lower parasitism levels. These results from the glasshouse study provide evidence of the repellent properties of deltamethrin, which in this circumstance exhibited a greater deterrent effect to wasps than the attractant properties of aphid honeydew. However, these results were obtained with fresh insecticide residues, with no inclusion of weathering effects of either honeydew or insecticide deposits and in an enclosed environment, with minimal circulation of air. A more realistic situation was achieved in the field study, despite the small size of treated plots, which showed that the attraction/arrestment stimuli associated with the artificial honeydew, and perhaps oviposition of aphids, competed with the repellent effects of deltamethrin. This lead to similar numbers of *Aphidius* parasitoids caught on the sticky traps in the honeydew-only and honeydew+insecticide plots. Similarly, Perera (1982) showed for *Encarsia formosa* Gahan that the deterrent effects of a pyrethroid insecticide were overcome when host density was sufficiently high. In these type of studies, relative concentrations of kairomones and insecticide are important. If the kairomones prove highly volatile, it is possible they could create a more concentrated odour cloud in the short-term than the less volatile pyrethroid.

The experiments carried out in this chapter simulated situations where a sprayed crop has low post-spray pest populations relative to the amount of arrestment stimuli present from pre-spray populations. Results indicate there is likely to be a decrease in parasitoid searching efficiency (in addition to sublethal pesticide effects on foraging behaviour (Chapter 3)) as parasitoids continue to be arrested by kairomones on plants housing few or no live hosts. The survival of aphid hosts on cereal crops, and subsequent location by parasitoids, after insecticide applications, are investigated further in the following chapter.

CHAPTER FIVE

CONSEQUENCES OF REDUCED-DOSE APPLICATIONS OF DELTAMETHRIN FOR APHID CONTROL

INTRODUCTION

Several authors have drawn attention to the possibilities of increasing the selectivity of pesticides in a range of cropping systems by reducing dose rates (e.g. van den Bosch & Stern, 1962; Madsen & Williams, 1968; Kiritani, 1976; Metcalf, 1980; Poehling, 1988). In cereal systems, such policies have been demonstrated to offer a more economic means of aphid control in addition to having potential environmental benefits (e.g. Poehling, 1989, 1990; Mann *et al.*, 1991). Reducing the concentration of insecticide, achieved by increasing the dilution of the active ingredient in water, may permit a greater survival rate of natural enemies and also maintain a residual population of aphids as food for the conserved natural enemy fauna. Natural enemies may then remain in the crop and prevent any further pest resurgence (Chapter 1; Poehling, 1989).

Estimates of the efficiency of current application methods for insecticides in most crops suggest that only a small percentage of the applied active ingredient applied contacts the target pest itself (Metcalf, 1980). In the case of winter wheat, the attenuation of spray through the canopy provides for relatively uncontaminated areas of foliage (Chapter 2; Çilgi & Jepson, 1992). By using reduced dose-rate applications of, for example, the pyrethroid insecticide deltamethrin, which is a contact insecticide with no systemic action, static aphid colonies may survive in these refuges. This phenomenon has been reported by Poehling (1987a, 1987b) who recorded elimination of *Sitobion avenae* (F.) from wheat ears when using reduced dose rates of fenvalerate, but persistence of *Metopolophium dirhodum* (Wlk.) under flag leaves, in areas not covered by insecticide residues.

Considerable information is available about the fine-scale distribution patterns of aphid populations on plants (e.g. Wratten, 1974; Hodgson, 1978; Takeda, 1980; Harrington & Taylor, 1990). However, despite the observations by Poehling (1987a, 1987b), no other studies have investigated the spatial distribution of surviving aphid colonies on insecticide-treated plants. Information of this kind is important for the understanding of where residual populations may remain after application of reduced dosages, and how soon depleted

populations may recover. The spatial redistribution of aphids could prove detrimental for biological control programmes because many parasitoids are reported to be limited by their ability to find hosts (Price, 1980). Studies therefore need to determine whether parasitoids (and predators) can successfully locate any surviving residual aphid populations before the use of reduced-dosage insecticide programmes in cereals.

Parasitoid survival in insecticide-treated crops also needs to be known and assessments of the toxicity of foliar residues after treatment are needed to determine the risks posed to newly colonising parasitoids.

The specific aims of this chapter were to:

(1) assess the efficacy of the pyrethroid insecticide, deltamethrin, as a summer applied cereal aphicide at a range of different concentrations;

(2) measure the stratification of spray deposition through the crop canopy. This was to determine the degree of direct exposure of aphid colonies to spray and measure the levels of residues which may be potentially contacted by foraging parasitoids;

(3) record the spatial distribution of aphids on winter wheat plants and the resulting survival/redistribution of populations over the following days after application with reduced dose rates of deltamethrin;

(4) assess the residual toxicity of different deltamethrin concentrations on flag leaves by exposing parasitoids on subsequent days after treatment.

EXPERIMENTAL METHODS

Plot layout

The field experiments were conducted in a mature winter wheat crop (cv. Brigadier, g.s. 73 (Tottman & Broad, 1987), with a tiller density of 470/m^2) on the Manydown Estate near Basingstoke during June/July 1994. Four 20m x 1m plots were marked out with 8m of crop left between them to avoid cross-contamination when spraying. Two different treatments were applied: (i) water, to act as a control, and (ii) the pyrethroid insecticide, deltamethrin (Decis, 2.5% E.C.).

Meteorological measurements

Daily rainfall and hourly temperature readings were recorded for the duration of the study on open ground in an adjacent field using a Delta-T electronic data logger.

Spray application

The spray applications were made using a Chesterford Miniature Logarithmic Sprayer (MDM Engineering Ltd) operating at 2 bar, and giving at swath width of 1m. Walking speed was adjusted to give a precise volume application rate of 200l/ha following individual calibration of the nozzles.

The logarithmic dilution principle of the sprayer involves the chemical concentrate being forced out of the vessel at a constant rate and being replaced at the same rate with a diluent (water) which is intimately mixed with it, causing the concentration of the chemical to decrease in an exponential manner. When the liquid is sprayed and the walking speed kept constant, then the chemical dosage rate is proportional to both time and distance.

Calibration of the sprayer was carried out using a 1% w/v red dye solution (Kenacid Scarlet) and Whatman 9cm-diameter filter papers placed at 1m intervals along a 20m transect on a flat tarmac surface. The deposition of dye on each filter paper was measured colorimetrically (see below). It was calculated that for every 3m along the transect, with a constant walking speed of 1m/sec, the dose of concentrate halved.

Solutions of deltamethrin at double the recommended field concentration (i.e. equivalent of 2 x 6.25g AI/ha in 200 litres of water) were applied along three of the 20m transects. Walking commenced, and was maintained at a constant speed, immediately the spray emerged from the nozzles. The sprayer was set up to logarithmically dilute the initial concentrate to a final concentration of 1/100th of the original over the 20m transect. The dose rates of deltamethrin applied ranged from 2 x to 1/50th of field concentration along

the transect. The fourth transect was sprayed with water.

Spray deposition through the crop canopy

To make a direct measurement of the volume of spray solution landing on different parts of the plant at the time of spray application, a 1% w/v red dye solution (Kenacid Scarlet) was applied using the logarithmic sprayer, with dye solution in both the concentrate and diluent bottles, to maintain constant dye strength. The spray solution was applied to a $10m^2$ (10m x 1m) area of wheat, as described above.

Twenty five strips of white paper (3.25cm x 1cm) were positioned at five different levels throughout the crop canopy. The papers were pinned vertically along the length of wheat ears, and horizontally along the central abaxial and adaxial sections of flag leaves and first leaves. Separate plants were selected randomly for each paper/position combination. After the spray solution had dried (approximately 30 mins) the pieces of paper were individually removed with fine forceps and transferred into dry, labelled tubes and stored in cold (6°C), dark conditions until analysis. A sample of the spray formulation in the tank mix was taken immediately after spraying and stored in identical conditions to the sample tubes. For the analysis, individual papers were placed in 6ml of distilled water, left to soak for 20 mins, then shaken for 1 min to ensure all the dye was washed off into the solution. The volume of dye in solution was determined by analysis in a Gallenkamp colorimeter. The optical density readings were corrected by using a sample of distilled water as a blank comparison. To construct a calibration curve, enabling the amount of dye contained in the samples to be expressed as microlitres of the original spray formulation, a range of volumes of the original tank mix were accurately micro-pipetted into 6ml of distilled water and analysed in the colorimeter. A linear regression analysis was undertaken to obtain the calibration curve. The volume of spray formulation in microlitres was converted to volume per cm^2 by division with the surface area of one side of the paper ($3.25cm^2$).

Aphid assessments

Twenty four hours prior to spray application, groups of five randomly selected tillers were carefully cut at ground level at 1m intervals down a 20m insecticide-treated transect. The numbers and location of aphids on the plants were recorded. In addition, at each 1m interval in another transect replicate, five wheat tillers supporting aphid colonies on ears and leaves were marked using labelled jewellers tags. Twenty tillers were marked in the control transect. Again, the numbers and location of aphids were recorded. Further aphid assessments on randomly selected plants and marked tillers were conducted at 1, 3 and 5 days after spraying.

Parasitoid bioassay

To obtain sufficient numbers of aphid primary parasitoids for the bioassays, D-vac suction sampling was carried out in an adjacent crop. Live female parasitoids of the genus *Aphidius* (identified by their characteristic wing venation, see Powell (1982)) were collected and kept individually for 24h prior to the experiments in 2cm x 1.75cm ventilated clip cages. They were fed with drops of 50% honey solution smeared on the mesh covering.

After spray residues of water and deltamethrin had dried (approximately 1h), 10 clip cages, each containing a single live *Aphidius* female, were positioned onto the upper surfaces of randomly selected flag leaves at 1m intervals along the 20m insecticide-sprayed transect. Ten individual wasps were clip-caged onto flag leaves in the control transect. Parasitoids were in contact with the upper leaf surface and fed with a 50% honey solution. The mean leaf area enclosed by these chambers was 3.1cm^2. The weight of the clip cages drew the leaves down into the dense crop canopy, thus minimising the likelihood of parasitoid mortality through desiccation. Parasitoid condition was assessed after 24h and recorded as either dead/affected or live. The above procedure of exposing new parasitoids to foliage for 24h was repeated on days 3 (72-96h) and 5 (120-144h) after spraying.

Statistical analysis

(1) The volumetric data of spray deposition for each position in the crop was tested for homogeneity of variance using the Levene test (Day & Quinn, 1989). Tukeys HSD multiple comparisons were calculated following one-way ANOVA in order to identify which means differed significantly.

(2) The mean numbers of aphids on the five tagged plants at each location along the deltamethrin-treated transect was calculated. A $\log_{10}$ (n+1) transformation was applied to all aphid data then the post-treatment transformed value for each group of tagged plants was subtracted from the pre-treatment count of the same plants, to form a "log-difference" value (Sotherton *et al.*, 1987).

(3) Probit analysis was performed on aphid mortality data, corrected for control effects using the Abbott's formula (Abbott, 1925), from the first post-treatment assessment to obtain dose-response statistics (Finney, 1971).

(4) Log-difference values were calculated for aphid numbers on individual plant structures (i.e. ear, flag leaf and first leaf) to identify whether aphid survival/mortality varied significantly over the plants.

(5) Dose-response mortality trends were calculated for parasitoids on the days following insecticide treatment. Probit analysis was performed on parasitoid mortality data obtained from the first post-treatment bioassay.

Meteorological data

Daily temperatures ranged from 8.4-31.2°C over the seven experimental days. No rainfall was measured (Table 5.1).

Table 5.1. Temperature and rainfall measurements over the experimental period.

Date relative to treatment	Date	Mean daily temperatures (°C)		Rainfall (mm)
		Minimum	Maximum	
-1	29/06	11.1	28.7	0
0	30/06	8.4	29.4	0
+1	01/07	9.2	29.4	0
+2	02/07	14.0	30.6	0
+3	03/07	12.4	31.2	0
+4	04/07	9.4	25.8	0
+5	05/07	9.0	28.7	0

Spray deposition in the crop canopy

Statistical analysis of deposition rates of spray formulation on different plant surfaces revealed significant heterogeneity between positions in the canopy ($P<0.001$) (Table 5.2). Deposition rates were significantly higher ($P<0.05$) on the upper surface of flag leaves than any other plant surface. The ear received the next highest deposition rate, though not significantly different from the upper surface of the first leaves. Very low levels of spray formulation were found on the lower leaf surfaces.

Aphid distribution over plants

The mean number of aphids on tagged control plants (sprayed with distilled water), over the four sample dates, revealed similar numbers on the ears and flag leaves, with fewer aphids colonising the first leaves (Table 5.3). The lower leaf surfaces were the preferred location for aphid colonisation, compared to upper surfaces. Numerical changes occurred in aphid colonies on each plant structure of tagged control plants on each post-

treatment sample date (Table 5.4). Aphid numbers on the ears and flag leaves decreased over the five days after treatment (DAT), whereas numbers on the first leaves increased. Despite these redistributions of aphids around the plant, the overall changes of aphid numbers on the plant as a whole showed little difference from the pre-treatment numbers.

Table 5.2. Deposition rate of spray tracer solution (µl/cm^2) on winter wheat. Data from Çilgi & Jepson (1992) given for comparison purposes (c.v. Rendevous; growth stage 73; application rate 220l/ha).

Canopy position	Volume of tracer (µl/cm^2 ± S.E.). Data from the current study	*Volume of tracer (µl/cm^2 ± S.E.). Data from Çilgi & Jepson (1992)*
Ear	0.27 (± 0.019) **a**	*0.23 (± 0.01)*
Flag leaf (upper surface)	0.43 (± 0.054) **b**	*0.305 (± 0.02)*
Flag leaf (lower surface)	0.03 (± 0.0001) **c**	*(upper and lower combined)*
First leaf (upper surface)	0.19 (± 0.024) **a**	*0.298 (± 0.02)*
First leaf (lower surface)	0.04 (± 0.0001) **c**	*(upper and lower combined)*

ANOVA with additional Tukey HSD test ($F=8.02$, d.f.=37, $P<0.001$). Different letters in the column indicate significant statistical separation ($P<0.05$).

Table 5.3. Mean number of aphids, over the four sample periods, on different plant positions on the tagged control wheat plants (sprayed with water).

Crop position		Mean number per plant (± S.E.)		Number expressed as percentage of total
Ear			**11.75 (± 0.68)**	38.5%
Flag leaf	upper	2.13 (± 0.16)		
	lower	10.35 (± 1.03)	**12.48 (± 1.05)** (combined)	40.9%
First leaf	upper	0.98 (± 0.17)		
	lower	5.30 (± 0.65)	**6.28 (± 0.75)** (combined)	20.6%

Table 5.4. Calculated log-difference values for changes in aphid numbers on different plant structures on the tagged control wheat plants on days 1, 3 and 5 after treatment with water. Positive values indicate a decrease in aphid numbers compared to pre-treatment numbers, a negative value indicates a numerical increase.

	Days after treatment		
Plant structure	1	3	5
Ear	0.006	0.015	0.103
Flag leaf	0.076	0.182	0.127
First leaf	-0.040	-0.077	-0.089
Whole plant	0.019	0.068	0.053

Location of mummified aphids

A large proportion of the aphid mummies detected during the project were located on the ear (Table 5.5), with the awns and lower flag leaves also harbouring a significant proportion.

Table 5.5. Number and location of mummified aphids found on wheat plants during the experimental period.

Canopy position	Number of mummies	Percentage of total
Awn	15	12%
Ear	53	43%
Flag leaf (upper surface)	11	9%
Flag leaf (lower surface)	22	18%
First leaf (upper surface)	11	9%
First leaf (lower surface)	11	9%

Aphid numbers on untagged wheat plants

Pre-treatment aphid counts on randomly selected wheat plants showed a mean of between 4 and 11 aphids per plant along the spray transect (Figure 5.1). On day 1 after treatment, aphid numbers began to fall below the pre-treatment values at a dose rate of 1g of active ingredient (A.I.) (equivalent of 1/6th of the recommended field concentration). At insecticide concentrations below this there were no visually detectable changes in aphid numbers over the whole plants. At higher concentrations of A.I., aphid numbers declined, resulting in complete elimination at dose rates equivalent to 1/2 the recommended field concentration or greater. Aphid numbers recorded on days 3 and 5 after treatment showed similar trends.

Aphid numbers on tagged wheat plants

The log-difference values, indicating changes in aphid numbers over whole tagged plants, indicated that the main population decreases occurred again at deltamethrin concentrations greater than 1g A.I. of deltamethrin (Figure 5.2). Very little change in numbers was observed between the sample dates. Probit regression analysis on estimated mortality rates gave an LD_{50} of 1.33g A.I. (≡1/5 of the recommended field concentration of deltamethrin). The LD_{10} was calculated at 0.31g A.I. (≡1/20 field concentration) and the LD_{90} at 5.62g A.I. (≡1/1.1 field concentration).

When aphid data for tagged plants were separated according to plant structure, log-

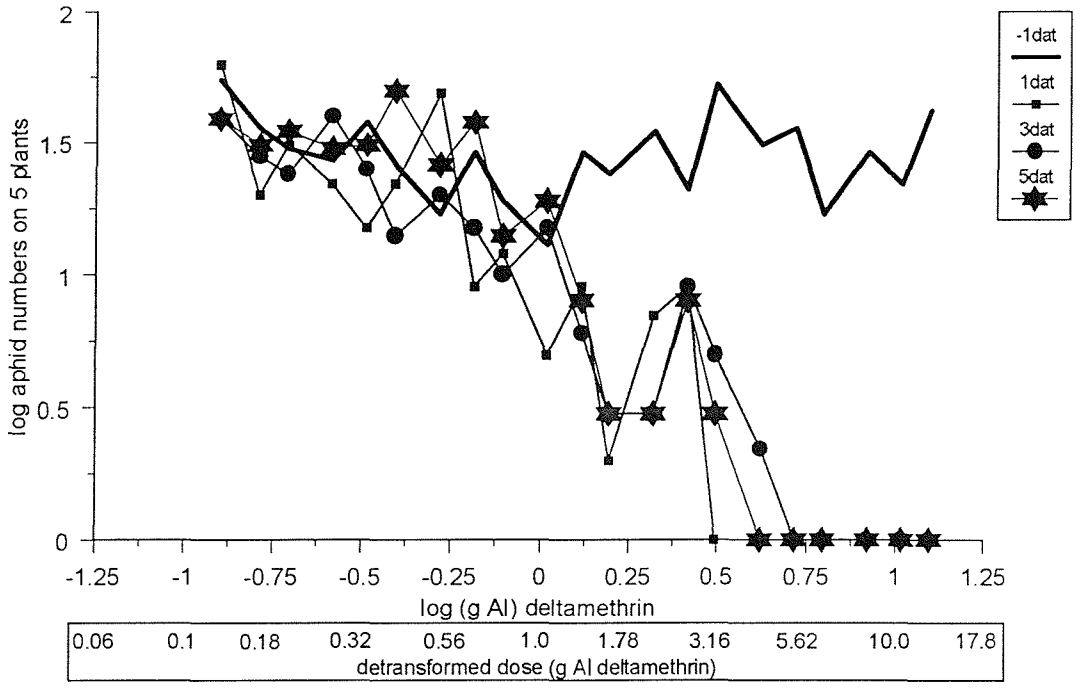


Figure 5.1. Number of aphids on untagged wheat plants along the deltamethrin-treated transect, showing pre-treatment numbers (-1) and numbers on 1, 3 and 5 days after treatment (dat).

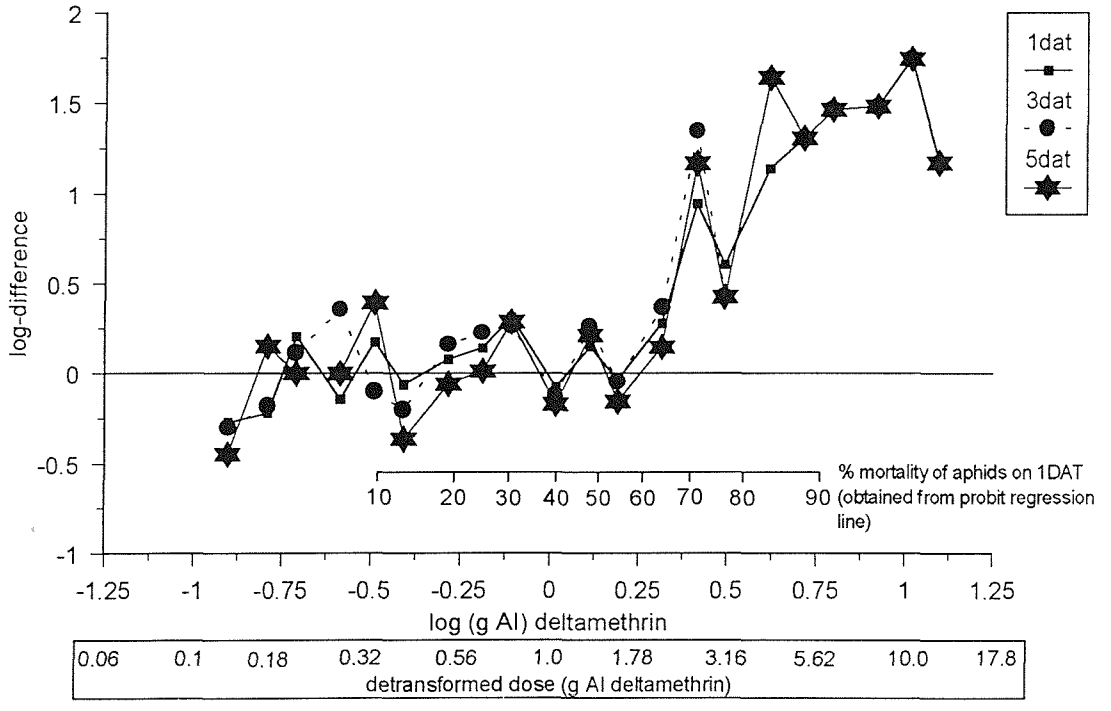


Figure 5.2. Changes in aphid numbers (expressed as log-difference values, see text for details) on tagged wheat plants, on 1, 3 and 5 days after treatment (dat). Percentage mortality estimates for aphids on 1dat are given, based on probit regression equation (y=-0.248+1.7778X; goodness of fit χ^2=3.721, d.f.=13, N.S.).

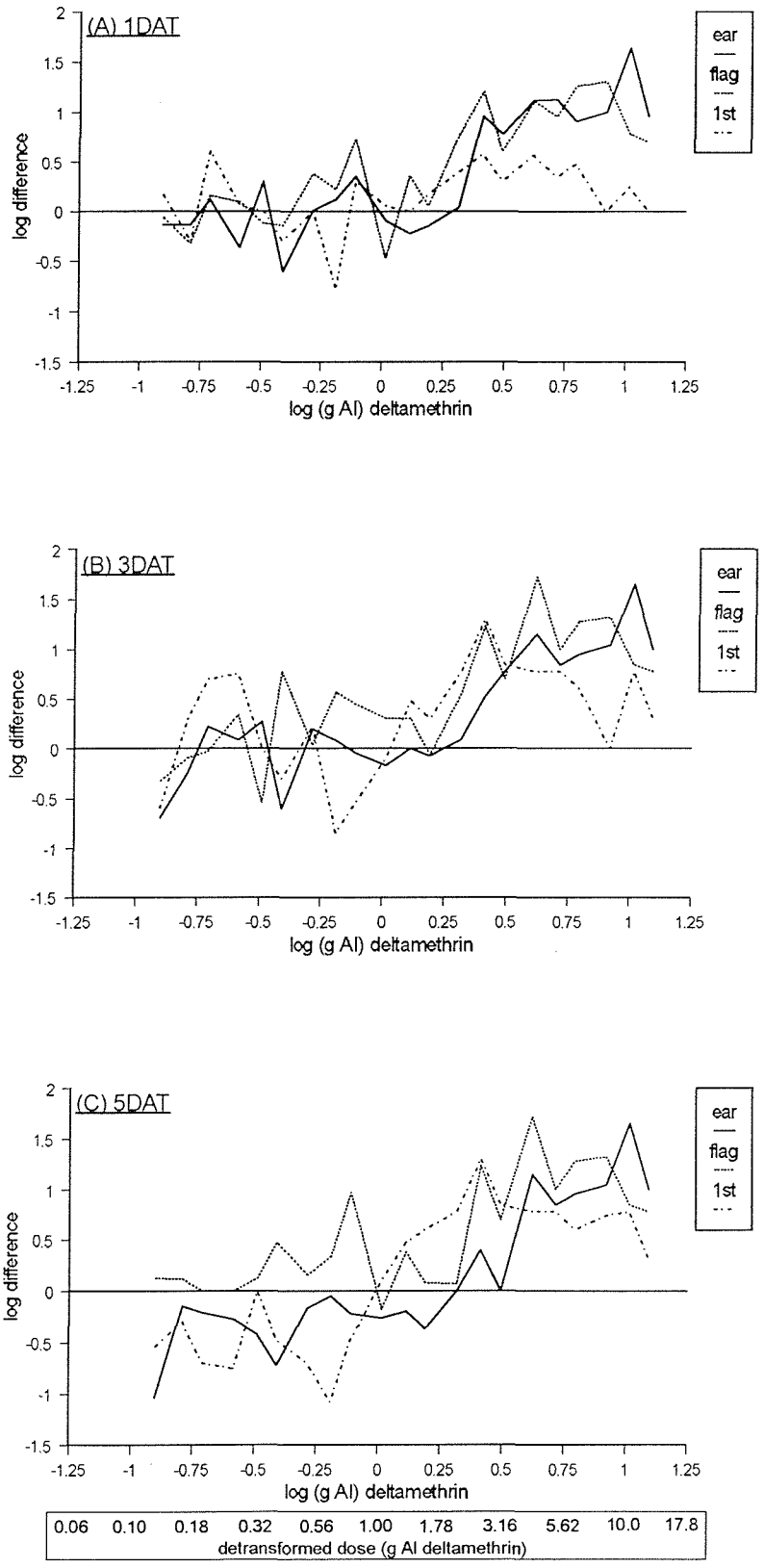


Figure 5.3. Changes in aphid numbers (expressed as log-difference values, see text for details) on different plant structures on tagged wheat plants, on (A) 1, (B) 3, and (C) 5 days after treatment (DAT).

difference values indicate differing degrees of numerical change in aphid populations (Figure 5.3). On day 1 after treatment, aphids survived lower concentrations of deltamethrin to an equal extent on all plant structures (Figure 5.3a). However, at the higher concentrations, causing >60% aphid mortality, the greatest rates of aphid decline occurred on the ears and flag leaves, with lower mortality (i.e. greater survival) occurring on the first leaves. At 3 and 5 DAT, the levels of aphid reduction on ears and flag leaves remained high where the strongest concentrations of deltamethrin were applied (Figures 5.3b&c). On plants sprayed with the lowest concentrations of insecticide, larger negative log-difference values, indicating that population increases, relative to pre-treatment densities, occurred in the ear and first leaf positions.

Parasitoid bioassay

Percentage mortality of parasitoids in the control areas was very low (day 0 (0%), day 3 (10%) and day 5 (0%)). Data from the insecticide treatments were corrected for control mortality using Abbott's correction (Abbott, 1925). Percentage mortality trends of parasitoids exposed to deltamethrin residues on flag leaves, for the first 24h after application (Day 0), showed greater mortality at the highest insecticide concentrations (Figure 5.4a). Subsequent exposure on days 3 and 5 revealed shallower trends in mortality, with higher concentrations causing less parasitoid mortality at increasing times after application (Figures 5.4b&c).

Probit regression analysis of mortality data from the first bioassay (0-24h after treatment) revealed that an LD_{50} dose was achieved at 0.81g A.I. (≡1/8 of the recommended field concentration of deltamethrin) (Figure 5.4a). The LD_{10} was calculated at 0.18g A.I. (≡1/33 field concentration) and the LD_{90} at 3.45g A.I. (≡1/1.8 field concentration).

A comparison of dose response statistics (between LD_{10} and LD_{90}) for aphids and parasitoids indicates that for a given deltamethrin concentration, parasitoids exhibit greater toxicity than their hosts, under the given bioassay conditions (Figure 5.5). For comparison, LD_{50} values of 1.33g A.I. of deltamethrin (≡1/5 field concentration) for aphids and 0.81g A.I. (≡1/8 field concentration) for parasitoids were recorded.

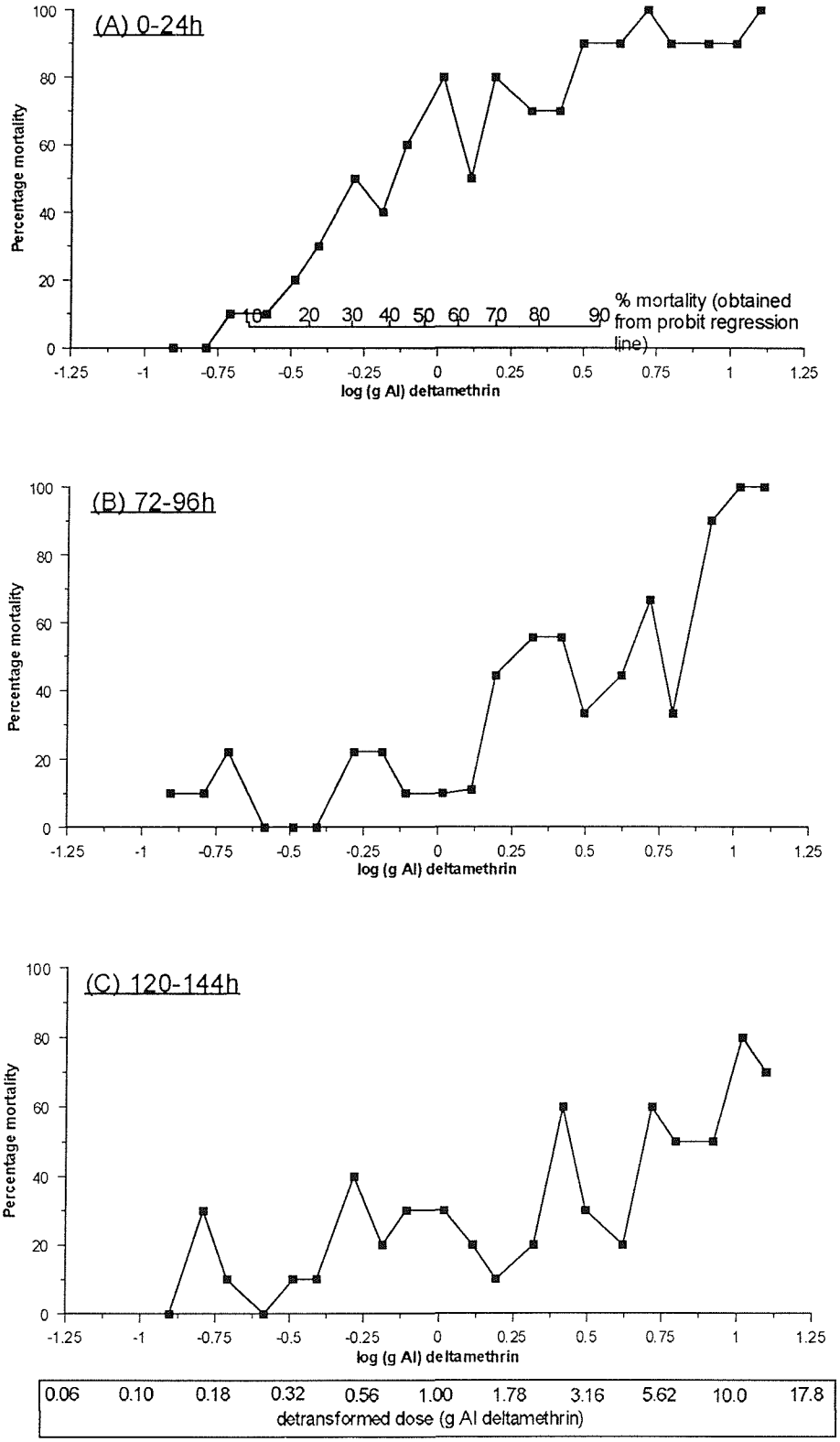


Figure 5.4. Percentage mortality of parasitoids, corrected for control changes using Abbott's formula (Abbott, 1925), exposed to deltamethrin residues on flag leaves on (A) 0-24h, (B) 72-96h and (C) 120-144h after spray application. Percentage mortality estimates are given for parasitoids during the 0-24h bioassay (A), based on the probit regression equation ($y=0.189+2.024X$; goodness of fit $\chi^2 = 9.028$, d.f.=14, N.S.).

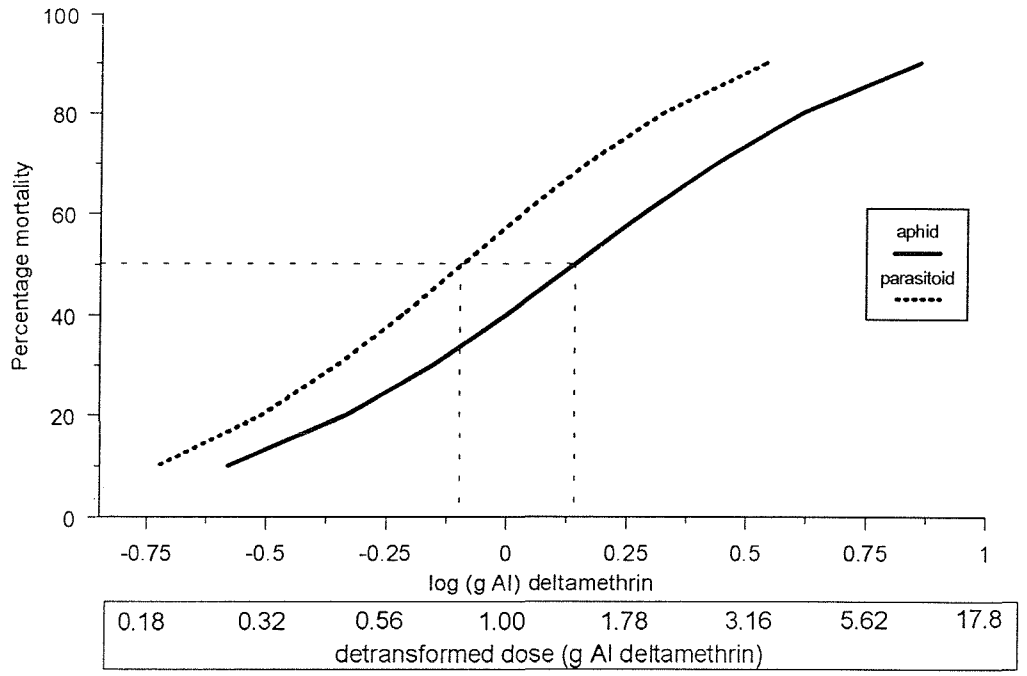


Figure 5.5. Comparison of dose response statistics for aphids and parasitoids obtained from data and probit equations given in Figures 5.2. and 5.3a. respectively.

DISCUSSION

Aphid colonies were found positioned on different plant structures of winter wheat, with greatest numbers on ears and flag leaves. These are the most preferred feeding sites for *S. avenae* and *M. dirhodum* (Vickerman & Wratten, 1979). Overall small changes and redistributions of aphid numbers were recorded on the control tagged plants over the six day experimental period. This may have resulted from aphids becoming restless, or disturbed by predators/parasitoids, and wandering/falling off the plant (Chapter 4), or from the action of plants brushing together, dislodging them. In addition, rainfall has been implicated in dislodging aphids from plants (Carter *et al.*, 1980), however none was recorded during the experimental period. Total aphid numbers on control plants did not change significantly between sample dates, therefore any significant changes in aphid numbers on plants following insecticide applications may be attributed solely to the treatment effect.

High levels of aphid control over the whole plant were achieved with concentrations of deltamethrin exceeding 1g A.I./ha in 200l of water (≡1/6 of the recommended field concentration). These results agree with Turner (1994) and Wiles & Jepson (1995) who found similar reductions in aphid numbers in field plots of wheat sprayed with deltamethrin at reduced dose-rates of 1.25g and 1.56g A.I./ha in 200l water respectively.

Little indication was given of any recovery of aphid numbers on either tagged or untagged plants at the highest dose rates over the experimental period. However, percentage mortality trends for parasitoids exposed to these concentrations of insecticide residues on flag leaves declined over time. This may suggest that the residual activity of deltamethrin was of relatively short duration under the given conditions of high daily temperatures and no rainfall. Further bioassays are needed to consider the residual activity under a wide range of different conditions, because the decline in bioavailability of a pesticide on foliage has been shown to be determined by: (i) crop type, which determines the degree of adsorption of the active ingredient to plant cuticular waxes: the waxer the cuticle, the greater the adsorption for a given octanol:water partition coefficient (Ford & Salt, 1987), (ii) crop growth stage, which determines the surface area over which the chemical is spread: the greater the area, the more rapid the dissipation to the atmosphere for a given leaf type (Boehncke *et al.*, 1990), and (iii) climatic conditions such as wind and temperature, which have a direct impact upon volatilisation rate (Arnold & Briggs, 1990).

The trend for a decay in the toxic effect of deltamethrin residues on flag leaves with time provides a basic guide to the probability of harm to parasitoids invading the treated

area at different times following spray application. This will have important consequences for the rate of reinvasion, mediated by diffusion processes, which determine the rate of population recovery (Chapter 7).

Invertebrate exposure to insecticides is determined by a number of biological factors, including the seasonal phenology of the organism and its temporal and spatial distribution in the treated area. A comparison of the dose-response statistics for aphids and parasitoids indicated that for a given concentration of deltamethrin, the natural enemy will suffer greater mortality under the given conditions. This represents an undesirable situation from the point of view of an integrated pest management program, suggesting that physiological selectivity in favour of the parasitoid, through dose reductions is unlikely to be achieved. However, these estimates were of intrinsic toxicities of deltamethrin concentrations to pest and parasitoids determined by their degree of exposure in the bioassays. Aphid populations, being relatively non-mobile, were mainly exposed to the non-systemic insecticide through direct contact at the time of spraying. In contrast, the parasitoids, normally highly mobile both between and upon plants (Chapter 3), were potentially exposed to the insecticide through direct, residual and dietary routes (Chapter 1). Therefore, the parasitoid bioassays in this study represented a worst-case scenario, with wasps confined for 24h on the highest deposits of fresh insecticide residues. By incorporating more 'realistic' features into laboratory and semi-field bioassays, as outlined in Chapters 2 and 3, (i.e. exposing parasitoids to residues for different durations of time and on different residue concentrations on the plant), more realistic risk predictions of parasitoid and pest populations could be made.

The spray deposition trend showing an attenuation down through the crop canopy agreed with previous findings (Chapter 2; Jepson *et al.*, 1987; Unal & Jepson, 1991; Çilgi & Jepson, 1992). The deposition measurements were also in close agreement with those reported by Çilgi & Jepson (1992) using a tractor-mounted sprayer in a winter wheat crop at the same growth stage (Table 5.2). Data of this sort is important for risk predictions because the hazard which an insecticide poses to a particular invertebrate species will be a function of the relative distributions of the target population and the pesticide throughout the crop canopy. Species which frequently inhabit the upper crop canopy, i.e. parasitoids and the majority of aphids on a wheat plant, are likely to be more at risk than species inhabiting the soil surface or lower leaves, all other things being equal (Jepson, 1989).

Certain regions of the wheat plants, i.e. the first leaves, and particularly the lower surface of leaves, received significantly less spray than other areas of the plant. With the

application of reduced-dose rates below 1/6th of the field concentration, these relatively uncontaminated plant parts acted as refuges for surviving aphid populations. The level of spray deposition within individual plant parts has also been shown to vary, due to differences in the shape and curvature of leaves (Last & Parkin, 1987; Moyle, 1994). Therefore, more detailed studies of aphid location upon individual leaves, and spray deposition trends spanning all the phases of crop growth when sprays are applied, may help in assessing the extent of aphid survival and their subsequent location by foraging parasitoids after spray applications.

A parasitoid's residual exposure to pesticides is determined by the extent of searching activity, which is dependent upon prey density and distribution (Chapter 3 & 4). Within an insecticide-treated environment, a pest's food supply (the crop) remains unlimited, whereas, as demonstrated in this study, a natural enemy's food supply (the pest) is often significantly reduced and/or its distribution changed in space (Trumble, 1985). If the pesticide reduces pests to a large extent, natural enemies starve, emigrate or have limited reproduction following spraying. This effect may be minimised by using reduced-dose insecticide applications. In this study, no assessment was made of the degree of foraging success of parasitoids searching for residual aphid populations on plants. This was due to the 11-15 day time period between parasitoid oviposition and mummification (Hågvar & Hofsvang, 1991) which delays possible assessments of parasitism. However, it has been demonstrated that aphid parasitoids search all parts of wheat plants (Chapter 3; Gardner & Dixon, 1985), and that mummies are commonly found on lower leaves (Table 5.5). It could therefore be assumed that these residual aphid colonies would be located by parasitoids, provided honeydew and residues of low insecticide concentration posed minimal interference with the parasitoids normal foraging patterns (Chapter 3 & 4). To overcome the problems of immigration and emigration of aphids and the tendency of certain parasitised aphids to wander away from the site of oviposition (Chapter 2), future studies need to employ large treated plots to answer the above questions. In addition, the dissection of randomly selected aphids from surviving colonies could be used to provide estimates of percentage parasitism.

Overall, this chapter has outlined a general test method for estimating optimum dose-rates of aphicides which can provide effective control yet leave residual aphid populations for parasitoid and predator populations. Comparisons of different classes of insecticides would prove interesting, because physiological selectivity may be achieved by using systemic compounds, such as the organophosphate insecticide, dimethoate. These

would remove the 'pest refuge' phenomenon demonstrated with contact insecticides and may therefore enable further reductions of insecticide active ingredient applied to crops. In addition, dimethoate residues on foliage have been shown to dissipate more rapidly than deltamethrin over time (Jepson *et al.*, 1994), therefore posing less long-term risk to foraging parasitoids. Further experimental studies are required to determine pesticide distribution on crops and the influence of environmental factors in altering toxicity over time. Knowledge of the rate of uptake by beneficial invertebrates, using residue analysis, would also be of value in determining the underlying factors which control toxicity. This is explored in the following chapter.

CHAPTER SIX

QUANTIFICATION OF PESTICIDE UPTAKE BY APHID PARASITOIDS IN LABORATORY BIOASSAYS

INTRODUCTION

Attempts at ranking the level of risk posed by pesticides to non-target invertebrates in arable crops based on their presence or absence at the time of spray application, their position in the crop canopy, and their diurnal activity patterns have been made (Jepson, 1989). However, such rankings are limited by the lack of quantification of important factors that mediate short-term effects, including the susceptibility and exposure of individual species (Jepson, 1988). An arthropod acquires a toxic dose of pesticide by initial contact, followed by transfer of the toxicant to the target site or sites of action within its body (Gerolt, 1983). The three main routes of exposure for adult aphid parasitoids are uptake after direct exposure to spray droplets, uptake of residues by contact with contaminated surfaces, e.g. soil or vegetation, and oral uptake from contaminated food sources, e.g. honeydew (Chapter 1; Croft, 1990). These routes of exposure are not necessarily of equal importance, but each pose different threats to parasitoid survival through time. Therefore to extrapolate from laboratory studies to field conditions, quantitative information is needed on the relative importance of these different routes of uptake.

The primary goal of most research to date has been to determine whether natural enemies are killed by exposure to pesticides rather than to identify the sources of exposure. A simulation model developed by Salt & Ford (1984) has attempted to investigate factors such as encounter and transfer of pesticide from treated plant surfaces that determine the residual toxicity to insects. Jepson *et al.* (1990) and Wiles & Jepson (1993a) further developed this model for short-term hazard prediction for terrestrial invertebrates exposed to pesticides in cereal crops. 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 have an important influence on its susceptibility to pesticide residues. This approach of modelling residual toxicity of pesticides to non-target insects has been applied successfully to relatively large invertebrates (i.e. carabids and coccinellids) (Wiles & Jepson, 1993a), however it becomes impractical when using aphid parasitoids due to their small size and periodic flight behaviour. Therefore, in order to quantify the routes of pesticide exposure

available for aphid parasitoids, a series of novel experiments were designed and carried out at Washington State University, U.S.A.

Laboratory bioassays were conducted to quantify the topical, residual and oral exposures of two aphid parasitoid species, *Aphidius colemani* Starý and *A. ervi* Haliday to the organophosphate insecticide diazinon. Methodologies were developed for processing both ^{14}C-labelled and radioinert samples of the insecticide. Diazinon was chosen as the test chemical due to the availability of a ^{14}C-labelled sample at the time of experimentation. The use of the organophosphate insecticide prevents direct comparison and interpretation of other results in this thesis, however, the aim of this chapter was to develop techniques and identify the importance of the different routes of insecticide exposure for parasitoids.

The specific aims were to:

(1) quantify the degree of diazinon uptake by adult aphid parasitoids by residual exposure for different time periods and to different concentrations of chemical;

(2) record parasitoid behaviour on treated substrates to determine the influence of different behaviours on exposure levels;

(3) quantify the degree of diazinon uptake via parasitoid feeding on contaminated food sources;

(4) determine the level of exposure (a combination of residual and oral routes) of parasitoids foraging on diazinon-treated plants;

(5) quantify the degree of protection provided by an aphid mummy case to developing parasitoids, against topical applications of diazinon.

This type of information could be used to improve hazard indexes for parasitoids exposed to pesticides and aid the interpretation of ecotoxicological data obtained from field studies.

EXPERIMENTAL METHODS

Insect material

A. colemani were purchased from Plant Sciences Inc., California, U.S.A. *A. ervi* were cultured on pea aphids, *Acyrthosiphon pisum* (Harris), maintained on broad bean plants, *Vicia fava* (cv. Banner), in an environmental chamber (25±1°C; 78±5% R.H.; 16:8 light:dark photoperiod). Female parasitoids were used in bioassays within 48h of emergence, and unless otherwise stated, fed on a 50:50 honey:water solution on cotton wool prior to use.

Insecticides

The organophosphate insecticide, Diazinon Ag500 (399.1mg A.I./ml) was obtained through commercial sources, and hereafter referred to as the radioinert sample. A ^{14}C-labelled sample of technical Diazinon (0.150mCi) was obtained from the Ciba Geigy Corporation (reference number: JAK-V-93; Δ-2-^{14}C-Diazinon; 98.8% purity; specific activity=37.2μCi/mg). This was combined with formulated radioinert diazinon to make a solution of radio-labelled diazinon of field concentration (1.25mg/ml).

Spray apparatus

A horizontal spraying apparatus was designed and constructed, according to the dimensions of the conventional vertical Potter spray tower (Potter, 1952). The horizontal design was necessary to allow the spraying device to be enclosed within a large polythene bag and housed inside a fume cupboard in order to control external contamination by spray drift. A Thomas atomiser, connected to an air compressor, was separated from the object to be sprayed by a rigid transparent acetate column (61.5 cm length, 12 cm diameter). The spray was delivered at 15 p.s.i. for a 3 sec period. The time period was chosen arbitrarily as it provided a fine, even, deposit over the glass surface. A repeatable mean spray deposit of 7.14ng/mm^2 was achieved.

In all bioassays, quantities of radioinert and radio-labelled diazinon in parasitoid tissue was analysed using gas chromatography and liquid scintillation counter techniques, respectively. A description of each technique is given below.

Gas chromatography sample preparation

To kill and preserve the wasps prior to analysis, 1ml of acetone was added to a 2ml

glass vial containing 5 wasps. The contents were transferred into a 4ml Wheaton v-vial and 4 x 250µl rinses of acetone were quantitatively transferred from the first vial to the Wheaton vial. The contents of the vial were evaporated using a gentle stream of air within a fume cupboard. After an addition of 1ml of ethyl alcohol, the vials were placed in a Bransonic Ultrasonic cleaner for 30 mins to extract diazinon. Afterwards, the insect mixture was allowed to settle for 10 mins and then transferred into a 10ml syringe filter fitted with an Acrodisc 3CR PTFE (3mm HPLC certified 0.45µm) and filtered into an autosampler vial. 1µl of the filtrate, a mixture of diazinon and ethyl alcohol, was injected into a gas chromatography column (details in Table 6.1). A calibration curve was established by injecting a range of known quantities of radioinert diazinon, enabling subsequent readings from bioassays to be converted to µg of active ingredient of diazinon.

Table 6.1. Details of the gas chromatography column.

Apparatus description:	HP5890 Series II Gas Chromatograph equipped with Nitrogen-Phosphorus Detector (NPD), automatic sampler and ChemStation program.
Column description:	Phase: DB-17; Length: 30 metres; Internal diameter: 250mm; Film thickness: 0.25 microns.
Temperature of heated zones:	Oven: 90°C to 260°C, at 35°C/min; Injector: 260°C; Detector: 275°C (NPD).
Flow rates of gases:	Carrier gas: Helium, 5ml/min; Make-up gas: Helium, 25ml/min; Detector gases (1) Air: 110ml/min; (2) Hydrogen: 3ml/min.
Injection volume:	1µl

Scintillation counter sample preparation

Batches of five parasitoids were placed into scintillation vials and kept frozen until analysis. Once thawed, wasps were dissolved in 250µl of tissue solubiliser (Tissue Solubiliser TS-2, Research Products International), and left in a waterbath at 50°C for 12h. 20ml of Aquasol-2 (Packard Instrument Company) was added to each scintillation vial and the solution was radioassayed. Radiocarbon in the parasitoid samples was determined by use of a Searle Delta 300LSC model 6890 (Searle Analytic Inc., Illinois, U.S.A.). The data was converted from counts per minute (cpm) to disintegrations per minute (dpm) by correcting for background radiation, dilution, quench and counting efficiency. A calculation of dpm per quantity of diazinon of the original insecticide solution enabled the subsequent conversion of dpm readings to µg/active ingredient of diazinon in parasitoid samples.

Bioassays

A series of bioassays were conducted to determine parasitoid exposure to diazinon via residual, oral and direct routes.

(1) To determine the quantity of insecticide uptake via residual exposure

Solutions of radioinert diazinon representing the recommended field concentration (1.25mg in 1ml of water), and 1/2 x field concentration were made. These solutions were sprayed onto the inner surfaces of glass Petri-dish bases and lids (9cm-diameter) using the spray apparatus described above. Control dishes were sprayed with distilled water.

For each insecticide concentration, female *A. colemani* were individually transferred using an aspirator onto a treated Petri-dish, once the spray solution had dried (approximately 30mins), and left exposed to residues for either 0.5, 1, 2.5, 4, 5.5 or 8 mins. Ten wasps were used for each insecticide concentration and exposure time combination. Initial observations indicated these exposure times covered the full range of observable toxic effects in parasitoids exposed to a field concentration of diazinon. At the end of the set exposure period, wasps were promptly removed using clean forceps, and transferred to glass vials for preparation of chemical analysis using gas chromatography, as described above. For a comparison of analytical techniques, an identical set of bioassays were performed using solutions of radio-labelled diazinon. Chemical analysis was performed using a scintillation counter, as described above.

Behavioural observations

Observations of individual *A. colemani* were made over a 7 min period in glass Petri-dishes sprayed with either the recommended field concentration of radioinert diazinon, or distilled water. Twenty wasps were observed for each treatment. An event-recording computer software package ("Observer"; Noldus, 1990) was used to record durations and sequences of walking, resting, and grooming behaviours. Walking included brief stops to probe the substrate with antennae. Resting was a motionless state with no antennal or mouthpart contact, and grooming described the cleaning of any body part using the front or back legs.

Toxicity assessments

A. colemani were exposed for 8 mins to glass Petri-dishes sprayed with solutions of the recommended field rate and diluted concentrations representing 1/2, 1/4, 1/8, 1/16 and 1/32 of the field rate of radioinert diazinon. Fifteen parasitoids were tested for each treatment. Immediately after the exposure period, parasitoids were transferred using an

aspirator to clean, aerated vials and offered a food source (50:50 honey:water solution on cotton wool). Their condition (either affected/dead or live) was assessed at 1, 24 and 48h after exposure.

In addition, groups of 10 *A. colemani* were exposed to residues of field concentration radioinert diazinon for either 10, 20 or 30 secs. They were transferred to clean, aerated vials, offered a food source and their condition (either live, affected, knocked down or dead) was assessed at 10, 15, 30, 45, 60, 75 and 90 mins after exposure.

(2) To determine the quantity of insecticide uptake via oral exposure

Individual *A. colemani* and *A. ervi*, previously denied access to food for 24h, were offered a food source combining a 50:50 mixture of honey and a solution of radioinert diazinon at field concentration. Twenty wasps of each species were tested. The honey solution was presented through the narrow end of a disposable pipette tip (1-200µl volume range). This ensured that only the head and mouthparts of wasps contacted the solution. Time spent feeding (taken as time spent with head inserted in end of pipette tip) was recorded. Once feeding had ceased, wasps were immediately removed using forceps and processed for chemical analysis as described above. For comparison, identical bioassays were conducted, this time substituting a field rate solution of the radio-labelled diazinon into the food source.

To aid the interpretation of results, a comparison of the differences in size of *A. colemani* and *A. ervi* was made. Ten dead individuals of each species, air-dried at room temperature for 6 days, were weighed using a Mettler AE160 balance.

(3) To determine the quantity of insecticide uptake during parasitoid foraging (residual and oral exposure)

Clean broad bean plants, grown in 9cm-diameter pots, of similar growth and structure (approximately 25cm height, 6-7 open leaves) were selected for the bioassays.

All spray applications were made with a hand-held Thomas atomiser, connected to an air compressor operating at 15 p.s.i., held 20cm above a plant for a 3 sec period. This method ensured a fine deposition of spray over all leaf upper surfaces.

Two different bioassays were conducted:

(i) Six plants were sprayed with a solution of radioinert diazinon representing a concentration of 1/8th of the recommended field rate. Previous bioassays indicated that this

dose permitted the survival of parasitoids for the duration of a 1h bioassay of the type described below. After residues had dried, acetate cylinders were placed over the plants and 5 female *A. colemani* were released into each chamber. After 1h, the 5 wasps from each plant chamber were removed using clean forceps and placed into an individual 2ml glass vial. They were subsequently processed according to the gas chromatography sample preparation method described above.

(ii) Six plants were sprayed with a 50:50 honey:water solution. Once dried (approximately 30 mins) they were sprayed with the diazinon solution, and parasitoids exposed, as described in (i).

(4) To determine the degree of insecticide diffusion through a mummy case (direct exposure)

150 aphid mummies containing late larval stages of *A. ervi* were positioned onto glass cover slips with double-sided adhesive tape. Single 0.5µl droplets of a radio-labelled solution of field concentration diazinon, with a 0.2% solution of a wetting agent (Triton X100), were applied to the dorsal surface of the mummies using a Hamilton repeating microapplicator fitted with a 25µl Hamilton syringe. At time intervals of 1, 2, 4, 8, 16 and 24h after treatment, 25 mummies were randomly selected and their dorsal surfaces removed using fine insect pins. Parasitoids were carefully extracted using clean forceps. Subsequently, two separate chemical analyses were conducted:

(a) internal extraction - involved determining the quantity of chemical that had penetrated through the mummy case and become absorbed in the developing parasitoid. For this, batches of five extracted pupae were placed into single scintillation vials;

(b) external extraction - involved determining the quantity of chemical that remained unabsorbed on the mummy case. Each batch of five coverslips, empty mummy cases and adhesive tape from (a) were placed in a single scintillation vial. Vials from (a) and (b) were processed and analysed in a scintillation counter as described above.

To assess the toxicity of different concentrations of diazinon applied topically to aphid mummies, single 0.5µl droplets of radioinert diazinon at concentrations representing field concentration and 1/3, 1/10, 1/30 and 1/100 of field concentration were applied to aphid mummies containing pupal stages of *A. ervi*. Distilled water was applied to batches of mummies as a control. Twenty mummies were used for each treatment and kept in ventilated chambers. An assessment of successful parasitoid emergence was made 3 days after treatment.

Residual exposure

The uptake of diazinon by *A. colemani* was dependent on the concentration of the residues and exposure time (Figures 6.1a&b). Uptake increased progressively with exposure time, with periods of most rapid uptake occurring during the first 4 mins and 5.5 mins of exposure to field concentration and half field concentration residues, respectively. Beyond these time periods, total uptake either slowed down or ceased. The two different analytical techniques retrieved similar quantities of diazinon from parasitoids tested on both radioinert and radio-labelled residues.

To identify large overall changes in parasitoid behaviour patterns during the exposure period, behavioural data was divided into seven separate minute intervals (Figures 6.2a&b). On the control Petri-dishes (treated with distilled water), parasitoids spent greater than 75% of their total time undertaking walking behaviour during each of the minute intervals of exposure (Figure 6.2a). Grooming occupied a small proportion of time, with resting bouts continuing for similar lengths of time throughout the minute exposure periods. On the diazinon-treated dishes, parasitoids spent a decreasing amount of time walking during consecutive minutes of exposure (Figure 6.2b). A small degree of resting was recorded during the first minute period, after which the behaviour became absent. Grooming behaviour became more predominant during each minute interval after exposure.

The onset of uncoordinated behaviour patterns of parasitoids on the diazinon-treated dishes was recorded at 2.50 ± 0.11 mins (mean ± S.E.) after release. Behavioural changes were characterised by an increase in walking speed (unquantified) and uncoordinated walking behaviours, e.g. walking backwards and turning in circles, with antennae dragging on the substrate and wings raised. The mean time to knockdown of parasitoids was recorded at 5.55 (± 0.12) mins.

Exposure for 8 mins to radioinert diazinon residues of concentrations greater than 1/16th of the field rate resulted in 100% mortality of parasitoids after 24h post-exposure (Table 6.2). Greater survival was achieved with lower insecticide concentrations, however only the 1/32 x field rate concentration permitted a degree of parasitoid survival after 48h post-exposure.

Assessments of *A. colemani* exposed to residues of radioinert diazinon at field concentration indicated that after a 10 sec exposure, all wasps were knocked down 45 mins later, subsequently resulting in 100% mortality after 90 mins (Table 6.3). Longer exposure times to residues resulted in shorter times to knockdown and mortality after exposure.

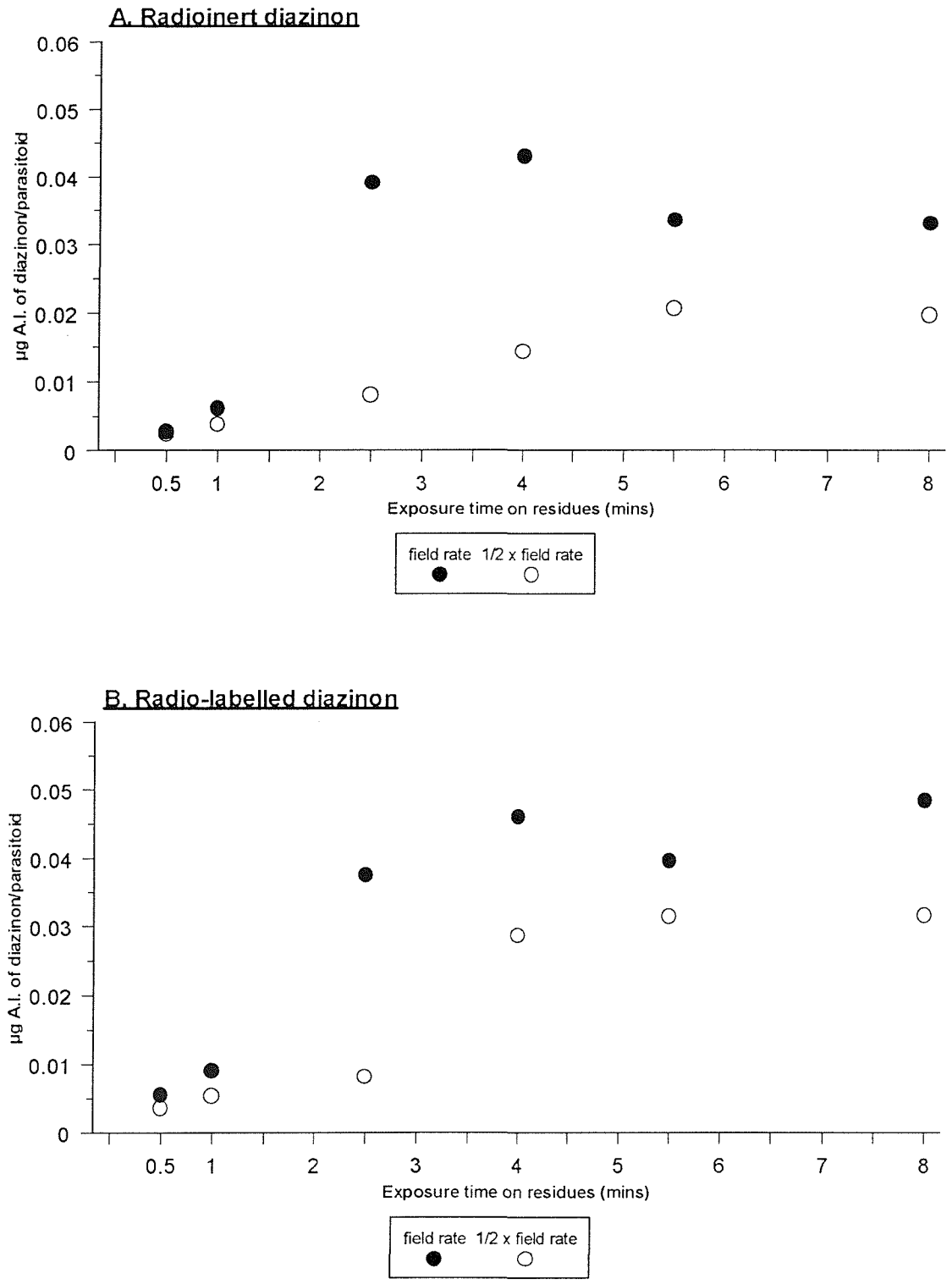


Figure 6.1. Quantity of active ingredient of diazinon taken up by *Aphidius colemani* via exposure for different time periods to residues of field concentration and 1/2 field concentration of (A) radioinert and (B) radio-labelled diazinon.

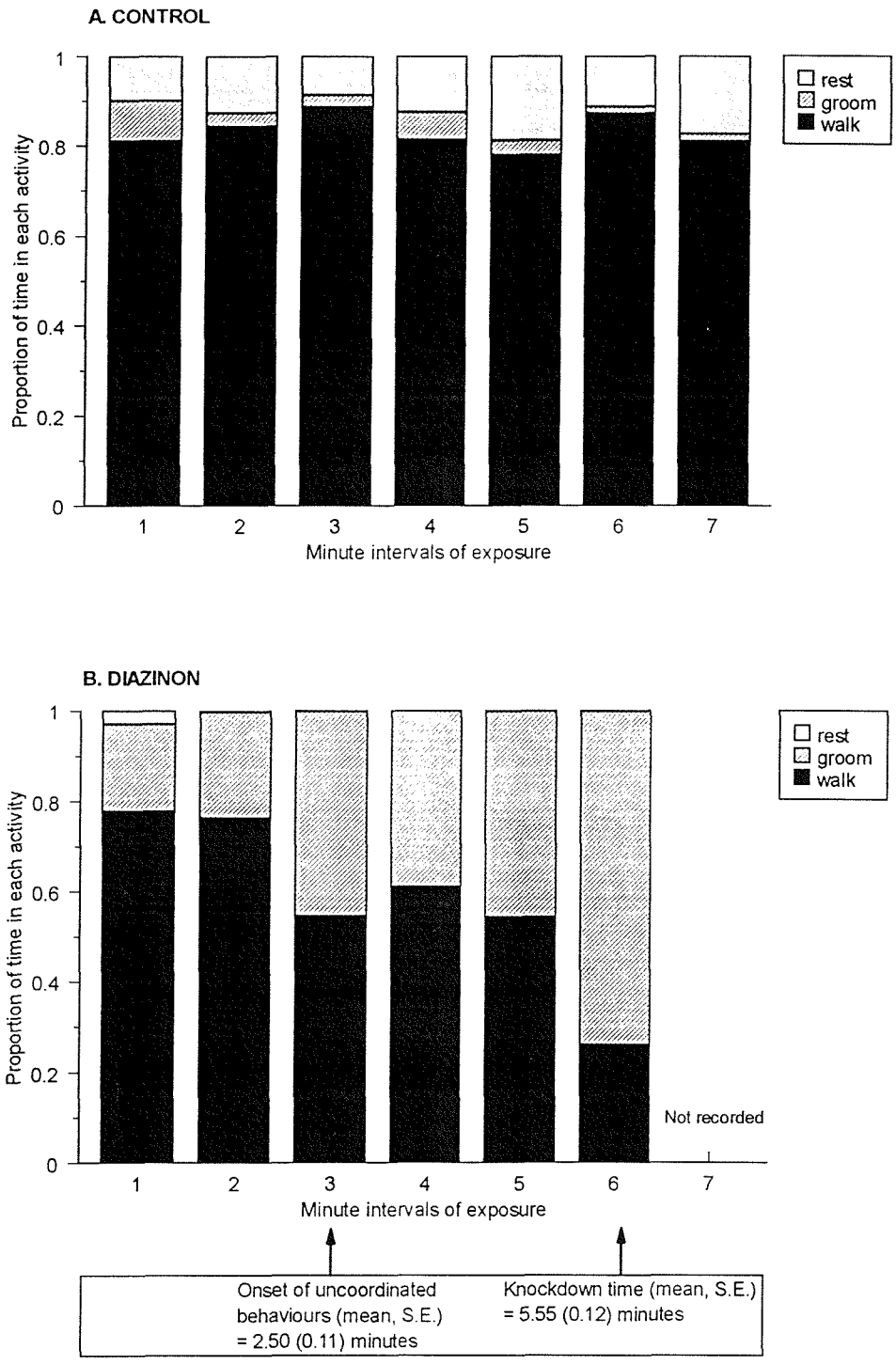


Figure 6.2. Mean proportions of time spent by *Aphidius colemani* (n=20) undertaking walking, grooming and resting behaviours on (A) control (distilled water) and (B) diazinon (field rate concentration) treated glass substrates. Time after exposure is divided into separate minute intervals to highlight changes in behaviour patterns. Mean times of the onset of uncoordinated behaviours and knockdown are given for parasitoids on diazinon-treated surfaces.

Table 6.2. Percentage mortality of *A. colemani* (n=15/treatment) at time intervals after an 8 min exposure period to residues of radioinert diazinon of different concentrations, and distilled water (control).

	Mean percentage mortality at time (hours) after exposure		
Concentration (FR=field rate)	**1h**	**24h**	**48h**
Control	0	0	7
1/32 x FR	0	40	67
1/16 x FR	7	100	100
1/8 x FR	40	100	100
1/4 x FR	100	100	100
1/2 x FR	100	100	100
FR	100	100	100

Table 6.3. Percentage of *A. colemani* (n=10/treatment) recorded as either affected (a), knocked down (k) or dead (d) at time intervals (mins) after different time exposures to residues of field rate radioinert diazinon, or distilled water (control).

Exposure period to residues	Minutes after exposure						
	10	**15**	**30**	**45**	**60**	**75**	**90**
Control	0	0	0	0	0	0	0
10 sec	0	60k	20a +60k	100k	100k	100k	100d
20 sec	20a +80k	100k	100k	100k	100d	--	--
30 sec	100k	100d	--	--	--	--	--

Oral exposure

The quantity of diazinon taken up by the two parasitoid species was positively correlated with time spent feeding (Figures 6.3a&b). Overall, individuals of *A. ervi* showed a greater degree of uptake per unit time of feeding compared to *A. colemani.* This may be attributed to their differences in size, with a 1:4.8 ratio of dry weights of *A. colemani* and *A. ervi* used in these bioassays (Table 6.4). A comparison of the two different analytical techniques suggests that the use of radio-labelled diazinon samples enabled a greater detection of quantities of active ingredient uptake per unit time of feeding (Figures 6.3a&b).

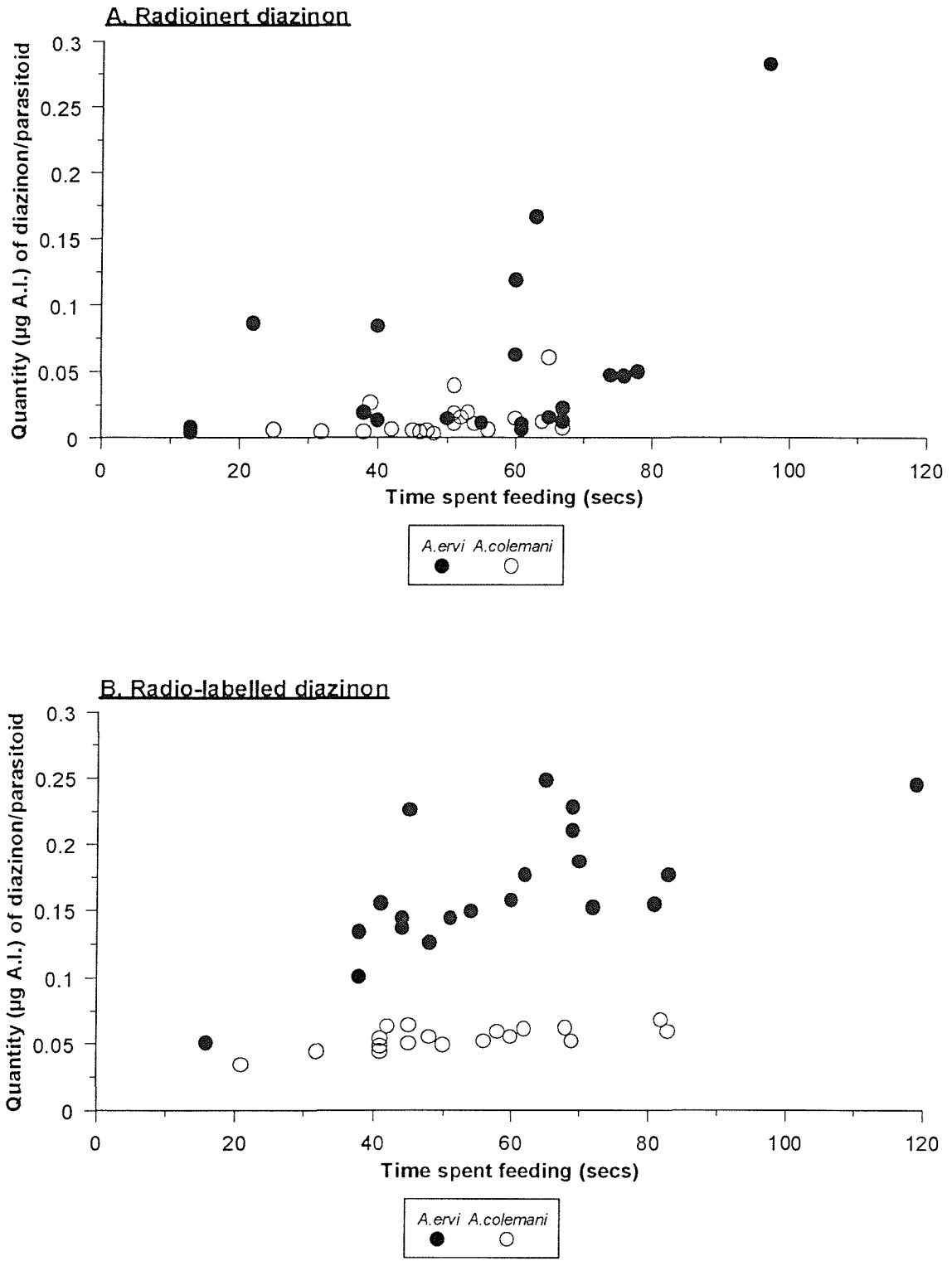


Figure 6.3. Quantities of active ingredient of diazinon ingested by *Aphidius ervi* and *A. colemani* during periods of feeding on honey solutions containing field concentrations of (A) radioinert diazinon and (B) radio-labelled diazinon.

Table 6.4. Dry weights of *Aphidius colemani* and *A. ervi*.

	Total dry weight of 10 individuals	Ratio of weights (A. colemani : A. ervi)
Aphidius colemani	0.60mg	1 : 4.8
Aphidius ervi	2.90mg	

Residual and oral exposure through foraging behaviour

Greater uptake of diazinon was acquired by parasitoids foraging on treated broad bean plants when honey solution was present on the foliage, although statistical analysis showed a non-significant difference (P>0.05) (Table 6.5).

Table 6.5. Quantity of active ingredient of diazinon taken up by *Aphidius colemani* foraging in the presence and absence of dried honey residues for up to 1h on broad bean plants treated with a field concentration of radioinert diazinon.

	Mean quantity of diazinon (µg A.I./insect)	
Replicate[a]	Foraging on plants sprayed with diazinon	Foraging on plants sprayed with diazinon & honey solution
1	0.034	0.050
2	0.056	0.048
3	0.021	0.049
4	0.029	0.081
5	0.080	0.071
6	0.030	0.039
Mean (±S.E.)	0.042 (±0.009)	0.056 (±0.007)
Statistical analysis:	No significant difference between means (T-test, t=1.33, d.f.=11, P>0.05)	

[a]each replicate included 5 parasitoids

Topical exposure to parasitoids in aphid mummies

Topical applications of a field concentration of radioinert diazinon resulted in 100% mortality of *A. ervi* housed within mummies (Table 6.6). Greater parasitoid survival and subsequent emergence was achieved with increased dilutions of the diazinon concentration.

Table 6.6. Percentage mortality (assessed as a failure of successful emergence at 3 days post-treatment) of *Aphidius ervi* housed within mummies (n=20/treatment) topically treated with 0.5µl droplets of radioinert diazinon of different concentrations.

Treatment	Field rate (FR)	1/3 x FR	1/10 x FR	1/30 x FR	1/100 x FR
Mortality[a]	100	60	30	0	0

[a]corrected for control mortality (10%) using Abbotts formula (Abbott, 1925)

The quantities of active ingredient of radio-labelled diazinon penetrating through the mummy case and taken up by the parasitoid was dependent on time after insecticide application (Table 6.7). The proportion of total insecticide applied to mummies, which was extracted from parasitoids by chemical analysis, showed a progressive increase over time (Figure 6.4). However, percentages remained below 6% throughout the 24h post-treatment period.

Table 6.7. Quantity of active ingredient retrieved from parasitoids and mummy cases at time intervals after topical applications of 0.5µl droplets of field concentration ^{14}C-labelled diazinon. Means based on 5 replicates.

Time after treatment (h)		Mean (± S.E.) µg A.I. of diazinon per 5 parasitoids/mummies
1	Parasitoid	0.0022 (±0.00049)
	Mummy	0.147 (±0.0067)
2	Parasitoid	0.0056 (±0.00041)
	Mummy	0.137 (±0.0169)
4	Parasitoid	0.0060 (±0.00108)
	Mummy	0.174 (±0.0218)
8	Parasitoid	0.0046 (±0.00040)
	Mummy	0.096 (±0.0230)
16	Parasitoid	0.0088 (±0.00179)
	Mummy	0.179 (±0.0136)
24	Parasitoid	0.0061 (±0.00111)
	Mummy	0.102 (±0.0084)

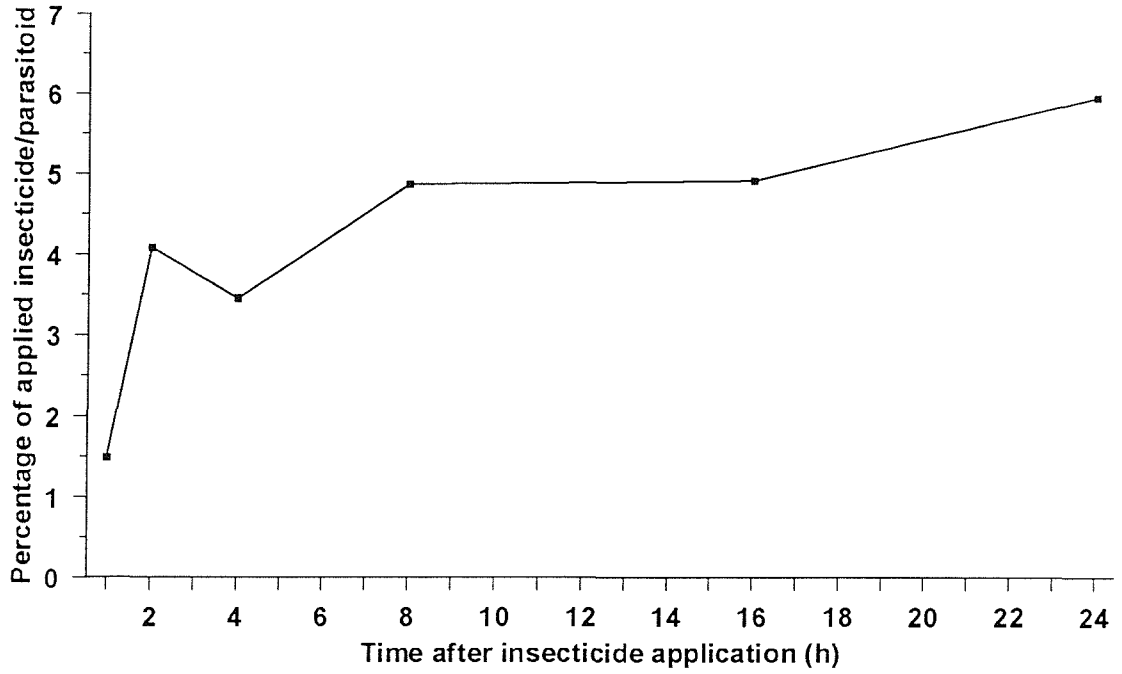


Figure 6.4. Percentage of the total insecticide applied to mummies (field concentration of ^{14}C-labelled diazinon) retrieved from internal parasitoids (*Aphidius ervi*) at time intervals after application. Raw data given in Table 6.7.

DISCUSSION

This study has shown that aphid parasitoid life-stages are extremely vulnerable to the organophosphate insecticide diazinon, and are exposed, by differing degrees, via topical, residual and dietary routes.

Despite remaining unquantified in this study, adult parasitoids are also likely to be exposed to aerial sprays of pesticides. However, the level of this exposure is seldomly monitored because of the difficulty in studying flying arthropods (Rabb & Kennedy, 1979) and the shortness of its duration. Although residual exposure may be of equal or lesser importance than direct fallout immediately following a pesticide application (Croft, 1990), it does represent a more persistent phenomena and is important in determining patterns of recovery and reinvasion of invertebrates into treated crops.

Residual exposure

The complex behavioural processes involved in residual uptake of pesticides by parasitoids include foraging movements over treated foliage and grooming behaviours. In addition, pesticide deposition, redistribution, weathering, the nature of the treated surface and the dynamics of substrate growth or change influence residual uptake of pesticides (Croft, 1990). In this study, the rate of diazinon uptake by parasitoids via residual contact was shown to be dependent on exposure time and insecticide concentration. The majority of insecticide uptake occurred during the first four mins of exposure to field concentration residues on the glass dishes. This was accompanied with increasing bouts of grooming which have been implicated in spreading chemical particles, picked up on the tarsi, around an insects body (Gratwick, 1957; Kühner *et al.*, 1985b). However, on further exposure to residues, the dominant behaviour category switched from walking to grooming but resulted in very little further uptake of diazinon. The sites of uptake of residual deposits, mainly the tarsal receptors or setae (Hartley and Graham-Bryce, 1980), may have reached maximum loading and/or the grooming actions may have subsequently removed insecticide particles from the exterior of the parasitoid. The results from this study suggested that the penetration of diazinon into the parasitoids was a relatively slow process. For example, a 10 sec exposure to diazinon residues of field concentration proved sufficient time to accumulate a toxic dose, with resulting mortality recorded 90 mins post-exposure. However, it took 5.5 mins of continuous exposure to the residues for parasitoids to show knockdown.

Future studies could quantify the insecticide actually penetrating into the parasitoid over time to determine the quantity of insecticide causing a toxic dose. If using a radio-

labelled insecticide, the radioactivity picked up could be separated into an outer fraction by rinsing off the insect, subsequently followed by an inner fraction which had penetrated through the cuticle, extracted by grinding up the tissues as described in this study.

When using results from laboratory bioassays to make risk predictions for the field it must be borne in mind that bioavailability of an insecticide, which determines the uptake and hence the dose of toxicant to which an organism is exposed, is dependant on the substrate on which it is applied (Chapter 2; Jagers op Akkerhuis & Hamers, 1992). For example, on leaf surfaces, bioavailability is negatively correlated with the octanol/water partition coefficient of the compound and the thickness of the cuticular wax layer (Ford & Salt, 1987). Insecticide transfer from a leaf to an insect is also influenced by the droplet size, viscosity of the formulation, concentration and the architecture of the wax layer (Salt & Ford, 1984). Therefore "correction factors" such as those detailed in Chapter 2 need to be quantified to allow extrapolation of glass plate-derived data to field conditions.

The results obtained suggest that the locomotion and grooming behaviours of a parasitoid are important factors determining the degree of insecticide uptake from a treated surface. It may prove useful in the future to model pesticide uptake by a representative parasitoid species under known conditions and then calibrate this model with crucial species-dependent factors to predict uptake in other parasitoid species on other substrates. Behavioural studies are therefore needed to accompany toxicological data for interpretation purposes. For example, any increase in the frequency of walking by a parasitoid will serve to increase the rate of pesticide encounter and therefore the probability of acquiring a toxic dose. For predictions relating to the real world, complex behavioural patterns on different plant architectures need to be considered (e.g. Chapter 3).

Future applications of the analytical techniques devised in this chapter could be to quantify the effects of pesticide residues on a whole parasitoid-host complex enabling risk predictions for use in integrated pest management systems. For example, it is believed that natural enemies have a greater susceptibilty to pesticides than their prey/hosts due to their generally small size and longer search times on treated substrates (Hoy & Dahlsten, 1984; Waage *et al.*, 1985). Quantification of residual exposure to insecticides by primary parasitoids, hyperparasitoids and aphids moving around treated plants could be undertaken in assocation with behavioural studies to aid in the interpretation of toxicological data and the possible choice of selective compounds.

Dietary exposure

A high degree of dietary exposure was demonstrated by parasitoids feeding on diazinon-contaminated honey solution. However, the importance of this route of exposure remains unquantified in the field, and was presumably overestimated in the laboratory bioassays due to the food source being in a readily available liquid form and the parasitoids being previously denied food for 24h. The results do suggest, however, that the presence of field concentrations of diazinon showed little or no repellent properties to feeding *A. colemani* and *A. ervi* and therefore could represent an important route of exposure to these parasitoids in the field. The study of *A. colemani* foraging on diazinon-treated plants indicated that the presence of dried honey solution on the foliage resulted in greater quantities (though not statistically significant, $P>0.05$) of insecticide uptake, compared to parasitoids foraging in its absence. The availability of honey residues may have prompted feeding (dietary exposure) and/or an increase in the retention of foraging parasitoids on the foliage (residual exposure) (Chapters 2 & 3). The results suggest that under the given conditions, residual exposure was of greater importance than dietary exposure in determining the quantity of diazinon taken up by parasitoids. This same ranking of the importance of exposure routes was made by Mullié & Everts (1991) measuring the uptake of ^{14}C deltamethrin by the linyphiid spider *Oedothorax apicatus* (Blackwall).

Topical exposure in mummy cases

A study by Hsieh & Allen (1986) found that of 50 aphid mummies, containing *Diaeretiella rapae* M'Intosh, sprayed with a field concentration of diazinon, 100% mortality was recorded. Of these, 13% of unemerged parasitoids were killed in the late larval, prepupal and pupal stages, 20% killed in the adult stage without full wing development and 67% killed in the adult stage with full wing development. The results from this current study agree, with 100% mortality recorded from field rate applications of diazinon. This suggests that diazinon was effective at penetrating the mummified aphid cuticle to kill the developing parasitoids. Results indicated that the amount penetrating through the mummy into the developing wasp gradually increased over a period of 24h, however remained a small percentage of the total amount applied to the mummy (between 1.46% - 5.61%). Additional mortality to parasitoids may have resulted from exposure to insecticide vapours within the mummy, though this was unquantified.

Previous findings have indicated that there can be a wide variation in the activity of insecticides from several classes against aphid parasitoids within mummies (e.g. Hsieh & Allen, 1986; Abo El-Ghar & El-Sayed, 1992). For example, organophosphate insecticides are reported to generally prove more toxic to developing parasitoids than pyrethroids. Their

increased uptake by insects may result from their generally lower octanol/water partition coefficients and higher vapour pressures compared to pyrethroids (Graham-Bryce, 1987). Further comparative evaluations are needed to determine the degree of penetration of insecticides of different classes through a mummy case. In addition, the quantity of chemical ingested during the process of cutting an emergence hole in the dorsal surface of the mummy, and the extent of residual exposure as the adult emerges from the mummy could be incorporated in predictions of risks posed to developing and emerging parasitoids. Toxicological interpretation of results from laboratory and field studies regarding adult survival after emergence and subsequent longevity and fecundity of surviving females (Chapter 2) is important as latent effects on parasitoids of insecticides applied to mummies have been assessed through two successive generations (Abo El-Ghar & El-Sayed, 1992).

The routes of exposure and the role of behavioural patterns in determining the degree of pesticide uptake by parasitoids is still poorly understood. This area of research is clearly very complex, but the studies reported in this chapter have shown that the quantities of uptake can be determined and linked with behaviour, albeit in a simplified system.

CHAPTER SEVEN

TEMPORAL AND SPATIAL CHANGES IN APHID AND PARASITOID POPULATIONS FOLLOWING FIELD APPLICATIONS OF DELTAMETHRIN

INTRODUCTION

Pesticide impact in the field is mediated by ecological, behavioural, climatic and habitat-related factors which act on the exposed organism and the active ingredient in question (Jepson, 1993a). Although laboratory bioassays provide valuable information on intrinsic toxicity of pesticides, the artificial conditions prevent many factors, that are important in the field, from being considered. Laboratory-derived toxicological data alone are therefore a poor indicator of the level of toxicity in the field. It is therefore recognised that field studies are an important follow-up for laboratory results (Hassan, 1985; Felton *et al.*, 1986), with data reflecting the effects of pesticides on the population dynamics of both target and non-target species. Previous chapters have shown how pesticidal effects on aphid parasitoid species can be manifested through: (i) direct mortality (Chapter 2); and (ii) sublethal effects, such as reduced fecundity/longevity (Chapter 2) and alteration of foraging patterns (Chapters 3 & 4). Within the field, further indirect effects of pesticide application, such as changes in host distribution (Chapter 5) or the complete removal of host populations can prolong the observed detrimental impact upon foraging parasitoids.

The principal aim of field studies is to evaluate the impact of a commercial application of pesticides. However, despite representing greater realism than laboratory and semi-field bioassays, the scale of treatment in field trials cannot truly simulate the area of commercial treatment. In 1992, the area of cereals in Great Britain treated with insecticides alone totalled 2.3 million hectares (Davis *et al.*, 1992). These insecticide treatments usually cover entire fields and contiguous blocks of fields, with up to several km^2 being treated simultaneously (Aebischer, 1990). Despite this, the majority of field-scale studies to monitor the impact of pesticides upon beneficial organisms have been conducted in small plots within single fields and over a single cropping season (Table 7.1). These field studies incorporating the assessment of pesticide effects on Hymenoptera in cereal crops each involve different plot sizes, test chemicals and sampling techniques, thereby making comparisons between them difficult.

Hymenopteran aphid parasitoids have been categorised as having high dispersal

Table 7.1. Summary of literature concerning effects of pesticides upon Hymenopteran parasitoids in cereal crops.

Author	Pesticide treatment (applied at the recommended field concentration, unless otherwise stated)	Plot size used	Notes
Vickerman & Sunderland (1977)	dimethoate	6 ha	75% reduction in numbers of Hymenoptera. 30% reduction still evident after 2 months, compared to unsprayed control.
Ba-Angood & Stewart (1980)	dimethoate, pirimicarb	0.0004 & 0.0005 ha	Both insecticides caused reduced parasitoid populations 1 week after application. However, pirimicarb showed a relatively reduced effect compared to dimethoate after 2 weeks.
Inglesfield (1984)	pyrethroid WL85871, triazophos	0.1 ha	No control treatment. Braconidae numbers were reduced by both treatments, with the pyrethroid causing a greater impact.
Powell *et al.* (1985)	dimethoate, pirimicarb	0.025 ha	Primary and hyperparasitoids of aphids were significantly reduced by both insecticides. Effects on hyperparasitoids were still evident after 2 weeks, effects on primary parasitoids still evident after 3 weeks.
Shires (1985)	cypermethrin, demeton-s-methyl	0.04 ha	Hymenopterous parasitoids significantly reduced in cypermethrin-treated field immediately after treatment, but rapid recovery to control levels. Demeton-s-methyl caused significant reduction in parasitoid numbers 3 to 4 weeks after treatment.
Fisher & Chambon (1987)	deltamethrin, dimethoate	2 ha	Most Hymenoptera showed little or no difference in numbers before and after insecticide treatments.
Poehling (1987a)	fenvalerate, pirimicarb (applied at field concentration and 1/3 and 1/6 concentrations)	0.27 ha	Pirimicarb at full concentration caused significant reduction in parasitoid numbers. The effects were reduced by using the lower dose rates. Initial toxicity of fenvalerate was lower compared to pirimicarb, with no differences found with the reduced doses. Long-term sampling showed that both insecticides had detrimental effects on parasitoid populations only in the high dose rate plots.
Smart *et al.* (1989)	cypermethrin, demeton-s-methyl, pirimicarb	0.84 ha	Both cypermethrin and demeton-s-methyl reduced Hymenopteran numbers, with greater recovery occurring 1-2 weeks later in the cypermethrin treatment. Selectivity of pirimicarb in favour of parasitoids demonstrated.

capacity between years, with a large spring dispersal into cereals and low activity once within the crop (Carter, 1987; Jepson, 1989). None of the reported field studies have investigated the degree to which the dispersive movement of these insects controls the rate or process of reinvasion into treated plots. Information of this kind is needed to aid the interpretation of pesticide effects in field studies. This is because scale of treatment has been shown to influence the extent and duration of pesticide effects on other invertebrate species (e.g. Jepson, 1989; Duffield, 1991; Thacker, 1991).

A diagrammatical representation of the population changes of a susceptible invertebrate population in pesticide field trials is given in Figure 7.1. Following the pesticide treatment, susceptible populations often undergo two phases of numerical change compared with an untreated control population. Initially there is a period of rapid population reduction (the level of effect), followed by a period when numbers gradually increase, resulting in a population recovery (duration of effect). The spatial scale of pesticide treatment is unlikely to affect the level of initial reduction in population abundance, however it can affect subsequent rates of population recovery (Duffield & Aebischer, 1994).

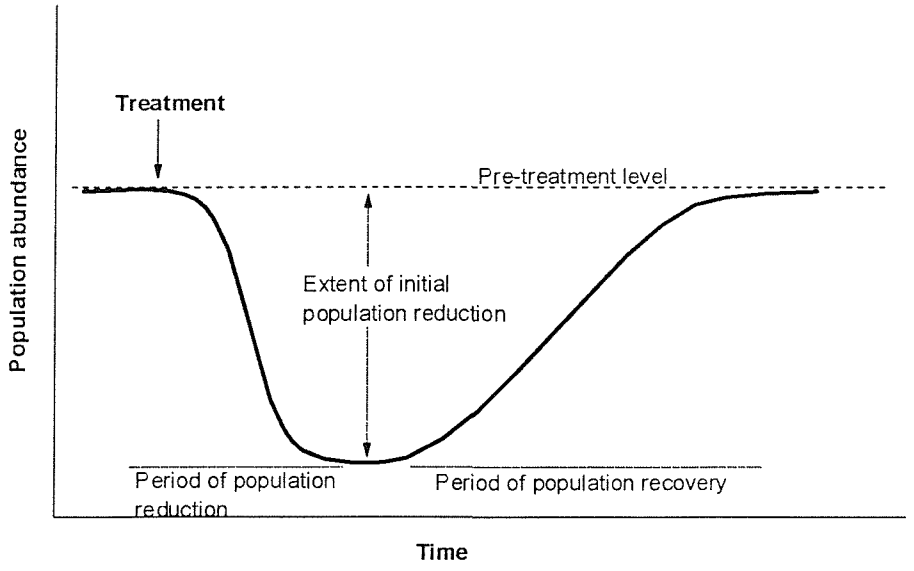


Figure 7.1. Numerical changes of a susceptible field invertebrate population following treatment.

Recovery by non-target invertebrate populations following a pesticide treatment may be mediated via a number of mechanisms. These include:

(1) Reproduction of the surviving population;

(2) Revival of knocked-down individuals;

(3) Horizontal recruitment, which is the movement of individuals into the treated area from

external, untreated areas;

(4) Vertical recruitment, which can involve the emergence of individuals from sub-surface refugia or, in the case of parasitoids, from aphid mummies.

(5) Natural mortality in control areas, i.e. where comparisons are made with populations in control areas, then an artificial recovery may be recorded.

Spatial scale of treatment has a large influence on horizontal recruitment. By increasing the area treated, this increases the distance an immigrating individual has to travel, hence prolonging the duration of pesticide effects.

The actual design of field trials for the evaluation of pesticide side-effects is important because it will influence many of the factors controlling the extent and duration of the observable effects. For example, the developmental growth stage of the crop at the time of spray application affects the amount of pesticide contacting the foliage and reaching the ground through the crop strata (Çilgi & Jepson, 1992). Date of treatment will also influence which species are present and their phenological stages. This is particularly important when assessing the effects of pesticides applied to cereal crops, because primary and hyperparasitoids of aphids show distinct abundance cycles throughout the crop growing season.

The aims of this chapter were to investigate the impact of the pyrethroid insecticide, deltamethrin upon aphids and their associated parasitoids in a winter wheat crop. By using large field plots (4ha in size), the scale of treatment was closer to commercial practice than most previously reported field studies. The following specific questions were addressed:

(1) What level of reduction in aphid, primary parasitoid and hyperparasitoid populations results from applications of a recommended field concentration and a reduced concentration (representing a concentration of 1/20th of field rate) of deltamethrin? This information can be used to assess the potential for using reduced dose rates of insecticide to provide pest control via both chemical and biological means.

(2) Can the degree of recovery of invertebrate populations be measured in the insecticide-treated areas over the following weeks after treatment? This would provide information on the duration of detectable insecticide effects for the given area of crop treated, enabling predictions of any resurgence by pest species.

(3) If recovery rates of invertebrate populations are measurable, could the mechanism of reinvasion be determined? This would provide an insight into how both pest and parasitoid species recolonise treated areas, thereby aiding the design and interpretation of future field sampling programmes.

(4) What effects do the insecticide applications have upon the spatial and temporal

aggregation of these invertebrate populations within the treated area? This has important consequences for the successful location of surviving pest populations by foraging parasitoids.

Field site

An experiment was carried out during June and July 1993 in a winter wheat crop (cv. Mercia) at South Allenford farm, Hampshire. The crop had a mean density of 448 tillers/m^2 and was at decimal growth stage 73 (Tottman & Broad, 1987) at the time of spray application. Three experimental plots each measuring 200m x 200m (4ha) were marked out with at least 20m of unsprayed crop left between them to avoid cross-contamination (Figure 7.2). The treatments used were: the recommended field concentration of deltamethrin (Decis 2.5% E.C.; 6.25g/AI/ha in 200l of water), a reduced dose-rate of deltamethrin representing 1/20 of the recommended field concentration, and an unsprayed control. Insecticide treatments were applied using a tractor-mounted sprayer (details in Table 7.2).

Table 7.2. Details of the spray equipment used.

Spray equipment:	Chafer Tramliner SP 2000l
Boom width:	24m
Number and type of nozzles:	72 hollow cone
Spray tank pressure:	1.7 bar
Tractor speed:	10 km/h
Volume application rate:	200l/ha

Meteorological conditions

Daily temperatures (minimum and maximum) and rainfall records were recorded for the duration of the study period. Precipitation was measured at the farm using a Delta-T electronic data logger, fitted with a tipping bucket rain gauge. Temperature records were obtained from the Meteorological Office at their nearest recording site (Hurn Airport, Bournemouth, approximately 20 miles away from the study site).

Sampling programme for determining spatial distributions and horizontal recovery trends

Within the plots, a grid of transect lines was marked out at 20m intervals. At selected sites, visual counts of aphid numbers, aphid mummies, and D-vac suction samples and sticky trap catches of hymenopterous parasitoids were made. Each sampling method varies in their efficiency of estimation, therefore brief descriptions of each are given below for comparison.

(a) Aphid counts

A pre-treatment survey of aphid numbers was conducted over the whole field. *Sitobion avenae* (F.) were present in large colonies on wheat ears, with only very small numbers of *Metopolophium dirhodum* (Wlk.) found on leaves. Subsequent counts of aphids were restricted to wheat ears, thereby assessing only *S. avenae*.

On each assessment date, 25 randomly selected wheat ears from each of the 45 sites (shown in Figure 7.3a) were carefully excised and placed in labelled polythene bags. They were returned to the laboratory for cold storage prior to counting.

Aphid assessments were taken on -9, -2, +2, +8, +14, +21, +27 and +36 days before and after treatment (Table 7.3). Aphid mummies found on ears were carefully removed and placed in aerated labelled tubes to allow subsequent emergence, for identification purposes. For a true assessment of parasitism, ears containing live aphids were removed from the centre of all plots on each assessment date, and maintained in agar within aerated glass tubes, to encourage any subsequent mummification and parasitoid emergence. Unfortunately, fungal infection on the ears caused high aphid mortality and therefore this method was discontinued.

Visual counts of aphids are the most accurate method of determining the numbers present on cereals (Dewar *et al.*, 1982). As this represented a laborious task to conduct in this field trial, wheat ears were excised and aphids counted in the laboratory. This method had the obvious disadvantage of only sampling the aphids present on the ears, and also only providing an mean number of aphids per 25 ears.

(b) D-VAC sampling

At each of the 45 sites indicated in Figure 7.3a, sampling with the Dietrick suction sampler was carried out between 11.00 and 15.00 British Summer Time (B.S.T.) in dry, warm weather on -3, +1, +5, +12, +19, +28 and +37 days before and after treatment (Table 7.3). At each site, five areas of 0.1 m^2 were sampled for 5 sec each. The collected material was placed in labelled polythene bags and immediately frozen on return to the laboratory. Taxonomic identification covered the Hymenoptera associated with cereal aphids (using the key in Powell (1982)), and also total counts of Linyphiidae. Other groups of invertebrates such as Aphididae, Staphyliniidae, Coccinellidae, Carabidae and Syrphidae were present in too few numbers for analysis.

The D-vac comprises a "squirrel cage" fan which draws air through a flexible duct fitted with a removable nylon fine mesh net (Dietrick, 1961). Placement of the air duct nozzle on the ground while the machine is in operation causes surface material to be drawn into the net and the collected material can be transferred into a polythene bag by removing

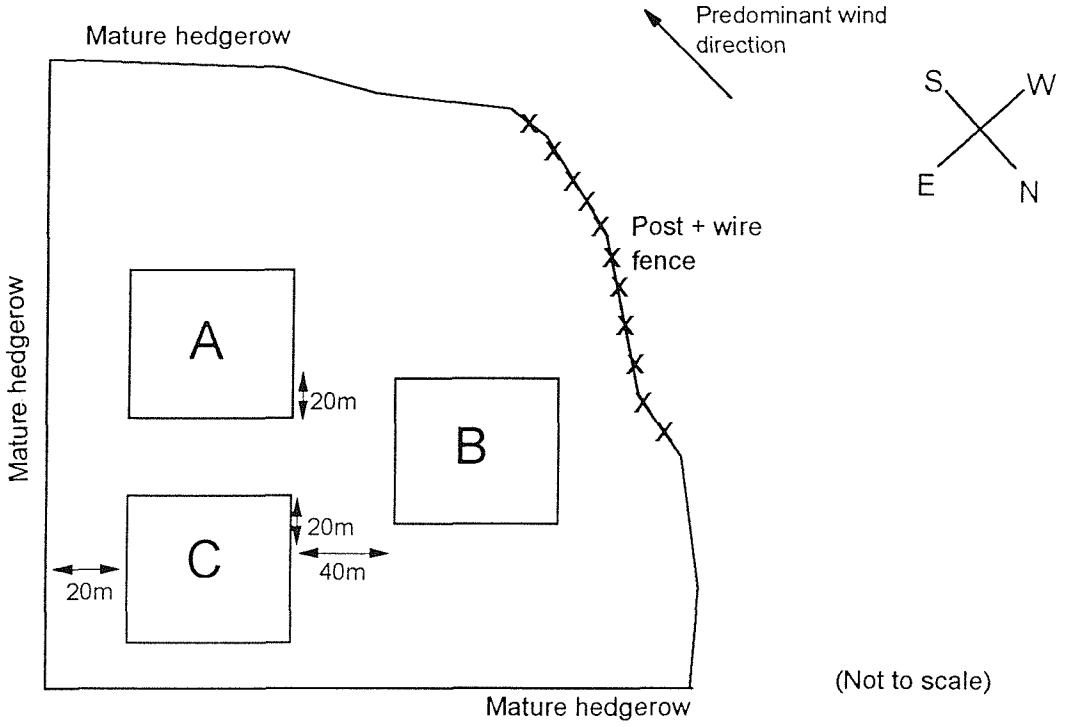


Figure 7.2. Plot layout for the three treatments used in the 1993 field experiment. Plot A = unsprayed control; plot B = deltamethrin at a concentration of 1/20 field rate; plot C = field rate deltamethrin.

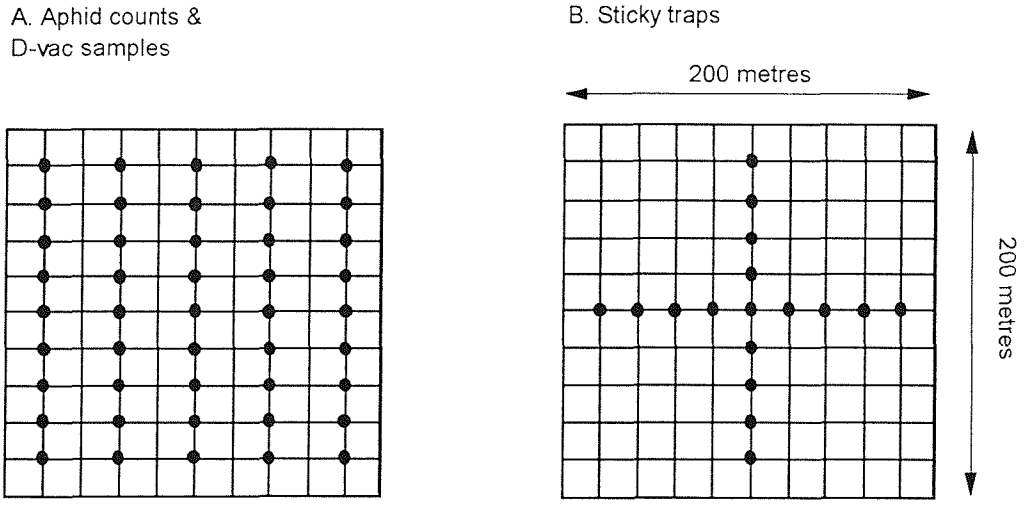


Figure 7.3. Location of the sampling positions (dots) within a plot for (A) aphid counts and D-vac sampling, and (B) sticky traps.

Table 7.3. Timetable of sampling dates

Date	Growth stage(*)	Days before/after treatment	Aphid count	D-vac	Sticky trap
June 14	72	-9	✓		
15		-8			
16		-7			
17		-6			
18		-5			
19		-4			✓
20		-3		✓	✓
21		-2	✓		✓
22		-1			
23	73	0			✓
24		+1		✓	✓
25	74	+2	✓		✓
26		+3			
27		+4			
28		+5		✓	✓
29		+6			✓
30		+7			✓
July 01	77	+8	✓		
02		+9			
03		+10			
04		+11			✓
05		+12		✓	✓
06		+13			✓
07	84	+14	✓		
08		+15			
09		+16			
10		+17			
11		+18			
12		+19		✓	✓
13		+20			✓
14	86	+21	✓		✓
15		+22			
16		+23			
17		+24			
18		+25			
19		+26			✓
20	87	+27	✓		✓
21		+28		✓	✓
22		+29			
23		+30			
24		+31			
25		+32			
26		+33			
27		+34			
28		+35			
29	90	+36	✓		
30		+37		✓	

* Growth stage based on Tottman & Broad (1987)

the net. For this study, an extraction period of 5 secs for each sub-sample was considered sufficient (Coombes, 1987).

The sampling efficiency of the D-vac is dependent on the timing and duration of sampling, the type of vegetation, the fauna sampled, weather conditions and the performance of the D-vac operator. These factors were standardised in this study by sampling between the same hours on dry, sunny days, for a standard length of time, and sampling weed-free areas of crop of similar plant density.

(c) Sticky traps

Traps were made from transparent acetate sheets (14cm x 19.5cm). Water-resistant glue ("Trappit" insect glue, Agrisense-BCS Ltd.) was evenly applied to give a total sticky area of 12cm x 26cm (= 312cm^2). The sheet was stapled into a cylindrical shape measuring 8.5cm in diameter and 14cm long.

At each of the 17 sites shown in Figure 7.3b, two sticky traps were attached to a vertical fibreglass cane using wooden clothes pegs. One trap was placed about 60cm above the ground in the region of the flag leaves, the other at a height of 20cm above the top of the crop canopy (125cm above the ground). Traps were left exposed in the field for a 48h period and then removed in labelled polythene bags. Insect taxonomic identification was carried out as above.

The 48h sampling periods covered -4 to -2, 0 to +2, +5 to +7, +11 to +13, +19 to +21, and +26 to +28 days before and after treatment (Table 7.3).

Transparent traps, as opposed to coloured ones, were used to prevent attraction of insects. Capture relied instead on catching insects by impaction as the airstream moved over and around the trap. The cylinderical trap design has been shown by various workers (e.g. Broadbent *et al.*, 1948; Heathcote, 1957; Lewis, 1959) to be more efficient than flat sticky traps for sampling various insect populations. Flat traps have more air current eddies around their sides and only sample efficiently from the two main surfaces.

Weather can affect trap catches indirectly through the effect on insect activity, with greater numbers of small insects flying on warm, calm days than during periods of wet weather with high wind speeds (Juillet, 1964). Penman & Long (1960) conducted a survey of the microclimate in wheat fields and found that in calm weather the motion of air inside the crop, though not completely absent, was very much suppressed with negligible turbulence compared to above the crop canopy.

In this study, the sticky traps were positioned to sample at two different heights, with sticky traps above the crop assumed to sample migratory insects entering or leaving the area, and the lower traps collecting insects conducting "trivial flight" (Southwood, 1962)

within the crop canopy whilst searching for food, shelter, mates or oviposition sites.

Choice of statistical analysis

A review of the literature concerning the analysis of the side-effects of pesticides in field trials reveals an inconsistency in the statistical methods used and the standards of scientific rigour adopted. As a consequence, data comparisons between different studies are prevented. Brief descriptions of the various statistical methods used in previous field trials and in the current study are given below.

In the simplest form, without any statistical analysis, data have been presented by showing the time trends in species population numbers within the individual treatment and control plots (e.g. Zoebelein, 1988). Other studies have simply expressed the numbers sampled in the treatment areas as a percentage of the numbers in the control areas (e.g. Vickerman & Sunderland, 1977). To incorporate statistical analysis into the data of field trial studies, the use of ANOVA has been widely used to determine differences between treatments. However, the level of variation between different plots can result in significant pre-treatment differences (Sotherton *et al.*, 1988). This phenomenon can occur as a result of: (i) the heterogenous distribution of many non-target invertebrates in agricultural land (Wratten & Thomas, 1990); (ii) an inability to carry out sampling before the experimental design is set up (Vickerman, 1985); and (iii) a need to conduct the trial on a large scale to prevent rapid recolonisation masking any treatment effects (Sotherton *et al.*, 1988).

Various attempts have been made to resolve this problem. For example, the pre-treatment differences may be included as a covariate within the ANOVA (Vickerman *et al.*, 1987a). Alternatively, relative changes within the individual treatment and control plots may be analysed by the 'log-difference' method which takes into account differences in pre-treatment numbers (Sotherton *et al.*, 1987). However, this analytical method has various restrictions/criticisms, because the analysis is carried out on differences between increasingly remote sample dates, and only permits the use of one pre-treatment sampling date to be used in all the calculations.

The statistical analysis used in the current study follows that used by Duffield & Aebeischer (1994), which overcomes many of the criticisms levelled at the other methods. The method minimises the effect of pre-treatment heterogeneity in invertebrate numbers between plots by comparing the treatment and control plots for each sampling occasion rather than over increasingly remote periods. The analysis consequently allows for phenological change within the population and also the effects of weather and various field effects by measuring changes relative to the corresponding controls.

Spatial and temporal density maps of invertebrates

The number of aphids per 25 ears and total *Aphidius* spp. per D-vac sample, collected at each of the 45 sampling points over the three experimental plots, were transformed and analysed using the geostatistical kriging technique to produce contour maps connecting points of equal density (Surfer v3.0, Golden Software Inc.). This technique enables the estimation of values at unsampled locations by taking a weighted linear average of available samples (Liebhold *et al.*, 1993).

(1) Analysis of overall population trends

Aphids

The total number of aphids recorded within the control plot throughout the season remained relatively low, with mean numbers remaining below two aphids per wheat ear (Figure 7.4). The overall aphid populations declined from mid June to leave very low numbers at the time of insecticide application (23rd June) in the treatment plots. Subsequent recovery of aphid numbers in the control plot occurred to give a peak during the third week of July followed by a crash in numbers by the 29th of July. The decline in aphid numbers coincided with the main periods of recorded rainfall, between the 18-20th June and 9-19th of July (Figure 7.6). Maximum and minimum temperatures remained stable throughout the season, with slightly lower readings during days when rain fell.

A degree of heterogeneity in pre-treatment aphid numbers were recorded between plots, with slightly greater numbers found within the 1/20th field rate plot on 21st June (Figure 7.4). Therefore, to remove this bias, aphid numbers are also expressed as a proportion of the pre-treatment numbers (sampled two days prior to spraying) for that particular plot (Figure 7.5). Aphid numbers in the control plot showed a slight decline between the 'pre-treatment' and first 'post-treatment' assessments (Figure 7.5). Within the plot sprayed with the 1/20th field concentration of deltamethrin, pre-treatment aphid numbers were reduced by 40% on the first post-treatment sample, after correction for changes in numbers in the control plot (Table 7.4). A slow recovery of aphid populations followed, with a small peak in numbers (half of that recorded in the control plot), occurring on 20th July (Figure 7.5). Aphid numbers in the plot sprayed with the full-field concentration of deltamethrin were heavily depleted by the insecticide treatment (a reduction of 78%) (Table 7.4). Again, a slow recovery of aphid populations occurred, with a peak in numbers recorded on 20th July, at a time when aphid numbers in the control plot were in decline (Figure 7.5).

Total primary parasitoids

(i) D-vac samples. The total number of aphid primary parasitoids caught during D-vac sampling in the control plot showed an increase between the first two sample occasions (Figure 7.7a). Subsequent assessments revealed a declining trend of parasitoid numbers over the following 37 days. The 1/20th field concentration of deltamethrin caused a 60%

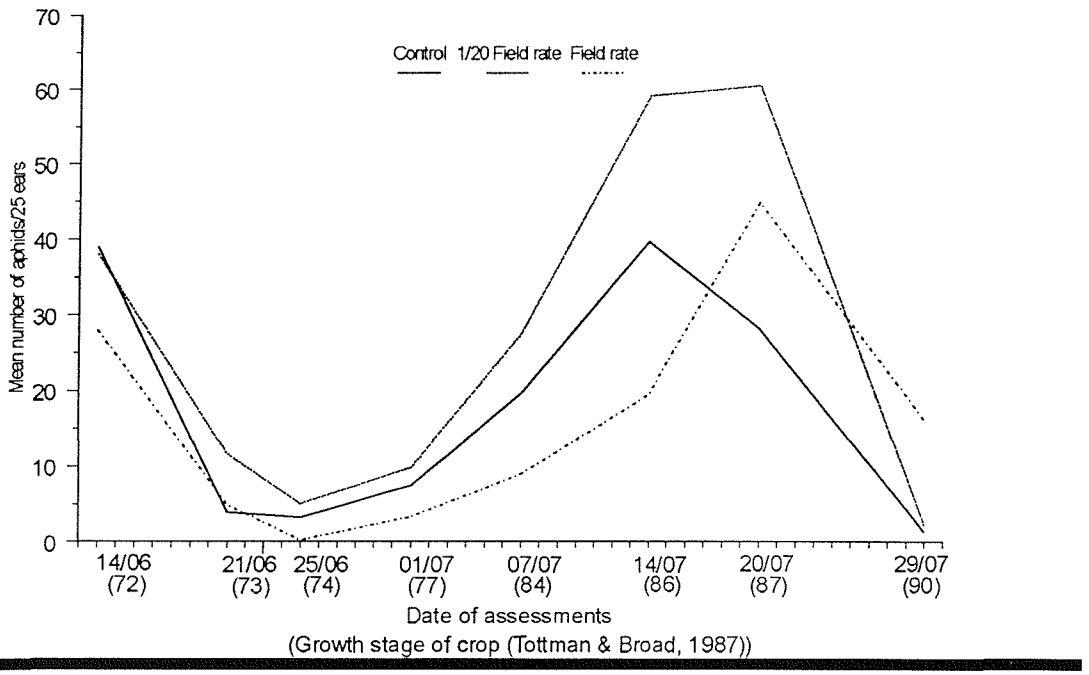


Figure 7.4. Trends in aphid numbers in the three treatment plots throughout the sampling period, with sample dates and growth stages of the crop indicated.

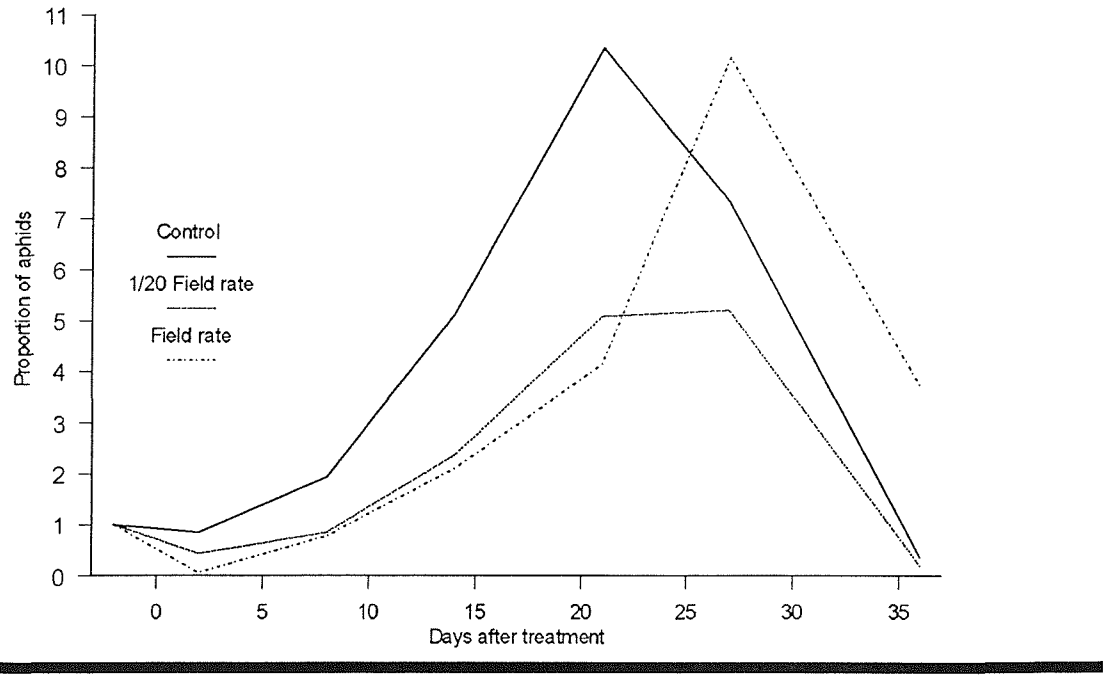


Figure 7.5. Aphid numbers, expressed as a proportion of the pre-treatment numbers, in each treatment plot.

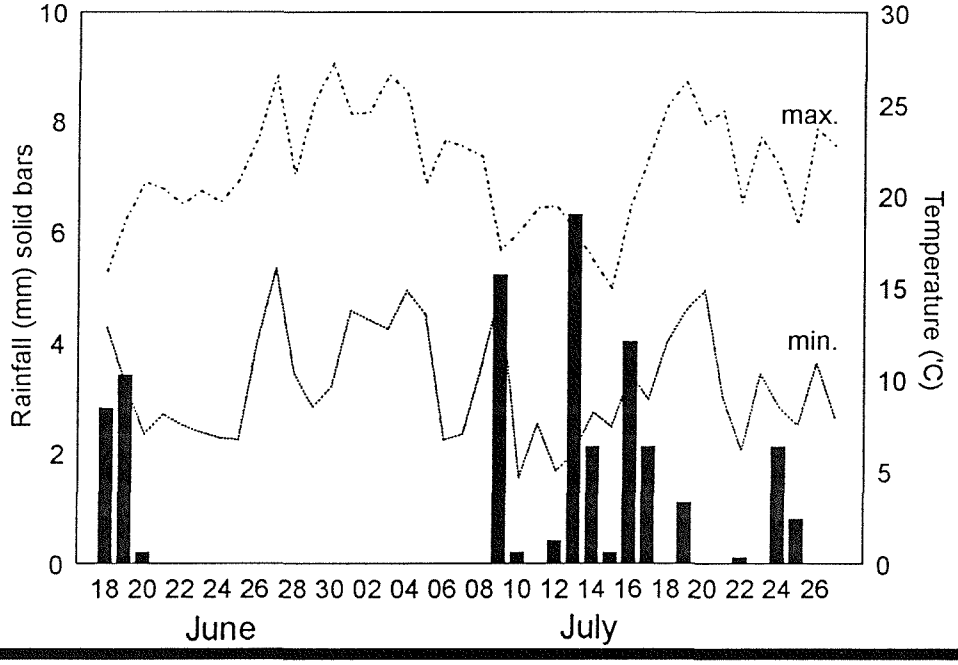


Figure 7.6. Meteorological measurements (rainfall and minimum and maximum temperatures) throughout the experimental period.

Table 7.4. Percentage reduction in treated populations between the pre-treatment and the first post-treatment sampling date for the three sample methods. Percentages corrected for population changes in the untreated control, (percentage treated population change subtracted from the percentage change in the control).

		Percentage reduction			
		Aphid count / D-Vac		Sticky trap	
		1/20 Field rate	Field rate	1/20 Field rate	Field rate
Hemiptera					
	Aphididae	**40**	**78**	---	---
Hymenoptera					
Primary parasitoids					
	Aphidius spp.	75	97	23	71
	Toxares/Ephedrus spp.	30	82	79	102
	Aphelinus spp.	+13[2]	33	---	---
	Total parasitoids[1]	**60**	**90**	**28**	**75**
Hyperparasitoids					
	Dendrocerus spp.	42	+33[2]	---	---
	Corunal/Asaphes spp.	44	104	---	---
	Alloxysta spp.	64	43	16	14
	Total hyperparasitoids	**54**	**47**	**17**	**17**
Other groups					
	Total Linyphiidae	**117**	+7[2]	**17**	**106**

[1] Total parasitoid category also includes small changes in numbers of *Diaeretiella rapae* and *Praon volucre*.
[2] Positive values indicate an increase in populations after correction for control numbers.

reduction of pre-treatment numbers of primary parasitoids when assessed on the first post-treatment sample date (Figure 7.7a; Table 7.4). The full field concentration of deltamethrin resulted in a 90% reduction in primary parasitoid numbers on the first sample date after spraying (Figure 7.7a; Table 7.4).

Aphidius spp. represented the most abundant group of primary parasitoids found in all D-vac samples. The percentage reduction of these species alone on the first post-treatment sample was 75% and 97% in the 1/20th and full field concentration plots respectively (Table 7.4).

(ii) Sticky traps. In general, fewer primary parasitoids were caught per sticky trap during their two day exposure period compared to a single D-vac suction sample (Figures 7.7a and 7.7b). The same trend of insecticide effects by the two dose rates of deltamethrin was evident, but to a lesser extent than that shown with the D-vac sample data. The total primary parasitoids in the control plot showed a small steady decline over the sample intervals. The 1/20th field concentration of deltamethrin caused a reduction of 28% in total primary parasitoid numbers (23% in *Aphidius* spp. numbers) assessed during the first post-treatment sample period, when corrected for control numbers (Table 7.4). The full field concentration of deltamethrin caused a reduction of 75% in total parasitoid numbers (71% of *Aphidius* numbers)(Table 7.4).

Total hyperparasitoids

(i) D-vac samples. Numbers of hyperparasitoids collected within the control plot remained low throughout the sampling season (Figure 7.8a). The 1/20th field concentration of deltamethrin caused an initial reduction of 54% of pre-treatment numbers of total hyperparasitoids, after correction for control numbers (Table 7.4). With the full field concentration, the percentage reduction was similar, at 47% (Table 7.4).

(ii) Sticky traps. In general, fewer hyperparasitoids were caught per sticky trap during their 2 day exposure period compared to a single D-vac suction sample (Figures 7.8a & 7.8b). Both concentrations of deltamethrin gave an initial 17% reduction in the number of pre-treatment hyperparasitoids, after correction for control numbers (Table 7.4).

Total linyphiids

(i) D-vac samples. Total numbers of linyphiids collected within the control plot showed an initial increase followed by a gradually declining trend over the following 36 days (Figure 7.9a). The 1/20th field concentration of deltamethrin resulted in a large initial reduction of pre-treatment numbers by 117%, after correction for changes in control numbers (Table 7.4). The application of the full field concentration of deltamethrin produced

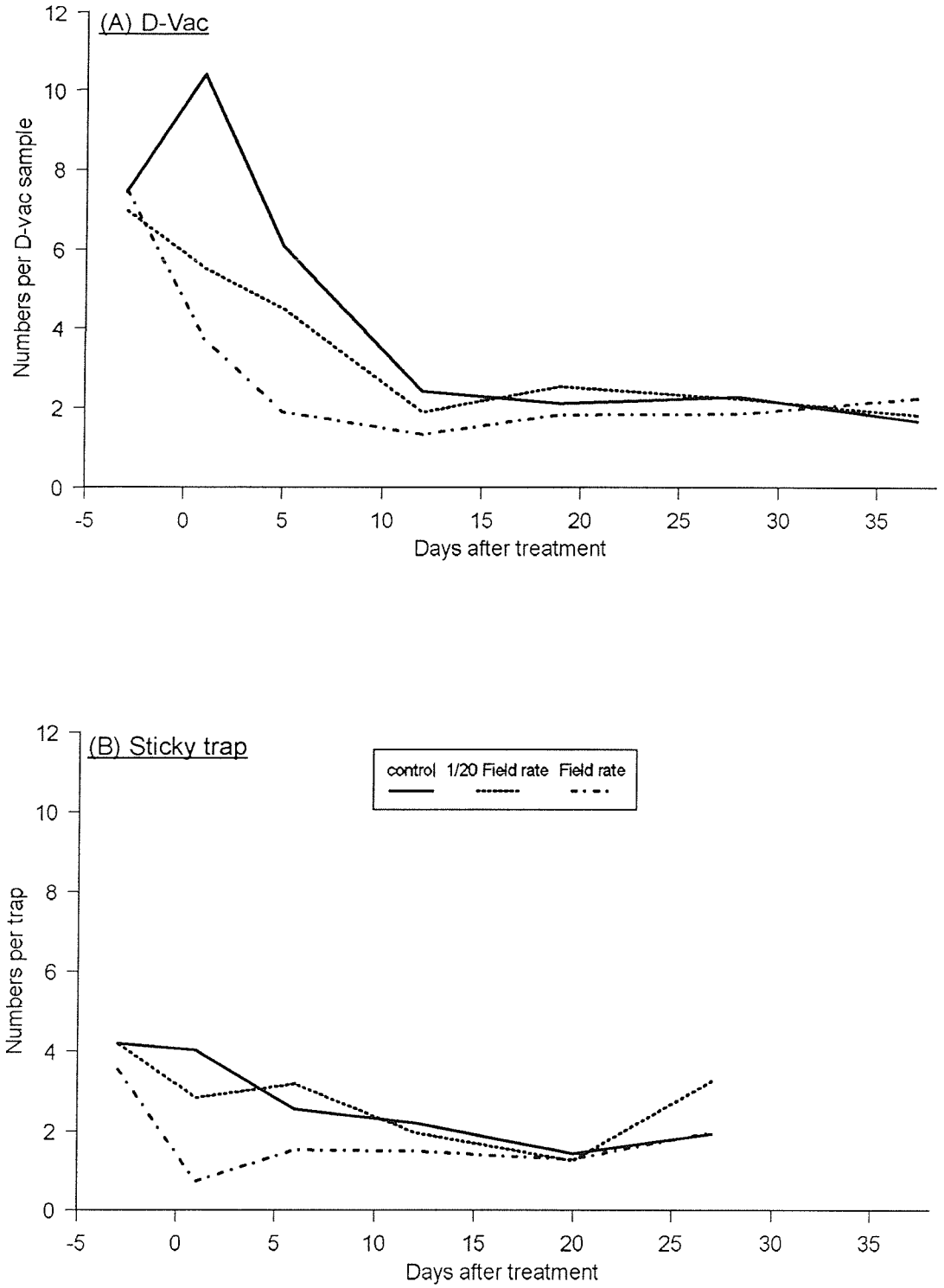


Figure 7.7. Total number of aphid primary parasitoids caught on days before and after insecticide treatment by (A) D-Vac and (B) Sticky trap (upper and lower traps combined) sampling in the three treatment plots.

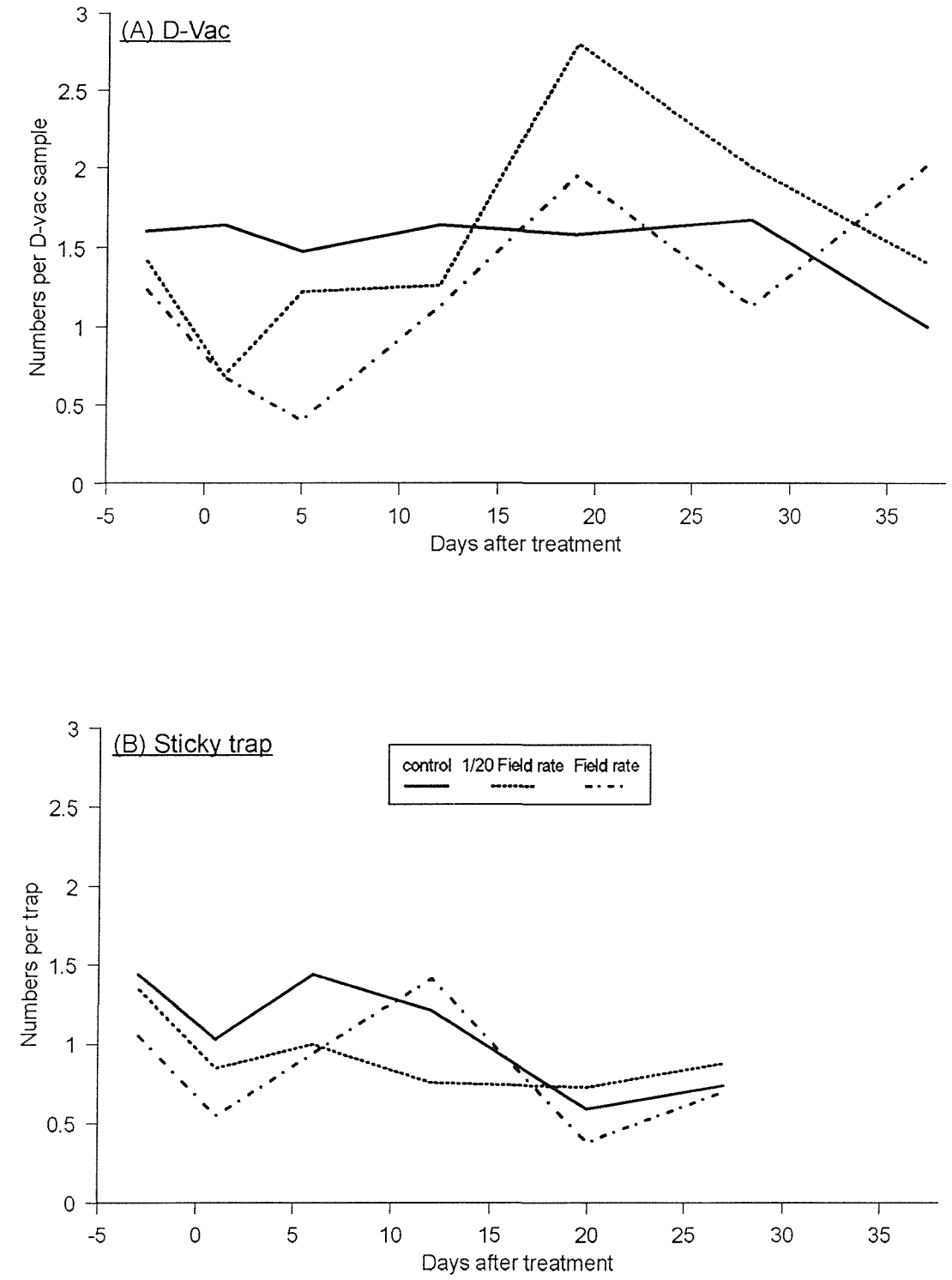


Figure 7.8. Total number of aphid hyperparasitoids caught on days before and after insecticide treatment by (A) D-Vac and (B) Sticky trap (upper and lower traps combined) sampling in the three treatment plots.

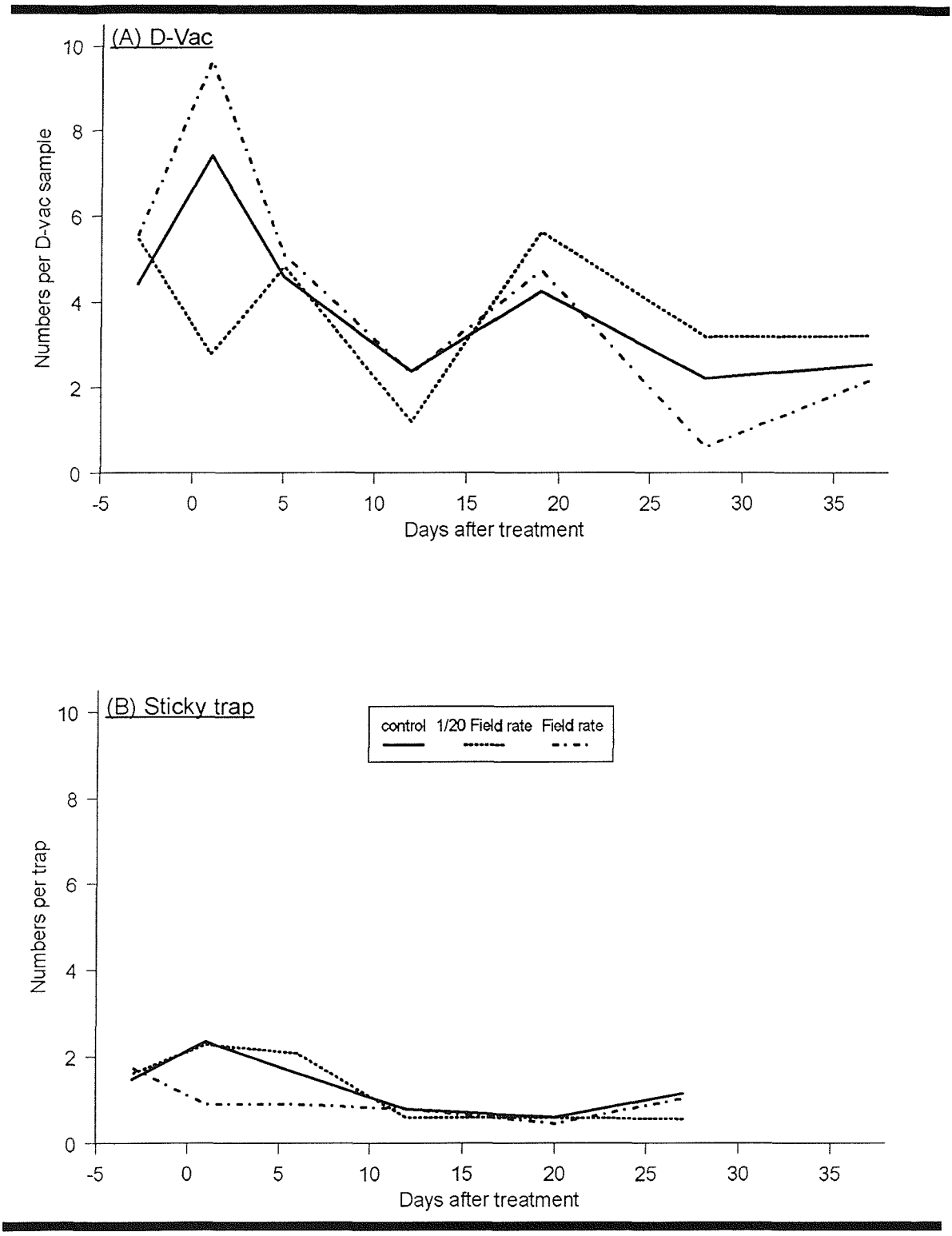


Figure 7.9. Total number of linyphiids caught on days before and after insecticide treatment by (A) D-Vac and (B) Sticky trap (upper and lower traps combined) sampling in the three treatment plots.

an unexpected result, with an increase of pre-treatment numbers by 7%, recorded during the first post-treatment sample, after correction for control numbers (Table 7.4).

(i) Sticky traps. In general, fewer linyphiids were caught per sticky trap during their two day exposure period compared to a single D-vac suction sample (Figures 7.9a & 7.9b). The numbers of linyphiids caught in the control plot showed the same trend as that for the D-vac data (Figures 7.9b). However, the 1/20th field concentration of deltamethrin resulted in only a 17% reduction of pre-treatment numbers, and the full field concentration causing a large reduction (106%), after correction for control numbers (Table 7.4).

Effect of sticky trap position on catch size

The number of insects, in each taxonomic group, caught on sticky traps varied not only with sample date but also according to the trap position. Significantly higher numbers of aphid primary parasitoids were found on the lower sticky traps (around flag leaf height), compared to upper traps (above crop canopy), during the first three sampling periods in the control plot (Figure 7.10a). Subsequent sampling periods during July showed no significant differences in parasitoid numbers between the two different trap heights. The total number of female *Aphidius* spp. on the lower traps remained relatively constant throughout the sample periods (varying from 50% to 64% of the total *Aphidius* caught) (Table 7.5). In contrast, the percentage of *Aphidius* spp. identified as females on the upper traps over the sampling intervals were greater (63% to 94%), with the highest percentages occurring during the last three sample periods.

Table 7.5. Percentage of *Aphidius* parasitoids identified as females on upper and lower sticky traps throughout the sampling season in the control plot. Numbers in parenthesis represent numbers of *Aphidius* sexed.

Sample period	Upper trap	Lower trap
19-21 June	69% (13)	55% (67)
23-25	63% (8)	64% (78)
28-30	75% (8)	61% (41)
04-06 July	85% (13)	50% (18)
12-14	94% (16)	55% (11)
19-21	87% (15)	50% (22)

The total number of aphid hyperparasitoids caught in the control plot showed no significant differences between trap heights, during any sample period, despite a trend for higher numbers on the upper traps (Figure 7.10b).

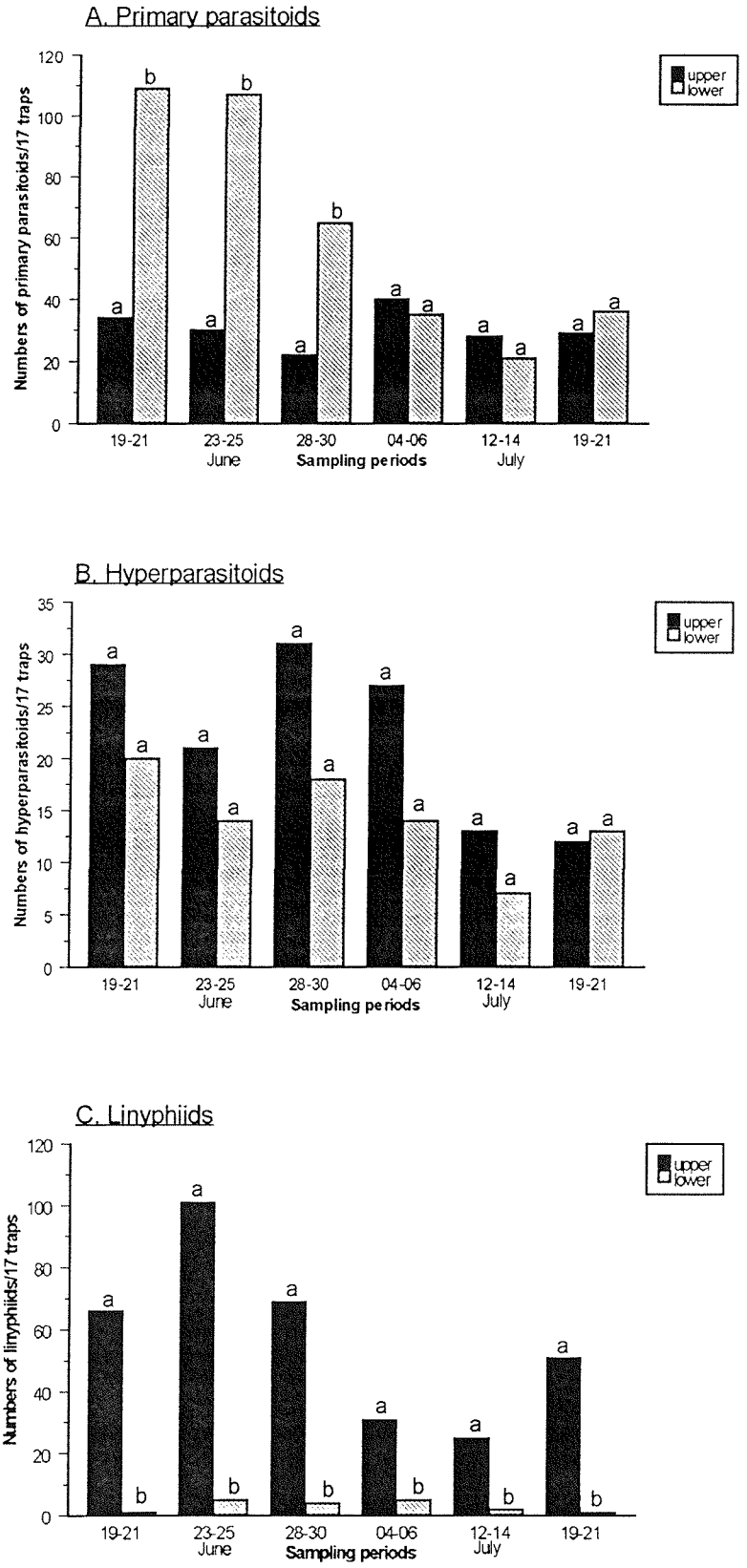


Figure 7.10. *Total number of (A) primary parasitoids, (B) hyperparasitoids and (C) linyphiids caught on upper* and lower sticky traps in the control plot. Significant difference shown if columns in the same sample period have different letters (one-way ANOVA, Tukey HSD $P<0.05$).

Linyphiid spiders caught in the control plot showed significantly higher numbers on the upper traps, compared to lower traps, on each sample date (Figure 7.10c). Very low numbers were caught on the lower traps.

Collection of mummies

The trend of the total number of mummies collected over the season in the three treatment plots was similar (Figures 7.11a, b & c). These numbers correlate with the trend in aphid numbers found in the plot (Figure 7.5). Throughout the season, proportions of primary and hyperparasitoids varied, with hyperparasitoids becoming more abundant at the end of July (Figures 7.11a, b & c).

Aphidius spp. were the most common primary parasitoids to emerge from the collected mummies, with species of hyperparasitoids spread amongst the three main groups identified (Table 7.6).

Table 7.6. Total numbers of mummies collected, and subsequent primary and hyperparasitoid emergence, from the treatment plots on each sample date. FR=Field rate concentration of deltamethrin.

			Primary parasitoids			Hyperparasitoids		
Date	Treatment	Total number of mummies	*Aphidius* spp.	*Ephedrus* spp.	*Praon* spp.	*Alloxysta* spp.	*Dendrocerus* spp.	*Corunal Asaphes* spp.
21 June	Control	30	17	3	1	2	2	5
	1/20 FR	34	25	2		3	2	2
	FR	19	13	1		5		
25 June	Control	12	6			2	2	2
	1/20 FR	10	6	2				2
	FR	8	4	1		1		2
01 July	Control	1	1					
	1/20 FR	9	4	2		2	1	
	FR	2	2					
07 July	Control	6	2			2		2
	1/20 FR	13	8			3	2	
	FR	9	4	2		1	2	
14 July	Control	8	7			1		
	1/20 FR	16	6	2	2	1	3	2
	FR	12	6		2	3		1
20 July	Control	18	4			6	3	5
	1/20 FR	31	8	6		7	8	2
	FR	41	20	3	3	8	5	2
29 July	Control	10	4	1		4	1	
	1/20 FR	24	10	1		9	1	3
	FR	40	12	3	3	13	7	2

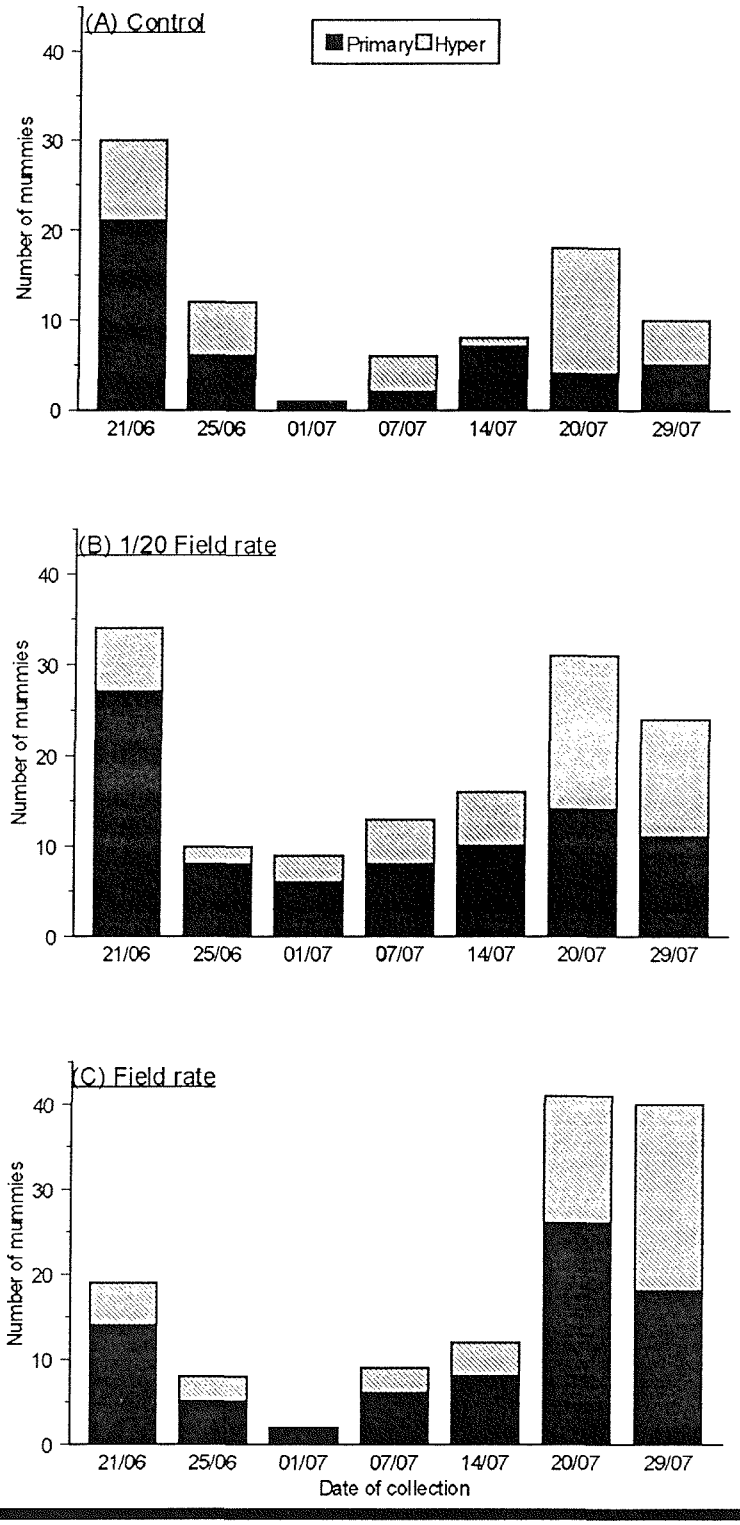


Figure 7.11. Number of mummies containing primary and hyperparasitoid species collected from (A) Control, (B) 1/20 Field rate and (C) Field rate plots during the field season.

(2) Analysis of population recovery

Statistical analysis

A $\log_{10}(n+1)$ transformation was applied to all the invertebrate data (Table 7.7; step 1), then the post-treatment transformed value for each position in the plot was subtracted from the pre-treatment one to form a 'log-difference' (Table 7.7; step 2). To determine whether recovery had occurred on each post-treatment date, the log-difference data from each sample point at a particular distance into the plot (i.e. 20m, 40m, 60m or 80+m) were tested for significance (one-way ANOVA, $P<0.05$) against the control log-differences from the equivalent plot positions.

Further analysis was performed to determine whether gradients of recovery were detectable. On each sample date, the log-difference for each treated position was subtracted from the control log-difference for that date and plot position, to form a modified '*k*-value' (Varley & Gradwell, 1960) (Table 7.7; step 3).

For graphical representation of recovery and reinvasion trends, the rates of change of the modified *k*-values with respect to distance into the treated plot on a given date were calculated. The regression coefficient of *k*-values against $\log_{10}$ (distance) was termed the 'gradient' (Figure 7.12). A positive gradient indicates that the number of insects (relative to the control) decreased as distance into the treated plot increased, i.e. more insects at the plot edge than at the centre, and vice-versa for a negative gradient.

Table 7.7. Example of the transformations used in the statistical analysis of raw data (from Duffield, 1991). Log-difference data (step 2) was used in the ANOVA calculations for measuring spatially explicit recovery. K-values (step 3) were used in the calculation of reinvasion gradients.

		Treated		Control	
		Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
	Initial data	99	9	99	31
Step 1	⇓				
	Log (n+1)	2.0	1.0	2.0	1.5
Step 2	⇓				
	Pre-treatment - Post-treatment ('log-difference')		1.0		0.5
Step 3	⇓				
	Treated - Control (*k*- value)		0.5		

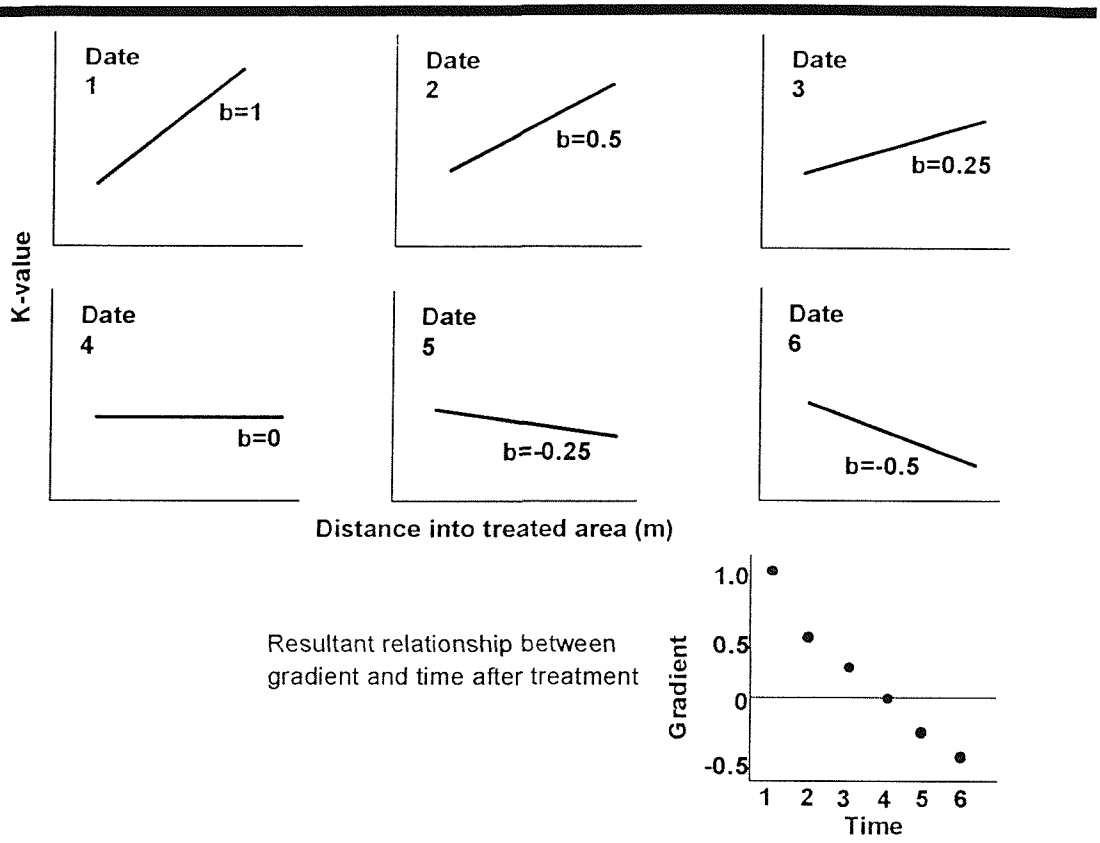


Figure 7.12. Hypothetical data used to show the derivation of "gradient" and "time after treatment" figures (from Duffield, 1991).

The results of these two analyses are presented together for aphids, primary parasitoids, hyperparasitoids, and linyphiids using: (a) figures displaying gradients of modified *k*-values and $\log_{10}$-transformed distance into treated area, against days after treatment and (b) a tabulated summary of the results of the ANOVA between treated and control log-difference data. This permits a visual assessment of the spatial trends in parasitoid numbers, once control effects are removed, and a statistical assessment of the extent of the treatment effects at each trapping coordinate in the sampling grid.

(3) Analysis of gradients within the treated area and recovery trends (based on D-vac assessments)

Aphids

Initially after treatment, the gradient in the 1/20 field rate plot was significantly ($P<0.05$) less than zero (Figure 7.13a (graph)), indicating greatest aphid densities at the

centre of the plot. During subsequent sampling dates no significant gradients were detected. Significant reductions ($P<0.05$) were detected at the 20m position on both 2 and 8 days after treatment (DAT) (Figure 7.13a (table)). By 14 DAT no significant reductions were detected at any of the distances into the plot.

Within the field rate plot, no significant gradients were detected on any post-treatment date (Figure 7.13b (graph)). Significant reductions of aphid numbers were detected at all the sample positions at 2 DAT (Figure 7.13b (table)). On the two subsequent sample dates (8 and 14 DAT) significant reductions were only detected at the 20m and 80+m sample positions. All distances, except 40m, showed significant reductions during the assessments on day 21 after treatment. By 27 DAT no significant reductions were detected at any of the distances into the plot.

Primary parasitoids

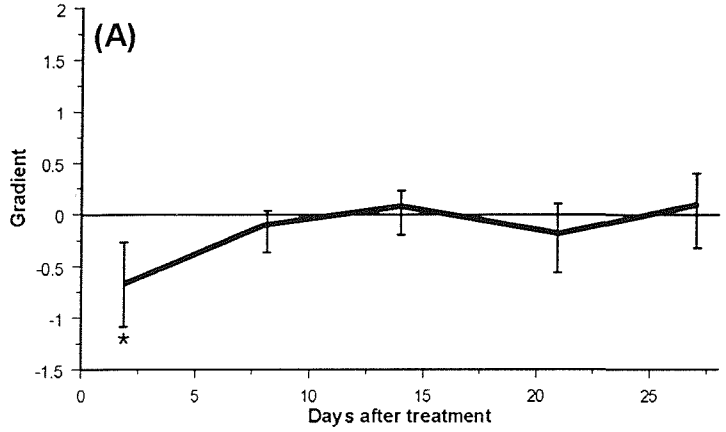
In the 1/20 field rate plot there was a trend for negative gradients upto 28 DAT, though only significantly ($P<0.05$) different from zero on day 5 after treatment (Figure 7.14a (graph)). Significant reductions were detected at 20m and 60m into the plot on day 1 after treatment (Figure 7.14a (table)). Subsequent sampling dates showed no significant reductions at any distances into the plot.

Within the field rate plot, the gradient was initially significantly ($P<0.05$) greater than zero on 1 DAT (Figure 7.14b (graph)), indicating greater densities at the plot edges compared to the centre. Further positive gradients, though non-significant, remained until 19 DAT. Significant reductions were found at all distances on both 1 and 5 DAT (Figure 7.14b (table)). On 12 DAT, significant reductions were only found at 60m and 80+m distances into the plot. Later sampling dates indicated no significant reductions at any distances into the plot.

Hyperparasitoids

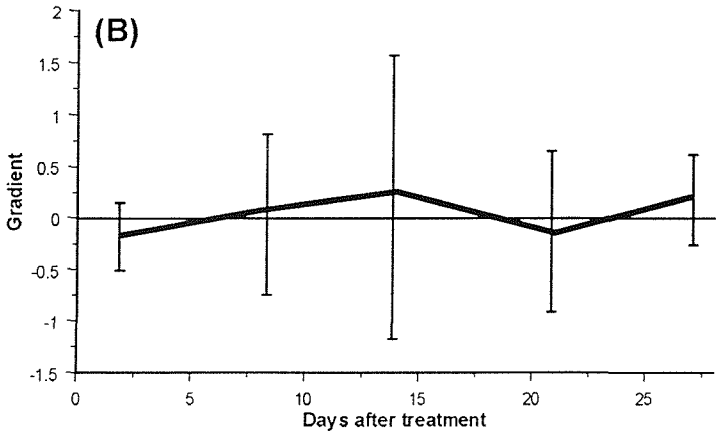
Initially after treatment, the gradient in the 1/20 field rate plot showed no significant difference from zero (Figure 7.15a (graph)). It subsequently increased to give a positive value, differing significantly ($P<0.05$) from zero on day 5 after treatment. Subsequent sampling dates indicated negative gradients which differed significantly ($P<0.05$) from zero on days 19 and 28 after treatment. Significant reductions were detected at all distances on day 1 after insecticide application (Figure 7.15a (table)). At 5 DAT, significant reductions were only detectable at distances of 60+m. Subsequent sampling dates indicated no further statistically significant reductions at any of the sample distances.

The gradient was initially significantly ($P<0.05$) greater than zero in the field rate plot



Days after treatment

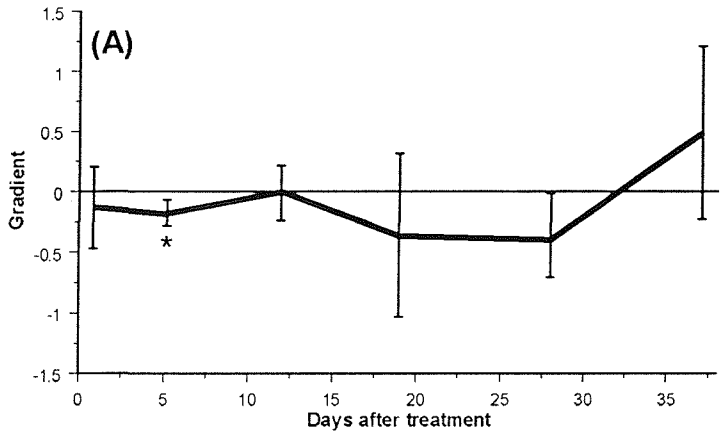
Distance into treated plot (m)	2	8	14	21	27
20	*	*	-	-	-
40	-	-	-	-	-
60	-	-	-	-	-
80+	-	-	-	-	-



Days after treatment

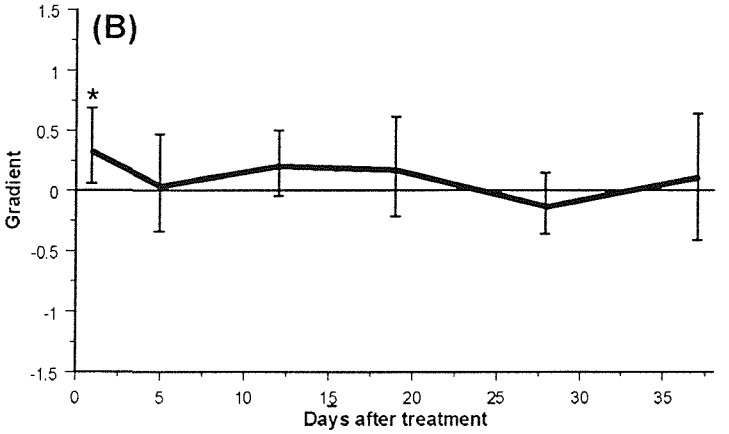
Distance into treated plot (m)	2	8	14	21	27
20	*	*	*	*	-
40	*	-	-	-	-
60	*	-	-	*	-
80+	*	*	*	*	-

Figure 7.13. Aphid data expressed as the gradient (k-value against log10 distance into treated plot) (± 95% confidence limits) against time after treatment; where * P<0.05. Summary tables give ANOVA results for date analysis of log-difference values at each treated position compared with the control; where * P<0.05 significant treated population reduction and - P>0.05, for (A) 1/20 field rate plot and (B) field rate plot.



Days after treatment

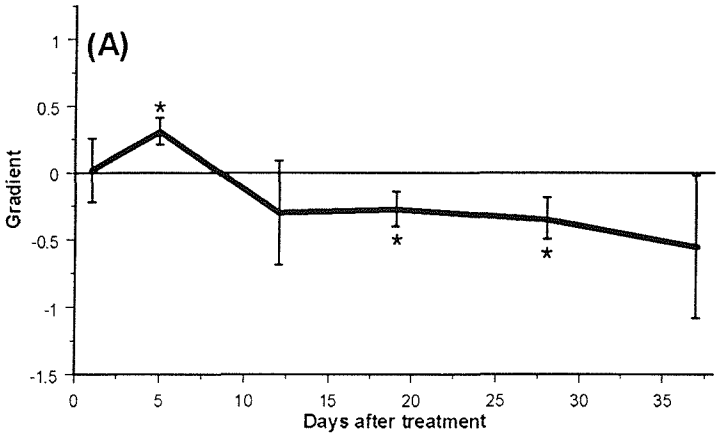
Distance into treated plot (m)	1	5	12	19	28	37
20	*	-	-	-	-	-
40	-	-	-	-	-	-
60	*	-	-	-	-	-
80+	-	-	-	-	-	-



Days after treatment

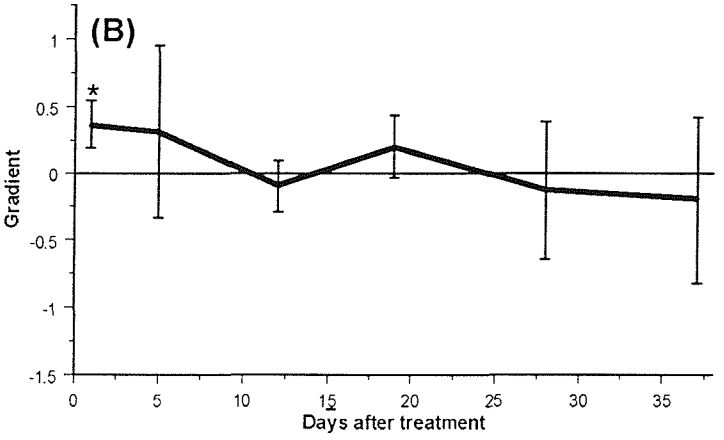
Distance into treated plot (m)	1	5	12	19	28	37
20	*	*	-	-	-	-
40	*	*	-	-	-	-
60	*	*	*	-	-	-
80+	*	*	*	-	-	-

Figure 7.14. Primary parasitoid data expressed as the gradient (k-value against log10 distance into treated plot) (± 95% confidence limits) against time after treatment; where * P<0.05. Summary tables give ANOVA results for date analysis of log-difference values at each treated position compared with the control; where * P<0.05 significant treated population reduction and - P>0.05, for (A) 1/20 field rate plot and (B) field rate plot.



Days after treatment

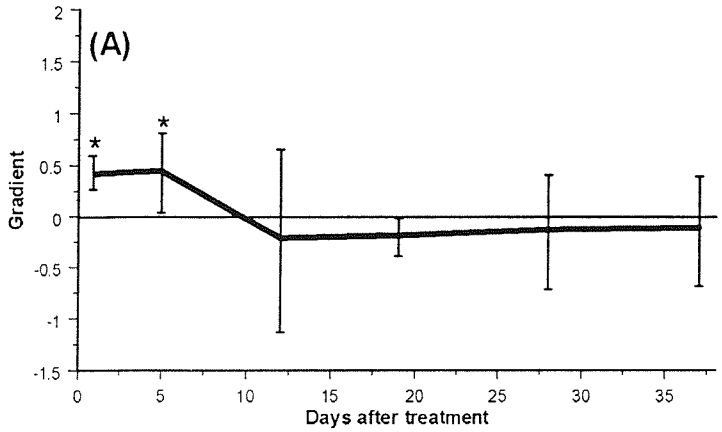
Distance into treated plot (m)	2	8	14	21	27
20	*	-	-	-	-
40	*	-	-	-	-
60	*	*	-	-	-
80+	*	*	-	-	-



Days after treatment

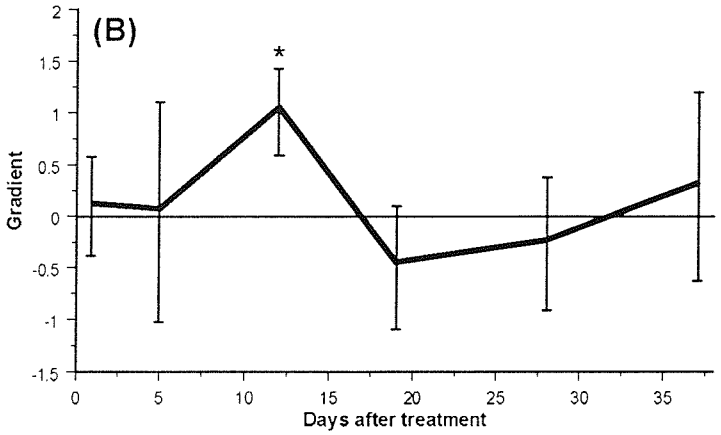
Distance into treated plot (m)	2	8	14	21	27
20	*	-	-	-	-
40	*	*	-	-	-
60	*	*	*	-	-
80+	*	*	-	-	-

Figure 7.15. Hyperparasitoid data expressed as the gradient (k-value against log10 distance into treated plot) (± 95% confidence limits) against time after treatment; where * P<0.05. Summary tables give ANOVA results for date analysis of log-difference values at each treated position compared with the control; where * P<0.05 significant treated population reduction and - P>0.05, for (A) 1/20 field rate plot and (B) field rate plot.



Days after treatment

Distance into treated plot (m)	1	5	12	19	28	37
20	*	*	-	-	-	-
40	*	*	-	-	-	-
60	*	-	-	-	-	-
80+	*	*	-	-	-	-



Days after treatment

Distance into treated plot (m)	1	5	12	19	28	37
20	-	-	-	-	-	-
40	-	*	-	-	-	-
60	-	-	*	-	-	-
80+	-	-	*	-	-	-

Figure 7.16. Linyphiid data expressed as the gradient (k-value against log10 distance into treated plot) (± 95% confidence limits) against time after treatment; where * P<0.05. Summary tables give ANOVA results for date analysis of log-difference values at each treated position compared with the control; where * P<0.05 significant treated population reduction and - P>0.05, for (A) 1/20 field rate plot and (B) field rate plot.

(Figure 7.15b (graph)). Gradients then showed a declining trend over subsequent sampling dates, with no further significant differences from zero detected. Significant reductions were detected at all sampling distances in the field rate plot on day 1 after treatment (Figure 7.15b (table)). At 5 DAT, significant reductions remained detectable at distances of 40+m. Only the 60m position showed a significant reduction at 12 DAT, with subsequent sampling dates indicating no further significant reductions at any of the sample distances.

Linyphiids

Significant positive gradients were detected on the first two sample dates after treatment in the 1/20 field rate plot (Figure 7.16a (graph)). No further significant gradients were detected. Significant reductions were detected at all the sample positions on 1 DAT, and all, except the 60m position, on 5 DAT (Figure 7.16a (table)). No further reductions were detected at any sample position on subsequent dates.

The only significant positive gradient detected in the field rate plot was at 12 DAT (Figure 7.16b (graph)). Significant reductions were only recorded at 40m on day 5, and 60+m on day 12. Subsequent sample dates revealed no significant reductions at any plot position (Figure 7.16b (table)).

(4) Spatial and temporal distribution patterns

Aphids

The spatial and temporal patterns of aphid numbers in the three experimental plots were mapped using the kriging technique (Figure 7.17). Each individual square represents the 4ha plot and the isoclines differentiate between areas of equal aphid density, estimated from the 45 sampling locations over the whole plot (Figure 7.3). The isopleth gradient was set at intervals of 5 aphid units, which was chosen arbitrarily to highlight areas of different aphid density.

Aphid numbers were low and relatively homogenous over the three experimental plots before insecticide treatments (Figure 7.17, column 1). Within the control plot, aphid numbers continued to increase in density over the following 14 days (Figure 7.17, row 1). The application of the 1/20 field concentration of deltamethrin resulted in reduced densities of aphids on 2 DAT, followed by an increase in density over the subsequent two sampling dates (Figure 7.17, row 2). The application of the full field concentration of deltamethrin resulted in a virtual elimination of aphid densities at 2 DAT (Figure 7.17, row 3). At 8 DAT, "hot spots" of aphid outbreaks are evident, which continue through 14 DAT, as aphid

populations recover.

***Aphidius* spp. parasitoids**

The spatial distributions of *Aphidius* spp. in the three experimental plots, during the pre-spray assessment, were comparably heterogenous (Figure 7.18, column 1). In the control plot, the densities increased over the following days, with greater homogeneity of densities. On 12 DAT the populations had started to decline (Figure 7.18, row 1). The application of the 1/20th field concentration of deltamethrin caused a decline in densities on 1 DAT, with the highest numbers occurring around the edge of the plot (Figure 7.18, row 2). Subsequent reinvasion resulted in increased densities on 5 DAT. In the plot sprayed with the full field concentration of deltamethrin, the parasitoid densities were considerably reduced and remained low over the following 12 DAT, again with the highest densities occurring around the plot edges (Figure 7.18, row 3).

A comparison of the aphid and mummified aphid (i.e. parasitism rates) aggregation patterns could not be made as parasitism levels remained low throughout the experiment.

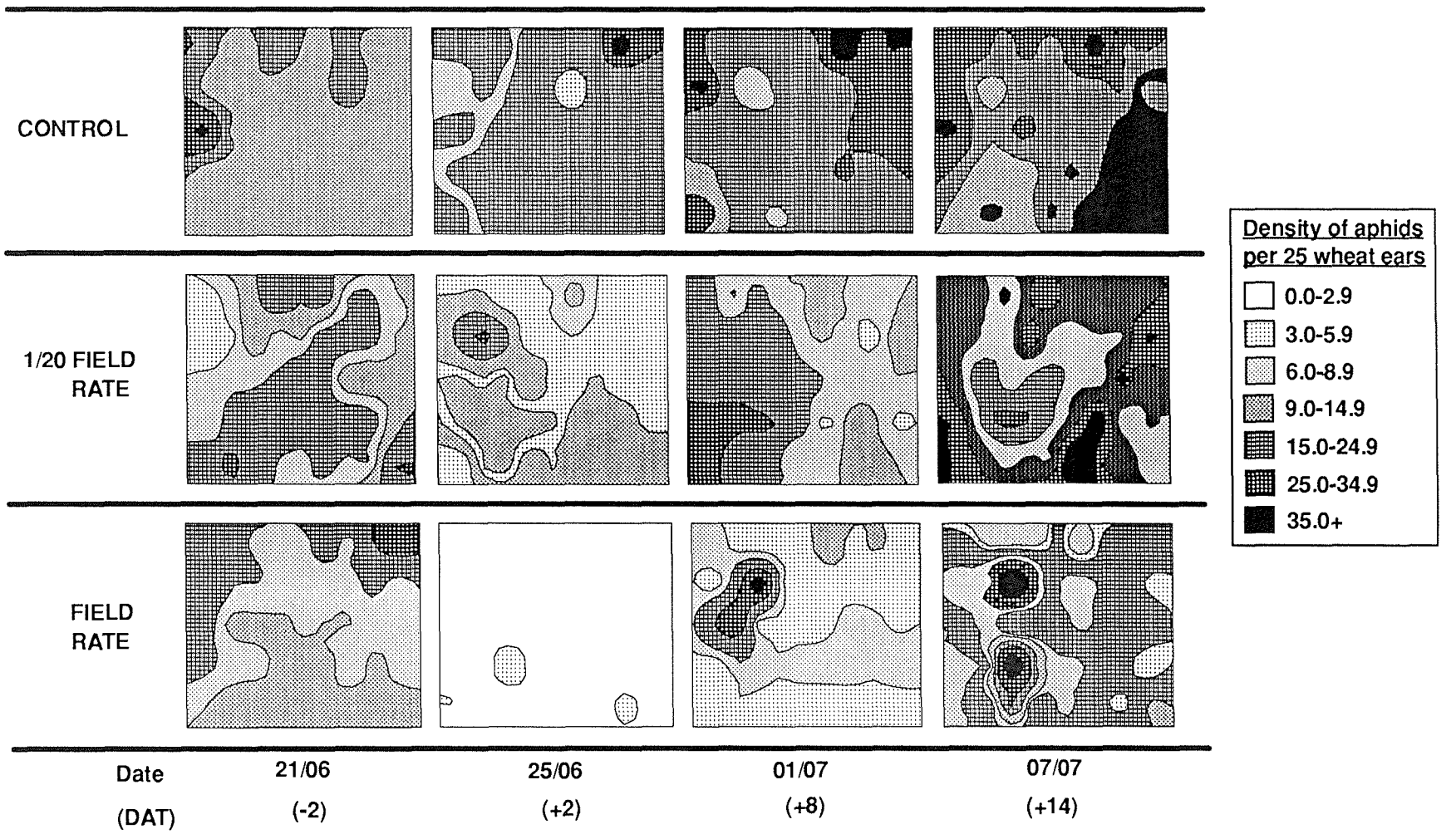


Figure 7.17. Density distribution maps of aphids in the three treatment plots on days before (-) and after (+) treatment (DAT). Each square represents the 4ha plot. Maps generated using the kriging technique (see text for details) show isopleths linking areas of equal aphid density.

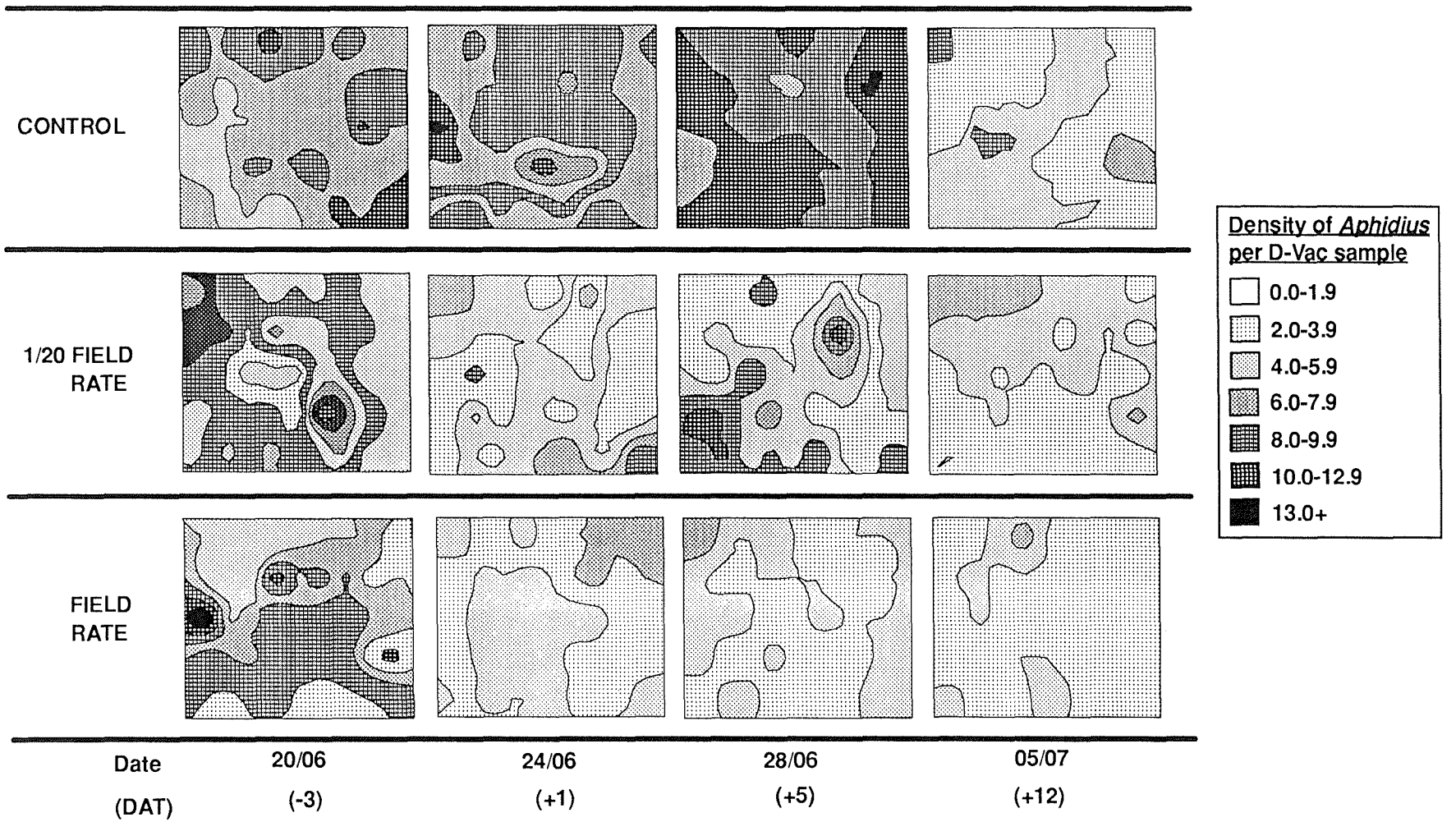


Figure 7.18. Density distribution maps of *Aphidius* parasitoids in the three treatment plots on days before (-) and after (+) treatment (DAT). Each square represents the 4ha plot. Maps generated using the kriging technique (see text for details) show isopleths linking areas of equal parasitoid density.

DISCUSSION

The overall effectiveness of deltamethrin as an aphicide, applied at the label recommended field rate was confirmed. However, its broad-spectrum nature was also demonstrated with initial reductions detected in all the invertebrate groups studied. Each group is treated separately below, to explain the observed overall trends in their numbers within the different plots, and subsequent recovery patterns. The post-treatment period was characterised by the establishment of gradients of *k*-value within the treated area between sites at different distances into the treated area. Differences in the recovery patterns of each invertebrate group may be explained in terms of differences in susceptibility to the insecticide, behaviour and predominant dispersal mechanism used by each group during the study period. Three possible patterns of recovery were possible. Firstly, invertebrate groups can exhibit a positive gradient between *k*-value and distance into the treated area, indicating greater numbers at sites close to the untreated edge compared with numbers at the centre. Secondly, the opposite can occur, with negative gradients indicating more at the plot centres. Thirdly, no significant gradients can occur, indicating that the process of reinvasion/recovery is either absent or occurring through a random pattern.

Aphids. The mid-season population crash of aphid numbers in the control plot may be linked to the period of heavy rainfall recorded during this period (Figure 7.6). Rainfall has been implicated in dislodging aphids, especially nymphs, and thereby reducing population numbers in wheat crops (Jones, 1979; Dean, 1978; Carter *et al.*, 1980). Subsequent recovery of numbers occurred during the period of high temperatures and low rainfall and whilst the crop was at the milky growth stages 73-80 (Tottman & Broad, 1987), giving a peak during the third week of July. A crash in numbers by the end of July was again correlated with high rainfall and lower temperatures, accompanied with an advanced growth stage of crop (hard dough development of the grain), which becomes unsuitable for aphid feeding (Dixon, 1987).

The application of the reduced dose rate of deltamethrin (representing 1/20th of the field concentration) provided a 40% reduction of pre-treatment aphid numbers, when corrected for control changes. This degree of aphid control using a considerably diluted concentration of insecticide would provide substantial economic rewards for farmers (Mann *et al.*, 1991). Further investigation is needed however, as this recorded reduction may be artificially high as it only represents aphids positioned on wheat ears, i.e. *S. avenae*, and not other species inhabiting different plant structures, such as *M. dirhodum*, that are known

to survive on lower leaves at reduced dose rates (Chapter 5; Poehling 1987a, 1987b).

Aphid numbers in the field rate plot were virtually eliminated. A slow recovery of numbers followed with a peak late in the season. This delayed peak may indicate some potential resurgence which may have been of economic importance if spray application had occurred earlier in the season. Resurgence can be defined as an increase in the numbers of insecticide-treated insects over and above the numbers on unsprayed plants. Possible explanations for resurgence are: (i) the reduction by the insecticide of the natural enemies and competitors of the pest species, (ii) an insecticide-induced improvement to plant nutritive quality, and (iii) the direct stimulatory effect of the pest themselves. The phenomenon of post-pyrethroid resurgence is well documented with mites (e.g. Hoyt *et al.*, 1978; Zwick & Fields, 1978; Hall, 1979) and brown-plant hoppers (Chelliah *et al.*, 1980). Reports of resurgence in aphids are less common, however cases have been noted in France and Canada (Cancelado & Radcliffe, 1979) and New Zealand (Penman & Chapman, 1980) and also demonstrated in laboratory and glasshouse studies (Jackson & Wilkins, 1985).

The speed and extent of reinvasion and recovery of predatory invertebrate groups has been implicated in determining the survival and recovery of aphid populations after insecticide application (Duffield & Aebischer, 1994). Rates of reduced feeding (Wiles & Jepson, 1993b) and increased locomotion and repellency (Heneghan, 1992) of certain predatory groups (i.e. carabids, staphylinids and linyphiids) have been recorded in insecticide-treated areas. The extent of these factors on invertebrate groups remained unquantified in this study, however they would have been less severe and of shorter duration on the predatory groups in the reduced dose rate plot, compared to the area treated with the full concentration of deltamethrin.

A significantly negative gradient was observed for the aphids present in the plot sprayed with the reduced concentration of deltamethrin two days after treatment (DAT). This result indicated greater recovery and/or survival at distances furthest from the untreated edge. A possible explanation of this phenomenon may be given by the patterns of abundance of predatory invertebrate groups in the plot. Of these, only linyphiids, which are known to be important predators of cereal aphids (Sunderland *et al.*, 1986; Sopp & Chiverton, 1987) were monitored in this study. In the 1/20th field concentration plot, significant positive gradients of linyphiid numbers were detected up to 5 DAT, indicating a progressive reinvasion of the depleted areas from the undepleted surroundings. Linyphiid dispersal is by two mechanisms; ballooning over long distances at certain times of the year (Vugts & Wingerden, 1976) and by cursorial movements over the soil surface (Sunderland *et al.*, 1980). With low numbers of spiders recorded in the plot centre soon after spraying,

a resultant "linyphiid-depleted" area was established in which aphid populations may have had the potential to increase at a greater rate than those near the plot edges. This phenomena was also demonstrated for aphids in the study by Duffield & Aebischer (1994) using a field recommended concentration of the organophosphate insecticide, dimethoate. They hypothesised that the broad-spectrum insecticide created a predator-depleted area which then progressively recovered from the edge to the centre. The aphids recovered most rapidly in the areas with the greatest reduction in predator numbers, i.e. the plot centre.

In this study, a recovery trend of this nature was not detected for aphids in the plot sprayed with the field concentration of deltamethrin. No significant gradients (either positive or negative) were found on any post-treatment sampling date. This is indicative of a pattern of virtual elimination of aphids within the plot, accompanied by a random pattern of recovery and/or survival occurring over the whole plot.

Due to the distance between sampling points, the distribution maps linking aphid densities of equal size reveal spatial changes on a large scale. They do not show aphid distribution changes on a smaller scale (i.e. between plants, within plants), which have been shown to occur after insecticide treatment (Chapter 5). The fairly uniform build-up of aphid populations over time in the control plot is demonstrated, with a more patchy recovery of aphids evident in the deltamethrin-treated plots. Alate cereal aphids are exceedingly mobile in relation to the size of the areas treated in this experiment (Tatchell *et al.*, 1988); consequently the treated areas may have received a uniform "rain" of alates rather than a progressive invasion from the untreated surroundings by the less mobile apterae and nymphal aphids.

Hymenopteran parasitoids. A high degree of toxicity/disruption to aphid primary parasitoid populations was recorded with the field rate application of deltamethrin. Similar findings have been recorded using other pyrethroids in field studies (e.g. Inglesfield, 1984; Shires, 1985; Poehling, 1987a; Smart *et al.*, 1989). The application of the reduced concentration of insecticide resulted in smaller initial reductions of parasitoid numbers. The resultant lower residual toxicities on crop foliage would have permitted a greater degree of survival (Chapter 5) and/or re-invasion of parasitoids, compared to in the field rate plot. Recorded initial reductions in numbers after spray application could have resulted from a combination of direct mortality, as a consequence of (i) direct exposure to spray droplets, (ii) exposure to residual deposits (Chapters 2 & 6), and (iii) dietary exposure (Chapter 6), and also to repellency/dispersal from the treated area (Chapters 3 & 4). The overall percentage reductions of hyperparasitoid populations were lower than that recorded for primary parasitoids. This may indicate they are less vulnerable to insecticide residues through their

behaviour (Chapter 3), or their increase in abundance throughout the season may potentially mask some degree of the pesticidal effects.

Significantly positive gradients were identified in the initial post-treatment distribution of both primary and hyperparasitoids in the plot sprayed with the full field concentration of deltamethrin (Figures 7.14b & 7.15b). Such a pattern of recovery is consistent with that expected from the reinvasion of the depleted areas from undepleted surroundings. A similar pattern of invertebrate recovery following depletion by pesticide application in cereals has been identified for Carabidae (Smart *et al.*, 1989; Jepson & Thacker, 1990; Duffield & Aebischer, 1994), Coccinellidae (Duffield & Aebischer, 1994), Linyphiidae (Thomas *et al.*, 1990; Duffield & Aebischer, 1994), and Staphylinidae (Jepson & Thacker, 1990; Duffield & Aebischer, 1994). In addition to this "horizontal recruitment", the process of "vertical recruitment" of parasitoids (i.e. emergence of adults from mummies) could have occurred, as protection against deltamethrin is afforded inside the aphid mummy case (Chapter 1). This process may have been a further unmeasured variable affecting the recover trends observed at each sampling distance into the treated plots over time.

The modes of dispersal, governing reinvasion and recovery patterns, available to hymenopteran adults are flying and walking, with aphid primary parasitoid larvae/pupae transported within aphid hosts by flight within alatae (Force & Messenger, 1968; Chua, 1975, 1977) or through the walking of apterae (Vater, 1971). Once within a suitable environment, male aphid parasitoids have a tendency not to disperse from the general area in which they emerge (van den Bosch *et al.*, 1967; Vater, 1971). However, they undergo more active flight than females, but only confined to short distances in the air strata near the ground (Vater, 1971). The females show a tendency to stay on plants, resting, feeding or searching for hosts, and then under certain conditions take off upwards to either make a short flight to a nearby plant or away from the crop to reach a range of air currents enabling dispersal over a greater distance. Increased dispersal by parasitoids away from deltamethrin-treated foliage under laboratory conditions has been demonstrated (Chapter 3). This phenomenon would have important consequences for dispersal behaviour, and hence reinvasion/recovery rates, in insecticide-treated crops, presumably resulting in greater time spent in flight.

The sex ratio of *Aphidius* populations caught on sticky traps throughout the season was biased in favour of females, with the strongest bias found on traps positioned above the crop canopy (i.e. wasps dispersing away) during July (Table 7.5). This agrees with previous reports of an increased emigration of female *Aphidius* species later in the season (Vorley & Wratten, 1987; Höller *et al.*, 1993). One proposed explanation is that female primary parasitoids leave areas of high hyperparasitoid density (found late in the season),

possibly due to repellent effects of pheromones produced by hyperparasitoids (Höller *et al.*, 1993). This may explain why large numbers of migrating cereal aphid primary parasitoids caught in the 12.2m high Rothamsted suction traps during summer have a female-bias of more than 80% (Dean, 1974; Dean *et al.*, 1981).

The density distribution maps for *Aphidius* spp. show their relatively homogenous distribution over the control plot. As noted previously, due to the spatial separation of sample points, these patterns indicate changes over a large scale only. The uniformity in parasitoid distribution may result from their active search for aphid hosts for egg deposition, causing them to spread out to cover the host population, leading to a less aggregated distribution than their hosts.

Unfortunately, due to low levels of parasitism and failure to successfully rear-on parasitised aphids in this study, the spatial association between aphids and parasitoids could not be ascertained.

Linyphiids. Due to their plant-active behaviour, and comparatively small, soft bodies, linyphiid spiders have been shown to suffer high mortality when exposed to deltamethrin (Rzehak & Basedow, 1982; Basedow *et al.*, 1985; Thomas *et al.*, 1990). As a result they have been proposed as good indicators of the side-effects of pesticides (Everts *et al.*, 1989; Everts, 1990). In the current study, an anomalous result was found with increased numbers of linyphiids collected in D-vac samples on 1 DAT, compared to pre-treatment numbers, in the plot sprayed with a field concentration of deltamethrin (Figure 7.9a). One possible explanation is that these linyphiids may have represented dead specimens that were collected from foliage and soil which, by using a freeze-kill method before counting, could not be distinguished from live individuals. The expected high mortality of linyphiid spiders with the field concentration of deltamethrin was however indicated in the sticky trap catches covering the 48h period after spray application. Significantly higher numbers of linyphiid spiders were caught on sticky traps positioned above the crop canopy throughout the field season, compared to lower traps. This can be explained by their substantial aeronautic dispersal activity, termed 'ballooning' (Weyman, 1993).

Implications for future field studies

Mapping techniques

To help interpret further field trials, the use of geostatistics for mapping spatial trends of invertebrates can be used, as demonstrated in this study. This method is relatively new

in ecological studies with only a few published papers describing this application in agricultural systems (e.g. Schotzko & O'Keeffe, 1989; Schotzko & Knudsen, 1992). The scope for using such techniques in the future is enormous, enabling detailed studies of spatial associations between pest insect and predators/parasitoids, and also recovery and reinvasion trends after insecticide applications. However, this would require the use of closely associated sampling areas to enable detection of changes in invertebrate numbers over a small scale. The choice of inter-sample distance is determined by the behaviour and distribution of the species in question. In addition, the choice of sampling methodology may also prove influential in the collection of parasitoids and the subsequent post-treatment conclusions from pesticide studies. For example, the use of both D-vac suction samples and sticky traps provided different information. D-vac samples were more efficient at collecting Hymenoptera than sticky traps exposed for 48h, however the latter provided information on the movement of parasitoids within and above the crop canopy. As a result, a combination of these two sampling methods is recommended for future studies on parasitoid populations.

Choice of plot size

In most field studies there is a trade-off between plot size and the extent of replication which is possible, therefore there must be a compromise between the need for statistical rigour and ecological factors when determining the scale of the experiment (Sotherton *et al.*, 1988). A study by Smart *et al.* (1989) to develop a methodology for the assessment of insecticidal effects on parasitoids and predators of aphids in a true field situation concluded that small replicated plots (ranging from 0.02 ha to 0.13 ha in size) were useful in estimating pesticide effects on the static pest species (aphids) much better than for the more mobile and less abundant beneficial species. Therefore in order to assess the latter, it was suggested that the use of the largest practicable plot size at the expense of plot replication would be most preferable. In the current study, the three plots were made as large as possible to fit within the available field, in order to monitor reinvasion effects of the highly dispersive insects. The use of large plot sizes allow more intensive sampling, using a range of different methods, and hence more opportunity to gain statistically significant results from small populations. The large plot layout decreases statistical sensitivity when compared to replicated plot trials, but Smart *et al.* (1989) found it did not mask obvious differences between treated and untreated areas, with small changes in populations of beneficial insects being probably of little importance (Dempster, 1984).

Various studies have involved treating single large plots with insecticide and using the untreated surrounding crop as a comparison (e.g. Jenkyn & Bainbridge, 1974; Smart

et al., 1989; Duffield & Aebischer, 1994). This type of experiment involves the use of pseudoreplication (Hurlbert, 1984), whereby replicated samples are taken from the same plot. Constraints are therefore imposed upon the statistical analysis. The validity of using this kind of unreplicated treatment experiment relies on the experimental plots being identical at the time of spray application and on their remaining identical to each other after treatment, except insofar as there is a treatment effect (Hurlbert, 1984).

One approach to overcome the problems posed by pseudoreplication is the use of a BACI design (Stewart-Oaten *et al.*, 1986) which requires samples, replicated in time, to be taken in a plot **B**efore the treatment begins and **A**fter it has begun, at both the **C**ontrol and **I**mpact sites, thus allowing a statistical separation of treatment and any natural phenomenon effects (e.g. weather, crop growth) to be separated. This approach was not deemed suitable for the current study, as it is arguable that the short summer activity period of parasitoids and rapid changes in host density in the cereal system render time-series analysis inappropriate.

The treated areas in this study were within-field plots and as a consequence each treated area was surrounded by a source of potential colonists in the untreated portion of the field. For the purposes of this study, recovery was determined by comparing relative invertebrate populations in the control and treated areas within a single field. Observed "statistical recovery" could therefore have resulted from a redistribution of the surviving population leading to an overall lowering of the field population (Thomas *et al.*, 1990; Duffield & Aebischer, 1994). With commercial applications of insecticide covering entire fields, the reservoir of potential recolonists would be separated from the depleted areas by field boundaries. These obstructions have been shown to inhibit movement of certain invertebrate populations between adjacent fields (e.g. carabids, Jepson & Thacker, 1990). Information is lacking on the movement of parasitoids during the field season, and the impact of field boundaries upon their dispersal to neighbouring crops. However, it can be surmised that reinvasion and subsequent recovery of parasitoid populations into insecticide-treated fields may proceed at a slower rate due to the greater areas treated and the spatial separation of potential colonists.

At present the Ministry of Agriculture, Fisheries and Food have recommended the use of minimum plot sizes of 1ha for assessing the effects of pesticides upon the whole range of beneficial invertebrate species in cereal crops, as the first step towards registering the compounds for commercial use (Working document 7.7: Guideline to Study on Within-season Effects of Insecticides on Beneficial Arthropods in Cereals in Summer, MAFF/PSD). However, the results from this current study suggest that the use of such small-scale within-field trials may underestimate the true impact upon highly dispersive species such as

parasitoids, as reinvasion would occur too rapidly. Further work is therefore needed to determine the role of size and proximity of reservoir populations in the recovery processes on the scale of experimental field trials.

CHAPTER EIGHT

GENERAL DISCUSSION

The series of laboratory, semi-field and field experiments that were carried out in this study have provided an insight into the toxic risks posed by summer-applied deltamethrin sprays to aphid parasitoid species inhabiting temperate cereal crops. The implications of these findings have been discussed in detail in each respective chapter. The following section will therefore only discuss the overall implications of the results and how they may be used for aiding and improving risk assessment to parasitoids in laboratory and semi-field bioassays, and designing and interpreting the results of field trials. Areas for future research are also discussed.

Short-term risk assessments

Until recently, risk analysis has tended to exploit toxicological data collected on the "micro" scale (over hours following treatment and on the scale of the diel activity range of the organism) to extrapolate to effects on populations and communities at the "meso" (over weeks to months, on the field scale) or "macro" scales (over months to years, on the farm scale and beyond) (Chapter 1, Figure 1.2). To enable more realistic predictions and an assessment of which circumstances lead to significant hazard, ecological ideas are required (Jepson, 1993a). This would enable the movement away from the principle of worst-case scenarios presented by current tests, to a situation of "realistic worst-case". For this purpose, modifications were made, in the present study, to current published laboratory and field methods assessing pesticide effects on parasitoid species, to incorporate more realistic variables (Table 8.1). The usefulness of laboratory-derived toxicological data for parasitoids, from glass plate studies, may be improved by incorporating correction factors obtained from measurements of relative toxicity of pesticides on substrates that the organisms are likely to encounter, e.g. crop foliage.

The toxicity of fresh residues of deltamethrin, at a range of concentrations, to *A. rhopalosiphi* was estimated to be on average 1.5 times greater on glass plates than on wheat flag leaves. Standard test methods therefore overestimate the toxic effects that are likely to be found in the field. Further improvements to current tests were investigated by exposing parasitoids to insecticide residues at the range of concentrations potentially encountered in the field. This was achieved by using diluted deltamethrin concentrations

Table 8.1. Summary of the modifications made in this study to existing laboratory and field test methods for assessing pesticide effects on aphid and parasitoid populations.

Common features of existing test methods (Hassan, 1989)	*Modifications used in this study*
(A) LABORATORY	
Adult parasitoids	
1. Exposure to pesticide residues on glass plate	1. Exposure to pesticide residues on more realistic substrates, i.e. cereal foliage, to account for substrate-mediated toxicity
2. Only recommended dose rate of pesticide used	2. Range of concentrations used, ranging from field rate (FR) to 1/64 x FR, to determine optimum dose rates for parasitoid survival and simulate toxicities of weathered residues
3. Fixed exposure period on residues	3. Variable exposure periods ranging from 2 to 45 mins to represent more realistic levels of parasitoid exposure in the field
4. Only residual exposure to pesticide assessed	4. Quantification of residual and dietary exposure to pesticides to determine their relative importance
Pupal stages of parasitoids	
1. Only recommended dose rate of pesticide used	1. Range of concentrations used to determine optimum dose rates for parasitoid survival.
2. Maximum application rate	2. Quantification of actual exposure of mummies in field to enable correction factors to be applied to laboratory toxicity data
3. No assessment of different exposure routes	3. Quantification of direct, residual and dietary exposure to pesticide to determine their relative importance and the degree of pesticide penetration through a mummy case
4. Only mortality assessments made	4. Additional sub-lethal (longevity and fecundity) assessments made to determine longer-term effects of pesticides
(B) FIELD	
1. Only recommended dose rate of pesticide used	1. Range of concentrations used to determine optimum dose rates for aphid control and parasitoid survival
2. Total counts of aphid and parasitoid numbers made	2. Additional quantification of spatial and temporal distributions of aphid, primary parasitoid and hyperparasitoid populations made. Reinvasion and recovery patterns determined to assess the importance of the spatial scale of insecticide treatment on aphid and parasitoid populations. Assessment of residual toxicity of pesticide deposits on cereal foliage to investigate the toxicity to reinvading parasitoids. Measurement of spray deposition trends through crop canopy to determine location of insecticide-free refuges where aphid populations may survive post-treatment

in laboratory bioassays, and more realistic exposure times, derived from behavioural observations of parasitoids foraging on insecticide-treated substrates. At low concentrations and short exposure times, parasitoids showed lower mortality and recovered from knockdown when removed from the contaminated area.

The measurement of spray deposition on mummies positioned throughout a winter wheat crop suggested that current laboratory bioassays, using pesticides delivered in a spray or applied topically to the mummy case, could grossly overestimate the quantity of active ingredient impinging upon the mummy in the field (Chapter 2). Insecticide doses that cause lethal/sublethal effects in the laboratory may therefore prove harmless in the field. Greater realism for risk predictions and insecticide screening could be achieved by the further determination of "true" exposure levels in the field for larval/pupal stages of parasitoids within mummies in a variety of crops and at different growth stages throughout the pesticide spraying season. Through this work, the possibility of achieving differential insecticide selectivity on the pest/natural enemy complex by applying insecticides when pupal stages of parasitoids are protected inside the mummy could be assessed.

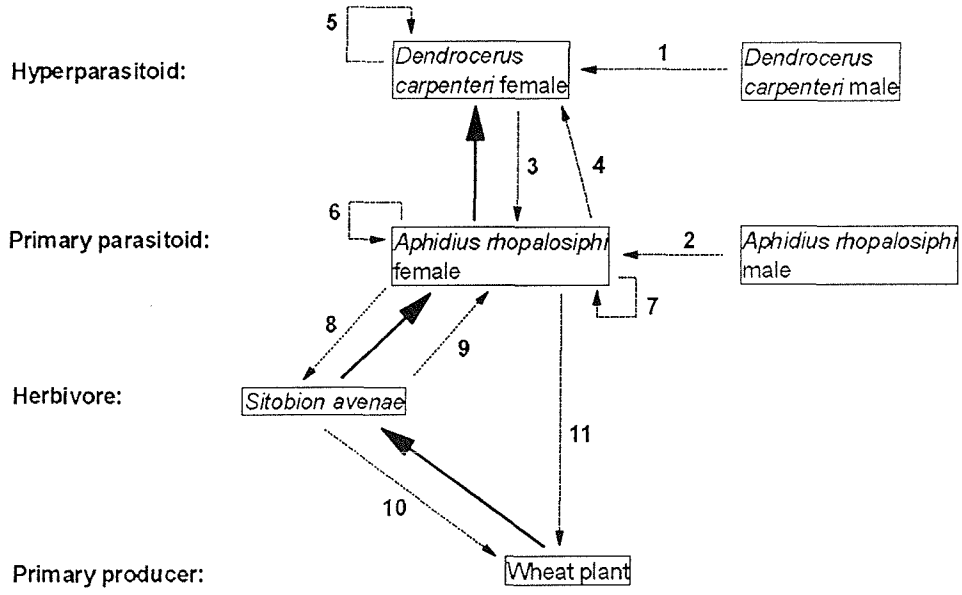
In order to further improve risk predictions it may be necessary to take into account other routes of exposure, such as dietary exposure. It was demonstrated that this route may potentially be an important cause of mortality for aphid parasitoids after an insecticide application. The effects of this exposure, however, remain unquantified in the field and require further investigation to determine its importance.

Further work using radio-labelled pesticides and/or residue analysis techniques are needed to improve the accuracy of risk predictions for invertebrates through the various routes of exposure (e.g. Chapter 6). Also toxicological information is required concerning toxic interactions of pesticide doses received by different routes of exposure so that mortality derived from laboratory studies may be used to predict overall effects on given invertebrate species. This more mechanistic approach has excellent potential for feedback to integrated pest management programs and the setting of optimum dose rates.

Compared with mortality studies where the insect is classified simply as "dead" or "alive", sublethal effects on physiology and behaviour can prove exceedingly subtle and multifaceted. For example, residues of deltamethrin resulted in altered foraging behaviours of primary parasitoids and hyperparasitoids on honeydew-contaminated substrates, leading to changes in distribution on plants (Chapter 3) and reduced parasitism of hosts by the primary parasitoids (Chapter 4). It was concluded that the greater attraction of honeydew kairomones over the repellent properties of deltamethrin could ultimately result in greater residual exposure of foraging parasitoids to insecticides. This has important implications for

aphid parasitoids entering honeydew- and insecticide-contaminated crops, and should therefore be further quantified and incorporated into future risk predictions.

The changes in behaviour and attraction of parasitoids to honeydew kairomones represents only one component of the complex chemical interactions within an aphid parasitoid's food-web (a part of which is presented in Figure 8.1) that could potentially be disrupted by the presence of pyrethroid insecticides. The impact of deltamethrin residues upon the other chemical links remains unquantified but may prove important for the success of biological control. For example, an insecticide may disrupt the host location process of



Chemical links
1, **2**: female sex pheromones, attracting males
3: kairomones associated with primary parasitoid, attracting hyperparasitoid
4: kairomones associated with hyperparasitoid, causing dispersal of primary parasitoid
5, **6**: host marking pheromones, preventing super- or multiparasitism
7: interspecific interference pheromones, causing dispersal
8: kairomones associated with host, attracting primary parasitoid
9: aphid alarm pheromones, causing dispersal
10: kairomones associated with host plant, attracting aphid
11: kairomones associated with host plant, attracting primary parasitoid

Figure 8.1. A summary of the chemical (broken line) and trophic (solid line) relationships between members of an aphid parasitoid food-web.

hyperparasitoids, thereby indirectly improving primary parasitism. Alternatively, the success of host plant location by primary parasitoids may be affected, or the female sex pheromones masked, subsequently resulting in reduced aphid parasitism. The interpretation of laboratory and field results would therefore benefit from further research investigating insecticide effects on these other chemical associations. For this purpose, specific parasitoids need their behavioural repertoires sufficiently characterised to enable researchers to analyse subtle changes in behaviour in the presence of semiochemicals and insecticides.

An investigation of optimum dose rates of deltamethrin applied to winter wheat for effective aphid control indicated that dose rates as low as 1/6th of the recommended field rate of deltamethrin (≡1.04g AI ha^{-1}) may provide a significant degree of aphid control, whilst preserving a high percentage of adult primary parasitoids contacting crop foliage after spraying. This suggests that there is scope for reducing dose rates of deltamethrin in cereals as an approach to promote biological control and reduce pesticide inputs and costs to the farmer. However, before such policies are advocated, the effects of deltamethrin applied at reduced dose rates upon other natural predators in the field, and the reliability of these parasitoids/predators in providing control of residual pest populations needs to be determined. In addition, investigations of pesticide degradation on plants and accumulation and degradation of the pesticide in the insect are needed to foster greater levels of selective pest control and improve risk predictions. An ultimate goal would be to predict the level of risk to organisms entering the treated crop over time and hence predict rates of recolonisation and recovery of parasitism.

Long-term risk assessments

A ranking system of relative risk of suffering long-term effects for selected beneficial invertebrate groupings in cereals has been previously devised (Table 8.2). Within this system, Braconidae are classified as being at relatively low risk because of their high fecundity, high dispersal potential and short duration of exposure to insecticides during the cereal growing season.

These rankings are in accordance with the general findings from recent long-term studies in this crop (e.g. Burn, 1989, 1992). However, it has been suggested that the effects on dispersive species such as Braconidae may be underestimated because of the limited experimental scale over which field-based studies have been based (Jepson, 1993a). The relative importance of each guild of beneficial invertebrate as biocontrol agents of aphids also remains unquantified. This factor may however be of importance because, for example,

Table 8.2. Qualitative scoring system to rank guilds of beneficial invertebrates in terms of their susceptibility to long-term pesticide side-effects (from Jepson, 1993a). High scores indicate higher relative risk.

	Carabidae							
	A	B	C	*Staphylinidae*	*Linyphiidae*	**Braconidae**	*Coccinellidae*	*Syrphidae*
1.Dispersal rate	7	6	5	4	2	**3**	1	1
2. Diet range	3	2	2	2	2	**1**	1	1
3. No. sprays per annum	2	2	3	2	3	**1**	1	1
4. Voltinism	2	2	2	2	1	**1**	1	1
5. Fecundity	3	3	3	2	2	**1**	1	1
Total score	17	15	15	12	10	**7**	5	5
Rank	1	2	2	4	5	**6**	7	7

Key: *Dispersal rates*: relative rates ranked (species with limited dispersal more at risk). *Diet range*: pest-specific 1, mainly consuming pest 2, polyphagous 3 (polyphagous species likely to complete life cycle in sprayed area and are more at risk than species entrained to the seasonal of pest species). *Number of sprays per annum*: exposed to 1-3 of the annual spray campaigns (the larger the number, the greater the risk). *Voltinism*: univoltine 2, multivoltine 1. *Fecundity*: low 3, intermediate 2, high 1. Carabid group A = autumn breeding, field overwintering species: group B = spring breeding, field overwintering species: group C = autumn or spring breeding, field overwintering species.

even though aphid parasitoids may be classed as low risk in terms of long-term susceptibility to pesticides, they may be of much greater importance for aphid control within a given season than certain carabid beetles that suffer relatively high risk. The ranking system could therefore move from being environmental-protection focused, to being more crop protection focused by taking beneficial capacity into account.

Results from the large field trial indicated that primary parasitoids and hyperparasitoids showed a progressive reinvasion into deltamethrin-treated plots from untreated areas in the same field (Chapter 7). This gradual equilibration may not represent dynamics under real agricultural practice where whole fields are sprayed and reservoirs of reinvaders are more distant. At present, the only practical way to correct for this is to ensure plots are as large and square as possible, with sampling occurring at the centre and for a limited duration after treatment (Sotherton *et al.*, 1988). The current recommendation by the Ministry of Agriculture, Fisheries and Food (Working document 7.7: Guideline to Study the Within-season Effects of Insecticides on Beneficial Arthropods in Cereals in Summer, MAFF/PSD) for minimum plot sizes of 1ha are likely to underestimate the pesticidal effects on Hymenoptera, because of their high dispersive capabilities.

Future risk assessments based on laboratory tests of susceptibility (e.g. Chapter 2; Hassan, 1989) could be improved by the incorporation of indices of dispersal rate as an important parameter in the estimation of pesticide hazard to selected beneficial organisms. From this current study, it could be predicted that aphid parasitoids, though intrinsically

highly susceptible to deltamethrin (Chapter 2), would be less likely to suffer serious side-effects in the field, compared to other beneficial invertebrates (e.g. Carabidae), as a result of their greater dispersal capabilities. However, data from such studies need to be interpreted with care as apparent "recovery" of populations through immigration may be rapid, but negative effects of the pesticide may be detectable in the next generations some months after spraying (Waage *et al.*, 1985; Godfray & Chan, 1990).

In this study, parasitoid reinvasion trends were crudely measured by the use of suction sampling and sticky trap catches. Results highlighted the importance of selecting appropriate sampling methodologies for the study in question. Precise measurements of parasitoid dispersal would present formidable technical problems. However, various attempts have involved releasing certain parasitoid species into a habitat where they do not occur naturally and plotting the distribution of recaptures (Papaj & Vet, 1990), and marking adult parasitoids with trace elements which can be detected on recapture (Hopper & Woolson, 1991). Such studies would prove expensive and time-consuming, however could provide opportunities in the future of accurately measuring parasitoid movements into insecticide-treated areas.

At present, large field-based experiments may quantify short-term hazard to parasitoids, however the realism of these within-field studies and scale of treatment requires further investigation with respect to patterns of reinvasion by different species. In addition, the role of field boundaries in the invertebrate recovery process needs to be taken into account. A further tier of testing, such as long-term monitoring during the initial period of pesticide registration approval, supplemented by models (e.g. Sherratt & Jepson, 1993) which incorporate pesticide applications to whole fields and whole farms might then provide a realistic prediction of hazard.

In conclusion, the continued goal of achieving an effective and robust approach to ecotoxicological risk analysis is important in order to provide a means of selecting appropriate chemicals and for using them in the most selective way, thus minimising non-target side-effects. The continued role of organisations such as the International Organisation of Biological Control (IOBC) in the design of laboratory, semi-field and field studies to screen a wide range of pesticides, efficiently and economically, remains crucial. However, before pesticides are accepted or rejected for use in certain cropping systems, the incorporation of more ecological characteristics of associated pests and natural enemies into screening tests may provide greater insight into the potential risks posed. Unfortunately, this entails greater time and effort which may be limiting factors within current pesticide screening/research organisations.

More research is urgently needed to aid the organisation of, and predict the consequences of, the likely reduction of pesticides within agriculture in the near future. For example, by the year 2000, Sweden plans to reduce pesticide usage to 50% of a base-line level, France is aiming to achieve supervised control and Spain is trying to reduce pesticide residues in export commodities (Jansma *et al.*, 1993). In other countries, the economic pressure on farmers to avoid the use of full rate insecticides is likely to be high, with a likely movement towards using chemicals at much lower rates simply to keep aphids in check rather than to clear fields completely (Mann *et al.*, 1991). It is hoped that through defining optimum dose rates and timings of pesticide applications, and understanding susceptibility and behaviour of pest and beneficial invertebrates, it may be possible to successfully incorporate chemicals and beneficial organisms in integrated pest management programmes of the future.

Overall, the approaches developed in this study to aid and improve current laboratory, semi-field and field toxicological experiments for aphid parasitoids in the cereal ecosystem (Table 8.1) may be adapted to many other chemicals, parasitoid species and cropping systems. A greater understanding of basic parasitoid behaviour and pesticide-parasitoid interactions is still needed in order to identify which parameters are most important. Further information should allow us to enter a more predictive mode from which results from laboratory bioassays can be applied to field situations.

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