INTERACTIONS BETWEEN APHIDS, THEIR INSECT AND FUNGAL NATURAL ENEMIES AND THE HOST PLANT

by

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ABSTRACT

Multitrophic and intraguild interactions influence the success of biological control. The interactions between *Acyrthosiphon pisum*, three natural enemies (*Pandora neoaphidis*, *Coccinella septempunctata* and *Aphidius ervi*) and the host plant, *Vicia faba*, were assessed. Volatiles released from aphid-damaged plants had a direct effect on *P. neoaphidis* indicating they may act as synomones. However, volatiles did not increase efficacy of the fungus suggesting it is not a bodyguard species. Transmission was greatest during plant colonisation by aphids and was not affected by plant condition. Infection by *P. neoaphidis* had a direct negative effect on the fitness of the aphid through reduced reproduction and early host death.

Avoidance of infected colonies by predators and parasitoids could reduce the effectiveness of guilds of natural enemies for biological control. However, at the laboratory scale *A. ervi* and *C. septempunctata* did not detect infection and entered and foraged in infected aphid colonies. *Aphidius ervi* spent longer searching for hosts on plants that had been damaged by aphid feeding (and were emitting aphid-induced species-specific volatiles) and this may increase fungal transmission. *Coccinella septempunctata* and *A. ervi* significantly reduced populations of *A. pisum* when introduced as individual species whereas *P. neoaphidis* had no effect on aphid population size. Foraging by both *C. septempunctata* and *A. ervi* increased the abundance and distribution of *P. neoaphidis* which may be sufficient to initiate an epizootic. The benefits of increased transmission by *C. septempunctata* outweighed the fitness costs to the fungus of intraguild predation. *Pandora neoaphidis* was associated with a decrease in the reproductive success of *A. ervi*, which was further reduced as the competitive advantage of the fungus increased. This could result in competitive exclusion of the parasitoid. Polytunnel experiments confirmed that *A. ervi* did not discriminate between infected and uninfected aphid colonies at this spatial scale. However, *A. ervi* did not incur a fitness cost from foraging in patches containing the fungus.

These results indicate that *C. septempunctata* and *P. neoaphidis* may be effective as multi-species biocontrol agents. In contrast, competition between *P. neoaphidis*

and *A. ervi* may reduce their overall effectiveness as control agents. Further work is required at larger spatial scales and over several generations of both the pest and natural enemy species to confirm these interactions. The implications of these results for the use of *P. neoaphidis* as part of a multi-species biological control program are discussed.

CHAPTER 1: INTRODUCTION

1.1 APHIDS AND NATURAL ENEMIES

1.1.1 Biology of aphids

There are in excess of 4000 species of aphids, infesting one in four plants in temperate regions (Dixon, 1998). Most aphid species have a similar morphology, with the majority of species being green or brown and having a narrow head and bulbous abdomen (Figure 1.1). The wings of alate aphids are usually clear with the front pair being longer than the hind pair. Aphids belonging to the Aphididae, the largest insect family in the United Kingdom, are characterised by having at least four oblique veins in the forewing and paired abdominal cuticles which secrete wax (Chinery, 1993).

Although most aphid species are autoecious (live on one host plant species), about 10% are heteroecious (over-winter on a primary host, and move to a secondary host during summer) (Dixon, 1998) (Figure 1.2). For example, the currant-lettuce aphid *Nasanovia ribisnigri* (Mosely) over-winters on the blackcurrant plant before moving to its summer host, lettuce, where it is regarded as a pest species. Parthenogenetic viviparous reproduction allows 'telescoping' of generations, i.e. embryos start developing before their mothers are born. This reduces the generation time of aphids resulting in high reproductive rates (Blackman and Eastop, 2000). For example, during a single season a hectare of field beans can give rise to 4000 million alate black-bean aphids, *Aphis fabae* (Scopoli) (Dixon, 1998). However, aphid populations are regulated by natural enemy species including, hymenopteran parasitoids, predators and entomopathogenic fungi.

Aphids feed by inserting their stylets into the plant and 'tapping' the sap. Different aphid species feed in different ways. *Aphis fabae* settles on veins and feeds on phloem sap whereas *Myzus persicae* (Sulzer) settles away from veins and feeds on both phloem sap and the contents of the spongy mesophyll (Lowe, 1967). Spatial distribution of individuals also differs between aphid species, for example, *A. fabae*

and *Acyrthosiphon pisum* (Harris) are found in compact aggregations on a plant whereas *M. persicae* are dispersed over the whole plant (Lowe, 1967).

Aphids cause direct damage to crops through feeding and indirect damage through the spread of plant viruses. In 1990 it was estimated that aphid transmitted barley-yellow-dwarf-virus caused crop loss to the value of US$40million p.a. in Australia and New Zealand alone (Johnstone *et al.*, 1990). Although aphids were controlled successfully using insecticides, intensive application of insecticides has lead to a global increase in the incidence and severity of insecticide resistance. For example, in Europe *M. persicae* has developed resistance to organophosphate and carbamate insecticides via elevated carboxyl esterase, to pyrethroid insecticides via knock-down resistance and to pirimicarb and triazamate insecticides via Modified AcetylCholinEsterase (MACE) resistance (Foster *et al.*, 1998, 1999). Resistance to insecticides and increased concerns of their impact on the environment has highlighted the need for alternative methods to control pest aphid species.

1.1.1.1 *Biology of* Acyrthosiphon pisum

The pea aphid, *Acyrthosiphon pisum*, is one of 250 species of aphids in the Superfamily Aphidoidea (Subfamily: Aphidinae) (Blackman and Eastop, 2000) (Figure 1.3). *Acyrthosiphon pisum* originated in the Palaearctic but is now found world-wide, and is mainly a pest of leguminous plants e.g. lucerne, sweet pea and broad bean (Milner, 1982). Like many other aphid species it is a vector of plant disease, vectoring more than 30 plant viruses (Blackman and Eastop, 2000; Milner, 1982).

Acyrthosiphon pisum is a heteroecious aphid species (Figure 1.2). Its primary hosts include vetch and crimson clover whilst its secondary hosts are mainly pea, bean and alfalfa. *Acyrthosiphon pisum* is a medium to large aphid (apterae 2.5-4.4mm, alate 2.3-4.3mm) which is usually green, although some genotypes are pink (Blackman and Eastop, 2000). *Acyrthosiphon pisum* are characterised by pale green or brown antennae which have a dark band between segments 3 and 4. Their siphunculi have dark tips and their legs, which are usually pale green or brown, have a dark band in the middle joint (HortFact, 1998) (Figure 1.1). Alate morphs

are very similar to apterous morphs, however, they have two pairs of wings that attach to the thorax and extend beyond the abdomen (HortFact, 1998). At 20-25°C, *A. pisum* takes seven days to pass through four instars before becoming an adult (HortFact, 1998). During summer, *A. pisum* reproduces parthenogenetically and produces between 5-15 nymphs per day for up to 15 days (HortFact, 1998).

1.1.2 Aphid natural enemies

1.1.2.1 *Biology of* Pandora neoaphidis

Pandora neoaphidis (Remaudière and Hennebert) Humber (Zygomycetina: Entomophthoraceae) is the most common fungal pathogen of aphids in temperate regions, and does not infect any other groups of arthropods (Glare and Milner, 1991) (Figure 1.4). *Pandora neoaphidis* has been recorded infecting over 70 species of aphids (Pell *et al.* 2001). Some authors refer to *P. neoaphidis* as *Erynia neoaphidis*, however, for the purpose of this thesis the name *Pandora neoaphidis* (as described by Humber, 1981) will be used. *Pandora neoaphidis* attacks many different species of pest and non-pest aphids and is capable of causing epizootics in field populations (Feng and Johnson, 1991; Feng *et al.*, 1999; Pell *et al.*, 2001; Pickering and Gutierrez, 1991; Shah, Clark and Pell, 2004). For example, epizootics have been recorded in populations of *A. fabae* and *M. persicae* (Pell *et al.*, 2001). In some cases, *P. neoaphidis* epizootics are more important than predators and parasites at controlling aphid numbers (Butt, Beckett and Wilding, 1990; Dean and Wilding, 1973). *Pandora neoaphidis* infections usually occur between April and December, being most common between May and August (Butt *et al.*, 1990; Keller, 1991; Wilding, 1970; Wilding and Perry, 1980).

The spores, or conidia, of *P. neoaphidis* are nearly ellipsoid in shape with a mean diameter of approximately 20.5μm (see section 3.4) (Hemmati *et al.*, 2001). Conidia are forcibly discharged from the host at a speed of $8.0ms^{-1}$ to a height of approximately 2mm (Hemmati *et al.*, 2001). This allows the conidia to escape the boundary layer and pass into free air where they can be dispersed via air currents (Hemmati *et al.*, 2001). Although conidia produced *in vivo* and *in vitro* are slightly different in size, with *in vivo* conidia being smaller, they are equally infectious

(Hemmati *et al.*, 2002; Milner and Bourne, 1983). A single *A. pisum* infected with *P. neoaphidis* can produce up to 400 000 conidia, with conidia number increasing as humidity increases (Wilding, 1969, 1981).

When conidia land they use topographical and chemical signals to determine whether they are on a suitable host (Hajek and St. Leger, 1994). Conidia that land on an unsuitable host remain infective for up to 14 days, however, infectivity decreases as time increases (Brobyn, Wilding and Clark, 1985). These conidia may form secondary conidia, which are more resistant to adverse abiotic conditions (Brobyn *et al.*, 1985). *Pandora neoaphidis* forms two types of secondary conidia. At lower temperatures (5°C) the secondary conidia are lemon shaped whereas at higher temperatures (21°C) they are more rounded, however, the reason for this is unknown (Brobyn *et al.*, 1985; Morgan *et al.*, 1995).

Conidia that land on a suitable host produce a germ tube within 6 hours, which then forms an appressorium that penetrates the host's cuticle (Butt *et al.*, 1990). The ability to invade directly through the host cuticle makes entomopathogenic fungi the most appropiate entomopathogen for microbial control of sucking pests (Poprawski, Legaspi and Parker, 1998). Once the fungus has penetrated the host cuticle it forms protoplasts that colonise the aphids body tissue, eventually leading to death (Butt *et al.*, 1990). Shortly before the aphid dies the fungus forms rhizoids that emerge from the ventral surface of the cuticle and anchor the cadaver to the leaf (Dean and Wilding, 1971). This prevents the cadaver from falling off the plant (which would remove it from the environment of transmission) and maximises the probability that the conidia are taken up in to the air current (Butt *et al.*, 1990). Under favourable conditions sporulation takes place.

Pandora neoaphidis requires a saturated atmosphere (usually greater than 90% relative humidity) in order to sporulate, germinate and penetrate the host cuticle (Dean and Wilding, 1971; Milner and Bourne, 1983; Morgan *et al.*, 1995; Wilding, 1969; Wilding, 1973). The optimum temperature for *P. neoaphidis* infection is approximately 20°C, with inhibition of infection occurring at 30°C (Hajek, 2001; Wilding, 1973). As temperature decreases, time to kill increases. For example, *P. neoaphidis* will infect and kill an adult *A. pisum* in 4 days at a constant temperature

of 20°C whereas at 5°C the fungus will take 17 days to infect and kill the aphid (Wilding, 1970). Temperature and humidity are therefore the major factors that influence whether or not an epizootic will occur (Hall and Bell, 1960; Wilding, 1970). Missionier, Robert and Thoizon (1970) showed that epizootics of entomopthoralean species occurred when the humidity was greater than 90% for 8 hours or, when it rained for more than 5 hours a day at a temperature of greater than 20°C. A field study by Wilding (1981) supported these findings. During the warm dry summers of 1975 and 1976, entomopthoralean species including *P. neoaphidis* that were introduced into fields to control the black bean aphid, *A. fabae*, failed to establish and spread. In contrast, during the summers of 1977 and 1978, which were cooler and moister summers, transmission of the fungus occurred and reduced the population size of *A. fabae* by approximately 45%. However, heavy rain can also be detrimental to the fungus. Pell, Tydeman and McCartney (1998b) demonstrated that rainfall removed conidia from leaves, and that heavy rainfall was capable of knocking cadavers off plants and on to the soil where they were destroyed.

At present it is not known how *P. neoaphidis* over-winters. No resting spores have been found that can survive winter months (Feng *et al.*, 1999). Feng *et al.* (1992) found 'spherical bodies' in cadavers of *A. pisum* infected with *P. neoaphidis* and hypothesised that these may be a mechanism for over-wintering. More recently Nielsen *et al.* (2003) suggested that *P. neoaphidis* may survive 'unfavourable conditions' in the form of thick walled conidia (loricoconidia), and that the production of these conidia was stimulated by changes within the host.

Susceptibility to *P. neoaphidis* is dependent on the species of host aphid, with there being some examples of resistance to *P. neoaphidis* (Shah *et al.* 2004). Milner (1982, 1985) found that in Australia there were two biotypes of *A. pisum*, one being susceptible and the other being resistant to *P. neoaphidis*. Bioassays showed that the susceptible strain of *A. pisum* was reduced by 94% when inoculated with *P. neoaphidis* whereas the resistant strain remained uninfected. Further bioassays indicated that the LC_{50} of the resistant strain was ten times greater than the susceptible strain. However, the resistance mechanism is unknown. Breise (1981) hypothesised that resistance may be due to either differences in the aphid's cuticular

biochemistry or the presence of metabolites that were toxic to the fungus. As *P. neoaphidis* is the most common pathogen of *A. pisum* in Australia and causes widespread epizootics, resistance could lead to problems in controlling aphid numbers in field populations (Milner, 1985). Resistance to *P. neoaphidis* is not limited to Australia, for example, Ferrari *et al.* (2001) found significant differences in the susceptibilities of different *A. pisum* clones to *P. neoaphidis* in UK field populations. A potential fitness cost to resistance was identified by Pickering and Gutierrez (1991) in a field population of *A. pisum* and the closely related species *Acyrthosiphon kondoi* (Shinji). In alfalfa fields, 34% of *A. pisum* were found to be infected with *P. neoaphidis* whereas only 6% of *A. kondoi* were infected with the fungus. However, in the absence of the fungus *A. pisum* was found to out-compete *A. kondoi.* Pickering and Gutierrez (1991) hypothesised that resistance to *P. neoaphidis* reduces the intrinsic fitness of *A. kondoi.*

1.1.2.2 *Biology of* Coccinella septempunctata

The seven-spot ladybird, *Coccinella septempunctata* (L.), is a generalist predator of aphids, and will predate all the species it encounters (Ferran and Dixon, 1993) (Figure 1.5). *Coccinella septempunctata* originated in the Paleoarctic but has the ability to disperse over large distances (Schaefer, Dysart and Specht, 1987). For example, *C. septempunctata* was first identified in the USA in 1958. As a result of natural spread and introductions for biological control it can now be found in 34 eastern and central states (Schaefer *et al.*, 1987). Wind systems aid the dispersal of *C. septempunctata* and may enable individuals to travel long distances. For example, *C. septempunctata* has been found on the island of Sable, Nova Scotia, which lies 175 km from the mainland (Schaefer *et al.*, 1987).

Coccinella septempunctata disperse from their hibernation sites during spring and search extensively for prey. After mating, females lay batches of yellow oval eggs attached to a substrate, for example a leaf (Hodek, 1973). After hatching the larvae pass through four instars. A pupa then forms which is attached to the substrate by its cauda (Hodek, 1973). If irritated, the head region of the pupa can move up and down (Hodek, 1973). Although the reason for this is unclear, it may be a defence against predation from other foraging arthropods. After pupation the adult beetle

emerges with soft yellow elytra which harden and attain their characteristic red and black aposomatic colouration. Aggregation and mating of *C. septempunctata* occurs during the summer before the return to hibernation sites in the autumn (Ferran and Dixon, 1993). *Coccinella septempunctata* usually hibernates in aggregations of 5-50 individuals in leaf litter, at the bases of plants and under stones (Schaefer *et al.*, 1987).

Adult *C. septempunctata* are aposomatically coloured and, when attacked, release the bitter tasting alkaloid coccinelline through femero-tibial pores in their legs (Agarwala and Dixon, 1992). The release of coccinelline is referred to as 'reflex bleeding'. Larvae are not aposomatically coloured and are preyed upon by conspecific and non-conspecific species, with the threat of cannibalism increasing as aphid numbers decrease (Agarwala and Dixon, 1992). Cannibalism decreases as larval instar increases, with unhatched eggs and freshly emerged larvae (that are attached to their egg by an anal disc) being the most under threat (Agarwala and Dixon 1992; Dixon, 1959). Inter-specific predation occurs between *C. septempunctata* and the two-spotted ladybird *Adalia bipunctata* (L.). To defend against this threat, *A. bipunctata* produces eggs which are toxic to *C. septempunctata.* Whereas *C. septempunctata* larvae that have consumed *A. bipunctata* eggs are likely to die, consumption of *C. septempunctata* eggs have no negative effects on the fitness of *A. bipunctata* (Agarwala and Dixon, 1992).

Coccinella septempunctata is positively phototactic, negatively geotactic and uses visual and chemical cues whilst foraging (Dixon, 1959; Ferran and Dixon, 1993; Fleschner, 1950; Obata, 1986). *Coccinella septempunctata* can detect prey visually from a distance of 7-10mm. Volatiles, such as aphid alarm pheromone ((*E*)-ß-farnesene) and those released from aphid-infested wheat plants, can be detected, and aphid honeydew has been shown to arrest foraging individuals (Al Abassi *et al.*, 2000; Carter and Dixon, 1984; Nakumata, 1984; Ninkovic, Al Abassi and Pettersson 2001; Stubbs, 1980) (see section 1.3.2.3).

When foraging, *C. septempunctata* searches extensively and intensively, utilising veins and surface irregularities to increase their chance of locating prey (Dixon, 1959; Ferran and Dixon, 1993). On capturing prey, *C. septempunctata* feeds

continuously whilst its maxillary palps are in contact with body fluid (Nakumata and Saito, 1985). Following consumption of prey, searching changes from extensive to intensive, which is characterised by an increase in turning rate, increased scanning movement and decreased searching speed (Carter and Dixon, 1982; Nakumata, 1982, 1984). If another prey item is not found within 70 seconds, the ladybird returns to extensive searching (Nakumata, 1982). Ladybirds have the ability to assess and respond to the quality of their surrounding environment and, although the response of individual ladybirds to aphid density is weak, the response of large numbers of ladybirds to aphid population density is strong (Hemptinne, Dixon and Coffin, 1992; Hemptinne *et al.*, 1993; Ives, Kareiva and Perry, 1993). As hunger increases, the handling time per prey item increases (Dixon, 1959). However, unlike parasitoids, there is no evidence that ladybirds can assess prey density through the use of volatile cues (Carter and Dixon, 1982).

1.1.2.3 *Biology of* Aphidius ervi

Aphids are attacked by hymenopteran parasitoids belonging to the families Aphidiinae and Aphelinidae (Müller *et al.*, 1999). The economic importance of parasitoids has increased with the development of agriculture and, since 1945, research has focused on the use of parasitoids for biological control (Stary, 1970). *Aphidius ervi* (Haliday) (Aphidiinae) is a hymenopteran parasitoid indigenous to Eurasia (Takada and Tada, 2000) (Figure 1.6). It has been introduced to many other parts of the world for biological control and, along with the closely related species *A. colemani* (Viereck), is commercially available for biological control (Poppy, Powell and Pennacchio, 1997b; Powell *et al.*, 1998). For example, *A. ervi* is used in Europe for the biological control of *Macrosiphum euphorbiae* (Thomas) on tomato and *Aulacorthum solani* (Kaltenbach) on sweet pepper in glasshouses (Poppy *et al.*, 1997b; Takada and Tada, 2000). *Aphidius ervi* has been recorded attacking over 20 aphid species on legumes, brassicas and cereals, including; *A. pisum, M. persicae* and *Sitobion avenae* (Fabricius) (Du *et al.*, 1997; Pennacchio *et al.*, 1994; Stary and Delfino, 1986; Takada and Tada, 2000).

Aphidius ervi is a koinobiont endoparasite (host continues to develop after oviposition) and a free-living wasp. The following is a simplified lifecycle of an

Aphidiinae parasitoid taken from Stary (1970). When a female parasitoid finds a suitable host aphid, oviposition takes place. The female inserts her ovipositor into the host aphid and deposits a single egg. For *A. ervi* this process takes approximately one second. Following oviposition the embryo develops and hatches to produce a larva. The larva passes through four instars. During instars I, II and III the larva feeds on liquid food and does not affect the vital organs of the host. It is during instar IV that the larva uses its mandibles to cause injury to the vital organs of the aphid before completely consuming the inside of the host. The larva then cuts a small hole in the aphid's cuticle and uses a secretion from its silk glands to attach the aphid to the substrate (usually a leaf). This prevents the aphid from falling to the ground and being destroyed. The larva then spins a cocoon using its silk glands. The cocoon is often referred to as the 'mummy'. A pre-pupa then forms inside the cocoon and transformation of larval tissue takes place. This is followed by the pupal stage during which pigmentation occurs. Finally, the adult parasitoid cuts a circular hole in the cocoon with its mandibles and emerges. At 18°C, the process takes approximately 10 days. The cycle then repeats, with there being 4-5 generations of *A. ervi* during a single field season (Cameron, Powell and Loxdale, 1984).

The searching behaviour of hymenopteran parasitoids is a non-random process and involves responses to environmental cues at varying spatial scales (Umoru, Powell and Clark, 1996). Whilst searching for suitable hosts, parasitoids go through five distinct steps; host habitat location, host location, host suitability (antennation and ovipositor probing) host acceptance (oviposition) and host regulation (Doutt 1964; Storeck *et al.*, 2000; Vinson, 1975). In order to reproduce successfully parasitoids must distinguish between suitable and unsuitable host species (Storeck *et al.*, 2000). Chemical cues originating from both host aphids and host-aphid infested plants play a role in both host finding and recognition (Storeck *et al.*, 2000; Wickremasinghe and Van Emden, 1992). Section 1.3.2.2 gives a detailed review on how *A. ervi* uses plant-derived volatile cues to locate hosts. Aphid honeydew is a short-range chemical cue used by several parasitoid species, including *A. ervi*, during host location. Current research indicates that honeydew acts as an arrestant, not an attractant. Wickremasinghe and Van Emden (1992) found that the response of *A. ervi* to plant odour was stronger than the response to the host aphid, honeydew, or

the host aphid and honeydew together. Therefore, under natural conditions honeydew may have an insignificant effect on the attraction of a foraging parasitoid to a plant. However, once parasitoids have reached a plant, honeydew has been found to have an effect on parasitoid foraging. Bouchard and Cloutier (1984) recorded longer search times for *Aphidius nigripes* (Ashmead) on honeydew contaminated plants, indicating that the honeydew was acting as an arrestant. Honeydew can also directly benefit foraging parasitoids and is, along with nectar and pollen, a food source for non-host feeding parasitoids (Budenberg, 1990; Hajek, 2004).

Once *Aphidius ervi* has found a plant containing suitable hosts it uses a variety of short-range chemical and visual cues during host examination (Battaglia *et al.*, 1993; Pennacchio *et al.*, 1994). Battaglia *et al.* (1993) induced an attack response from *A. ervi* on glass beads coated with host-aphid cornicle secretion, however, control glass beads were never attacked. Battaglia *et al.* (2000b) also demonstrated that *A. ervi* exhibits an attack response to host-aphid exudates, indicating that contact kairomones which elicit an attack response are present in the aphid's cuticle. A combination of host-aphid cornicle secretion and contact kairomones therefore appear to be used by *A. ervi* to detect suitable hosts. Although visual cues are not as specific as those received from chemical cues, they do have an effect on the oviposition behaviour of the parasitoid (Battaglia *et al.*, 2000b; Goff and Nault, 1984). Several aphid species occur in different colours morphs and these suffer from different levels of parasitism (Ankersmit Acreman and Dijkman, 1981; Michaud and Mackauer, 1994, 1995). For example, pink morphs of the alfalfa aphid *Macrosiphum creeli* (Davis), are attacked less frequently than green morphs (Michaud and Mackauer, 1995). Experiments have demonstrated that *A. ervi* showed a strong attack response to green *A. pisum* or crushed *A. pisum*, but not to *A. pisum* blackened by chemical treatments (Battaglia *et al.*, 2000a). Further experiments indicated that *A. ervi* attacks aphids if their colour reflects light with a wavelength of 560-700nm (Battaglia *et al.*, 2000a).

Parasitoids that enter aphid colonies and locate aphids show a preference for the host species on which they have been reared (Cameron *et al.*, 1984). It was demonstrated by Van Emden *et al.* (1996) that *Aphidius rhopalosiphi* (De Stephani-

Perez), used chemical cues from their mummy cases to recognise the plant cultivar on which they had been reared. Emergence conditioning may therefore also play a role in the attack response of *A. ervi.* However, this preference can be modified, and once a parasitoid changes its host, parasitism increases with time spent on the new host (Cameron *et al.*, 1984; Chow, 2000; Powell and Wright, 1988). For example, Cameron *et al.* (1984) found that *A. ervi* reared on *A. pisum* never attacked *Microlophium carnosum* (Buckton) and significantly fewer mummies were found on *S. avenae.* However, after 4-5 generations on *S. avenae*, the parasitisation of *A. ervi* was the same as that on its former host, *A. pisum.*

1.2 BIOLOGICAL CONTROL

1.2.1 Biological control

The first recorded successful example of biological control dates back to 1888 when the vedalia beetle, *Rodalia cardinalis* (Mulsant), was introduced to California from Australia by Albert Koebele to protect the citrus industry from pests including cottony-cushion scale, *Icerya purchasi* (Maskell) (Howath, 1991; Luck, 1990). Between 1888 and 1986 there were 1162 species introductions, with 25% giving successful regulation and 69% giving partial control (Luck, 1990). However, as early workers only recorded data from successful introductions, the actual number of species introductions are unknown (Howarth, 1991). Some authors claim a success rate of only 10-20% (Howarth, 1991).

Biological control is seen as a 'green approach' to pest management and may be the only affordable option for pest control in developing countries (Cory and Myers, 2000). The aim of biological control programmes is to create 'a balance between pests and their natural enemies which reduces the pest population to a non-pest level' (Bellows, 2001). The process involves the selection, screening and introduction of a biological control agent (Bellows, 2001). There are three major categories of biological control, classical, augmentation and conservation. Classical biological control is the introduction of an exotic (non-native) species, aiming to permanently control an exotic pest. Augmentative biological control is the use of mass produced individuals to supplement indigenous populations. Introductions

can be either inundative releases (use of a large quantity of the introduced species to control the pest) or inoculation releases (use of a small quantity of the introduced species to establish a population and their offspring used to control the pest). Conservation biological control is the identification and preservation of indigenous natural enemy species (Luck, 1990).

There are four main groups of natural enemies against insects: entomopathogenic nematodes, insect pathogens, parasitoids and predators. Species from these groups can be used as single species or multi-species control agents against herbivorous pests. Examples of biological control programmes using single and multiple species of entomopathogens, coccinellids (predators) and hymenopteran parasitoids are described in more detail in sections 1.2.2-1.2.4.

1.2.2 Use of entomopathogens for biological control

Research on the use of entomopathogens for biological control dates back to the late 19th century (Lacey *et al.*, 2001), however, in the past ten years the number of entomopathogens registered for biological control has risen greatly (James *et al.*, 1998). *Bacillus thuringiensis* (Berliner) is now widely used for the biological control of lepidopteran, coleopteran and dipteran pest species in orchards, glasshouses, forests and crops (Howarth, 1991; Lacey *et al.*, 2001). Although *B. thuringiensis* occupies the largest share of the entomopathogen biological control market (US$75-125 million/ year), entomopathogens only occupy 1.0-1.5% of the total crop protection market (Lacey *et al.*, 2001). Potentially, this share could decrease through both competition from transgenic plants and concerns that many entomopathogens may affect non-target organisms (James and Lighthart, 1994; Lacey *et al.*, 2001; Shah and Pell, 2003).

At present there are more than 700 described species of entomopathogenic fungi, however, there are few fungal-biopesticides commercially available in Europe. Current research is focusing on the use of entomopathogenic fungi for the control of a number of pest species (Lacey *et al.*, 2001). Available biopesticides include Vertalec®, which is based on *Lecanicillium longisporum* (Zimmerman) and is used widely to control aphids on chrysanthemum plants in glasshouses, and

BotaniGard®, which is a formulated isolate of *Beauveria bassiana* (Balsamo) Vuillemin, which is used to control grasshoppers, whiteflies, thrips and aphids (Milner, 1997; Shah and Pell, 2003). Once a suitable entomopathogen has been identified, it can be exploited in one of the following strategies; 1) epizootics of the pathogen can be encouraged from natural populations (conservation biocontrol), 2) the pathogen could be introduced into crops to initiate an epizootic (inoculative augmentation), 3) the pathogen could be used as part of a classical biological scheme, 4) the pathogen could be applied as a sprayable bioinsecticide (inundative augmentation) (Milner, 1997).

The majority of fungal entomopathogens are specific to particular species or related groups of organisms (Milner, 1997). Most entomopathogenic fungi enter insects directly through their exoskeleton. They are, therefore, the only significant entomopathogen to attack sucking insects (Lacey, Fransen and Carruthers, 1996; Latge and Papierok, 1988). Rapid infection and active discharge of infective conidia that have a short generation time maximise the potential of an epizootic occurring (Milner, 1997). However, an epizootic requires optimal abiotic and biotic conditions, which makes them unpredictable. Therefore, if entomopathogens of aphid pest species are to be used for biological control, detailed knowledge is required on the abiotic and biotic factors that govern epizootics, which include temperature and humidity, solar radiation, host behaviour, inoculum densities and pathogen vigour (Fuxa and Tanada, 1987; Harper, 1987; Wilding, 1981).

Several species of entomopathogenic fungi have been used successfully as biological control agents. *Entomophaga maimaiga* (Humber) was introduced as a classical biocontrol agent to the USA in 1910 and 1911 in an attempt to control the gypsy moth, *Lymantria dispar* (L.), which was responsible for the defoliation of up to 13 million acres of forest in north-eastern USA (Hajek, 2004; Lacey *et al.*, 2001). Epizootics of *E. maimaiga* caused significant decreases in populations of *L. dispar*, and in some cases, the complete collapse of populations (Hajek, 2004; Lacey *et al.*, 2001). A second example of a successful classical introduction was in Australia where *Zoophthora radicans* (Brefeld) Batko was introduced to control the spotted alfalfa aphid, *Therioaphis maculata* f. *maculata* (Busk) (Milner, 1997; Milner and Soper, 1981). Entomopathogenic fungi have also been used as part of augmentation

biological control programmes. *Metarhizium anisopliae* (Metschnikoff) has been formulated into the commercially available product 'Green Muscle' for use against outbreaks of locusts and grasshoppers in Africa (Shah and Pell, 2003). Wilding (1981) added laboratory produced *P. neoaphidis* and *Neozygites fresenii* (Nowakowski) to field populations of *A. fabae* in an attempt to supplement the naturally occurring fungus populations and initiate an epizootic. Although during the cool and moist seasons of 1977 and 1978 the fungi spread rapidy, this was not found during the warm dry seasons of 1975 and 1976, supporting the previous statement that abiotic conditions affect whether or not entomopathogenic fungi can be successfully used as biological control agents.

The entomopathogenic fungus *P. neoaphidis* is currently being developed for biological control. Once *P. neoaphidis* epizootics are initiated they have the potential to significantly decrease aphid populations. For example, Silcev (1992) found that 73.5% of diseased aphids sampled in Yugoslavia were infected with *P. neoaphidis* and, Feng, Johnson and Halbert (1991) found 43.9% of diseased aphids sampled in USA to be infected with *P. neoaphidis*. There are four natural sources of inoculum that could initiate a *P. neoaphidis* epizootic: soil, infected apterous aphids on crops, infected alate aphids and other aphid natural enemies (Roy and Pell, 2000; Roy, Pell and Alderson, 2001; Wilding, 1981). If the inoculum was introduced earlier or in greater quantities, *P. neoaphidis* epizootics may occur earlier and more predictably in the season (Wilding, 1981). At present *P. neoaphidis* cannot be efficiently mass-produced or stored and so the use of *P. neoaphidis* for biological control may not be cost-effective (Milner, 1997). However, through habitat manipulation and the formation of refugia (conservation biocontrol), both the concentration and early season activity of *P. neoaphidis* may be increased, and this may help control aphid populations in crops adjacent to the field margin (Shah and Pell, 2003).

1.2.3 Use of coccinellids and parasitoids for biological control

Predators and parasitoids are widely used in both classical and augmentative biological control programs (see Tables 1.1 and 1.2 for common predator and parasitoids released as augmentative biological agents). There are three major

groups of parasitoids: parasitic wasps (Hymenoptera), parasitic flies (Diptera) and parasitic beetles (Coleoptera), with parasitic wasps being the group most commonly used in biological control programmes, accounting for approximately 85% of classical releases. Predators used for biological control include ladybirds (Order Coleoptera), true bugs (Order Hemiptera), lacewings (Order Neuroptera) and predatory mites (Order Acarina) (Hajek, 2004). A detailed review on the use of predators and parasitoids for biological control is beyond the scope of this thesis, however, brief examples of the use of coccinellids and parasitoid wasps as biological control agents will now follow.

There have been more than 400 species of coccinellids and 1300 species of parasitoids released as biological control agents (Kindlmann and Dixon, 2001). Coccinellids and parasitoid wasps have been introduced as classical biological control agents against herbivorous pests in both glasshouses and the field. For example, seven species of coccinellids, including *R. cardinalis, Cryptognatha nodiceps* (Marshall) and *Hyperaspis pantherina* (Fürsch-Kenya) are used for the biological control of scales, three species (*Serangium parcesetosum* (Sicard), *Serangium* n. sp. and *Clitostethus arcuatus* (Rossi)) are currently being evaluated at control agents against whitefly in the USA and, numerous coccinellid species, including *C. septempunctata* and *Harmonia axyridis* (Pallas), have been released as control agents against psyllids and aphids (Obrycki and Kring, 1998). The parasitoid *A. ervi* was imported to Japan to control *M. euphorbiae* on glasshouse tomatoes, and the related species, *A. colemai*, is to be introduced into Japan to control *Aphis gossypi* Glover and *M. persicae* (Takada, 1998). In Europe *A. ervi* is used to control *M. euphorbiae* on tomato and *A. solani* on sweet pepper (Takada and Tada, 2000). Examples of classical control in the field include that of the parasitoid *Apoanagyrus lopezi* (De Santis), which was introduced from South America to central Africa to control the cassava mealybug, *Phenacoccus manihoti* (Matile-Ferrero), which had become a pest on cassava plants (Hajek, 2004).

In some cases a specific biotype of a parasitoid species is required to achieve the successful control of a pest. This is illustrated in the following example from Hajek (2004). The Walnut aphid, *Chromaphis juglandicola* (Kaltenbach), is a pest of walnut trees and, although it was native to the 'old world', is now found wherever

walnut trees are planted. The parasitoid *Trioxys pallidus* (Haliday), was introduced from France to control outbreaks of *C. juglandicola* in California. However, as the biotype failed to establish the initial introduction was a failure. In contrast to the French biotype, a second biotype of the same species was introduced from Iran and became an established and successful control agent. It was hypothesised that the weather conditions in Iran were similar to those in California (hot summers and cold winters) and, therefore, the Iranian biotype was better adapted to foraging and survival in California than the French biotype. Predators are still responsible for the majority of biological control in California (Frazer *et al.*, 1981). In the 1970's, the USDA imported the coccinellid, *C. septempunctata*, as a biological control agent (Chang, 1996; Howarth, 1991) and in 1994 the Asian ladybird *H. axyridis* was imported into orchard yards in eastern USA to control aphids (Cory and Myers, 2000).

The egg parasitoids *Trichogramma* spp. are widely used as part of augmentation biological control programmes against lepidopteran pests in numerous crops, including cereals, cotton and sugar cane (van Lenteren and Bueno, 2003). It is estimated that 10 million, 2 million and 1.5 million hectares of crops are under control using these species in the former USSR, China and Mexico respectively. Coccinellids are also used in augmentation biological control programmes in both glasshouses and the field. For example, *Hippodamia convergens* (Guerin-Meneville) is used to control aphids in field crops in California and Texas and a second coccinellid species, *Delphastus pusillus* (LeConte), is used to control *Bemisia* spp. in glasshouses and the field (Debach, 1974; Obrycki and Kring, 1998). As a result of several successful augmentation programmes, *D. pusillus* is now available from over 25 commercial sources (Obrycki and Kring, 1998).

Not all species released as biological control agents are successful and, without detailed knowledge of the biology of the introduced species, releases could end in failure. For example, coccinellids used as biological control agents can either be collected from over-wintering sites (e.g. *H. convergens*) or mass-reared in insectaries (e.g. *R. cardinalis* and *Stethorus picipes* (Casey)) (Obrycki and Kring, 1998). The ladybeetle *H. convergens* is collected from over-wintering aggregations in western USA and is sold to farmers and horticulturists in California and Texas

(Debach, 1974). However, these insects naturally disperse from over-wintering sites (and therefore from biological control release sites) and aggregate after ovarian diapause in order to mate and reproduce (Debach, 1974). Therefore, prior to

Table 1.1 Common predatory arthropods used for inoculative or inundative releases (taken from Hajek, 2004).

Order	Species	Active stages[1]	Use for control
True bugs	*Orius insidiosos*	N, A	Thrips
Beetles	*Cryptolaemus montrouzieri*	L, A	Mealybugs
	Carcinops pumilo	L, A	Fly larvae
Lacewings	*Chrysopa rufilabris*	L	Aphids
Flies	*Aphidoletes aphidimyza*	L	Aphids
Mites	*Hypoaspis miles*	N, A	Thrips, fungus gnats
	Mesoseiulus longipes	N, A	Spider mites
	Neoseiulus californicus	N, A	Spider mites
	Neoseiulus cucumeris	N, A	Thrips
	Neoseiulus fallacies	N, A	Spider mites
	Phytoseiulus persimilis	N, A	Spider mites

[1]N= Nymph, L= Larvae and A= adult

Table 1.2 Common parasitic wasps used for augmentative releases (taken from Hajek, 2004).

Group	Species	Host	Use area
Ichneumonoidea			
Aphidiidae	*Aphidius colemani*	Aphids	Indoors
	Aphidius matricariae	Aphids	Indoors
Chalcidoidea			
Aphelinidae	*Aphytis melinus*	Scale insects	Outdoors
	Encarsia formosa	Whiteflies	Indoors
	Eretmocerus eremicus	Whiteflies	Indoors
Pteromalidae	*Muscidifurax raptor*	House flies	Indoors
	Muscidifurax raptorellus	House flies	Indoors
	Muscidifurax zaraptor	House flies	Indoors
	Nasonia vitripennis	House flies	Indoors
	Spalangia cameroni	House flies	Indoors
Trichogrammatidae	*Trichogramma brassicae*	Moth eggs	Outdoors
	Trichogramma minutum	Moth eggs	Outdoors
	Trichogramma ostriniae	Moth eggs	Outdoors
	Trichogramma pretiosum	Moth eggs	Outdoors

purchase, insectaries that provide *H. convergens* beetles for biological control are required to feed the beetle a diet which ensures that ovarian diapause has finished and that the beetles are ready to reproduce. This will prevent subsequent dispersal and allow the ladybeetles to control the target pest. However, in a recent review of coccinellids used in biological control by Obrycki and Kring (1998), there was no evidence of the effectiveness of mass collected *H. convergen* to support their use as biological agents against aphids. Potentially, the use of coccinellids collected from over-wintering sites could have a negative effect on natural populations of coccinellids from both the collection and target site. For example, a reduction in the population size of the local population to a threshold from which it can not recover and, the increased distribution of parasitoids and microsporidian pathogens (Obrycki and Kring, 1998).

1.2.4 Negative impacts of biological control

Application of a biological control agent may not always be successful, and in some cases may have a negative impact. For example, introduced species may fail to control a pest, they may synergistically interact with other species to increase the pest problem, affect public health or attack non-target organisms (Howarth, 1991). To date, biological control has been implicated in the extinction of nearly 100 species (Howarth, 1991). However, some authors consider the impact on the environment as a result of biological control agents to be negligible relative to the effects of conventional agriculture, urban development and resource extraction (Meffe and Carroll, 1995; Primack, 1993). Four examples of species introductions that have resulted in a negative impact are described below:

As described in section 1.2.3, the parasitoid *T. pallidus* was introduced from Iran to California to control the walnut aphid, *C. juglandicola*. Although the parasitoid failed to control the aphid in the first season of its introduction, in subsequent seasons it successfully controlled aphid numbers. However, after the introduction of *T. pallidus*, fifteen natural predator species decreased in number (Frazer and van den Bosch, 1973). In this example, the parasitoid was more efficient than the native predator species. In 1994, the ladybird *H. axyridis* was released in the USA to control aphid numbers in orchard yards (Cory and Myers, 2000; Howarth, 1991).

Harmonia axyridis successfully controlled aphid numbers at the expense of the cerciclomid fly *Apdidoletes aphidmyza* (Rondani) and the coccinellid *C. septempunctata*, which was also introduced into the USA and has been implicated in excluding native species (Cory and Myers, 2000; Howarth, 1991). The seed-feeding weevil, *Rhinocyllus conicus* (Froelich), was introduced into northern USA to control exotic thistles of the genus *Carduus*. However, *R. conicus* also attacked thistles from the genus *Cirsium* and caused numbers of the picturewinged fly, *Paracantha culta* (Wiedemann) to decrease (Cory and Myers, 2000). In some cases it is not direct introductions that have detrimental effects on the local flora and fauna. Cactoblastic moths were successfully introduced from their native South America to Australia to control the prickly pear cacti. As a result of this success cactoblasic moths were introduced into the Caribbean where they were accidentally transported to Florida. Cactoblastic moth have been reported attacking five native species of cactus in the USA, including the rare semaphore cactus, *Optunia spinosissima* (Martyn) Mill (Cory and Myers, 2000). Commercial cacti growers are now under threat from cactoblastic moths (Cory and Myers, 2000).

1.3 TROPHIC INTERACTIONS

1.3.1 Intraguild interactions

A guild is made up of all the taxa in a community that use similar resources (Polis and Holt, 1992). Intraguild interactions occur between protagonists that occupy the same trophic level and compete for similar prey/hosts and may have synergistic, additive or non-additive effects on the species involved (Brodeur and Rosenheim, 2000; Ferguson and Stiling, 1996; Rosenheim *et al.*, 1995). Intraguild predation is the killing and eating of species within the same guild and is an extreme example of interference between natural enemy species (Hindayana *et al.*, 2001; Polis and Holt, 1992). The aggressor is the intraguild predator and the victim the intraguild prey (Meyhofer and Hindayana, 2000). Intraguild predation differs from classical predation in that it reduces competition for the aggressor species (Polis and Holt, 1992; Polis, Myers and Holt, 1989). For example, ladybirds and parasitoids compete for aphids, the ladybird using the aphid as a food source and the parasitoid using the aphid to complete its lifecycle. If the ladybird eats a parasitized aphid, it

not only gains a food source but also removes the developing parasitoid from the system. Intraguild predation may have indirect effects on other trophic levels. By removing a competing predator (competitive displacement), total predation may decrease, therefore increasing the population size of the herbivorous pest and reducing the fitness of the host plant (Rosenheim, 1998; Rosenheim *et al.*, 1995; Rosenheim, Wilhoit and Armer, 1993).

Intraguild interactions have been recorded between many taxa, including; multiple parasitoid species, nematodes and parasitoids, coccinellids and parasitoids, coccinellids and fungi, and, parasitoids and fungi (Colfer and Rosenheim, 1995, 2001; Ferguson and Stiling, 1996; Fuentes-Contreras, Pell and Niemeyer, 1998; Furlong and Pell, 1996; Meyhofer and Hindayana, 2000; Roy and Pell, 2000; Roy 1997; Sher, Parrella and Kaya, 2000; Silcev 1992). It should be noted that predator-parasitoid and predator-fungi intraguild interactions differ from those interactions between multiple parasitoid species or parasitoids and fungi. When predators consume parasitized or fungus-infected herbivores, intraguild predation occurs. As parasitoid-parasitoid and parasitoid-fungus intraguild interactions involve competition within a host, intraguild 'competition' is occurring.

1.3.1.1 *Intraguild predation*

Adult parasitoids and parasitoid larvae developing in aphid hosts are consumed by aphidophagous predators (Al-Rawry, Kaddou and Stary, 1969; Frazer and van den Bosch, 1973). As parasitoid mummies are sessile they have an increased susceptibility to attack (Meyhofer and Hindayana, 2000). Wheeler *et al.* (1968) found that mummified *A. pisum* were predated by eleven different predators belonging to the Neuroptera, Coleoptera and Hemiptera. Whereas aphidophagous predators consume their prey and remove them from the system, parasitized and unparasitized aphids are equally susceptible to predation (Lawton and Hassell, 1981). Therefore, predators remove the potential for parasitisation whereas parasitisation does not remove the potential for predation (Memmott, Godfray and Bolton, 1993). Predation of developing parasitoids by foraging predators from the same guild is referred to as asymmetric intraguild predation. For example unparasitized *A. fabae* and parasitized individuals containing developing larvae of

the parasitoid *Lysiphlebus fabrum* (Marshall) were found to be equally susceptible to intraguild predation from a variety of aphidophagous predators including; *C. septempunctata*, *C. carnea* and *Episyrphus balteatus* (De Geer) (Lawton and Hassell, 1981; Memmott *et al.*, 1993; Meyhofer and Klug, 2002; Wells, McPherson and Ruberson, 2001). Discrimination between unparasitised and parasitized aphids only occurred after mummification. The occurrence of intraguild predation is highly dependent on the species of the intraguild predator. Mummies of the cotton aphid, *A. gossypii*, containing *Lysiphlebus testaceipes* (Cresson) larvae were susceptible to predation by *H. convergens* but not predation by a second coccinellid species, *Scymnus ioewii* (Mulsant) (Wells *et al.*, 2001). Asymmetric intraguild predation may reduce the effectiveness of using guilds of predators and parasitoids for biological control. For example, mummies of the parasitoid *Aphidius floridaensis* (Smith) are consumed by the coccinellid *Cycloneda sanguinea* (L.) resulting in reduced control of the salt-marsh aphid, *Dactynotus* sp. (Ferguson and Stiling, 1996).

Predators themselves are susceptible to intraguild predation, becoming the intraguild prey. Intraguild predation between herbivore predators is influenced by several factors, including; the presence of alternate prey species and the developmental stage of the intraguild prey. The predatory mite *Typhlodromalus manihoti* (Moraes) is only an intraguild predator of *Typhlodromalus aripo* (DeLeon) larvae when there is a low density of the pest mite *Mononychellus tanajoa* (Bondar) (Onzo *et al.*, 2004). A second example is that of the spined soldier bug *Podisus maculiventris* (Say) which is an intraguild predator of eggs and larvae of the coccinellid *H. axyridis* and, a predator of eggs of a second coccinellid species, *Coleomegilla maculata* Lengi (De Clercq *et al.*, 2003; Mallampalli, Castellanos and Barbosa, 2002). In both these example *P. maculiventris* was not observed predating adult coccinellids, indicating that intraguild predation is developmental stage dependent. Both *P. maculiventris* and *H. axyridis* are predators of both the lepidoteran *Spodoptera littoralis* (Boisduval) and the aphid *M. persicae*. Although low levels of intraguild predation occurred in the presence of *S. littoralis*, it was found to increase in the absence of the lepidopteran (De Clercq *et al.*, 2003). In the absence of *S. littoralis*, the presence of the alternate prey species, *M. persicae*, did not reduce the intraguild predation of *P. maculiventris* on *H.*

axyridis (De Clercq *et al.* 2003). It was found that nymphs of *P. maculiventris* were unable to complete their development when fed on a diet of aphids alone. However, this developmental process could be successfully completed when fed on a diet of larval *H. axyridis* (De Clercq *et al.* 2003). Therefore, in the absence of a preferred prey species, intraguild predation may be required for a predator to complete its development.

A single intraguild predator species can have detrimental effects on several other predator species. The red imported fire ant, *Solenopsis invicta* (Buren), is an invasive species found in southern United States, and is increasing its range both east and westwards (Eubanks *et al.*, 2002). In glasshouse studies *S. invicta* had negative effects on 12 of 13 natural enemies of pests found on cotton, including; *C. septempunctata*, *H. convergens* and *Chrysoperla carnea* (Stephens) (Eubanks *et al.*, 2002). However, intraguild predation could be reduced though the suppression of *S. invicta* populations using fire ant bait (Eubanks *et al.*, 2002). In contrast, the introduction of an intraguild predator may benefit the native beneficial arthropod community. For example, the dominance of the exotic coccinellid *C. septempunctata* as a natural enemy of pest species in apple orchards in North America was reduced following the invasion of a second exotic coccinellid species, *H. axyridis* (Brown, 2003). The decreased dominance of *C. septempunctata* observed was a result of both interference competition and intraguild predation. This resulted in a resurgence of the native coccinellid population without decreasing the control of the pest species (Brown, 2003). Therefore, intraguild predators which have a negative impact on beneficial arthropods can be controlled through the application of an insecticide or the introduction of an alternative species.

Some species avoid intraguild predation by detecting and avoiding the presence of an intraguild predator/ competitor. The effectiveness of *A. ervi* for the biological control of *A. pisum* was found to be reduced due to the intraguild predation of *A. ervi* mummies by carabids (Snyder and Ives, 2001). However, *A. ervi* reduces the asymmetric competitive advantage of foraging predators by detecting and avoiding aphid colonies where a predator is present. For example, *A. ervi* avoids predation from *C. septempunctata* by spending less time searching in areas where it detects the presence of the coccinellid (Taylor, Müller and Godfray, 1998). Further

research has shown that foraging *C. septempunctata* larvae leave 'footprints' which contain the hydrocarbons n-tricosane ($C_{23}H_{48}$) and n-pentacosane ($C_{25}H_{52}$) and, that it is these footprints which have a repellent effect on *A. ervi* (Nakashima *et al.* 2004).

1.3.1.2 *Insect-fungus interactions*

Although most studies on interspecific competition are between closely related species, competition can occur between species from different kingdoms (Hochberg and Lawton, 1990). Inter-kingdom competition occurs between many taxa, including parasitoids and fungus, coccinellids and fungus and nematodes and parasitoids (Fuentes-Contreras *et al.*, 1998; Furlong and Pell, 1996, 2000; Lacey, Unruh and Headrick, 2003; Pell *et al.*, 1997; Roy and Pell, 2000; Sher, Parrella and Kaya, 2000).

Direct (fungus-parasitoid) and indirect (fungus-prey-parasitoid) intraguild interactions may occur between parasitoids and fungal pathogens (Brodeur and Rosenheim, 2000; Brooks, 1993). The fungal pathogens *M. anisopliae* and *B. bassiana* are under development to control grasshoppers and locusts, however, these fungi also show high levels of infectivity towards the parasitoids *Bracon hebetor* (Say) and *Apoanagyrus lopezi* (De Santis), which are also natural enemies of grasshoppers and locust (direct intraguild interaction) (Danfa and van der Valk, 1999). The parasitoids *Cotesia plutellae* (Kurdjumov) and *Diadegma semiclausum* (Horstmann) are both natural enemies of the diamondback moth, *Plutella xylostella* (L.). The entomopathogenic fungus *Z. radicans* is also a natural enemy of *P. xylostella*, and is being developed as part of an integrated pest management system against the moth (Furlong and Pell 1996, 2001; Furlong *et al.*, 1995; Pell *et al.*, 1993). *Diadegma semiclausum* was susceptible to *Z. radicans*, the fungus therefore having a direct effect on the parasitoid. Although *D. semiclausam* is 100 times less susceptible to *Z. radicans* than *P. xylostella* and, infected parasitoids were never found in the field, it still raises questions on the safety of using *Z. radicans* for the biological control of *P. xylostella* (Furlong and Pell, 1996). In contrast *C. plutellae* was not susceptible to *Z. radicans* (Furlong and Pell, 1996). Therefore, parasitoid species differ in their susceptibility to pathogens. It was also found that the

presence of *D. semiclausum* increased the transmission of *Z. radicans* whereas *C. plutellae* had no effect on the population size of the fungus. The increased transmission is thought to be a result of *D. semiclausum* increasing the movement of *P. xylostella* larvae and therefore increasing the potential of contact with infective conidia. Although *C. plutellae* increased the movement of larval *P. xylostella*, the increase was not sufficient to result in enhanced contact with infective conidia. A similar result to this was found by Fuentes-Contreras *et al.* (1998) where the parasitoid *A. rhopalosiphi* increased the transmission of *P. neoaphidis*, but did not act as a vector. In these examples the parasitoid is indirectly interacting with the fungus.

The outcome of indirect interspecific competition between a parasitoid and a fungal pathogen for a host insect is largely dependent on the life-cycle of the species involved. This was demonstrated by Powell *et al.* (1986) who studied the interactions between *A. rhopalosiphi* and *P. neoaphidis*. It was found that at 20°C *P. neoaphidis* took 3-4 days to complete its life-cycle, whereas *A. rhopalosiphi* took 8-9 days. If fungal infection occurred within four days of parasitisation the fungus out-competed the parasitoid. However, if fungal infection took place five or more days after parasitisation, the parasitoid out-competed the fungus. Although the fungus did not directly infect the developing parasitoid, it is thought that it deprives the parasitoid of food (Brodeur and Rosenheim 2000; Powell *et al.*, 1986). However, this competitive interaction will only take place if the parasitoid fails to detect the presence of the fungus within the host-aphid. Brobyn, Clark and Wilding (1988) found that *A. rhopalosiphi* would not attempt to oviposit in *P. neoaphidis*-sporulating cadavers and that the number of ovipositional attempts on aphids infected with *P. neoaphidis* for 3 days was significantly fewer than that on uninfected aphids and aphids infected with the fungus for up to 2 days. It was hypothesised that *A. rhopalosiphi* had a 'weakly developed' ability to detect the fungus and, that this ability may be due to fungal infection having a negative effect on the production of contact kairomones by the aphid.

Parasitisation may increase or decrease the susceptibility of an aphid to an entomopathogen. Fransen and van Lenteren (1994) found that competitive interactions occur between the entomopathogenic fungus *A. aleyrodis* and the

parasitoid *E. formosa* for the whitefly host, *T. vaporariorum.* If fungal infection occurred within three days of parasitisation there was a significant reduction in the number of parasitized hosts. However, if fungal infection occurred 4, 7 and 10 days after parasitisation there were no effects on the number of parasitized hosts. The decreased susceptibility of the parasitoid to the fungus coincided with the hatching of the parasitoid larva and it is thought that this may have induced changes in the host which altered its susceptibility to the fungus, for example, changes in the cuticle of the whitefly which reduced its susceptibility to the fungus or the production and release of compounds in response to the parasitoid larva which also have fungitoxic properties (Fransen and van Lenteren, 1994). In contrast, larvae of the bollworm *Heliothis zea* (Boddie) that have been parasitized by the braconid *Microplitis croceipes* (Cresson) have an increased susceptibility to infection by the fungus *Nomuraea rileyi* (Farlow) (King and Bell, 1978). Unlike the examples described previously where the outcome of competition for a host results in the successful development of either the parasitoid or the fungus, both the parasitoid and the fungus can develop successfully in *H. zea* that are inoculated with *N. rileyi* three to seven days after parasitisation (King and Bell, 1978).

Although parasitoids and fungi are intraguild competitors, they could still be used together for biological control. Hochberg, Hassell and May's (1990) model showing the effect of using parasitoids and pathogens for biological control indicated that the following four criteria were required for a successful biological control programme; the pathogen must have external stages that can bridge the intergenerational gap, attacks by the parasitoid and pathogen need to be clumped, both parasitoids and pathogens must have a high rate of searching/transmission, and there needs to be a degree of timing in the overlap of infection that is not one sided.

Entompathogenic fungi can have direct detrimental effects on predators from the same guild and are, in effect, intraguild predators. Sixteen genera of coccinellids have been recorded as being infected by the entomopathogenic fungus *B. bassiana*, including; *C. septempunctata*, *H. convergens* and *Coleomegilla maculate* (De Geer) (James and Lighthart; 1994; Magalhaes *et al.*, 1988; Yeo, 2000). For example, the coccinellid *Serangium parcesetosum* (Sicard) and *B. bassiana* are both natural enemies of whiteflies. However, *S. parcesetosum* is also highly susceptible to *B.*

bassiana (Poprawski *et al.*, 1998). Other species of entomopathogenic fungi have also been recorded as being intraguild predators of coccinellids. For example, the convergent lady beetle *H. convergens* and the fungal pathogens *Paecilomyces fumosoroseus* (Wize) and *M. anisopliae* are all natural enemies of aphids. However, *H. convergens* is susceptible to infection by both *M. anisopliae* and *P. fumosoroseus*, with observed mortality rates of 97% and 56% respectively (James and Lighthart, 1994).

Intraguild predation may not always be detrimental to the intraguild prey and in some cases may benefit the intraguild prey species. This is illustrated by the intraguild interactions that occur between *C. septempunctata* and *P. neoaphidis*. *Coccinella septempunctata* is an intraguild predator of *P. neoaphidis* (Roy and Pell, 2000; Pell *et al.*, 1997; Roy, 1997). However, *P. neoaphidis* may reduce the fitness of *C. septempunctata*. This is due to *C. septempunctata* having both detrimental and positive intraguild effects on *P. neoaphidis*. Both adult and larval *C. septempunctata* have been shown to prey upon living aphids infected with *P. neoaphidis*, as well as consuming sporulating cadavers (Pell *et al.*, 1997; Roy and Pell, 2000). Consumption of a cadaver will prevent sporulation whilst partial consumption decreases sporulation (Roy, 1997; Roy and Pell, 2000). However, in Petri dish bioassays, *C. septempunctata* consumed entire aphids but never consumed entire cadavers (Roy, 1997). An aphid is therefore a preferential food source over a cadaver. It also takes longer for *C. septempunctata* to consume infected aphids than healthy aphids (Pell *et al.*, 1997). The fungus, therefore, has a negative effect on the coccinellid by decreasing the quality of its food source. Roy (1997) demonstrated that the presence of *C. septempunctata* increased the transmission of *P. neoaphidis* to aphid hosts by increasing the movement of the aphids, and therefore increasing the frequency of which they encountered conidia. As *P. neoaphidis* conidia have a pre-formed covering of mucus which attaches them to the coccinellid, the fungus can be passively carried or vectored between aphid populations (Pell *et al.*, 1997). In experiments by Roy (1997) vectoring of *P. neoaphidis* by *C. septempunctata* caused a 10% infection rate in an aphid population. Although a 10% infection rate is low, it could lead to an epizootic (Roy, Pell and Alderson, 2001). The transmission of conidia from foraging coccinellids may make up for the decreased sporulation from predation (Roy and

Pell, 2000; Roy 1997). Roy (1997) also found that transmission from intact and damaged cadavers did not differ. Therefore, feeding damage may not significantly reduce sporulation. *Coccinella septempunctata* is, therefore, an intraguild predator of *P. neoaphidis* but also suffers a fitness cost in the form of a decreased quality of food supply.

1.3.2 Arthropod-plant volatile interactions

The first recorded example of an arthropod responding to volatiles released from a plant was in 1926 when McIndoo used an olfactometer to demonstrate that the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), responded to volatiles from potato plants (Dickens, 2000; McIndoo, 1926). Since 1926 there have been many studies on the responses of arthropods to plant volatiles. Taxa used include predatory and spider mites (*Phytoseiulus persimilis* (Athias-Henriot), *Tetranychus urticae* (Koch)), aphids (*Rhopalosiphum maidis* (Fitch), *A. pisum*) seven-spot ladybirds (*C. septempunctata*) and parasitoids (*A. ervi*, *A. colemani*) (Bernasconi *et al.*, 1998; Bolter *et al.*, 1997; Du, Poppy and Powell, 1996; Janssen *et al.*, 1997; Schaller and Nentwig, 2000; Storeck *et al.*, 2000; Venzon, Janssen and Sabelis, 1999).

Volatiles are one of the main information-conveying agents available to the natural enemies of herbivores and have been categorised depending on their function (Dicke *et al.*, 1990). The term 'infochemicals' incorporates all the chemical cues that occur between individuals of the same and of differing species. There are two major sub-classes, pheromones and allelochemicals. Pheromones convey information between individuals of the same species, e.g. alarm and sex pheromones, whereas allelochemicals convey information between individuals of different species. Allelochemicals are split into three sub-classes; allomones (from species one, affecting species two and benefiting species one), kairomones (from species one, affecting species two and benefiting species two) and synomones (from species one, affecting species two and benefiting species one and two). In some cases pheromones are also allelochemicals.

Foraging predators and parasitoids face the problem of reliably detecting the presence of suitable prey/ hosts. Although volatiles released from herbivores would be a reliable source of information used by their enemies to locate them, relative to their surroundings, herbivores have a low biomass and do not release large quantities of volatiles. Also, natural selection would favour herbivores that did not disclose their location to their predators (Dicke, 1999; Du *et al.*, 1997; Powell *et al.*, 1998; Vet and Dicke 1992). Therefore, volatile cues from herbivores are a reliable source of information but are released in small quantities and, therefore, are difficult to detect (Dicke and van Loon, 2000; Du *et al.*, 1996). Natural selection would favour plants that were able to attract entomophagous insects when infested with herbivores (Dicke and Sabelis 1988; Du *et al.*, 1997; Sabelis and Dejong 1988). Plants have a large biomass and therefore the systemic release of damage-induced volatiles by plants has a large odour plume (Du *et al.*, 1996; Vet and Dicke, 1992). Since the 1990's there have been many biochemical and molecular studies which indicate that plant volatile defences can be induced by exposure to herbivores and herbivore infested conspecifics, therefore increasing the odour plume and further aiding predators in locating prey (Guerrieri *et al.*, 2002; Vet and Dicke, 1992). The systemic release of herbivore-specific volatiles by plants is thought to be the solution to the detectability-reliability problem faced by foraging arthropods (Dicke and Van Loon, 2000; Powell *et al.*, 1998).

1.3.2.1 *Use of volatiles for the indirect defence of plants*

Until 1980, most research on chemicals that affect the behaviour of an arthropod had been done on bitrophic interactions, e.g. plant-herbivore or herbivore-predator (Dicke *et al.*, 1990). However, Price *et al.* (1980) argued that, to understand fully plant-insect interactions, multitrophic plant-herbivore-natural enemy interactions needed to be assessed, and not simple bitrophic plant-herbivore interactions. Since 1980 it has been demonstrated for several plant-herbivore-natural enemy systems that herbivory brings about cell damage and the release of volatiles that are attractive to herbivore natural enemies (Bolter *et al.*, 1997; Dicke *et al.*, 1998; Van Der Putton *et al.*, 2001; Vet and Dicke, 1992). The volatiles indirectly defend the plant from herbivore attack by attracting natural enemies of the herbivore. The responding natural enemies are often referred to as bodyguards (Elliot *et al.*, 2000).

Volatiles released in response to herbivore attack are an adaptive response by the plant to defend itself indirectly (Dicke *et al.*, 1999; Dicke and van Loon, 2000; Roland, 1990; Venzon *et al.*, 1999). Volatiles may be used by herbivore natural enemies during host location, as arrestant compounds, as indicators of prey density or to select oviposition sites. Volatiles can be released systemically (over the whole plant) and differ in blend and composition depending on whether the plant has been mechanically damaged or attacked by herbivores (Bolter *et al.* 1997; Dicke and van Loon, 2000). Volatile blends released in response to herbivore attack are usually composed of between 20 and 200 compounds (Dicke and van Loon, 2000).

The mechanism used by plants to detect and respond to herbivore attack is unclear. When gerbera plants are attacked by the spider mite, *T. urticae*, they release volatiles that attract the predatory mite *P. persimilis*. It is thought that herbivory induces a systemic elicitor that interacts with the plasma membrane leading to the activation of lipase, which releases the fatty acid, linolenic acid. Linolenic acid is converted to jasmonic acid via the octadecanoid pathway. Jasmonic acid then induces the production of volatiles that affect the third trophic level (Gols, Posthumus and Dicke; 1999). The presence of systemic elicitors has been found in a number of systems. Evidence to support this theory comes from Gols *et al.* (1999) who found that gerbera plants released similar volatiles when attacked by herbivores or when exposed to jasmonic acid.

The response of predators and parasitoids to plant volatiles may vary due to differences in experience, physiological state, environmental conditions, disease and genetic variation (Guerrieri *et al.*, 1999; Micha *et al.*, 2000; Rapusas, Bottrell and Coll, 1996; Vet *et al.*, 1990). The effects of herbivore and herbivore-induced plant volatiles on the behaviour of parasitoids, predators and non-arthropod species are described below.

1.3.2.2 *Response of parasitoids to volatiles*

Parasitoid foraging behaviour is influenced by genetic, physiological and environmental factors (Guerrieri *et al.*, 1999). Long-range olfactory cues are used

by parasitoids when locating their host's habitat (Powell and Zhang, 1983). Using an olfactometer, Powell and Zhang (1983) showed that the cereal aphid parasitoid *Aphidius uzbekistanicus* (Luzhetski) responded to volatiles released from the leaves of wheat plants, whilst *A. ervi*, which has a larger host range than *A. uzbekistanicus*, responded to volatiles from wheat and bean leaves. Following this finding, the role of plant volatiles during host location by parasitoids has been intensively studied. Experiments using both olfactometers and simple behavioural arenas have indicated that a variety of parasitoid species are attracted to volatiles released from both the host aphid and from host-aphid infested plants (Birkett *et al.*, 2003; Du *et al.*, 1996; Glinwood, Du and Powell, 1999; Mattiacci *et al.*, 2001; Micha *et al.*, 2000; Potting, Poppy and Schuler, 1999; Powell *et al.*, 1993; Vaughn, Antolin and Bjostad, 1996). The aphid parasitoids *Aphidius eadyi* (Stary) and *A. ervi* are attracted to components of aphid-sex pheromone (Glinwood *et al.*, 1999). This is an example of a pheromone also being used as a kairomone. A second example is that of Brussels sprout plants, *Brassica oleracea* (L.), infested with the caterpillar *Pieris brassicae* (L.). The quantity of green-leaf volatiles released from Brussels sprout plants that were fed upon by *P. brassicae* was greater than the quantity released from undamaged plants (Mattiacci *et al.*, 2001). The increased quantity of green-leaf volatiles attracted the parasitoid *Cotesia glomerata* (L.) which is a natural enemy of the caterpillar (Mattiacci *et al.*, 2001). The responding parasitoids may, therefore, be regarded as 'bodyguards'.

Previous experience of a host-plant complex may elicit a greater host-locatory response from a foraging parasitoid than from an individual with no previous foraging experience (Micha *et al.*, 2000; Potting *et al.*, 1999). This was demonstrated by Potting *et al.* (2000) who assessed the effect of foraging experience on the behaviour of the parasitoid *C. plutellae*, which is a natural enemy of the diamondback moth *P. xylostella*. Both *P. xylostella*-damaged and mechanically-damaged oilseed rape plants (*Brassica napus* L.) were attractive to foraging *C. plutellae*, and this attraction was found to increase if the parasitoid had gained oviposition experience or had been given prior contact with a *P. xylostella*-infested *B. napus* leaf (Potting *et al.*, 1999) (see section 1.1.2.3 for further information on host preference and learning in parasitoids).

It is possible to identify specific compounds released from herbivore-infested plants that attract the natural enemies of the herbivore (Birkett, *et al.*, 2003; Guerrieri *et al.*, 1999; Oldham and Boland, 1996). For example, infestation of the bean plant *Phaseolus vulgaris* (L.) by the whitefly *T. vaporariorum* elicits a host-locatory response by the parasitoid *E. formosa* (Birkett, *et al.*, 2003). Gas chromatography revealed that *P. vulgaris* plants infested with *E. formosa* release more than 20 compounds, four of which ((Z)-3-hexen-1-ol, 4, 8-dimethyl-1, 3, 7-nonatriene, 3-octanone and a fourth unidentified compound) were not released by uninfested plants (Birkett, *et al.*, 2003). These compounds were produced synthetically and their attractiveness to *E. formosa* assessed in a wind tunnel. Synthetic (Z)-3-hexen-1-ol, 4, 8-dimethyl-1, 3, 7-nonatriene, and 3-octanone all elicited orientated flight responses from *E. formosa* and, a mixture of (Z)-3-hexen-1-ol and 3-octanone elicited a greater response than either chemical alone (Birkett, *et al.*, 2003). Potentially, these chemicals could be used to manipulate the behaviour of herbivore natural enemies.

The response of *A. ervi* to *A. pisum*-infested *V. faba* plants has been intensely studied and the plant-aphid-parasitoid interactions derived. *Aphidius ervi* elicits a response to volatiles released from *V. faba* plants infested with the host aphid, *A. pisum*. However, *A. ervi* did not respond to undamaged *V. faba* plants, mechanically damaged *V. faba* plants or *V. faba* plants infested with the non-host aphid, *A. fabae* (Du *et al.*, 1996; Guerrieri *et al.* 1996; Poppy, Powell and Pennacchio, 1999). Therefore, the volatiles released from *V. faba* plants are influenced by both the presence and species of the herbivore causing the damage (Powell *et al.*, 1998). If the aphids were removed from the plant, the plants were still attractive to the parasitoid indicating attraction to the plant, not just the plant-herbivore complex (Du *et al.*, 1996; Guerrieri *et al.*, 1996). When samples of volatiles entrained from damaged and undamaged *V. faba* plants were placed in a wind tunnel, 73% of parasitoids elicited a response to the volatiles collected from damaged plants whereas only 16% responded to volatiles from undamaged plants (Powell *et al.*, 1998). Air entrainments from *V. faba* plants revealed that the volatiles released from *A. pisum*-infested plants were a different blend to those released from *A. fabae*-infested plants (Du *et al.*, 1996; Guerrieri *et al.*, 1996). Analysis of the volatiles using gas chromatography indicated that *A. pisum*-infested

V. faba plants released a greater quantity of 6-methyl-5-hepten-2-one compared to the amount entrained from uninfested bean plants (Du *et al.*, 1998; Powell *et al.*, 1998). Wind tunnel bioassays confirmed that *A. ervi* elicited a response to 6-methyl-5-hepten-2-one, and that this compound was released from *V. faba* plants after 48-72 hours of infestation by forty *A. pisum* aphids (Guerrieri *et al.*, 1999; Poppy *et al.*, 1997ab; Powell *et al.*, 1998). Further experiments showed that if the lower section of a *V. faba* plant was infested with *A. pisum* and volatiles were entrained from the upper, aphid free section of the plant, the entrained volatiles were still attractive to *A. ervi* (Guerrieri *et al.*, 1999; Poppy *et al.*, 1997b). Therefore, aphid infestation induces the systemic release of volatiles. The mechanism for this is still unclear (Poppy *et al.*, 1999). Guerrieri *et al.* (1999) hypothesised that the systemic release may be due to within plant circulation of aphid saliva, bioactive elicitors or synomones. Infestation by *A. pisum* also induces plant-plant communication. Guerrieri *et al.* (2002) demonstrated that root contact between an uninfested and an *A. pisum*-infested *V. faba* plant resulted in the uninfested plant becoming attractive to foraging *A. ervi.* It was suggested that the increased attraction may have been due to exudate from the roots of the infested *V. faba* plant inducing the inductive release of volatiles from the uninfested plant. To summarise, *V. faba* plants systemically release aphid species-specific volatiles in response to herbivory and these volatiles provide a detectable and reliable cue used by parasitoids to find suitable hosts.

1.3.2.3 *Response of predators to volatiles*

The response of arthropod predator species to both prey and plant derived volatiles has been assessed in both the laboratory and the field for a number of species, including mites, coccinellids, lacewings and hoverflies (Dicke *et al.*, 1998; Han and Chen, 2002b; Jamal and Brown, 2001; James, 2003; Ninkovic *et al.*, 2001). In a Y-tube olfactometer the coccinellid *Exochomus flaviventris* (Mader) showed a greater response to the odour of cassava plants infested with the cassava mealy bug, *P. manihoti*, than to those released from either the plant or the mealy bug alone (Le Ru and Makaya-Makosso, 1999). Behavioural experiments and electroanntenogram studies have shown that the coccinellid, *C. septempunctata*, and the lacewing, *Chrysopa sinica* (Tjeder), respond to volatiles released from both tea

aphids, *Toxoptera aurantii* (Boyer de Fonscolombe), and tea aphid-tea shoot complexes (Han and Chen, 2002ab). A final example is that of the twelve spotted ladybird, *C. maculate*, and the green lacewing *C. carnea*. Electroantennogram experiments showed that these species detected a variety of volatile compounds isolated from either the pea aphid, *A. pisum*, or from corn (Zhu *et al.*, 1999). The compounds (*E*)-β-farnesene (aphid-alarm pheromone) and 2-phenylethanol were particularly attractive, and in subsequent field tests attracted *C. maculata* and *C. carnea* into traps.

As described in section 1.3.2.2 for parasitoid-herbivore-plant interactions, it is possible to identify specific compounds released from herbivore-infested plants that attract predators of herbivores. The predatory mite *P. persimilis* responds to volatiles released from lima bean plants, *Phaseolus lunatus* (L.), infested with the spider mite *T. urticae* (Dicke *et al.*, 1998; Schutte *et al.*, 1998). Further studies indicated that the release of volatiles by an infested lima bean plant was systemic and that the volatiles from infested plants induced the release of volatiles from uninfested plants (Dicke *et al.*, 1998; Takabayashi and Dicke, 1992). Herbivory by *P. persimilis* caused a 200 fold increase in the release of several volatile compounds, including (*E*)-4, 8-dimethyl-1, 3, 7- nonatriene, linalool and methyl salicylate (Oldham and Boland, 1996). Janssen *et al.* (1997) found that *P. persimilis* was able to use volatile cues to determine whether a conspecific was present in a prey patch and, if so, to avoid it. However, if a non-conspecific competitor, for example *Neoseilus californicus* (McGregor), was present, *P. persimilis* did not avoid the patch (Janssen *et al.*, 1999).

There have been many studies on the responses of the generalist predator *C. septempunctata* to volatiles released from both aphids and aphid-infested plants. Although *C. septempunctata* has been shown to elicit a response to aqueous aphid extract, aphid alarm pheromone ((*E*)-β-farnesene) and to volatiles released from conspecific adults, it is unclear whether *C. septempunctata* uses aphid-induced plant volatiles to aid the detection of prey (Al Abassi *et al.*, 1998, 2000; Shonouda, 1999). Schaller and Nentwig (2000) found that *C. septempunctata* did not respond to the volatiles of 22 different plant species in a Y-tube olfactometer. In contrast, Ninkovic *et al.* (2001) found that *C. septempunctata* responded to volatiles released

from barley plants infested with *Rhopalosiphum padi* (L.) Although these results imply that *C. septempunctata* does respond to aphid-induced plant volatiles, it should be noted that a two-way olfactometer will indicate detection of a volatile but will not show whether an insect will make an orientated response towards the source of the volatile. Therefore, there is there is no conclusive evidence on whether or not *C. septempunctata* would make an orientated response to the volatiles from plants or prey when foraging.

The response of a predator to plant-derived volatiles may not always be consistant and changes within the plant, for example, the genotype, may affect its attractiveness. For example, the mirid predator *Cyrtorhinus lividipennis* (Reuter) is attracted to rice plants on which it can find its prey, the brown planthopper, *Nilaparvata lugens* (Stal) (Rapusas *et al.*, 1996). However, the odour of rice plants was only more attractive than that of clean air for 6 out of 15 plant genotypes (Rapusas *et al.*, 1996). Similar results have been found in parasitoid systems, for example, Kalule and Wright (2004) showed that *Aphidius colemani* shows a greater preference for the cabbage cultivar on which it had been reared. This indicates that both the results and conclusions drawn from experiments done to assess the responses of predator and parasitoids to uninfested and herbivore-infested plants are highly dependent on a number of factors, including the cultivar of the plant. Therefore, before predators and parasitoids are released as part of a biological control programme, the interactions between the natural enemies species and specific varieties of the host plant need to be assessed.

1.3.2.4 *Response of non-arthropod species to volatiles*

Although there has been extensive work on the interactions between plants and arthropods, there has been little work on interactions between plants and species from other kingdoms, for example, entomopathogenic fungi (Elliot *et al.*, 2000). Mutualistic interactions between plants and herbivore pathogens have received little attention (Elliot *et al.*, 2000). For a mutualistic interaction to occur, the plant must increase the abundance of the pathogen or increase its effectiveness either directly or indirectly (Elliot *et al.*, 2000). Abundance could be increased indirectly by the attraction of a fourth-party (Elliot *et al.*, 2000). For example, an arthropod that is

attracted to aphid-induced plant volatiles may also act as a vector of a fungus (see section 1.3.1.3). If *C. septempunctata* is attracted to aphid-induced plant volatiles as hypothesised by Ninkovic *et al.* (2001), the presence of aphid-induced plant volatiles may indirectly increase the encounter rate with fungal conidia and this may indirectly increase the abundance of the pathogen. Alternatively, aphid-induced plant volatiles may have a direct effect on the pathogen e.g. increased virulence. In this scenario the induced plant volatiles would have the same role as those described in the plant-aphid-parasitoid interaction described in section 1.3.2.2. The effect of plant derived volatiles on *P. neoaphidis* was studied by Brown *et al.* (1995) who showed that green-leaf volatiles released from tobacco plants *Nicotiana tabacum* (L.) inhibited both *in vivo* growth rate and conidial germination. Although this shows a negative effect of plant volatiles on a pathogen, it indicates that there could be the potential for mutualistic associations to occur. This interaction is discussed further in Chapter 3.

1.4 AIMS

Although multitrophic systems involving aphids, their insect natural enemies and the host plant have been studied in depth, there is little knowledge on the role of entomopathogenic fungi within these systems. The aim of this thesis is to further understand the multitrophic interactions that occur between *P. neoaphidis*, *A. pisum* and *V. faba* plants and the intraguild interactions that occur between *P. neoaphidis*, *C. septempunctata* and *A. ervi*. The knowledge gained from this study will have implications for the development of a multi-species biological control programme against aphids. The specific objectives of this thesis are:

1. To assess whether volatiles released from *A. pisum*-infested *V. faba* plants have a direct effect, either numerical or functional, on *P. neoaphidis*
2. To assess whether volatiles released from *A. pisum*-infested *V. faba* plants affect the interactions that occur between *P. neoaphidis* and *A. pisum*
3. To assess whether the presence of *P. neoaphidis* affects the foraging behaviour of *C. septempunctata* and *A. ervi*

4. To assess whether the presence of *C. septempunctata* and *A. ervi* affect the transmission of *P. neoaphidis*, both within and between aphid colonies

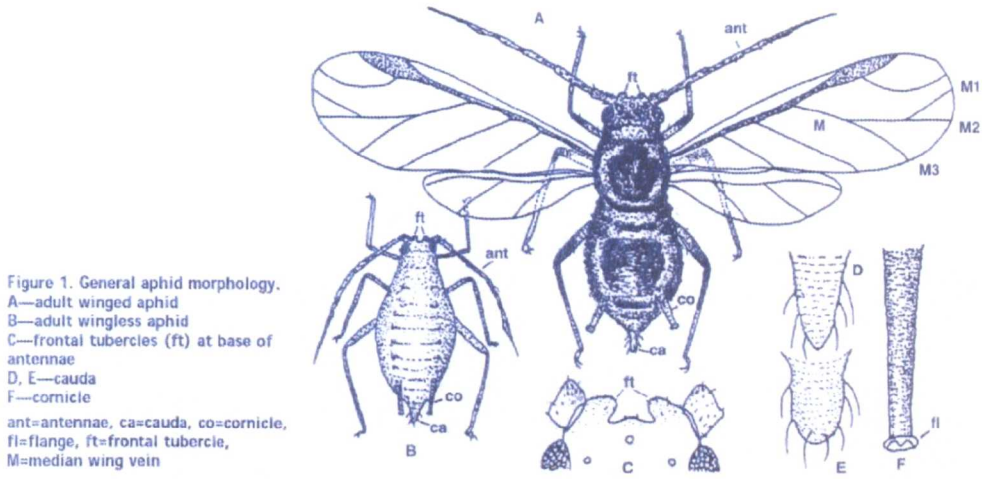

Figure 1.1 Aphid morphology (from Liu and Sparks).

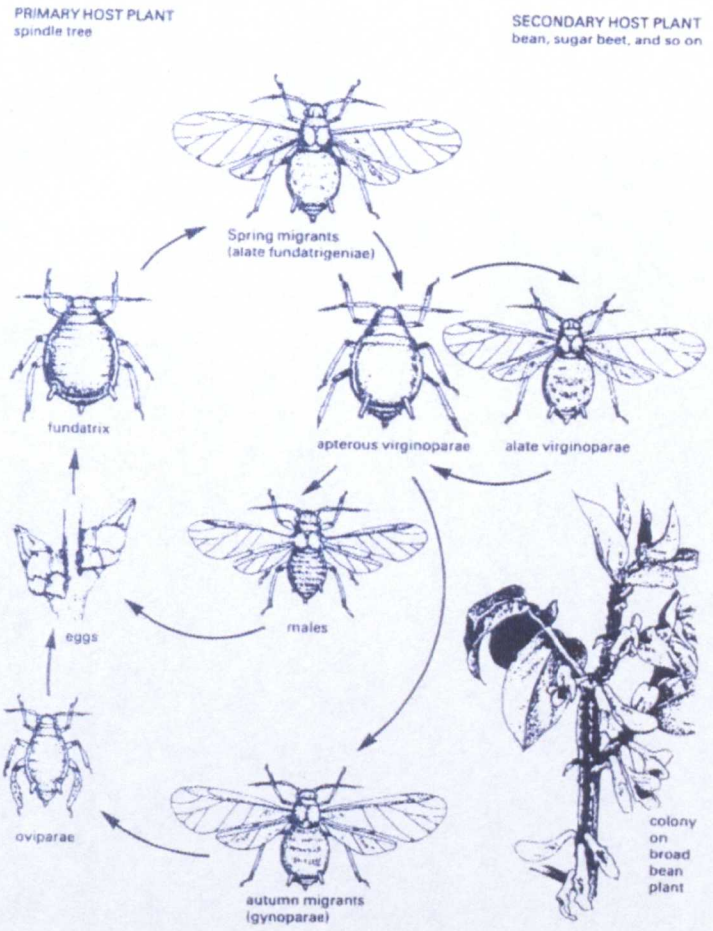

Figure 1.2 Life cycle of *Aphis* fabae, a heteroecious aphid species (from Blackman and Eastop, 2000).

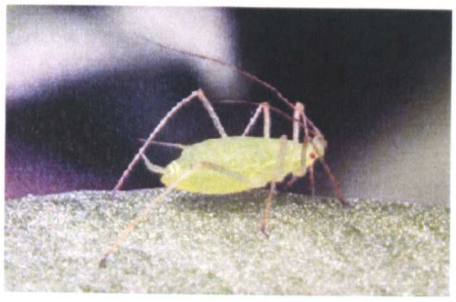

Figure 1.3 Adult *Acyrthosiphon pisum* (Pea aphid)

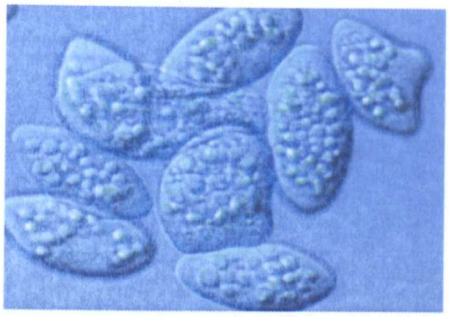

Figure 1.4 *Pandora neoaphidis* conidia

Figure 1.5 *Coccinella septempunctata* (Seven-spot ladybird)

Figure 1.6 *Aphidius ervi*

CHAPTER 2: GENERAL MATERIALS AND METHODS

2.1 PLANT CULTURES

2.1.1 *Vicia faba* plants

The dwarf broad bean plant, *Vicia faba* L. (cultivar The Sutton), was the only plant species used in this study. All plants were grown in the glasshouses at Rothamsted Research.

2.1.2 Production of aphid-damaged *Vicia faba* plants

Both previously undamaged and aphid-damaged plants were used during this study. Plants that were not manipulated prior to use in experiments will be referred to as being in an 'undamaged' condition. Plants that have been exposed to aphids will be referred to as being in a 'damaged' condition. To damage plants, forty *A. pisum* (mixed instars) were placed on a twelve-day-old bean plant and maintained in a controlled environment chamber (18°C, 16L:8D) for 72 hours. Unless stated otherwise, the aphids were removed from the plant immediately before the start of the experiment using a fine paint brush. As a result of aphid infestation, damaged plants would be releasing *A. pisum*-induced volatiles and contain both aphid honey dew and host exuviae.

2.2 INSECT CULTURES

All insects were reared in Perspex cages ($0.5m^2$ x 1m) at 18°C (16L:8D) within Rothamsted Research's insectary.

2.2.1 *Acyrthosiphon pisum*

Acyrthosiphon pisum (pea aphid) was the only species of aphid used in this study and was obtained from the culture held by Rothamsted Research. The aphids were reared on three four-week-old bean plants and were sub-cultured onto new plants as

necessary. The temperature and photoperiod of the insectary ensured an anholocyclic life cycle was maintained.

2.2.1.1 *Production of known-age* Acyrthosiphon pisum

A single leaf from a bean plant, approximately 60mm long, was placed abaxial side uppermost in a Petri dish (90mm diameter) containing 50ml of 1.5% tap-water-agar which was cool but still liquid. When the agar had solidified, fifteen adult *A. pisum* were placed on the leaf, the Petri dish covered with a tissue and the lid replaced. The Petri dish was then inverted and maintained within a controlled environment chamber (18°C, 16L:8D) for 48 hours. The adult aphids were then removed from the bean leaf using a fine paint brush (leaving a cohort of similarly aged first instar aphids). Finally the leaf was transferred to a plant-pot containing a 15-day-old bean plant and the nymphs allowed to move on to the plant. The plant was maintained in a controlled environment chamber (18°C, 16L:8D) until the aphids were required. Aphids became adults after 11 days ±1day.

2.2.2 *Aphidius ervi*

Aphidius ervi was the only species of parasitoid used in this study and was obtained from the culture held by Rothamsted Research (originally collected in 2001 from Colworth House Farm, Bedfordshire). Unless stated otherwise, this was the standard culture used in the experiments. In a limited number of experiments (indicated in text) a second culture, *A. ervi*$_{I2000}$, was used.

To rear *A. ervi*, sixty adults (mixed-sex) were placed in an insectary cage containing three two-week-old bean plants infested with a mixed-instar population of *A. pisum*. The cage was monitored for formation of aphid 'mummies', which were left to emerge. Both the standard culture and the *A. ervi*$_{I2000}$ cultures were reared independently of each other. Only female parasitoids up to five-days-old were used in experiments.

Female parasitoids were not separated from males during rearing and therefore may or may not have been mated. Unless stated otherwise, the parasitoids had gained

experience of foraging and oviposition from the culture cage. If naïve parasitoids (parasitoids with no prior foraging experience) were required, the leaves on which the mummies formed were removed from the rearing cage before emerging and placed in a clean insectary cage containing only a honey-water solution.

2.2.3 *Coccinella septempunctata*

Coccinella septempunctata was the only species of coccinellid used in this study. Mixtures of male and female coccinellids were used for the experiments. Field collected and laboratory-reared coccinellids were used in the experiments.

Adult *C. septempunctata* were collected regularly from the farm at Rothamsted Research between August 2000 and April 2004, and from the apple orchards at HRI-East Malling, Kent, during August 2001. The coccinellids were maintained on bean plants infested with mixed-instar *A. pisum*. The field-collected coccinellids were checked regularly for signs of parasitism. Any individual found to be parasitised was discarded. Field collected *C. septempunctata* were maintained within the insectary for a maximum of two months before use in experiments.

To rear *C. septempunctata*, a mixed-sex population (up to 80 individuals) was maintained in an insectary cage containing bean plants infested with mixed-instar *A. pisum*. When eggs were laid, the substrate on which they were attached, for example a leaf, was removed from the rearing cage and placed in an empty insectary cage. On hatching, the larvae were transferred using a fine paint brush to another insectary cage containing bean plants infested with mixed-instars *A. pisum*. The larvae were left to pass through their four developmental instars, pupate and become adults. Reared adult *C. septempunctata* were at least two-weeks-old and never older than 3 months before being used in experiments.

If starved individuals were required, adult *C. septempunctata* were transferred from the rearing cage to a Petri dish (90mm diameter) containing only a small piece of wet filter paper. A maximum of six individuals were placed in each Petri dish. The Petri dishes were maintained at 18°C (16L:8D) for 24 hours. This ensured the coccinellids were starved and would forage efficiently during the experiment.

2.3 FUNGUS CULTURES

Pandora neoaphidis isolate X4 (from the Rothamsted Research collection, original host = *A. pisum*), was the only species and isolate of fungus used in this study.

2.3.1 Production of *in vivo Pandora neoaphidis* cadavers

A piece of damp filter paper (70mm diameter) was placed in the lid of a Petri dish (90mm diameter). Seven dried cadavers were placed on the filter equidistance from each other. The lid was placed in a sealed plastic box (115mm wide x 170mm long x 60mm high) containing wet tissue (to create 100% relative humidity) and maintained at 10°C for 16 hours. This rehydrated the cadavers and initiated sporulation.

After 16 hours the lid was inverted and placed over a glass dish (70mm diameter, 40mm depth) containing approximately 100 eight-day-old *A. pisum* (4^{th} instar) and a microscope slide cover slip (22mm x 50mm). The dish was replaced in the plastic box containing the wet tissue and maintained at room temperature for 90 minutes.

After 90 minutes the cover-slip was examined using a Leitz Dialux 20 light microscope (x100 magnification) to ensure sporulation was ongoing and that there was no contamination. The aphids were then distributed between five two-week old bean plants. The bean plants were placed in a tray containing water, covered with a plastic bag (to create 100% relative humidity) and maintained within a controlled environment chamber (18°C, 16L:8D). After 24 hours, the plastic bag was removed and the plant maintained for a further 72 hours.

After 96 hours the newly formed cadavers (i.e. aphids that had died and turned yellow) were removed from the plants using forceps, placed in a ventilated box and air-dried over a fan within the controlled environment chamber. After a further 48 hours the cadavers were transferred to a ventilated glass tube and stored in a desiccator (20% relative humidity) at 4°C for up to 90 days.

2.3.1.1 *Re-hydration of dried* Pandora neoaphidis-*cadavers on tap-water-agar*

Sixteen hours prior to the start of the experiment, 25ml of 1.5% tap-water-agar was poured into a Petri dish (90mm diameter) and left to solidify. This gave an agar depth of approximately 3mm. Groups of dried *P. neoaphidis*-cadavers were then placed on the agar at least 10mm apart and the Petri dish put in a sealed plastic box (115mm wide x 170mm long x 60mm high) containing wet tissue (to create 100% relative humidity) and maintained at 10°C for 16 hours. After 16 hours, a size 5 cork borer (10mm diameter) was used to cut around the *P. neoaphidis*-sporulating cadavers to give '*P. neoaphidis*-agar discs' (Figure 2.1).

2.3.2 Production of *in vitro Pandora neoaphidis* cultures

2.3.2.1 *Making Sabouraud's-Egg-Milk-Agar (SEMA)*

In vitro P. neoaphidis cultures were grown on sterile Sabouraud's-Egg-Milk-Agar (SEMA). To prepare the sterile agar, the following items were autoclaved at 115°C for 20 minutes: one 500ml Duran bottle containing 20.8g of Sabouraud's dextrose agar (SDA) and 320ml of distilled water (lid loosely replaced), one 100ml Duran bottle containing 34ml of milk (lid loosely replaced), one empty 500ml Duran bottle (lid loosely replaced) and one Pyrex bowl (190mm diameter, 90mm depth) covered in tin foil. After autoclaving, the lids of the Duran bottles containing the SDA and the milk were tightened, the bottles transferred to a water bath and maintained at 55°C for at least 20 minutes or until required.

To ensure sterile conditions, the following was done using aseptic techniques within a laminar-flow hood that had been sterilized using 95% ethyl alcohol. An egg that had been surface sterilized (soaked in a mixture of 99% 95%-ethyl alcohol and 1% acetone for 2 hours prior to use) was flamed to remove residual alcohol. The egg was then cracked on the edge of the sterile Pyrex bowl, the white decanted into the Pyrex bowl and the yolk placed into the empty autoclaved 500ml Duran bottle. This was repeated with a second egg. The lid of the Duran bottle was replaced and

the bottle shaken to break the yolks. The milk and SDA were then added to the bottle containing the egg yolks, the lid replaced, and the bottle shaken to mix the ingredients. Twenty-four ml of the liquid SEMA was then measured into 90mm triple-vented-Petri dishes using a sterile pipette and allowed to solidify before the lids of the Petri dishes were replaced. Petri dishes that were not used immediately were inverted and stored within a sterile plastic bag for up to 30 days at 4°C.

2.3.2.2 *Removal of* Pandora neoaphidis *from liquid nitrogen and inoculation of SEMA plates*

A sachet containing *P. neoaphidis*-inoculated SEMA was removed from the liquid nitrogen storage Dewar and transferred to the laboratory within a transfer flask containing liquid noitrogen. The sachet was then plunged into a water bath and maintained at 37°C for two minutes. After two minutes the sachet was transferred to a laminar flow hood.

To ensure sterile conditions, the following was done using aseptic technique within a laminar-flow hood that had been sterilized using 95% ethyl alcohol. Using forceps, the sachet was immersed in 95% ethyl alcohol for 1 minute and then air-dried. One end of the sachet was removed using flamed scissors and a plug of *P. neoaphidis*-inoculated SEMA transferred to the centre of a SEMA plate using a flamed tungsten wire hook. The lid of the Petri dish was replaced and the dish put into a plastic box (115mm wide x 170mm long x 60mm high) containing an open ended glass tube (15mm diameter x 40mm long) that was half filled with distilled water. Finally, the box was sealed and maintained at 20°C in the dark until use or for up to 30 days. A *P. neoaphidis* colony growing on SEMA is shown in Figure 2.2.

2.4 STATISTICAL ANALYSIS

Figures and numerical data within the result sections are in the form of untransformed data. All error bars show standard errors. Methods used to transform data prior to statistical analysis are given in the relevant methods section. GenStat Release 6.2 (GenStat Sixth Edition (Service Pack 1), GenStat Procedure

Library Release PL14.1) was the only statistics programme used for data analysis in this study.

Figure 2.1 Agar disc containing five sporulating *Pandora neoaphidis* cadavers on the leaf of a 15-day-old *Vicia faba* plant.

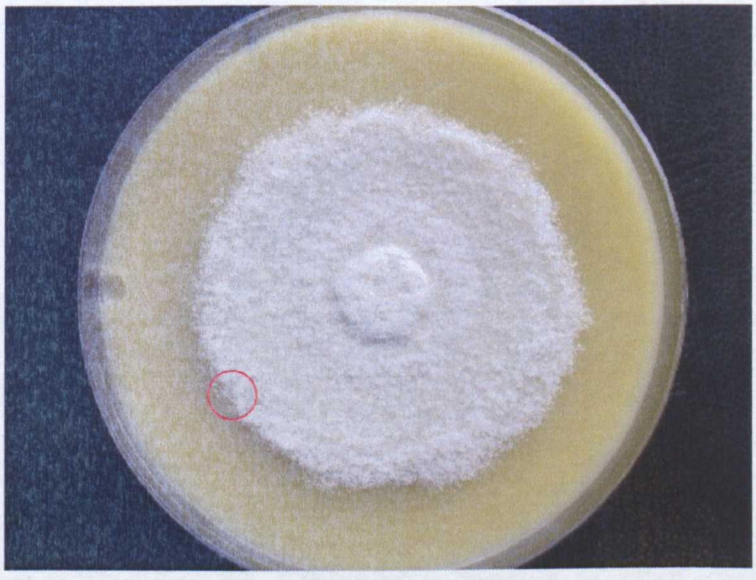

Figure 2.2 Petri dish containing a *Pandora neoaphidis* colony growing on SEMA. The red circle indicates the region sampled during sub-culturing.

CHAPTER 3: PLANT-FUNGUS INTERACTIONS: DOES *PANDORA NEOAPHIDIS* RESPOND TO HOST-APHID INDUCED PLANT VOLATILES?

3.1 INTRODUCTION

Numerous studies have shown that, in order to protect themselves, plants use allelochemicals to communicate directly with predators and parasitoids of herbivores (Bolter *et al.*, 1997; Dicke and van Loon, 2000; Dicke *et al.*, 1990; Powell *et al.*, 1998; Schutte *et al.*, 1998; Vet & Dicke, 1992). Synomones have been discovered in a number of plant-herbivore-natural enemy systems and are thought to be the solution to the detectability-reliabilty problem facing foraging predators and parasitoids. As described in section 1.3.2.2, bean plants infested with the aphid *A. pisum* release species-specific volatiles that attract the parasitoid *A. ervi*, and the parasitoid uses these volatiles as a detectable and reliable source of information for host-aphid location (Du *et al.*, 1996; Guerrieri *et al.*, 1996, 1999; Poppy *et al.*, 1997a; Poppy *et al.*, 1999). The behaviour of *A. ervi* is, therefore, influenced by the bean plant, resulting in the parasitoid becoming a bodyguard of the plant.

Bodyguards provide an indirect defence for the plant by reducing the herbivore population. The bodyguard does not have to benefit from the interaction; the effect on the bodyguard can be mutualistic, neutral or detrimental (Elliot *et al.*, 2000). Although there has been extensive work on the interactions between plants and arthropod natural enemies, there has been little work done on the interactions between plants and species from other kingdoms, for example, entomopathogenic fungi (Elliot *et al.*, 2000). For a fungal pathogen to be a bodyguard, the interaction between the plant and the fungus could either be numerical (altered population size of the fungus) or functional (increased effectiveness of the fungus) (Elliot *et al.*, 2000). A functional interaction would be a direct effect of the plant on the fungus and a numerical interaction could either be a direct interaction between the plant and fungus, for example, increased sporulation, or an indirect interaction such as enhanced transmission in the presence of a foraging arthropod.

Volatiles released from plants have both positive and negative functional effects on fungi. For example, the germination of spores of *Alternaria* sp. and *Fusarium* sp. is stimulated by volatiles released by aged pea seeds (Smith and Van Staden, 1995). A second example is that of the VA mycorrhizal fungus *Gigaspora gigantea* (Nicol. & Gerd.), where germ tubes grew both horizontally and vertically through air to make contact with the roots of both corn and bean plants (Koske, 1982). Volatile attractants released from roots are thought to provide a guidance mechanism for the germ tube (Koske, 1982). In contrast, volatile short-chain aldehydes are released as a defence mechanism against plant-pathogenic fungi (Lyr and Banasiak, 1983; Zeringue and McCormick, 1989). However, there is little evidence to suggest that plants use volatile cues to communicate with entomopathogenic fungi. The effect of volatiles released from tobacco plants (*N. tabacum*) on *P. neoaphidis* was studied by Brown *et al.* (1995). Tobacco plant green leaf volatiles (released from unmacerated and macerated leaves) and volatiles released as a result of infestation with the tobacco aphid *Myzus nicotianae* (Blackman) inhibited conidial germination. However, green leaf volatiles did not affect the proportion of aphids becoming infected with the fungus. From these data Brown *et al.* (1995) developed a conceptual model in which aphid-induced plant volatiles inhibited the production of secondary conidia, therefore increasing the viability of primary conidia and the potential for contact between ungerminated primary conidia and a host aphid. On contact with the host, plant volatile cues would be replaced by cues from the host aphid.

Potentially, volatiles released from host-aphid-infested bean plants may have a direct effect on *P. neoaphidis* and this may result in the fungus acting as a bodyguard species. This chapter examines the direct effects of volatiles released from aphid-infested bean plants on *P. neoaphidis*. More specifically:

- The effect of *A. pisum*-induced *V. faba* volatiles on conidia production (numerical interactions)
- The effect of *A. pisum*-induced *V. faba* volatiles on conidia size, conidial germination and mycelial growth (potential functional interactions)

3.2 MATERIAL AND METHODS

3.2.1 Sporulation monitor experiment to assess the effect of *Acyrthosiphon pisum*-induced *Vicia faba* volatiles on the sporulation rate of *Pandora neoaphidis*-infected cadavers

Three seed trays (155mm x 210mm x 45mm), each containing nine fifteen-day-old damaged bean plants still infested with 40 *A. pisum*, were placed in the bottom section of a sporulation monitor (Figure 3.1, see also Pell *et al.*, 1998a). The aphids remained on the plants throughout the experiment. The wells of a five-well sporulation monitor plate were filled with 1% tap-water-agar and a single dried *P. neoaphidis*-infected cadaver (that had been previously weighed) positioned on each agar-filled well. A mask, used to focus the sporulation of the cadavers, was then attached to the plate. The plate was inverted and placed above an acetate (180mm x 330mm) that was attached to the revolving drum of the monitor and would collect the ejected conidia. The lid of the monitor was then replaced. This was repeated at the same time in a second monitor containing undamaged bean plants. To avoid the transfer of plant volatiles from one monitor to another, the monitors were placed in separate sealed Perspex cages (1m wide x 1m wide x 1.5m high) adjacent to each other within the same controlled environment room (18°C, 16L:8D). The monitors remained in the same simulator cage throughout the experiment. The monitors ran for 48 hours.

After 48 hours the acetates were removed. The number of conidia was counted for a 12 minute period at the start of every fourth hour using a Reichert light microscope (x100 magnification). The experiment was repeated four times in total, with each treatment having been carried out in both monitors twice.

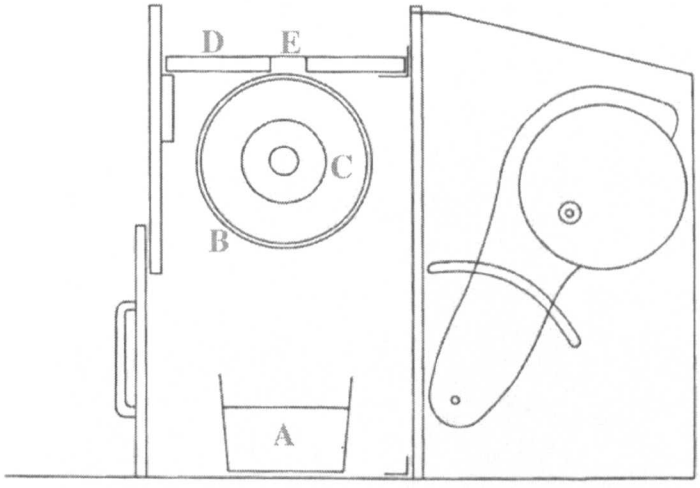

Figure 3.1 Cross section of a sporulation monitor (modified diagram from Pell *et al.*, 1998a). A= Seed tray, B= Acetate, C= Revolving drum, D= Plate and E= Well.

A linear regression analysis was used to assess whether there was a relationship between cadaver weight and total number of conidia counted per cadaver. To assess whether there was a difference in the number of conidia collected from the undamaged and damaged treatments, the total number of conidia counted from each replicate was derived and, after a log(n+1) transformation, the data analysed using ANOVA. To identify whether there was a difference in conidia production rate over time, conidia production rate between treatments or, an interaction between time and treatment, the data were transformed using a log(n+1) transformation and analysed using a Repeated Measures ANOVA.

3.2.2 *In vivo* conidia showering experiment to assess the effect of *Acyrthosiphon pisum*-induced *Vicia faba* volatiles on the conidia size of *Pandora neoaphidis*

The wells of a five-well sporulation monitor plate were filled with 1% tap-water-agar and a single dried *P. neoaphidis*-infected cadaver (that had been previously weighed) positioned on each agar-filled well. The plate was then inverted, suspended 10mm above a second plate and transferred to a Perspex cage ($0.5m^2$ x 1m) containing three damaged bean plants. The aphids used to damage the plants remained on the plant throughout the experiment. This was repeated at the same time using a second cage containing three undamaged bean plants. The cages were sealed to ensure no volatiles escaped, and were maintained at 18°C (16L:8D).

After 3, 6, 12, 24, 36 and 48 hours, a microscope slide was positioned on the plate below each sporulating cadaver and the conidia collected for one hour. The conidia were immediately fixed and stained with 10% cotton blue in lactophenol and covered using a cover slip (22mm^2). The lengths and widths of 20 conidia per slide were measured using 'Open Lab 2.2.3' imaging equipment. The experiment was repeated, with each treatment having been carried out in both Perspex cages.

A Repeated Measures ANOVA was used to assess whether there was a difference in conidia length or width over time, conidia length or width between treatments or an interaction between time and treatment that affected conidia length or width.

3.2.3 *In vivo* conidia showering experiment to assess the effect of *Acyrthosiphon pisum*-induced *Vicia faba* volatiles on the germination of *Pandora neoaphidis* conidia

One hundred adult *A. pisum* were inoculated with *P. neoaphidis* as described in section 2.3.1. Fifty aphids were then transferred to five previously damaged bean plants (10 per plant) that were planted individually in pots (85mm diameter). The remaining 50 aphids were transferred to five undamaged bean plants. Each plant was then covered with a lamp glass and sealed using Clingfilm (to create a relative humidity greater than 95% and prevent dispersal of volatiles) and placed in a controlled environment chamber (18°C, 16L:8D). Ten aphids were removed from each treatment after 3, 6, 9, 12 and 24 hours, placed dorsal side uppermost on a microscope slide, stained with 10% cotton blue in lactophenol and squashed, head-end first, using a cover slip (22mm^2). The total number of conidia and number germinating on the dorsal side of each aphid was assessed and the proportion of germinating conidia (germinating conidia / total conidia) derived.

Before analysis, the 'assessment time' was transformed to a log scale. A Generalised Linear Regression model was then constructed to assess whether there was an effect of time, treatment or a time-treatment interaction that affected the number of germinating conidia.

3.2.4 *In vitro* experiment to assess the effect of *Acyrthosiphon pisum*-induced *Vicia faba* volatiles on the growth rate of *Pandora neoaphidis*

An *in vitro* colony of *P. neoaphidis* was grown as described in section 2.3.2. To ensure aseptic conditions, all procedures were done in a laminar-flow hood that had been sterilized using 95% ethyl alcohol. Sixty plugs were cut from the growing edge of a 21-day-old colony using a size 5 cork borer (10mm diameter) (Figure 2.2). Each plug was transferred using a flamed tungsten wire hook to the centre of a triple vented Petri dish (90mm diameter) containing 24ml of SEMA, and the lid replaced. Each Petri dish was then placed within a second, larger, Petri dish (140mm diameter).

A polypropylene cap (33mm diameter) containing 5ml of distilled water was added to 15 of the replicate Petri dishes (140mm diameter) and the lids replaced and sealed using Parafilm to prevent volatiles escaping. This was repeated with either 2µl of re-distilled ether, volatiles entrained from damaged bean plants dissolved in ether (10ng/ml) (provided by Dr Keith Chamberlain, Rothamsted Research) or 6-methyl-5-hepten-2-one ($x10^{-4}$) added to the cap containing the distilled water. There were 15 replicates of each treatment in total. The positions of Petri dishes were randomised within an incubator and maintained at 18°C in darkness. Three replicates per treatment were destructively sampled after 5, 10, 15, 20 and 25 days and the radial growth (north, east, south and west) measured.

A Linear Regression model was constructed to assess whether there was an effect of time, treatment or a time-treatment interaction that affected the growth rate of *P. neoaphidis*.

3.3 RESULTS

3.3.1 Sporulation monitor experiment to assess the effect of *Acyrthosiphon pisum*- induced *Vicia faba* volatiles on the sporulation rate of *Pandora neoaphidis*-infected cadavers

There was a significant positive relationship between cadaver weight and total conidia produced (Linear regression: $F_{1, 39}= 15.37$, $p<0.001$) (Figure 3.2). Cadaver weight was, therefore, a covariate in subsequent analyses.

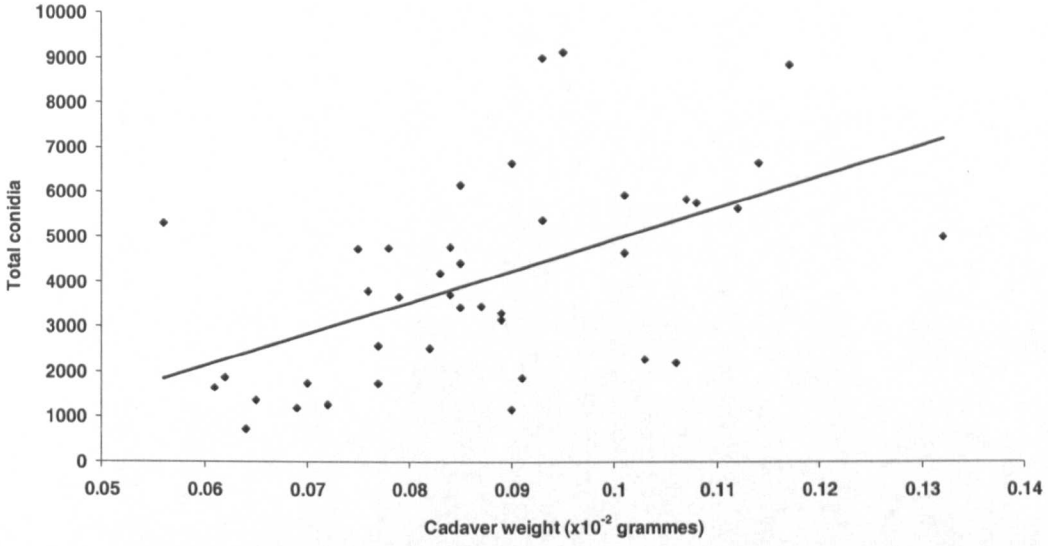

Figure 3.2 Relationship between the weight of *Pandora neoaphidis*-infected cadavers and the mean total number of conidia counted.

Sporulation had started in both treatments after four hours when the first count was made and continued throughout the experiment. There was a significant effect of time on sporulation over the 48-hour monitoring period (ANOVA: $F_{5, 219}= 55.20$, $p<0.001$), with the greatest number of conidia being produced after approximately eight hours (Figure 3.3). There were no significant differences in the number of conidia produced from cadavers exposed to volatiles from damaged bean plants infested with *A. pisum* compared to cadavers exposed to volatiles from undamaged bean plants over the 48 hour monitoring period (ANOVA: $F_{1, 479}= 0.88$, $p= 0.520$), nor was there a significant difference between the damaged and undamaged treatments in the total number of conidia produced (ANOVA: $F_{1, 39}= 0.02$, $p= 0.922$), with mean counts of 4117 and 3948 conidia respectively. There were no significant interactions between time and treatment that affected the number of conidia produced (ANOVA: $F_{5, 219}= 1.66$, $p= 0.146$) (Figure 3.3).

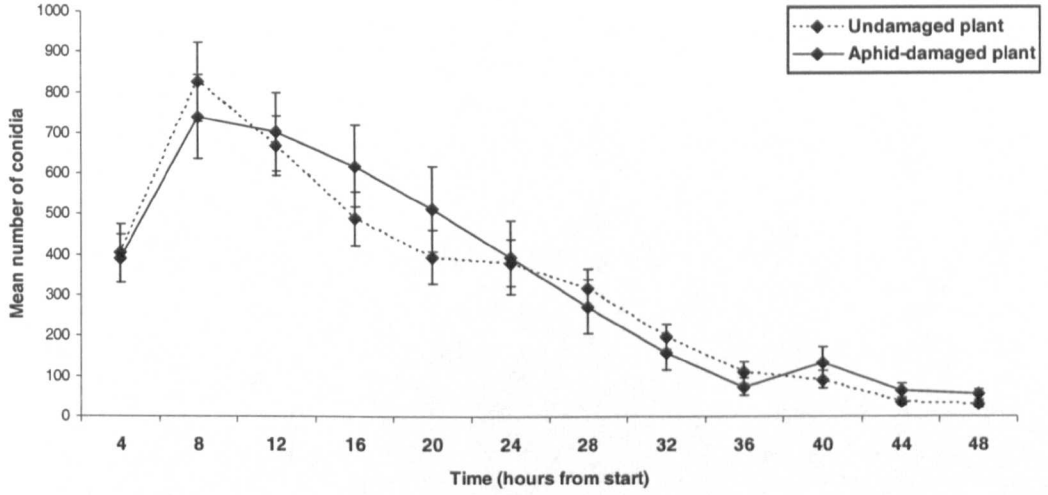

Figure 3.3 Mean number of conidia counted from *Pandora neoaphidis*-infected cadavers exposed to volatiles from undamaged and damaged bean plants over a 48 hour monitoring period.

3.3.2 *In vivo* conidia showering experiment to assess the effect of *Acyrthosiphon pisum*-induced *Vicia faba* volatiles on the conidia size of *Pandora neoaphidis*

There was a significant effect of time on the length (Repeated measures ANOVA: $F_{3,\ 1733}= 88.52$, $p<0.001$) and width (Repeated measures ANOVA: $F_{4,\ 1878}= 41.57$, $p<0.001$) of *P. neoaphidis* conidia over the 48-hour monitoring period (Figure 3.4). *Pandora neoaphidis* conidia exposed to volatiles from damaged bean plants were significantly longer than those exposed to volatiles from undamaged bean plants (Repeated measures ANOVA: $F_{1,\ 198}= 20.77$, $p<0.001$), with mean lengths of 24.6µm and 24.2µm respectively (Figure 3.4).

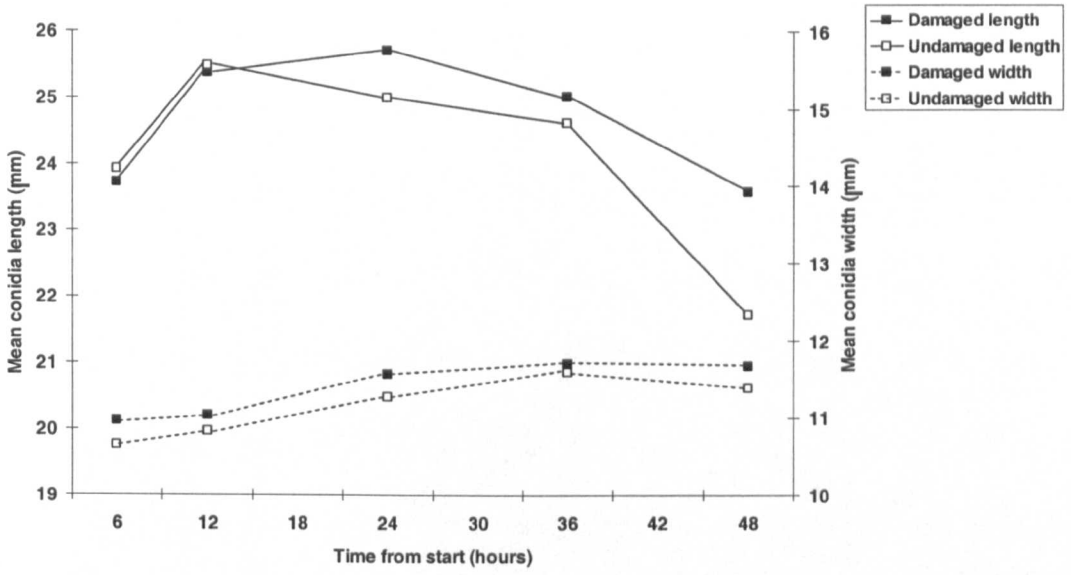

Figure 3.4 Mean lengths and widths of conidia collected from *Pandora neoaphidis*-infected cadavers exposed to volatiles from either undamaged or damaged bean plants over a 48 hour collection period.

Pandora neoaphidis conidia exposed to volatiles from damaged bean plants were significantly wider than those exposed to volatiles from undamaged bean plants (Repeated measures ANOVA: $F_{1,\ 198}= 15.99$, $p<0.001$), with mean widths of 11.4μm and 11.2μm respectively (Figure 3.4). There were significant interactions between time and treatment that affected the length of the conidia (Repeated measures ANOVA: $F_{3,\ 1733}= 10.85$, $p<0.001$) (Figure 3.4). There were no significant interactions between time and treatment that affected the width of the conidia (Repeated measures ANOVA: $F_{4,\ 1878}= 0.50$, $p= 0.726$) (Figure 3.4).

3.3.3 *In vivo* conidia showering experiment to assess the effect of *Acyrthosiphon pisum* induced *Vicia faba* volatiles on the germination of *Pandora neoaphidis* conidia

There were no significant effects of time on the number of germinating conidia (Logistic Regression: $F_{1,\ 87}= 0.37$, $p= 0.545$) (Figure 3.5). The number of conidia germinating was significantly greater on *A. pisum* feeding on previously damaged *V. faba* plants (that were releasing *A. pisum*-induced volatiles) compared to the number germinating on aphids feeding on previously undamaged control plants

(Logistic Regression: $F_{1, 87}$= 4.43, p= 0.038), with an average proportion of 0.31 and 0.26 germinating conidia respectively (Figure 3.5).

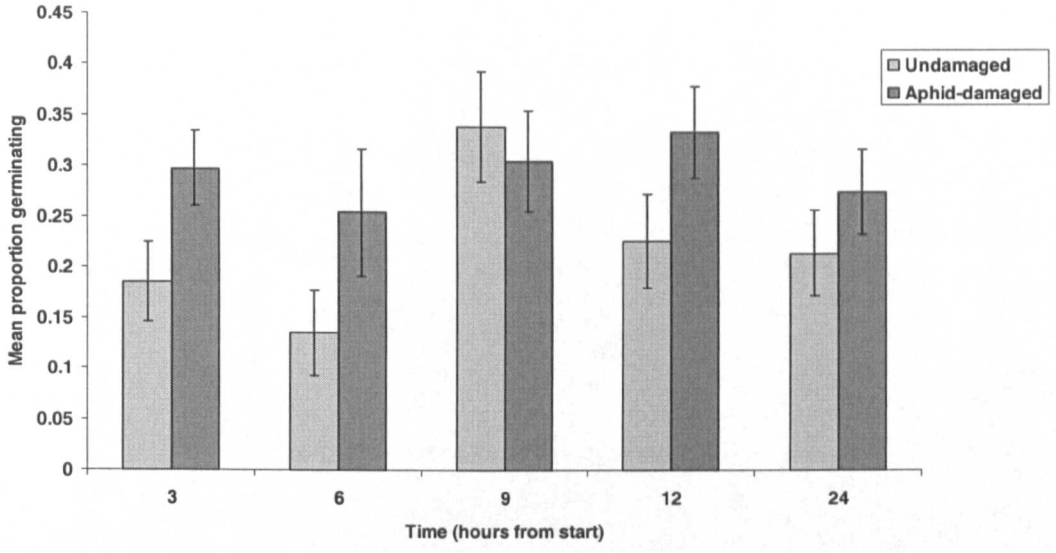

Figure 3.5 Mean proportion of *Pandora neoaphidis* germinating conidia on *Acyrthosiphon pisum* feeding on either undamaged or damaged bean plants over a 24 hour collection period.

The greatest difference in the number of germinating conidia appears to occur within 6 hours of inoculation, with more conidia germinating on aphids feeding on previously damaged *V. faba* plants. There were no interactions between time and treatment that affected the number of germinating conidia (Logistic Regression ANOVA: $F_{1, 87}$= 0.22, p= 0.639) (Figure 3.5).

3.3.4 *In vitro* experiment to assess the effect of *Acyrthosiphon pisum*-induced *Vicia faba* volatiles on the growth rate of *Pandora neoaphidis*

A single regression line provided an adequate description of the data. Colony size increased significantly with time (Linear regression: $F_{1, 51}$= 301.9, p<0.001). After 25 days the average radial growth of a colony was 13.76mm. Neither entrained damaged bean plant volatiles nor 6-methyl-5-hepten-2-one affected either the average colony size (Linear regression: $F_{1, 51}$= 0.45, p= 0.721; intercept= -1.583, S.E.= 0.99; slope= 1.0364, S.E.= 0.0607) or the rate of growth with time (Linear regression: $F_{3, 51}$= 2.14, p= 0.109) (Figure 3.6).

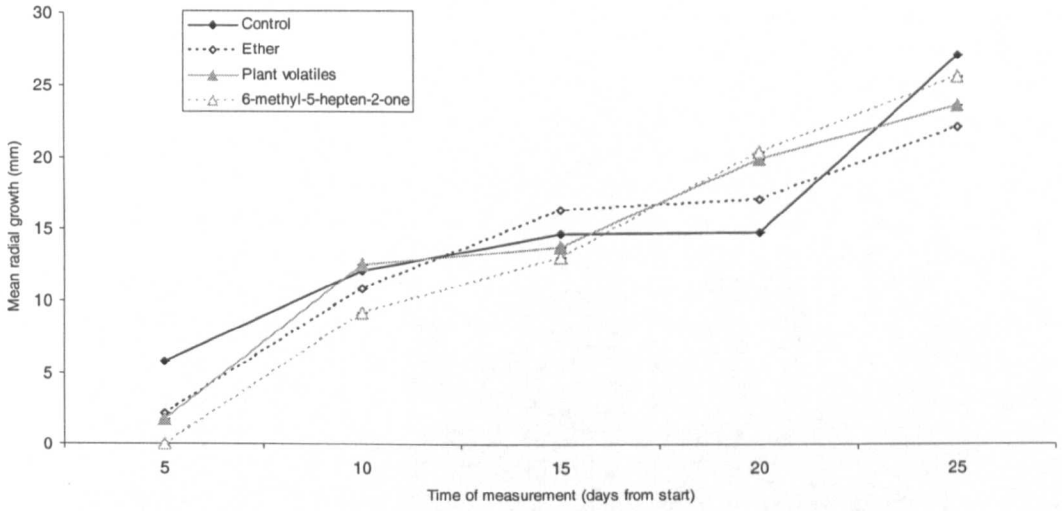

Figure 3.6 Mean *in vitro* radial growth of *Pandora neoaphidis* when exposed to volatiles from ether, entrained damaged bean plant volatiles dissolved in ether and, 6-methyl-5-hepten-2-one. The control treatment contained only distilled water.

3.4 DISCUSSION

The results presented here indicate that the proportion of *P. neoaphidis* conidia germinating on *A. pisum* is greater in the presence of volatiles released from damaged bean plants compared to the proportion germinating in the presence of volatiles released from undamaged bean plants. The presence of volatiles released from damaged bean plants also resulted in an increased conidial length and width. However, there were no effects of damaged bean plant volatiles on either the *in vivo* sporulation or *in vitro* growth of *P. neoaphidis*.

The absence of an effect of damaged plant volatiles on the mycelial growth may have been a result of the fungus: 1) not detecting the volatiles, 2) detecting but not responding to the volatiles or, 3) detecting but not being able to respond to the volatiles. In the study by Brown *et al.* (1995), green-leaf volatiles released from macerated tobacco leaves significantly reduced the *in vitro* growth rate of *P. neoaphidis* mycelia. These green leaf volatiles contained short chain aldehydes, which are used by plants as a defence against plant-pathogenic fungi (Lyr and Banasiak, 1983; Zeringue and McCormick, 1989). Brown *et al.* (1995) explained the reduced growth of *P. neoaphidis* as a result of the volatiles having a sub-lethal

effect on the fungus. When bean plants are infested with *A. pisum*, the blend of volatiles released also includes short chain aldehydes (Chamberlain, K. personal communication). The absence of an inhibitory effect of plant volatiles on mycelial growth during the experiment described here may have been a result of either; the volatile concentration not being great enough to have a lethal/ sub-lethal effect on the fungus or, the blend not containing volatiles detrimental to the fungus. However, from these data it was not possible to identify if either of these scenarios were occurring. Alternatively, the lack of an effect of damaged plant volatiles on mycelial growth may be a result of this process naturally occurring within the body of an aphid. The internal environment of the aphid is separated from external abiotic conditions by the cuticle, and this would prevent a direct interaction between plant volatiles and mycelial growth from occurring. It therefore seems unlikely that *P. neoaphidis* would show an adaptive growth response to plant volatiles and questions whether or not the effects observed by Brown *et al.* (1995) would occur under field conditions.

These data show that there is a significant positive relationship between cadaver weight and total conidia, and supports previous findings by Hemmati (1998). This positive relationship may be a result of larger aphids having more nutrients available to the fungus which allows a greater investment of energy into conidia production. Sporulation therefore appears to be limited primarily by host physiology and is unlikely to be influenced by volatile cues.

Pandora neoaphidis actively discharges conidia which are dispersed on wind currents (Hemmati *et al.*, 2001). The smaller the aerodynamic diameter (d_a; the diameter of a unit density (1g cm^{-3}) sphere that has the same fall speed as the conidium) of a conidium the greater the potential for dispersal (Hemmati *et al.*, 2002). For example, conidia ejected from *P. neoaphidis* have a smaller d_a than those released from *Conidiobolus obscurus* (Hall and Dunn), indicating that *P. neoaphidis* is better able to disperse and would travel further in air currents before being deposited (Hemmati, *et al.*, 2002). The results presented in this study show that conidia released in the presence of damaged plant volatiles are larger than those released in the presence of undamaged plant volatiles. Damaged plant volatiles may, therefore, indirectly reduce the ability of the conidia to disperse. Potentially,

P. neoaphidis may be responding to damaged plant volatiles by producing larger conidia that would settle nearby, i.e. on the plant which is being damaged by host aphids. However, the observed difference in conidial size may be statistically significant but not biologically significant. For example, Hemmati *et al.* (2002) found that although conidia released from field collected *P. neoaphidis*-infected cadavers were both longer and wider than those released from laboratory produced *P. neoaphidis*-cadavers (23.5 x 12.0μm and 18.8 x 10.2μm respectively), the dispersal characteristics of the conidia were similar. As the difference in conidial size found by Hemmati *et al.* (2002) is greater than the difference reported in this study (damaged = 24.6 x 11.4μm, undamaged = 24.2 x 11.2μm), it is unlikely that the dispersal characteristics of conidia exposed to volatiles from damaged and undamaged bean plants would differ significantly.

These results indicate that the proportion of conidia germinating on aphids feeding on previously damaged bean plants was greater than the proportion germinating on aphids feeding on previously undamaged plants. This does not support the findings of Brown *et al.* (1995), who found that germination was inhibited in the presence of both green leaf volatiles and aphid-induced volatiles released from tobacco plants. In the model by Brown *et al.* (1995), plant volatiles have a negative effect on germination which results in the inhibition of secondary conidia production and increases the time primary conidia remain on the leaf. This then resulted in an increased potential of viable primary conidia making contact with a suitable host. However, this is unlikely to occur as conidia that are deposited on leaves tightly adhere to the hydrophobic surface and require secondary conidia to be released for subsequent infection (Pell, J. K. personal communication). It is likely that the effects observed by Brown *et al.* (1995) are the result of toxic effects of the volatiles on both germination and growth of the fungus. Tobacco plants infested with aphids release nicotine and this has an insecticidal effect on aphids (Dixon, 1998). Nicotine may have a toxic effect on *P. neoaphidis* which results in decreased growth and germination. The results presented here indicate an alternative direct functional response of *P. neoaphidis* to aphid-induced plant volatiles to that found by Brown *et al.* (1995) and indicate a positive effect of plant volatiles on the fungus. The increased germination in the presence of damaged plant volatiles observed here may have been a result of the fungus detecting and

responding to the volatiles. This suggests a positive effect of plant volatiles on germination in which the fungus may use plant derived cues to indicate whether or not it is on a suitable host. This result complements the findings from plant-arthropod interactions in which herbivore-induced plant volatiles are used by predators and parasitoids as a detectable and reliable source of information to locate prey/hosts (Dicke, 1999; Du *et al.*, 1996; Guerrieri *et al.*, 1999). If the increase in the proportion of germinating conidia in the presence of host-aphid induced volatiles was an active response by the fungus to increase its reproductive potential and this resulted in an increased infection level, the fungus would, in effect, be acting as a bodyguard. Chapter 4 examines the infectivity of *P. neoaphidis* towards *A. pisum* feeding on damaged and undamaged bean plants.

For volatiles to affect the germination of a fungus directly, the fungus must be able to detect and respond to the volatiles. However, the observed increases in both the proportion of germinating conidia and the dimensions of the conidia may also be due to the microclimate of a plant previously infested with aphids providing abiotic conditions that favour germination. For example, the microclimate of the leaf surface of an aphid damaged plant may have a higher relative humidity than an undamaged leaf. Abiotic conditions have a direct effect on several species of entomopathogenic fungi (e.g. Dean and Wilding, 1971; Wilding, 1969; Yeo *et al.*, 2003). For example, as temperature increases, the *in vitro* germination and growth of isolates of *B. bassiana*, *L. lecanii*, *M. anisopliae* and *Paecilomyces fumosoroseus* (Wize) Brown also increases (Yeo *et al.*, 2003). Abiotic conditions therefore have a strong influence on the germination rate of entomopathogenic fungus and this has been linked to increased pathogencity (Altre, Vandenberg and Cantone, 1999; Jackson, Heale and Hall, 1985). Infectivity of *P. neoaphidis* is optimised in an environment with a relative humidity greater than 90% and a temperature of 20°C (Hajek, 2001; Wilding 1973). It may, therefore, be an indirect side effect of aphid infestation that results in increased germination and conidial size and this may affect the infectivity of *P. neoaphidis* (see section 4.4).

CHAPTER 4: PLANT-APHID-FUNGUS INTERACTIONS: DOES PLANT CONDITION HAVE AN EFFECT ON THE TRANSMISSION AND INFECTIVITY OF *PANDORA NEOAPHIDIS*?

4.1 INTRODUCTION

Pandora neoaphidis disperses and reproduces via the release of conidia (see section 1.1.2.1). Conidia that are released may infect aphids within the same aphid colony (within plant transmission) or in a second, previously uninfected, aphid colony (between plant transmission). There is a variety of ways in which conidia may move between aphid colonies. Conidia that are released from sporulating cadavers are forcibly ejected to a height of approximately 2mm (Hemmati *et al.*, 2001). This allows the conidia to escape the boundary layer of the plant and be dispersed in wind currents before potentially being deposited in another aphid colony (Hemmati *et al.*, 2001). Alternatively, transmission of fungal conidia may occur through the movement of infected aphids. In a survey by Chen and Feng (2002), 35% of alate *M. persicae* trapped from the air during migratory flight were infected with fungal pathogens, 66.1% of which were infected with *P. neoaphidis*. Movement of fungus-infected aphids into a second aphid colony may, therefore, result in the successful transmission of the pathogen. A final mechanism through which conidia can move between aphid colonies is through vectoring of conidia by other foraging arthropods. For example, whilst foraging for aphid prey, the coccinellid *C. septempunctata* can vector *P. neoaphidis* conidia to a second, previously uninfected aphid colony (Roy *et al.*, 1998) (see section 1.3.1.2). As the prey of the coccinellid is the same resource as that required by the fungus, the coccinellid is referred to as being a 'targeted vector'.

Once conidia have 'entered' an aphid colony, infection of suitable hosts and subsequent within plant transmission may occur. Conidia may become attached to a host aphid as a result of either direct deposition from the air currents or through the attachment of secondary conidia produced by primary conidia that have been deposited on the leaf surface. Increased aphid movement would increase the probability of the aphid coming into contact with the conidia and therefore increase

the transmission potential of the fungus. For example, the presence of the parasitoid *A. rhopalosiphi* increased the movement of the aphid *S. avenae* resulting in a greater proportion of the aphids becoming infected with *P. neoaphidis* (Fuentes-Contreras *et al.*, 1998). Other examples of enhanced fungal transmission in the presence of foraging arthropods include increased transmission of *Z. radicans* as a result of *D. semiclausam* searching for *P. xylostella* and increased transmission of *P. neoaphidis* as a result of *C. septempunctata* searching for *A. pisum* (Furlong and Pell, 1996; Pell *et al.*, 1997; Roy 1997; Roy *et al.*, 1998).

Transmission of a pathogen to a host may be influenced by the foraging behaviour of the host. Whilst foraging, aphids use both visual and chemical cues (Dixon, 1998). Although there is no direct ecological evidence confirming that aphids use plant volatile cues during host location, there is strong evidence from behavioural bioassays which suggests that these cues affect the foraging behaviour of aphids (Dixon, 1998). For example, *A. fabae* responded to volatiles released from undamaged bean plants but not to volatiles from damaged bean plants (Nottingham *et al.*, 1991). This finding was supported by Quiroz *et al.* (1997) who found that volatiles released from undamaged wheat seedlings had an arrestant effect on the aphid *R. padi* (L.) whereas *R. padi* was repelled by volatiles released from wheat plants infested with conspecific aphids. It is thought that the volatiles released from previously aphid-infested plants counteract the attractiveness of undamaged plant volatiles and that this resulted in a 'spacing behaviour' between aphids (Quiroz *et al.*, 1997).

The presence of intraguild competitors affects the foraging behaviour of aphids. Volatiles released from undamaged maize plants (*Zea mays* L.) attracted the aphid *R. maidis* (Fitch) in both olfactometers and whole plant behavioural studies, whereas volatiles released from mechanically damaged maize plants treated with regurgitate from the competitor, *S. littoralis*, repelled *R. maidis* (Bernasconi *et al.*, 1998). Analysis indicated that mechanically damaged plants treated with regurgitate from *S. littoralis* released a different blend of volatiles to those released from undamaged maize plants. It is thought that these repellent volatiles may convey information to the aphid: that the plant has initiated the release of toxic chemicals, that the plant is infested with a competitor or, that these volatiles may

attract predators and parasitoids/ increase the infectivity of entomopathogens (Bernasconi *et al.*, 1998; Brown *et al.*, 1995) (see section 1.3.2.2). The direct effect of a foraging arthropod may influence the foraging behaviour of an aphid. Aphids may avoid interacting with predator/ parasitoids/ pathogens by feeding in areas of a plant where their risk of encountering the natural enemy is lowest (i.e. enemy-free-space) (Hopkins and Dixon, 1997; Jeffries and Lawton, 1984). Alternatively, aphids may respond to the presence of natural enemies on encountering them, for example, by dropping from the plant or kicking (Chau and Mackauer, 1997; Hopkins and Dixon, 1997; Villagra, Ramirez and Niemeyer, 2002).

Aphids therefore respond to the presence of competitors and natural enemies whilst foraging. The foraging behaviour of *A. pisum* may be affected by changes in the condition of the host plant (i.e. damaged or undamaged) or the presence of a natural enemy, and this may increase the likelihood of contact with conidia. Alternatively, the responses of the host plant to aphid infestation may affect the infectivity of a fungal pathogen through a direct effect on the fungus, the aphid or both. This chapter examines whether the condition of the host plant influences the interactions between *A. pisum* and *P. neoaphidis* and assesses the direct effect of fungal infection on the reproductive potential of *A. pisum*. More specifically:

- The effects of *P. neoaphidis* and conspecific aphid-induced plant volatiles on the behaviour of *A. pisum* during host-plant selection
- The effects of conspecific aphid-induced plant volatiles on the transmission of *P. neoaphidis* during host-plant colonisation and *in situ* feeding
- The effects of conspecific aphid-induced plant volatiles on the infectivity of *P. neoaphidis*
- The effect of *P. neoaphidis* on the reproduction rate of *A. pisum*

4.2 MATERIAL AND METHODS

4.2.1 Dual choice whole plant experiment to assess whether *Acyrthosiphon pisum* colonises bean plants containing *Pandora neoaphidis*-sporulating cadavers

Seventy-two hours prior to the start of the experiment, two 15-day-old bean plants were transplanted into a seed tray (370mm x 230mm x 50mm) 180mm apart. The seed tray was then placed in a Perspex cage ($0.5m^2$ x 1m) within a controlled environment room (18°C, 16L:8D). Four replicate cages were prepared in total. Eighty *A. pisum* were added to each pair of plants (40 per plant) in two cages in order to damage them (as described in section 2.1.2) whilst the plants within the remaining two cages remained undamaged. All cages were maintained for 72 hours, after which time the aphids were removed from the damaged plants.

Twenty water-agar discs, each supporting two sporulating cadavers, were prepared as described in section 2.3.1.1. Five *P. neoaphidis*-agar discs were added to each cage to produce the following four treatments: undamaged plants plus *P. neoaphidis* on the left-hand-plant only, undamaged plants plus *P. neoaphidis* on the right-hand-plant only, damaged plants plus *P. neoaphidis* on the left-hand-plant only and damaged plants plus *P. neoaphidis* on the right-hand-plant only. A Petri dish (50mm diameter) containing twenty 8-day-old *A. pisum* (4^{th} instar) was then placed equidistant from each of the two plants and the lid removed. The number of aphids on each plant was assessed after 24 hours. The experiment was repeated four times using a Latin square design to ensure all four treatments were done in each cage once.

Before analysis, the data were transformed using a log transformation. ANOVA was used to assess whether there was an effect of damaged bean-plant volatiles, the presence of *P. neoaphidis* or an interaction between the bean-plant volatiles and *P. neoaphidis* on the number of aphids recovered.

4.2.2 Whole plant experiments to assess the effect of previous infestation by *Acyrthosiphon pisum* on the transmission of *Pandora neoaphidis* to *A. pisum*

4.2.2.1 *Transmission during colonisation*

Seventy-two hours prior to the start of the experiment, four 15-day-old bean plants, planted in individual plant pots (90mm diameter), were placed in a Perspex cage

($0.5m^2$ x 1m) within a controlled environment room (18°C, 16L:8D). Water was poured into the cage to a depth of 20mm to create a water trap between the plants. Four replicate cages were prepared. Forty *A. pisum* were added to each plant in two replicate cages in order to damage them (as described in section 2.1.2) whilst the plants within the remaining two cages remained undamaged. The cages were maintained for 72 hours, after which time the aphids were removed from the damaged plants. The soil around the base of each plant was then covered using a piece of filter paper (90mm diameter).

Forty water-agar discs, each supporting two sporulating cadavers, were prepared as described in section 2.3.1.1. Five water-agar discs were added to each plant within two cages to produce the following four treatments: undamaged plants, undamaged plants plus *P. neoaphidis*, damaged plants and damaged plants plus *P. neoaphidis*. Finally, fifteen 8-day-old *A. pisum* were placed on the filter paper at the base of each plant. The number of living aphids, dead aphids and *P. neoaphidis*-sporulating cadavers was assessed after 120 hours. The experiment was repeated four times using a Latin-square design to ensure all four treatments were done in each cage once.

Before analysis the data were transformed using a log(n+1) transformation. ANOVA was used to assess whether there was an effect of either damaged bean-plant volatiles or rehydrated *P. neoaphidis*-sporulating cadavers on the number of living aphids, dead aphids or *P. neoaphidis*-sporulating cadavers recovered. Any interaction between the bean-plant volatiles and rehydrated *P. neoaphidis*-sporulating cadavers that affected the number of living and dead aphids recovered was also assessed.

4.2.2.2 *Transmission during* in situ *feeding*

The method described in section 4.2.2.1 was repeated with the following modification. The fifteen 8-day-old *A. pisum* were placed at the base of the plant and left for 3 hours prior to the water-agar discs supporting two sporulating cadavers being added. This gave the aphids time to colonise the plant and start feeding before the fungus was added.

4.2.3 Whole plant experiment to assess the infectivity of *Pandora neoaphidis* to *Acyrthosiphon pisum* feeding on either undamaged or damaged *Vicia faba* plants

Seventy-two hours prior to the start of the experiment, eight 15-day-old bean plants, planted in individual plant pots (90mm diameter), were placed in a controlled environment chamber (18°C, 16L:8D). Forty *A. pisum* were added to each plant in order to damage them (as described in section 2.1.2). This was repeated using a second controlled environment chamber with bean plants that remained uninfested. Separate chambers were used to prevent volatiles released from undamaged and damaged plants mixing. The plants were maintained for 72 hours, after which the aphids were removed from the damaged plants. The soil at the base of each plant was then covered using a piece of filter paper (90mm diameter).

One hundred and twenty 10-day-old *A. pisum* (day-one adult) were inoculated with *P. neoaphidis* as described in section 2.3.1. The aphids were then divided into eight batches of 15 and placed on the filter paper at the base of four undamaged and four damaged plants. The plants were then covered with lamp glasses which were sealed using Clingfilm (to create >90% relative humidity) and placed back in the controlled environment chambers they had been taken from. This was repeated using uninfected aphids. Four treatments were prepared in total i.e. undamaged plants plus healthy aphids, undamaged plants plus *P. neoaphidis*-infected aphids, damaged plants plus healthy aphids and damaged plants plus *P. neoaphidis*-infected aphids.

After 24 hours, the cling film was replaced with netting and the plants maintained within the controlled environment chamber for a further 96 hours. After 96 hours the number of living aphids, dead aphids (not fungus infected) and *P. neoaphidis*-sporulating cadavers was assessed. The experiment was repeated four times in total, with each treatment having been done in both controlled environment chambers twice.

Before analysis the data were transformed using a log(n+1) transformation. ANOVA was used to assess whether there was an effect of either damaged bean-plant volatiles or inoculation with *P. neoaphidis* on the number of living aphids, dead aphids or *P. neoaphidis*-sporulating cadavers recovered. Any interaction between the bean-plant volatiles and inoculation with *P. neoaphidis* that affected the number of living and dead aphids recovered was also assessed.

4.2.4 Petri dish bioassay to assess the effect of *Pandora neoaphidis* on the reproductive rate of *Acyrthosiphon pisum*

To ensure 100% infection, the following method was used to inoculate *A. pisum* with *P. neoaphidis*. Three dried *P. neoaphidis* cadavers were positioned on a tissue that was placed on a damp sponge within a cap (20mm diameter). The cap was placed in a sealed plastic box containing wet tissue (to create >90 per cent relative humidity) and maintained in the dark at 10°C for 18 hours until the cadavers were sporulating profusely.

Twenty ten-day-old (day-1 adult) *A. pisum* were placed in an open ended glass tube (20mm diameter, 50mm long) which had one end sealed using netting (0.5mm diameter mesh) that was held in place with an elastic band. The open end of the tube was covered using a second piece of netting (2mm diameter mesh) that was held in place using the cap containing the cadavers. The tube was then positioned (cap end at the top) on a slide within a plastic box and maintained in darkness under ambient laboratory conditions for 1.5 hours. Conidia collected on the slide were examined to confirm they were *P. neoaphidis*.

The 20 *P. neoaphidis*-infected *A. pisum* were then transferred individually to separate bean leaves that were abaxial side uppermost on 1.5% tap-water-agar within a Petri dish (90mm diameter). This was repeated for 20 healthy one-day-old adult *A. pisum*. The dishes were then covered with a piece of tissue to reduce condensation and the lid replaced. Finally, the dishes were inverted and maintained at 18°C (16L:8D). The total number of nymphs produced was recorded after 24, 48, 72 and 96 hours. Replicates in which the aphid died without sporulating were removed from the analysis.

Before analysis the data were transformed using a log(n+1) transformation. ANOVA was used to assess whether there was an effect of *P. neoaphidis* on the total number of nymphs produced. A Repeated Measures ANOVA was used to assess whether there was an effect of *P. neoaphidis*, time or a *P. neoaphidis*-time interaction that affected the number of nymphs produced.

4.3 RESULTS

4.3.1 Dual choice whole plant experiment to assess whether *Acyrthosiphon pisum* colonises bean plants containing *Pandora neoaphidis*-sporulating cadavers

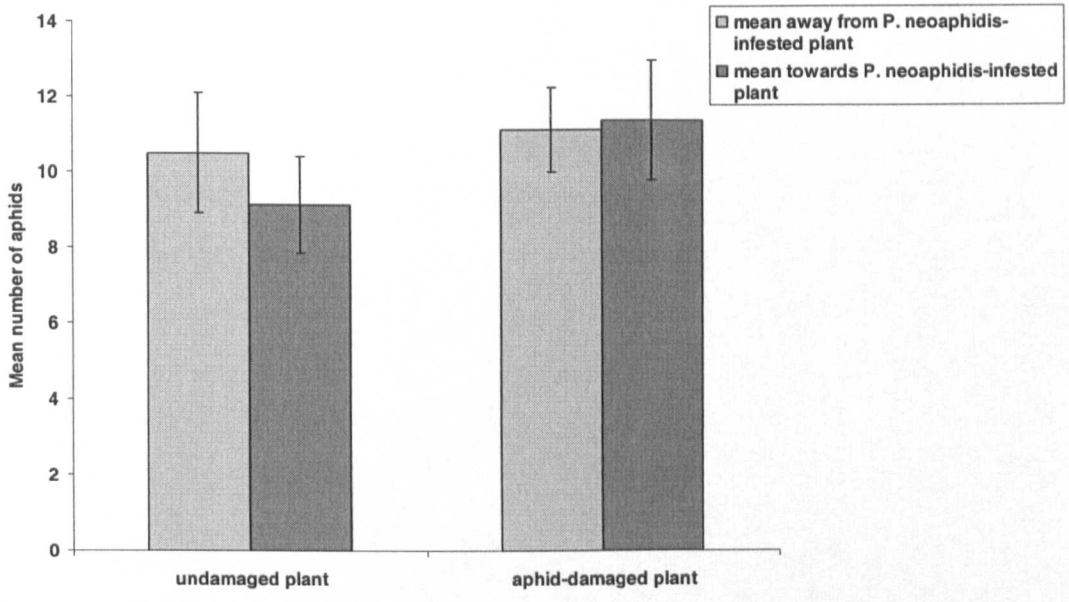

Figure 4.1 Mean number of *Acyrthosiphon pisum* recovered from undamaged and damaged bean plants either containing or not containing *Pandora neoaphidis*-sporulating cadavers.

There was a significant difference in the mean number of aphids recovered from the undamaged and damaged plant treatments (ANOVA: $F_{1, 31}= 6.56$, $p= 0.034$), with a mean of 11.25 aphids recovered from the damaged plant treatment and 9.81 aphids recovered from the undamaged plant treatment (Figure 4.1). There were no significant effects of *P. neoaphidis*-sporulating cadavers on the mean number of

aphids recovered (ANOVA: $F_{1, 31}$= 1.03, p= 0.327), with a mean of 10.81 aphids recovered from uninfested plants and 10.25 aphids from plants containing *P. neoaphidis*-sporulating cadavers (Figure 4.1). There were no significant interactions between plant condition and *P. neoaphidis*-sporulating cadavers that affected the mean number of aphids recovered (ANOVA: $F_{1, 31}$= 0.97, p= 0.341).

4.3.2 Whole plant experiments to assess the effect of previous infestation by *Acyrthosiphon pisum* on the transmission of *Pandora neoaphidis* to *A. pisum*

4.3.2.1 *Transmission during colonisation*

There were no significant effects of plant condition on the mean number of aphids recovered alive from the undamaged plant and damaged plant treatments (ANOVA: $F_{1, 63}$= 1.02, p= 0.352), with a of mean 8.44 aphids recovered from the undamaged plant treatments and 8.25 aphids recovered from the damaged plant treatments (Figure 4.2). There was a significant effect of *P. neoaphidis* on the mean number of aphids recovered alive (ANOVA: $F_{1, 63}$= 31.43, p= 0.001), with a mean of 9.94 aphids recovered from treatments not containing *P. neoaphidis*-sporulating cadavers and 6.75 aphids recovered from treatments containing *P. neoaphidis*-sporulating cadavers (Figure 4.2). There was no significant interaction between plant condition and *P. neoaphidis*-sporulating cadavers that affected the mean number of aphids recovered alive (ANOVA: $F_{1, 63}$= 0.78, p= 0.411).

There was a significant effect of *P. neoaphidis* on the mean number of sporulating cadavers recovered (ANOVA: $F_{1, 63}$= 156.18, p<0.001), with a mean of 0 sporulating cadavers recovered from treatments not containing *P. neoaphidis*-sporulating cadavers and 2.16 sporulating cadavers recovered from treatments containing *P. neoaphidis*-sporulating cadavers (Figure 4.2). However, there were no significant differences in the mean number of sporulating cadavers recovered from the damaged plant plus *P. neoaphidis* and undamaged plant plus *P. neoaphidis* treatments (ANOVA: $F_{1, 31}$= 0.04, p= 0.835), with 2.19 and 2.13 sporulating cadavers recovered respectively (Figure 4.2).

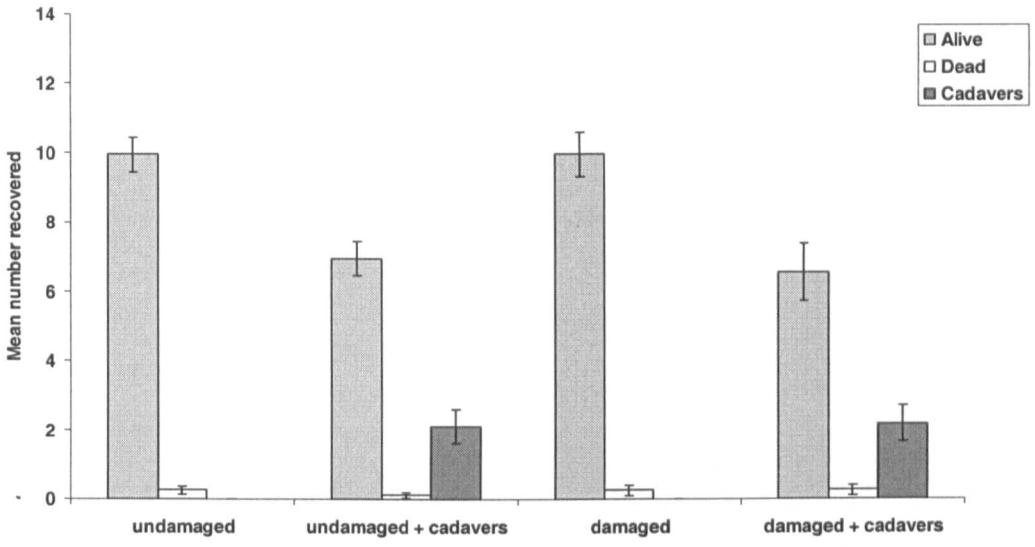

Figure 4.2 Mean number of *Acyrthosiphon pisum* recovered alive, dead or as *P. neoaphidis*-sporulating cadavers after colonising and feeding for 5 days on undamaged or damaged bean plants either containing or not containing *Pandora neoaphidis*-sporulating cadavers.

There were no significant effects of plant condition on the mean number of dead aphids (due to causes other than the fungus) recovered from the undamaged and damaged plant treatments (ANOVA: $F_{1, 63}= 0.06$, $p= 0.821$), with a mean of 0.19 dead aphids recovered from the undamaged plant treatments and 0.25 dead aphids recovered from the damaged plant treatments (Figure 4.2). There were no significant effects of *P. neoaphidis* on the mean number of dead aphids recovered (ANOVA: $F_{1, 63}= 0.16$, $p= 0.700$), with a mean of 0.25 dead aphids recovered from treatments not containing *P. neoaphidis*-sporulating cadavers and 0.19 dead aphids recovered from treatments containing *P. neoaphidis*-sporulating cadavers (Figure 4.2). There was no significant interaction between plant condition and *P. neoaphidis*-sporulating cadavers that affected the mean number of dead aphids recovered (ANOVA: $F_{1, 63}= 0.16$, $p= 0.700$).

4.3.2.2 *Transmission during* in situ *feeding*

There were no significant effects of plant condition on the mean number of aphids recovered alive from the undamaged and damaged plant treatments (ANOVA: $F_{1, 63}= 0.15$, $p= 0.714$), with a mean of 10.5 aphids recovered alive from the undamaged plant treatments and 10.3 aphids recovered from the damaged plant

treatments (Figure 4.3). There was a significant effect of *P. neoaphidis* on the mean number of aphids recovered alive (ANOVA: $F_{1, 63}$= 16.26, p= 0.007), with a mean of 11.3 aphids recovered from treatments not containing *P. neoaphidis*-sporulating cadavers and 9.4 aphids recovered from treatments containing *P. neoaphidis*-sporulating cadavers (Figure 4.3). There was no significant interaction between plant condition and *P. neoaphidis*-sporulating cadavers that affected the mean number of aphids recovered alive (ANOVA: $F_{1, 63}$= 0.7, p= 0.435).

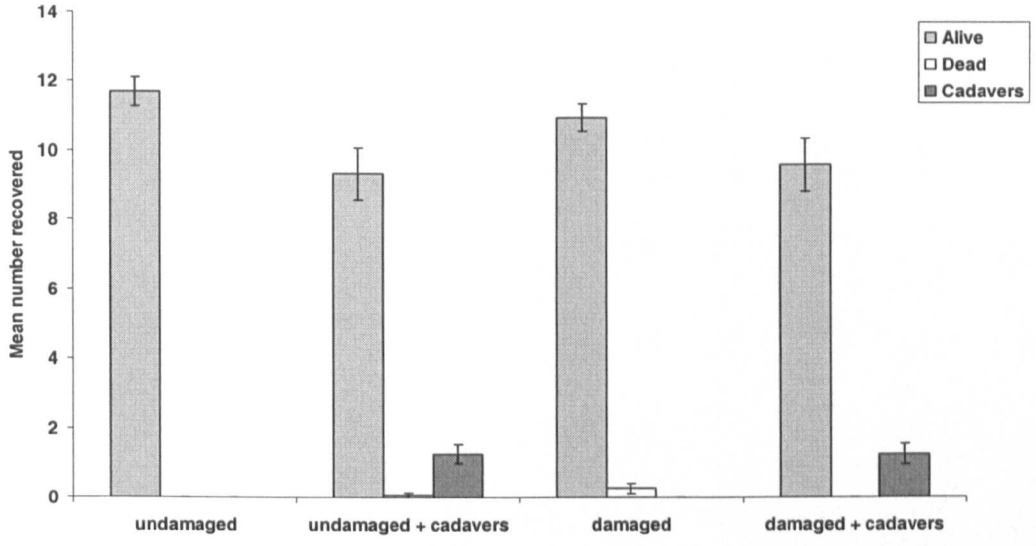

Figure 4.3 Mean number of *Acyrthosiphon pisum* recovered alive, dead or as *P. neoaphidis*-sporulating cadavers after feeding for five days on undamaged or damaged bean plants that were either containing or not containing *Pandora neoaphidis*-sporulating cadavers.

There was a significant effect of *P. neoaphidis*-sporulating cadavers on the mean number of sporulating cadavers recovered (ANOVA: $F_{1, 63}$= 32.86, p= 0.001), with a mean of 0 sporulating cadavers recovered from treatments not containing *P. neoaphidis*-sporulating cadavers and 1.25 sporulating cadavers recovered from treatments containing *P. neoaphidis*-sporulating cadavers (Figure 4.3). However, there were no significant differences in the mean number of sporulating cadavers recovered from the damaged plant plus *P. neoaphidis* and undamaged plant plus *P. neoaphidis* treatments (ANOVA: $F_{1, 31}$= 0.02, p= 0.893), with a mean of 1.25 sporulating cadavers recovered from both treatments (Figure 4.3).

There were no significant effects of plant condition on the mean number of dead aphids (due to causes other than the fungus) recovered from the undamaged plant and damaged plant treatments (ANOVA: $F_{1, 63}$= 1.30, p= 0.297), with a mean of 0.03 dead aphids recovered from the undamaged plant treatments and 0.13 dead aphids recovered from the damaged plant treatments (Figure 4.3). There were no significant effects of *P. neoaphidis*-sporulating cadavers on the mean number of dead aphids recovered (ANOVA: $F_{1, 63}$= 1.30, p= 0.297), with a mean of 0.06 dead aphids recovered from treatments not containing *P. neoaphidis*-sporulating cadavers and 0.03 dead aphids recovered from treatments containing *P. neoaphidis*-sporulating cadavers (Figure 4.3). There was no significant interaction between plant condition and *P. neoaphidis*-sporulating cadavers that affected the mean number of dead aphids recovered (ANOVA: $F_{1, 63}$= 4.10, p= 0.089).

4.3.3 Whole plant experiment to assess the infectivity of *Pandora neoaphidis* to *Acyrthosiphon pisum* feeding on either undamaged or damaged *Vicia faba* plants

There were no significant effects of plant condition on the mean number of aphids recovered alive from the undamaged and damaged plant treatments (ANOVA: $F_{1, 63}$= 9.70, p= 0.089), with a mean of 7.78 aphids recovered alive from the undamaged plant treatments and 6.81 aphids recovered from the damaged plant treatments (Figure 4.4). There was a significant effect of *P. neoaphidis* on the mean number of aphids recovered alive (ANOVA: $F_{1, 63}$= 80.57, p<0.001), with a mean of 10.3 aphids recovered from treatments containing uninoculated aphids and 4.31 aphids recovered from treatments containing inoculated aphids. There was no significant interaction between plant condition and inoculation with *P. neoaphidis* that affected the mean number of aphids recovered alive (ANOVA: $F_{1, 63}$= 0.02, p= 0.902).

There was a significant effect of inoculation with *P. neoaphidis* on the mean number of sporulating cadavers recovered (ANOVA: $F_{1, 63}$= 490.77, p<0.001), with a mean of 0 sporulating cadavers recovered from treatments containing uninoculated aphids and a mean of 6 sporulating cadavers recovered from treatments containing inoculated aphids. However, there were no significant

differences in the mean number of sporulating cadavers recovered from the damaged plant plus inoculated aphids and the undamaged plant plus inoculated aphids treatments (ANOVA: $F_{1, 31}$= 1.46, p= 0.351), with a mean of 6.06 and 5.94 sporulating cadavers recovered respectively.

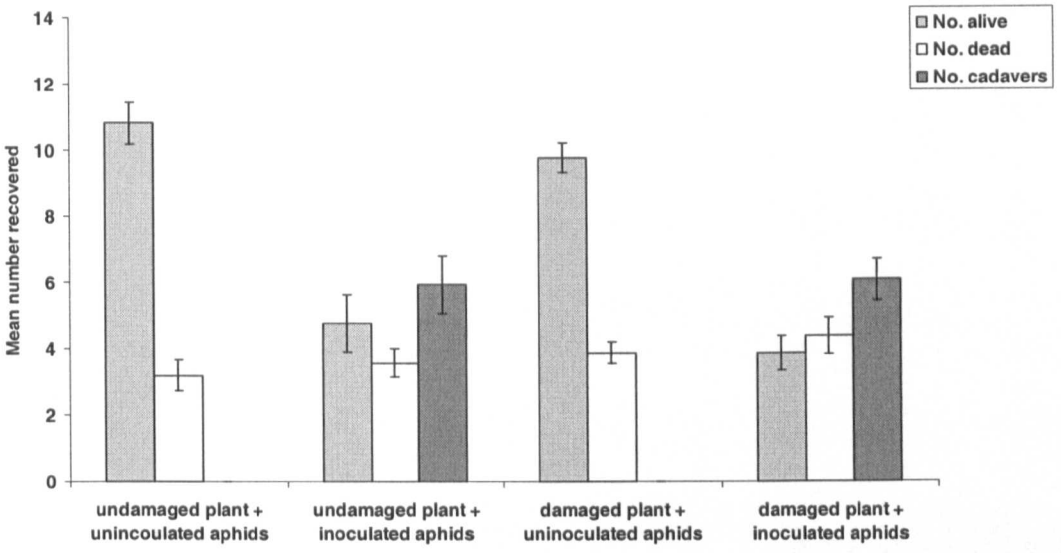

Figure 4.4 Mean number of *Acyrthosiphon pisum*, either previously uninoculated or inoculated with *P. neoaphidis*, recovered alive, dead or as *P. neoaphidis*-sporulating cadavers from either undamaged or damaged bean plants.

There were no significant effects of plant condition on the mean number of dead aphids recovered from the undamaged plant and damaged plant treatments (ANOVA:$F_{1, 63}$= 20.67, p= 0.045), with a mean of 3.38 dead aphids recovered from the undamaged plant treatments and 4.13 dead aphids recovered from the damaged plant treatments. There were no significant effects of inoculation with *P. neoaphidis* on the mean number of dead aphids recovered (ANOVA: $F_{1, 63}$= 0.62, p= 0.433), with a mean of 3.53 dead aphids recovered from treatments containing uninoculated aphids and 3.97 dead aphids recovered from treatments containing inoculated aphids. There was no significant interaction between plant condition and inoculation with *P. neoaphidis* that affected the mean number of dead aphids recovered (ANOVA: $F_{1, 63}$= 0.05, p= 0.821).

4.3.4 Petri dish bioassay to assess the effect of *Pandora neoaphidis* on the reproductive rate of *Acyrthosiphon pisum*

There was a significant effect of *P. neoaphidis* infection on nymph production by *A. pisum* (Repeated Measures ANOVA: $F_{1, 159}$= 238.30, p<0.001), with fewer nymphs produced from aphids infected with *P. neoaphidis* at all sample times compared to the uninfected control (Figure 4.5).

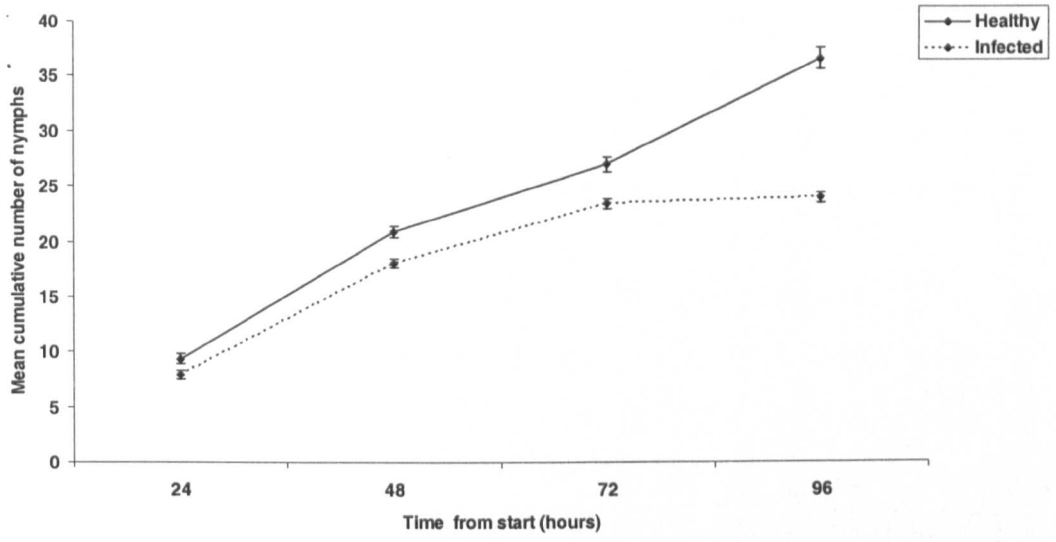

Figure 4.5 Mean cumulative number of nymphs produced by healthy and *Pandora neoaphidis*-infected *Acyrthosiphon pisum* over a 96 hour observation period.

There was a significant effect of time on nymph production by *A. pisum* (Repeated Measures ANOVA: $F_{2, 120}$= 102.24, p<0.001), with the greatest mean number of nymphs produced between 24 and 48 hours from uninfected aphids and the fewest mean number of nymphs produced between 72 and 96 hours from *P. neoaphidis*-infected aphids (Figure 4.5). There was also a significant interaction between *P. neoaphidis* infection and time that affected nymph production of *P. neoaphidis*-infected aphids (Repeated Measures ANOVA: $F_{2, 120}$= 89.97 p<0.001) (Figure 4.5). There was a significant difference in the total number of nymphs produced over the 96 hour observation period (ANONA: $F_{1, 39}$= 147.04, p<0.001), with a mean total of 23.85 nymphs produced from *P. neoaphidis*-infected aphids and 36.50 nymphs produced from healthy control aphids.

4.4 DISCUSSION

When colonising bean plants, *A. pisum* showed no preference between plants infested with *P. neoaphidis* and those not containing the fungus. Significantly more aphids were recovered from the damaged plant replicates indicating *A. pisum* was more successful at locating damaged plants compared to undamaged plants. Transmission of *P. neoaphidis* occurred during both plant colonisation and *in situ* feeding. There were no effects of damaged plants on either the transmission of *P. neoaphidis* or the infectivity of *P. neoaphidis* towards *A. pisum*. The reproductive potential of *P. neoaphidis*-infected *A. pisum* was reduced compared to that of uninfected *A. pisum*, and this was apparent after 24 hours of infection.

Previous studies have shown that aphids are repelled by volatiles released from host plants infested with conspecific aphids and that this may be due to the aphids detecting the presence of competitors, that the plant had released aphid-toxic chemicals or that the plant is more attractive to aphid natural enemies (Bernasconi *et al.*, 1998). However, the results of the 'dual choice colonisation' experiment showed that more *A. pisum* were recovered from damaged bean plants compared to the number recovered from undamaged bean plants. There are several reasons why this may have occurred. Potentially the volatiles released from damaged bean plants (or conspecifics) may indicate to foraging aphids that the plant is a suitable host. Volatiles released from damaged plants would, therefore, be more attractive to foraging *A. pisum* than those released from undamaged plants. However, this does not support previous studies which indicated that aphids were repelled by volatiles released from conspecific-aphid damaged plants (Nottingham *et al.*, 1991; Quiroz *et al.*, 1997). Alternatively, the volatiles released from undamaged bean plants may not be attractive to aphids on their own and additional cues, for example, volatile cues released from living aphids, may also be required. For example, *S. avenae* is attracted to volatiles released by wheat plants infested with *R. padi* but not to uninfested plants or plants that had been previously infested with *R. padi* (Johansson, Pettersson and Niemeyer, 1997). Although this result contradicts previous findings that suggest the presence of a competitor may repel foraging aphids, the feeding sites of *S. avenae* and *R. padi* on wheat plants are separated spatially (*R. padi* = bottom of plant, *S. avenae* = middle/ top of plant). Therefore, *S. avenae* and *R. padi* may not be direct competitors.

A final reason that may explain why more *A. pisum* were recovered from damaged plants compared to undamaged plants is that damaged plants may be easier to detect than undamaged plants. Previous published experiments to assess the response of aphids to plant volatiles used olfactometers which transport volatiles within an air-flow. However, in this experiment, the air in the cage was still. In these conditions aphids may require a greater quantity of volatiles in order to determine accurately the location of a host plant. This hypothesis is supported from air entrainment experiments in which the quantity of volatiles released from damaged bean plants was found to be greater than the amount released from undamaged plants (Chamberlain, K. personal communication).

The presence of *P. neoaphidis*-sporulating cadavers could have repelled, attracted or had no effect on foraging *A. pisum*. Repulsion by *P. neoaphidis* would benefit the aphid by reducing the probability of contact with conidia whereas attraction would benefit the fungus by increasing the probability of host contact with conidia. However, when selecting a host plant to colonise, *A. pisum* showed no preference between bean plants containing *P. neoaphidis* and those not containing the fungus. By colonising bean plants containing *P. neoaphidis*, foraging *A. pisum* increased the probability that they would come into contact with *P. neoaphidis* conidia, which may result in fungal infection followed by death. This raises the question of 'why does *A. pisum* not avoid *P. neoaphidis*?' Potentially *A. pisum* may not be able to detect the presence of the fungus. During the process of host recognition, aphids predominantly rely on sensilla within their antennae and proboscis to provide both tactile and chemical information on the suitability of the plant (Dixon, 1998). If *P. neoaphidis*-sporulating cadavers do not release any detectable volatile cues or do not alter the blend of volatiles released from the bean plant, *A. pisum* may not be able to perceive the presence of the fungus. The effect of *P. neoaphidis* infection on the blend of volatiles released from *A. pisum* is assessed in section 5.3.5. Alternatively, *P. neoaphidis* may be releasing volatile cues that could be detected by *A. pisum* but are masked by both undamaged and damaged plant volatiles. A final explanation may be that *A. pisum* is able to detect the presence of *P. neoaphidis* but not recognise it as a threat and therefore not modify their behaviour. *Acyrthosiphon pisum* have been shown to respond to the presence of foraging parasitoids by dropping from the plant (Villagra *et al.*, 2002). If *A. pisum* could

determine the presence of *P. neoaphidis* and associate it with being a threat, it would be expected to fall from the plant. However, as there are no significant differences in the number of sporulating cadavers and healthy aphids recovered from both the transmission during 'colonisation' and *'in situ'* feeding experiments, it appears that *A. pisum* does not respond to the presence of *P. neoaphidis*.

For transmission of *P. neoaphidis* to *A. pisum* to occur, the aphid is required to come into contact with conidia that have been deposited on the leaf surface. The encounter rate between *A. pisum* and *P. neoaphidis* conidia should increase with increased aphid movement and, therefore, the number of sporulating cadavers recovered should be positively correlated with aphid movement. As aphids move more during colonisation than through *in situ* feeding alone (the *in situ* data set is, in effect, a sub-set of the colonisation data set), it would be expected that the level of transmission would be greater during 'colonisation' than during '*in situ* feeding'. This is supported by the data where, although it can not be analysed statistically, there was a greater number of sporulating cadavers recovered from the transmission during 'colonisation' experiment than from the transmission during '*in situ* feeding' experiment. As the number of sporulating cadavers recovered does not differ between the undamaged and damaged treatments in either the transmission during 'colonisation' or '*in situ* feeding' experiments, it appears that there are no differences in the encounter rate between *A. pisum* and *P. neoaphidis*. This may indicate that the foraging behaviour of *A. pisum* is the same on both undamaged and damaged bean plants.

The results described in section 3.3 indicate that damaged plant volatiles can have a direct effect on *P. neoaphidis* through both increased conidia size and an increased proportion of germinating conidia. Although these results were statistically significant, further analysis was required to assess whether that had a biologically significant effect i.e. whether they affected the fungus-aphid interaction. Plants may have either a direct effect on the fungus, or, an indirect effect through affecting host quality (Shah, P. personal communication). For example, the species of the host plant indirectly influences the infectivity of *B. bassiana* towards the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Hare and Andreadis, 1983). Alternatively, the plant derived alkaloids solanine and tomatine have a direct effect

on *B. bassiana* through reducing *in vitro* colony formation and growth (Costa and Gaugler, 1989). The results from the infection level experiment show that the number of aphids becoming infected with *P. neoaphidis* did not differ between aphids feeding on previously damaged bean plants (and therefore exposed to damaged plant volatiles) and aphids feeding on undamaged bean plants. Therefore, although there was a direct effect of damaged plant volatiles on *P. neoaphidis*, this did not affect the interaction between *A. pisum* and *P. neoaphidis*. This supports the results of Brown *et al.* (1995) who found that volatiles released from undamaged and mechanically damaged tobacco plants had no effect on the proportion of *M. nicotianae* becoming infected with *P. neoaphidis*. It therefore appears that feeding on damaged plants does not affect the susceptibility of *A. pisum* to *P. neoaphidis* and, that plant volatiles do not play a significant role in host aphid identification by the fungus.

In both the transmission ('colonisation' and '*in situ* feeding') and infection level experiments there were no effects of plant condition on the number of living and dead *A. pisum* recovered. This may indicate that feeding on damaged bean plants does not have a negative effect on the fitness of *A. pisum*. Plants that are infested with herbivores have been shown to protect themselves by releasing compounds that are toxic or act as anti-feedents towards herbivores. For example, tobacco plants release nicotine from glandular hairs and this has an insecticidal effect on aphids (Dixon, 1998). However, as the response of plants to herbivores is complex and involves a number of plant defence/ wound genes and biochemical pathways, detailed molecular and biochemical analysis is required to accurately determine the direct defensive response of bean plants to infestation by *A. pisum* (Walling, 2000).

Pandora neoaphidis disrupted aphid reproduction within 24 hours of inoculation and this continued up to and including 96 hours post-inoculation. Overall *P. neoaphidis* decreased the reproductive potential of *A. pisum* by 34.7%. A similar result to this was found when *A. pisum* were inoculated with the entomopathogenic fungus *B. bassiana* (Roy *et al.* submitted). As *A. pisum* can produce live young up to 96h post-inoculation, it suggests that the vital organs and pathways required for parthenogenesis are not affected by the infection process. This supports previous findings that showed *P. neoaphidis* utilises non-essential body reserves during the

early stages of infection and only consumes vital organs immediately prior to death (Butt *et al.*, 1990). However, as the reproduction of the aphid is reduced within 24 hours of infection, this indicates that the fungus is utilising resources that would otherwise have been used for reproduction. Infection with *P. neoaphidis* therefore has a negative effect on the fitness of *A. pisum* through both reducing its host's reproductive potential during the infection process and through killing the host itself. In contrast, the fungus increases its own reproductive potential by allowing *A. pisum* to continue to produce nymphs which may become hosts themselves.

CHAPTER 5: FUNGUS-INSECT INTRAGUILD INTERACTIONS - PART 1: DO *COCCINELLA SEPTEMPUNCTATA* AND *APHIDIUS ERVI* DETECT AND RESPOND TO THE PRESENCE OF *PANDORA NEOAPHIDIS* DURING FORAGING?

5.1 INTRODUCTION

Foraging can be simply defined as 'location and selection of prey or hosts' (Gullan and Cranston, 1996). The foraging behaviour of insects ranges from a sit-and-wait strategy, which requires little energy, to active searching, which has an increased energy cost (Gullan and Cranston, 1996). Foraging arthropods and their prey are under opposing selection pressures where the prey maximises its own fitness by remaining undetected whereas the foraging predator/ parasitoid maximise its fitness by efficiently and accurately detecting its prey.

Both *C. septempunctata* and *A. ervi* actively search for their prey and use a combination of visual, chemical and tactile cues to detect suitable prey/ hosts (Battaglia *et al.*, 1993, 2000ab; Dixon, 1959; Ferran and Dixon, 1993; Fleschner, 1950; Majerus, 1994; Obata, 1986). Although there is strong evidence to suggest that *A. ervi* uses volatile chemicals released systemically from bean plants damaged by the host-aphid as a detectable and reliable cue during host finding, it is unresolved whether or not these cues are also used by *C. septempunctata* (Al Abassi *et al.*, 2000; Du *et al.*, 1998; Guerrieri *et al.*, 1999; Ninkovic *et al.* 2001; Ninkovic and Pettersson, 2003) (see section 1.3.2.2). Once *A. ervi* and *C. septempunctata* have entered an aphid colony both visual and olfactory cues are used to recognise and evaluate the host before either accepting the aphid and depositing a single egg or consuming/ rejecting the aphid (Batagglia *et al.*, 1993, 2000ab) (see section 1.1.2.3).

The potential fitness costs of selecting an unsuitable host/ prey is dependent on both the life-cycle of the species involved and the relative timing of the interaction. For example, the parasitoid *A. rhopalosiphi* incurs a severe fitness cost when foraging in aphid colonies infected by *P. neoaphidis* due to the ability of the fungus to

reproductively out-compete the parasitoid (Powell *et al.*, 1986) (see section 6.4). This is in contrast to *C. septempunctata* which, while preferring to consume healthy aphids to *P. neoaphidis*-infected aphids, will prey upon *P. neoaphidis*-infected aphids with no apparent direct detrimental effects (Pell *et al.*, 1997; Roy and Pell 2000). The parasitoid would, therefore, maximise its own reproductive success by avoiding aphid colonies in which *P. neoaphidis* was present whereas the coccinellid would incur no severe fitness cost by entering aphid colonies in which *P. neoaphidis* was present. *Aphidius ervi* has been documented avoiding the intraguild predator *C. septempunctata* (Nakashima and Senoo, 2003). It was found that *A. ervi* foraged for a shorter period in patches where both adult and larval *C. septempunctata* had foraged, and that this avoidance persisted up to 18 hours after the coccinellid had been removed from the patch.

Attraction of a foraging arthropod could increase the reproductive success of a fungus. For example, transmission of *Z. radicans* to *P. xylostella* is greater in the presence of the parasitoid *D. semiclausum* and transmission of *P. neoaphidis* to *S. avenae* was increased by the presence *A. rhopalosiphi* (Fuentes-Contreras *et al.*, 1998; Furlong and Pell 1996) (see section 1.3.1.2). In some cases foraging arthropods are able to vector fungus. For example, *P. neoaphidis* conidia that become attached to foraging *C. septempunctata* are passively vectored to aphid colonies not previously infected with the fungus (Roy *et al.*, 2001) (see section 1.3.1.2). Potentially, a fungus may increase its own reproductive success by attracting foraging arthropods. For example, dead female house flies, *Musca domestica* (L.), infected with the entomopathogenic fungus *Entomophthora muscae* (Cohn) Fresenius release volatile cues which attract male house flies (Zurek *et al.*, 2002). In contrast, volatile cues may be released from a fungus at low concentrations and, therefore, the presence of the fungus may go unrecognised. Alternatively, volatile cues may be released from the fungus which are repellent to foraging arthropods but are masked by plant derived volatile cues.

This chapter examines whether the foraging behaviour of *C. septempunctata* and *A. ervi* is modified in the presence of *P. neoaphidis* at various stages of host detection and selection and whether these interactions are affected by the presence of aphid-induced plant volatiles. More specifically:

- The effect of *P. neoaphidis* on the movement of *A. ervi* and *C. septempunctata* into aphid colonies
- The effect of *P. neoaphidis* on the foraging behaviour of *A. ervi* and *C. septempunctata* within aphid colonies
- The effect of *P. neoaphidis* infection on the ovipositional behaviour of *A. ervi*
- The effect of *P. neoaphidis* infection on the composition of volatiles released by *A. pisum*

5.2 MATERIALS AND METHODS

5.2.1 Y-tube experiments to assess the effect of *Pandora neoaphidis*-sporulating cadavers on the entry rate of arthropods into *Acyrthosiphon pisum* colonies in the presence and absence of *A. pisum*-induced *Vicia faba* volatiles

Two Y-tube olfactometers were set up (Figure 5.1) in the same Insect Behaviour Laboratory. The Insect Behaviour Laboratory provided uniform visual surroundings designed to minimise the response of insects to visual cues and was maintained at a constant temperature (19°C ± 1°C). The pump gave an airflow of 800cm^3/min through the olfactometer. The test insects were placed in the open end of the olfactometer and observed for 30 minutes. After 30 minutes the number of insects in each of the traps was assessed, removed and discarded.

The glassware was washed after each replicate using hot water containing detergent, sprayed with acetone, rinsed using cold water and dried. Non-glassware was washed using detergent, rinsed using cold water, sprayed with 95%-ethyl alcohol and dried. The olfactometer was then reconstructed. To control against a side bias within the room, the glass vessel that had previously been attached to the left arm of the Y-tube was attached to the right arm and vice-versa. At the end of each day the glassware was washed, sprayed with acetone and heated to 120°C. This ensured that any chemicals which had accumulated within the olfactometer during the experiment were removed. The glassware was then left to cool.

In treatments containing bean plants, two 15-day-old plants, either undamaged or damaged (still aphid infested), were placed in the glass vessel. In treatments containing *P. neoaphidis*, a Petri dish lid containing 80 sporulating cadavers was placed in the glass vessel. To prepare the cadavers, 16 hours prior to the start of the experiment, 80 dried cadavers were placed an equidistance from each other on damp filter paper in the lid of a Petri dish (90mm diameter). The lid was then transferred to a sealed plastic box (115mm wide x 170mm long x 60mm high) containing wet tissue (to create 100% relative humidity) and maintained at 10°C. This rehydrated the cadavers and initiated sporulation. A replicate Petri dish not containing *P. neoaphidis*-cadavers was placed in glass vessels in the non-fungus treatments.

5.2.1.1 Coccinella septempunctata

Forty adult *C. septempunctata* (mixed sex) were starved in batches of five as described in section 2.2.3. The coccinellids were transferred to the Insect Behaviour Laboratory containing the Y-tube olfactometer at least 30 minutes before the start of the experiments in order to acclimatise.

The response of the coccinellids to the following volatile cues was assessed: undamaged plants *vs.* clean air (day 1), undamaged plants plus sporulating cadavers *vs.* clean air (day 1), damaged plants *vs.* clean air (day 2) and damaged plants plus sporulating cadavers *vs.* clean air (day 2). The coccinellids were released in batches of five individuals. Each treatment was repeated four times on the same day, with each odour having been in both arms of the olfactometer twice. Days '1 and 2' do not indicate consecutive days.

The data were analysed using a Fishers Exact test to assess whether the coccinellids made an orientated response to the volatiles released from undamaged and damaged bean plants and whether the presence of *P. neoaphidis* affected this attraction.

5.2.1.2 Aphidius ervi

The parasitoids were removed from the rearing cage and transferred to the Insect Behaviour Laboratory containing the Y-tube olfactometer at least 30 minutes before the start of the experiments in order to acclimatise.

The response of the parasitoids to the following volatile cues was assessed: undamaged plants *vs.* clean air (day 1), undamaged plants plus sporulating cadavers *vs.* clean air (day 1), damaged plants *vs.* clean air (day 2), damaged plants plus sporulating cadavers *vs.* clean air (day 2), undamaged plants *vs.* undamaged plants plus sporulating cadavers (day 3) and damaged plants *vs.* damaged plants plus sporulating cadavers (day 4). The parasitoids used on days 1 and 2 were culture *A. ervi*$_{I2000}$ and were released in batches of 15 ±2 individuals. The parasitoids used on days '3 and 4' were the standard culture and were released in batches of ten individuals. Days '1, 2, 3 and 4' do not indicate consecutive days.

Each treatment was repeated six times on the same day, with each odour having been in each arm of the olfactometer three times. The data were analysed using a Chi^2 Test to assess whether the parasitoids made an orientated response to the volatiles released from undamaged and damaged bean plants in the presence and absence of *P. neoaphidis* and, whether the presence of *P. neoaphidis* affected this attraction.

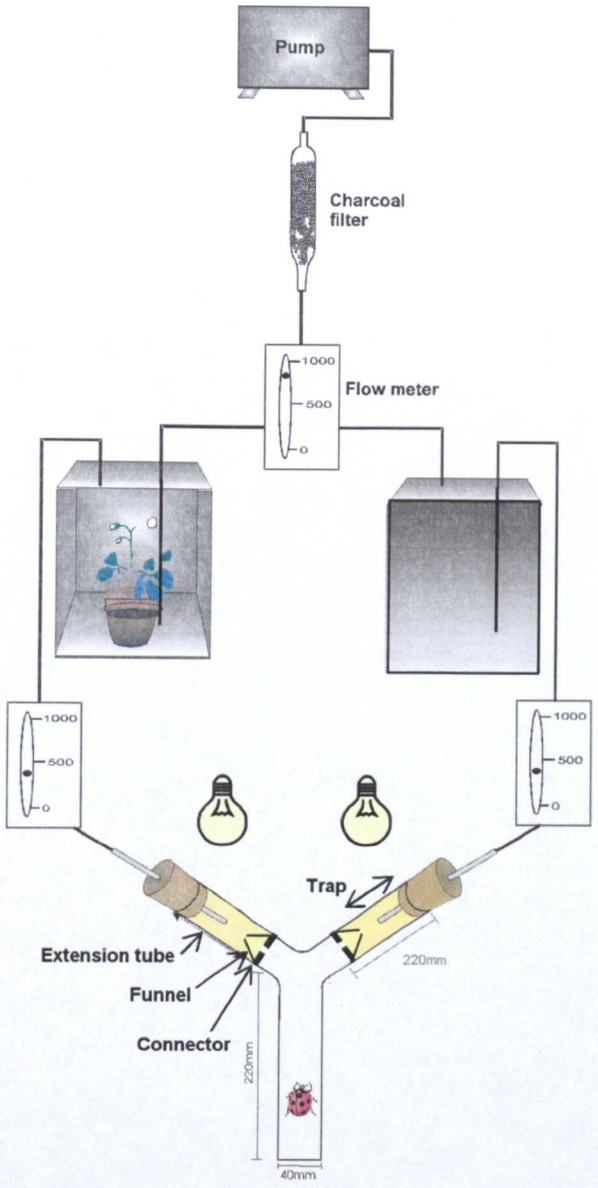

Figure 5.1 Design of horizontal Y-tube olfactometer. The olfactometer was made of a glass Y-tube (40mm internal diameter) which had been modified to create traps at the end of each arm. To make the traps, the arms of the Y-tube were attached to plastic connectors containing inverted funnels (5mm opening). The funnels were held in place using glass extension tubes (40mm internal diameter x 125mm). The rubber bungs which connected the Y-tube to the airflow sealed the open ends of the glass extension tube. This formed a trap into which insects could enter but not leave. Air was pumped through the activated charcoal filter to remove impurities and then passed through the flow meter where the total air flow was measured. The airflow was then split into two streams that were channelled to the bottom of the glass vessels containing the volatile odour source. This forced air containing the volatile odours out of the top of the glass vessels and through flow meters which were used to ensure each arm of the Y-tube received the same airflow. The airflow then passed through the arms of the olfactometer and mixed at the apex of the 'Y' before finally moving down the stem of the olfactometer and out through the opening. The test insects were placed in the opening of the Y-tube and were observed for 30 minutes. Insects that entered traps were scored as responding to the volatile cues. Insects that did not enter a trap were scored as not responding to the volatile cues.

5.2.2 Whole plant experiments to assess the effect of *Pandora neoaphidis*-sporulating cadavers on the foraging behaviour of arthropods on undamaged and damaged *Vicia faba* plants

5.2.2.1 Coccinella septempunctata

Twelve adult *C. septempunctata* (mixed sex) were starved in two batches of six individuals as described in section 2.2.3. The coccinellids were transferred to the Insect Behaviour Laboratory at least 30 minutes before the start of the experiments in order to acclimatise.

To prepare the cadavers, 16 hours prior to the start of the experiment, 60 dried cadavers were placed an equidistance from each other on damp filter paper in the lid of a Petri dish (90mm diameter). The lid was then placed in a sealed plastic box (115mm wide x 170mm long x 60mm high) containing wet tissue (to create 100% relative humidity) and maintained at 10°C. This rehydrated the cadavers and initiated sporulation.

The foraging behaviour of *C. septempunctata* was observed on three replicates of each of the following four treatments: undamaged plant infested with ten adult *A. pisum,* undamaged plant infested with ten *P. neoaphidis*-sporulating cadavers, damaged plant (aphids removed) infested with ten adult *A. pisum* and damaged plant (aphids removed) infested with ten *P. neoaphidis*-sporulating cadavers. The treatments were carried out in a random order to avoid any bias. In treatments containing *A. pisum*, the aphids were transferred onto the plant using a fine paint brush 30 minutes before the start of the experiment. This gave the aphids time to settle and start feeding. In treatments containing *P. neoaphidis*, the sporulating cadavers were placed in random positions on the plant immediately before the start of the experiment. This prevented the sporulating cadavers from desiccating.

A single coccinellid was transferred from the Petri dish to the apex of the plant using a fine paint brush and its behaviour observed for 30 minutes. The time coccinellids spent in each of the following behavioural categories was recorded: 'searching' (walking on the plant surface), 'feeding' (consuming either living

aphids or sporulating cadavers) or 'other' (e.g. grazing, grooming, resting). If the coccinellid left the plant before 30 minutes had elapsed, the total time spent on the plant was recorded and the replicate terminated. The total number of feeding attempts (incidents in which the coccinellid consumed or partially consumed a prey item) was also recorded. The plants and coccinellids were then discarded. The experiment was repeated on four occasions in total giving 12 replicates per treatment.

Before analysis, the data were adjusted to remove zero values using the following formulae (Aitchinson, 1986; Roy, 1997):

$$\delta(C+1)(D-C)/D^2 \text{ (added to all zero values)}$$
$$\delta C(C+1)/D^2 \text{ (subtracted from all non-zero values)}$$

where: δ= rounding error (0.5 seconds)
C= number of zero values per replicate
D= number of behavioural categories

After adjustment, the data were transformed using a log transformation and the 'eating/ searching' and 'other/ searching' ratios derived for each replicate. Multivariate analysis of variance (MANOVA) was used to assess whether there was an effect of damaged plant volatiles, *P. neoaphidis* or an interaction between damaged plant volatiles and *P. neoaphidis* that affected the foraging behaviour of the coccinellid. To assess whether there was a difference in the total number of feeding attempts among the four treatments, the data were transformed using a log(n+1) transformation and analysed using ANOVA.

5.2.2.2 Aphidius ervi

The experiment described in section 5.2.2.1 was repeated using naïve female *A. ervi* parasitoids with the following modifications. The time spent either 'searching' (walking on the plant surface) or doing 'other' behaviours (e.g. grazing, grooming, resting) was recorded. If the parasitoid left the plant before 30 minutes had elapsed, the total time spent on the plant was also recorded and the replicate terminated. The

number of ovipositional attempts was recorded. Two replicates per treatment were done on each of three occasions giving a total of six replicates per treatment. The data were adjusted and transformed as described in section 5.2.2.1. The 'other'/ 'searching' ratio was derived and ANOVA used to assess whether there was an effect of damaged plant volatiles, *P. neoaphidis* or an interaction among damaged plant volatiles and *P. neoaphidis* that affected the foraging behaviour of the parasitoid. To assess whether there was a difference in the number of ovipositional attempts between the four treatments, the data were transformed using a log(n+1) transformation and analysed using ANOVA.

5.2.3 Dual choice whole plant experiment to assess the leaving time of *Aphidius ervi* from aphid colonies containing *Pandora neoaphidis*-sporulating cadavers

Seventy-two hours prior to the start of the experiment, two 15-day-old bean plants were transplanted into a seed tray (370mm x 230mm x 50mm) 150mm apart. Six replicate seed trays were prepared. Forty *A. pisum* were added to each plant in order to damage them (as described in section 2.1.2). The cages were then maintained at 18°C (16L:8D) for 72 hours, after which time the aphids were removed.

Thirty minutes prior to the start of the experiment, ten adult *A. pisum* were transferred to the plant on the right of a replicate seed tray using a fine paint brush. Ten *P. neoaphidis*-sporulating cadavers were transferred to random positions on the plant on the left immediately before the start of the experiment. A single naïve female *A. ervi* parasitoid was then placed on the apex of the plant containing *P. neoaphidis*-sporulating cadavers and observed for 30 minutes. The number of times the parasitoid moved between the two plants was recorded along with the total duration spent on each plant. The parasitoid and seed tray were then discarded. A replicate was then run with both the plant on the left and the plant on the right infested with ten *A. pisum*.

This was repeated three times, with the fungus being on the left-hand plant twice and the right-hand plant once. For fungus replicates, the parasitoid was always released on the *P. neoaphidis*-infested plant. For the aphid-only replicates, the

parasitoid was released on the left plant twice and the right plant once. This experiment was repeated the following day using seed trays containing undamaged bean plants.

ANOVA was used to assess whether *P. neoaphidis* had a significant effect on the total time spent foraging in the experimental arenas.

5.2.4 Petri dish bioassay to assess the effect of stage of infection by *Pandora neoaphidis* on the attack rate of naïve *Aphidius ervi*

Experiment 1: Thirty ten-day-old *A. pisum* (day-1 adult) were inoculated with *P. neoaphidis* (as described in section 2.3.1) 120h, 96h, 72h, 48h, 24h and prior to the start of the experiment and were maintained on bean plants within a controlled environment chamber (18°C, 16L:8D) until required. At the start of the experiment, the 120h-infected aphids had died and were *P. neoaphidis*-sporulating cadavers.

Thirty minutes before the start of the experiment, a single leaf from a bean plant, approximately 50mm long, was placed abaxial side uppermost in a Petri dish (55mm diameter) containing 20ml of 2% tap-water-agar which was cool but still liquid. When the agar had solidified, six healthy adult *A. pisum* were placed on the leaf and the Petri dish's lid replaced. Six replicate Petri dishes were prepared. This was repeated using aphids infected with *P. neoaphidis* for 120h 96h, 72h, 48h and 24h.

A single naïve female *A. ervi* parasitoid was placed in a replicate Petri dish containing healthy *A. pisum*, the Petri dish's lid was replaced and the parasitoid observed for 5 minutes. The number of ovipositional attempts made by the parasitoid was recorded along with the time after introduction at which the first ovipositional attempt was made. An ovipositional attempt was deemed to have occurred when the abdomen of the parasitoid came into contact with an aphid in a 'stabbing motion'. After the five minute observation period had elapsed, the parasitoid was removed, the Petri dish covered with a tissue (to reduce condensation) and the lid replaced. The Petri dish was then inverted and maintained within a controlled environment chamber (18°C, 16L:8D) for up to 120h

to ensure that the inoculated aphids were infected with *P. neoaphidis*. A single replicate of each treatment was assessed. The order of the treatments was then randomised and the assessments repeated. This was done for six replicates of each treatment.

Before analysis, the data were transformed using a log(n+1) transformation. ANOVA was used to assess whether there was an effect of fungal infection on the number of ovipositional attempts made by *A. ervi* or the time taken until the first ovipositional attempt was made.

Experiment 2: The experimental protocol described above was repeated with the following two treatments: *A. pisum* that had been inoculated with *P. neoaphidis* for 1 hour prior to the start of the experiment and healthy aphids. The treatments were assayed in an alternating order and six replicates done for each treatment.

5.2.5 Entrainment of volatiles released by *Acyrthosiphon pisum* at different stages of *Pandora neoaphidis* infection

One hundred one-day-old adult *A. pisum* were inoculated with *P. neoaphidis* (as described in section 2.3.1) 120h, 96h, 72h, 48h and 24h prior to the start of the experiment. The aphids were maintained on bean plants within a controlled environment room (18°C, 16L:8D) until required. At the start of the entrainment, the 120h-infected aphids had died and were *P. neoaphidis*-sporulating cadavers.

A glass-chamber (approx. 50ml) containing an entry port at one end and an exit port at the opposite end was heated to 180°C to remove any volatile chemicals. Once cooled, 100 healthy adult *A. pisum* were transferred to the glass chamber and the entry port connected to a charcoal filter using PTFE tubing. The exit port was connected using PTFE tubing to a glass tube containing 50mg of Tenax which had been conditioned by heating at 220°C in a stream of charcoal-filtered nitrogen. The Tenax tube was itself connected to a pump (Figure 5.2). An air-flow of 100ml min^{-1} was drawn through the system for 2.5 hours. This was repeated on the same day using the 120h, 96h, 72h, 48h and 24h *P. neoaphidis*-infected aphids. The Tenax tube was replaced after each entrainment.

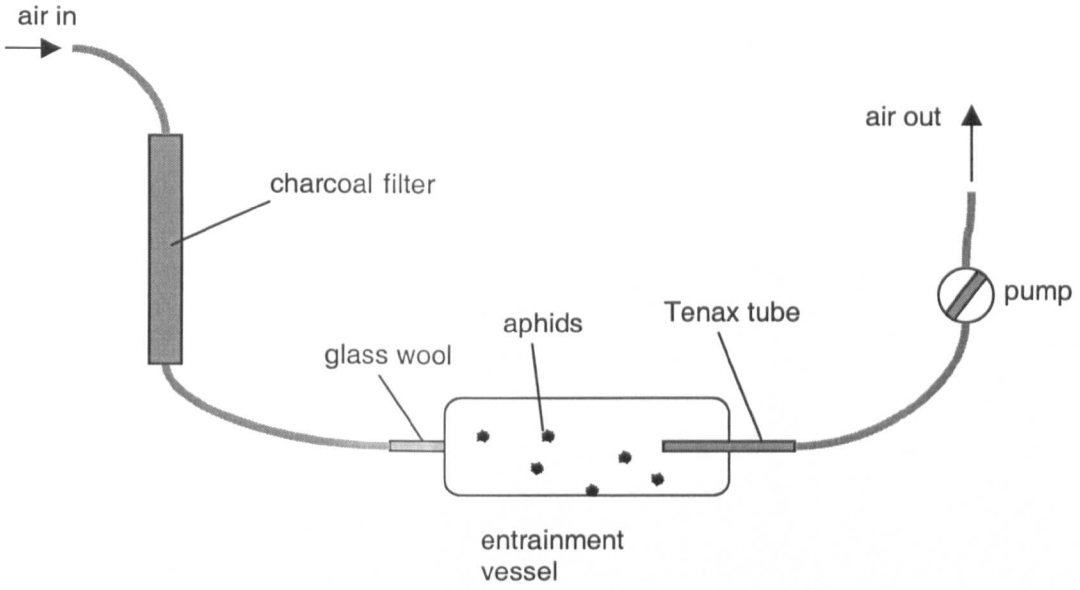

Figure 5.2 Design of air-entrainment equipment used to entrain volatile chemicals released from healthy *Acyrthosiphon pisum* and *A. pisum* inoculated with *P. neoaphidis* for 24, 48, 72, 96 and 120 hours (Diagram courtesy of K. Chamberlain).

The glass tubes containing the Tenax were then placed in the inlet of an Optic 2 thermal desorption unit. The temperature in the inlet was raised to 220°C to transfer the volatiles into the GC column (HP-1 50m x 0.32 ID x 0.5µm film thickness). The carrier gas used was hydrogen and the oven temperature increased from 30°C to 250°C at 10°C min^{-1}.

5.3 RESULTS

5.3.1 Y-tube experiments to assess the effect of *Pandora neoaphidis*-sporulating cadavers on the entry rate of arthropods into *Acyrthosiphon pisum* colonies in the presence and absence of *A. pisum*-induced *Vicia faba* volatiles

5.3.1.1 Coccinella septempunctata

A large percentage of *C. septempunctata* did not enter the trap at the end of either the treatment arm or the control arm in the undamaged plants *vs.* control (70%), undamaged plants plus sporulating cadavers *vs.* control (75%), damaged plants plus

aphids *vs.* control (40%) or damaged plants plus aphids plus sporulating cadavers *vs.* control (40%) treatments.

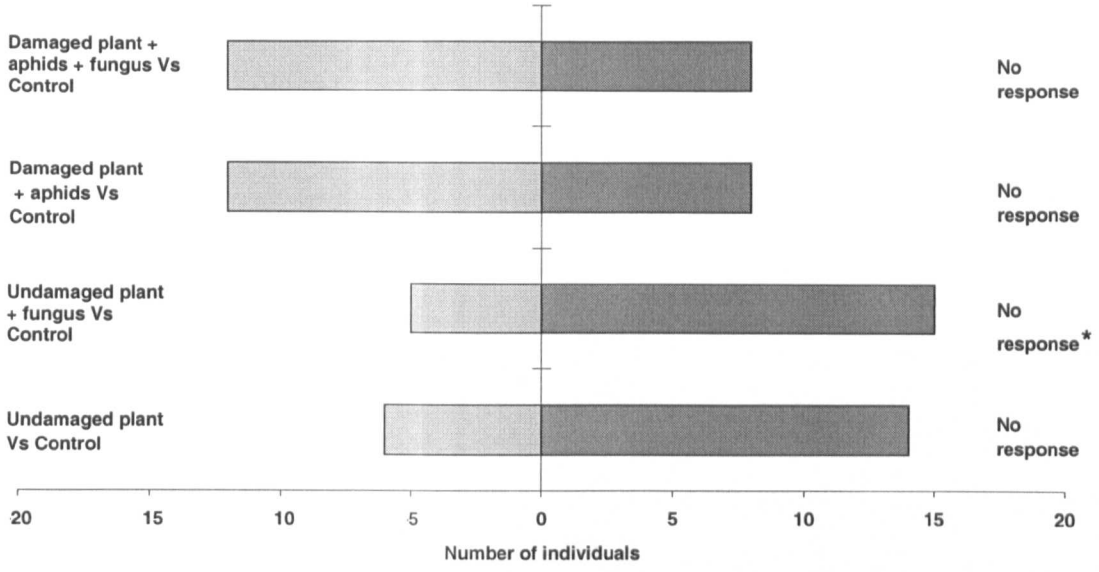

Figure 5.3 Number of *Coccinella septempunctata* entering either trap in a Y-tube olfactometer compared to those not making a decision when exposed to volatiles from undamaged and damaged bean plants infested with either *Acyrthosiphon pisum*, *A. pisum* plus *Pandora neoaphidis*-sporulating cadavers or purified air (control) (*$p < 0.05$).

There were no significant differences in the number of *C. septempunctata* walking up the Y-tube and entering either trap compared to those not making a decision when exposed to volatiles from undamaged plants (Fishers Exact Test: $p = 0.07$), damaged plants plus aphids (Fishers Exact Test: $p = 0.314$) or damaged plants plus aphids plus sporulating cadavers (Fishers Exact Test: $p = 0.328$) (Figure 5.3). Significantly fewer *C. septempunctata* walked up the Y-tube and entered either trap compared to those not making a decision when exposed to volatiles from undamaged plants plus sporulating cadavers (Fishers Exact Test: $p = 0.029$) (Figure 5.3).

5.3.1.2 Aphidius ervi

Only a small percentage of *A. ervi* did not enter the trap at the end of either the treatment arm or the control arm in the undamaged plants *vs.* control (7.7%), undamaged plants plus sporulating cadavers *vs.* control (16%), undamaged plants *vs.* undamaged plants plus sporulating cadavers (5%), damaged plants plus aphids

vs. control (4.5%), damaged plants plus aphids plus sporulating cadavers *vs.* control (8.2%) or damaged plants plus aphids *vs.* damaged plants plus aphids plus sporulating cadavers (10%) treatments.

There were no significant differences in the number of *A. ervi* entering the trap on the treatment arm compared to the control arm when exposed to volatiles from undamaged plants (Chi^2= 2.88, d.f.= 1, p= 0.09) or undamaged plants plus sporulating cadavers (Chi^2= 1.10, d.f.= 1, p= 0.295) (Figure 5.4). When given a choice of volatiles from undamaged plants and undamaged plants plus sporulating cadavers, the number of parasitoids recovered from the traps did not differ significantly (Chi^2= 0.15, d.f.= 1, p= 0.696) (Figure 5.4).

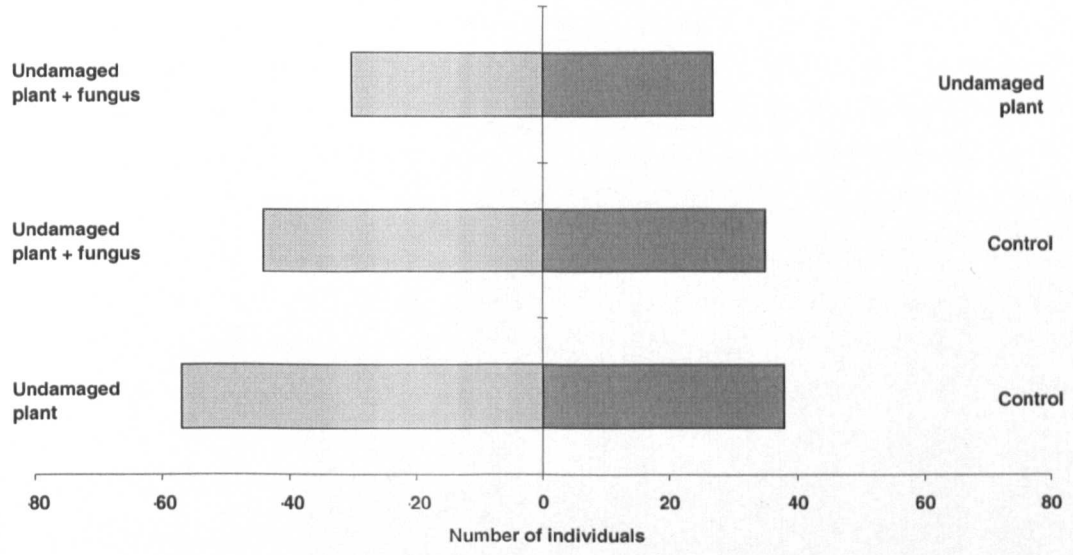

Figure 5.4 Number of *Aphidius ervi* entering the traps in a Y-tube olfactometer when exposed to volatiles from undamaged bean plants, undamaged bean plants infested with *Pandora neoaphidis*-sporulating cadavers or purified air (control) (*p< 0.05).

There were significant differences in the number of *A. ervi* entering the trap on the treatment arm compared to the control arm when exposed to volatiles from damaged plants plus aphids (Chi^2= 5.61, d.f.= 1, p= 0.018) or damaged plants plus aphids plus sporulating cadavers (Chi^2= 5.21, d.f.= 1, p= 0.022) (Figure 5.5), with more *A. ervi* entering the trap on the treatment arm. When given a choice of volatiles from damaged plants plus aphids or damaged plants plus aphids plus

sporulating cadavers, the number of parasitoids recovered from the traps did not differ significantly (Chi^2= 0.3, d.f.= 1, p=0.586) (Figure 5.5).

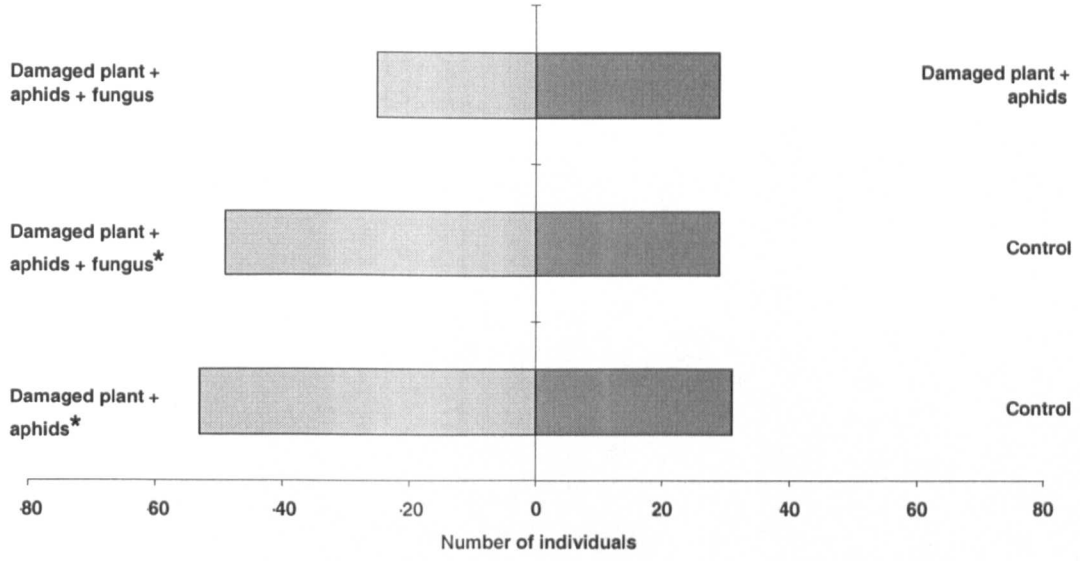

Figure 5.5 Number of *Aphidius ervi* entering the traps in a Y-tube olfactometer when exposed to volatiles from damaged bean plants infested with either *Acyrthosiphon pisum*, *A. pisum* plus *Pandora neoaphidis*-sporulating cadavers or purified air (control) (*p< 0.05).

5.3.2 Whole plant experiments to assess the effect of *Pandora neoaphidis*-sporulating cadavers on the foraging behaviour of arthropods on undamaged and damaged *Vicia faba* plants

5.3.2.1 Coccinella septempunctata

There were no significant effects of either plant condition (ANOVA: $F_{1, 47} = 2.89$, p= 0.097) or sporulating cadavers (ANOVA: $F_{1, 47} = 0.74$, p= 0.394) on the mean number of eating attempts made by *C. septempunctata*, with 0.58, 1.08, 1.67 and 2 eating attempts made on the undamaged plants plus aphids, undamaged plants plus sporulating cadavers, damaged plants plus aphids and damaged plants plus sporulating cadavers treatments respectively. There were no significant interactions between damaged plants and sporulating cadavers that affected the number of eating attempts made (ANOVA: $F_{1, 47} = 0.01$, p= 0.925).

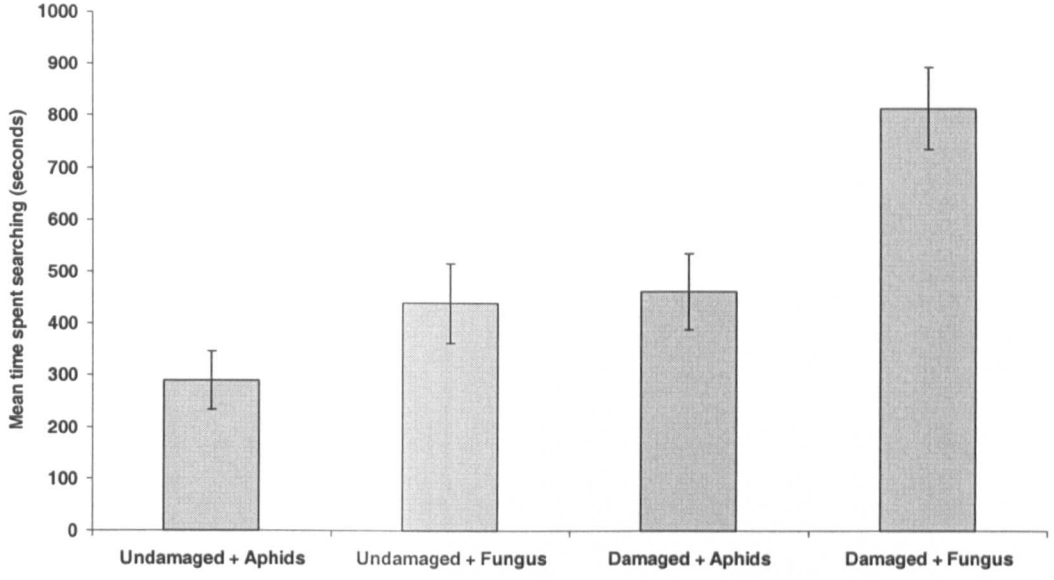

Figure 5.6 Mean time spent searching by *Coccinella septempunctata* on undamaged and damaged bean plants infested with either *Acyrthosiphon pisum* or *Pandora neoaphidis*-sporulating cadavers.

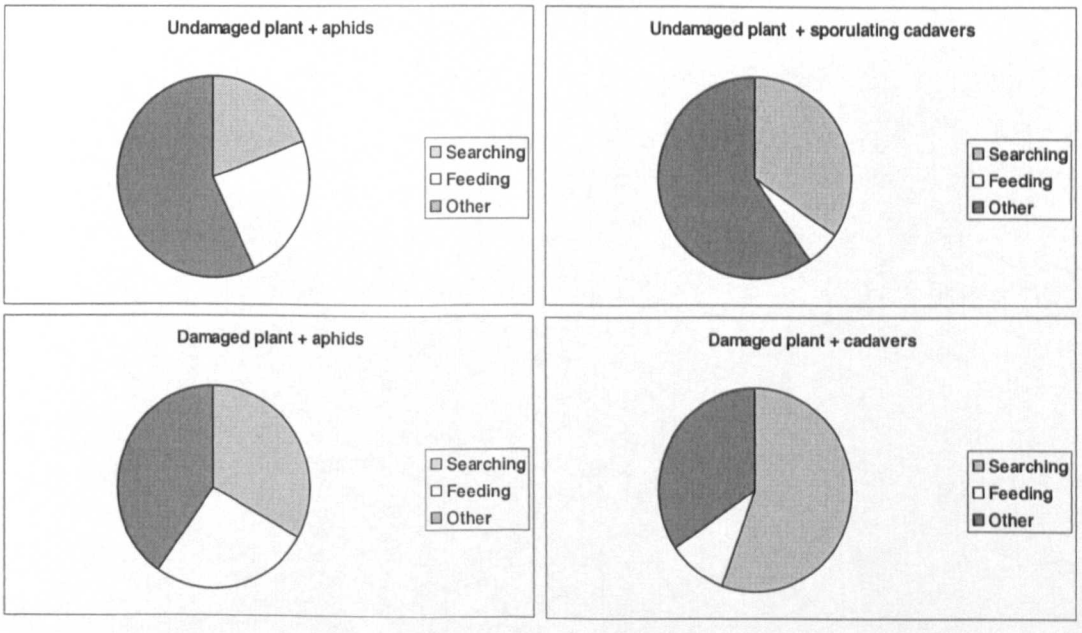

Figure 5.7 Mean proportional time spent either searching, feeding or doing other behaviours (resting, grooming, grazing) by *Coccinella septempunctata* on undamaged and damaged bean plants infested with either *Acyrthosiphon pisum* or *Pandora neoaphidis*-sporulating cadavers.

There were no significant effects of plant condition on the time spent searching by *C. septempunctata* (ANOVA: $F_{1, 47}$= 2.85, p= 0.099) (Figure 5.6). There were

significant effects of sporulating cadavers on the time spent searching by *C. septempunctata* (ANOVA: $F_{1, 47}$= 9.31, p= 0.004), with *C. septempunctata* spending longer searching on plants containing sporulating cadavers (Figure 5.6). There were no significant interactions between plant condition and sporulating cadavers that affected the time *C. septempunctata* spent searching (ANOVA: $F_{1, 47}$= 0.08, p= 0.784).

There were no significant effects of either plant condition (MANOVA: $F_{2, 42}$= 0.17, p>0.05) or sporulating cadavers (MANOVA: $F_{2, 42}$= 2.69, p>0.05) on the overall foraging behaviour (searching: feeding: other) of *C. septempunctata* (Figure 5.7). There were no significant interactions between plant condition and sporulating cadavers that affected the foraging behaviour of *C. septempunctata* (MANOVA: $F_{2, 42}$= 0.07, p>0.05) (Figure 5.7).

5.3.2.2 Aphidius ervi

There were significant effects of *P. neoaphidis* on the number of attacks made by *A. ervi* (ANOVA: $F_{1, 23}$= 18.12, p<0.001), with the mean number of attacks on the undamaged plants plus aphids and damaged plants plus aphids treatments being greater than the number on the undamaged plants plus sporulating cadavers and damaged plants plus sporulating cadavers treatments, with 2.33, 2.5, 0 and 0 attacks made respectively. There were no significant effects of plant condition on the number of attacks made by *A. ervi* (ANOVA: $F_{1, 23}$= 0.07, p= 0.800).

There were significant effects of plant condition on the time spent searching by *A. ervi* (ANOVA: $F_{1, 23}$= 16.47, p<0.001), with *A. ervi* spending longer searching on damaged plants than on undamaged plants (Figure 5.8). There were no significant effects of sporulating cadavers on the time spent searching by *A. ervi* (ANOVA: $F_{1, 23}$= 0.5, p= 0.487) nor was there a significant interaction between plant condition and sporulating cadavers that affected the time spent searching by *A. ervi* (ANOVA: $F_{1, 23}$= 0.01, p= 0.925) (Figure 5.8).

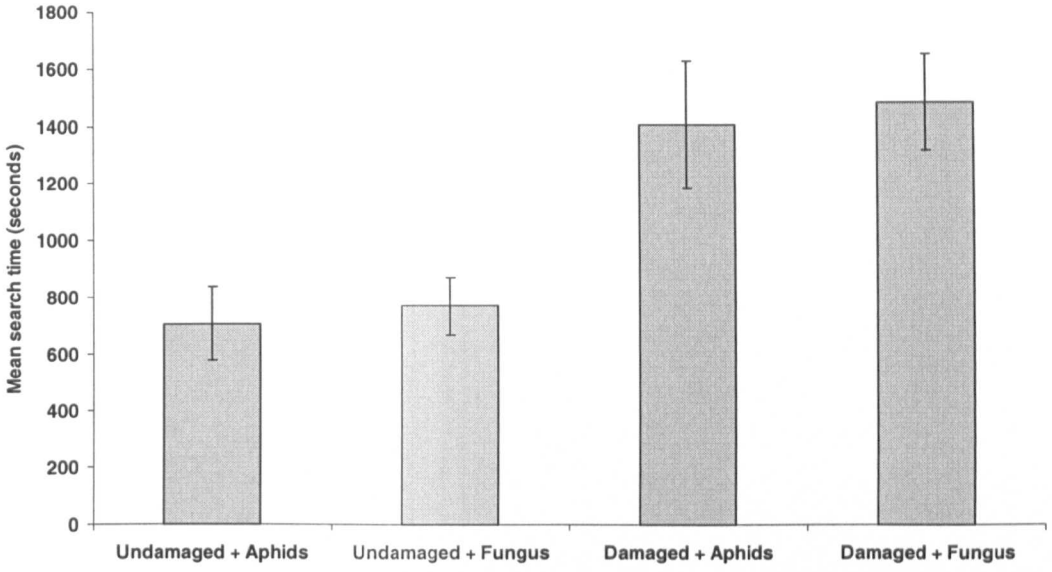

Figure 5.8 Mean time spent searching by naïve *Aphidius ervi* on undamaged and damaged bean plants infested with either *Acyrthosiphon pisum* or *Pandora neoaphidis*-sporulating cadavers

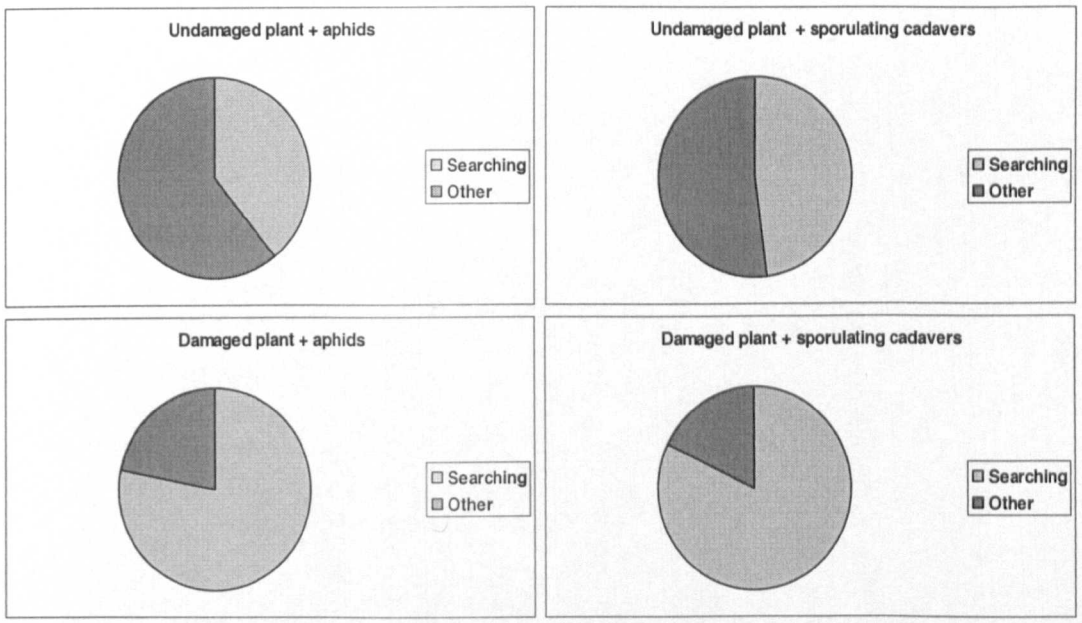

Figure 5.9 Mean proportional time spent either searching or doing other behaviours (resting, grooming, grazing) by naïve *Aphidius ervi* on undamaged and damaged bean plants infested with either *Acyrthosiphon pisum* or *Pandora neoaphidis*-sporulating cadavers.

There were significant effects of plant condition on the overall foraging behaviour (searching: other) of *A. ervi* (ANOVA: $F_{1, 23}$= 11.27, p= 0.004) (Figure 5.9). There were no significant effects of sporulating cadavers on the overall foraging

behaviour of *A. ervi* (ANOVA: $F_{1, 23}$= 0.71, p= 0.409) nor was there a significant interaction between plant condition and sporulating cadavers that affected the overall foraging behaviour of *A. ervi* (ANOVA: $F_{1, 23}$= 0.15, p= 0.706) (Figure 5.9).

5.3.3 Dual choice whole plant experiment to assess the leaving time of *Aphidius ervi* from aphid colonies containing *Pandora neoaphidis*-sporulating cadavers

Aphidius ervi was never observed moving from the starting plant to the second plant regardless of the treatment on the starting plant (Table 5.1). There were no significant differences in the total time spent by *A. ervi* on the starting plant in either the undamaged plant (ANOVA: $F_{1, 5}$= 1.03, p= 0.367) or damaged plant treatments ($F_{1, 5}$= 1.00, p=0.374).

Table 5.1 Movement of *A. ervi* between undamaged or damaged plants infested with either ten *A. pisum* or ten *P. neoaphidis*-sporulating cadavers

Starting plant	Starting side	Proportion on starting plant	Proportion on alternative plant	No. changes	Total time (seconds)
Undamaged *V. faba*					
+ *A. pisum*	Left	1	0	0	1800
	Right	1	0	0	131
	Left	1	0	0	1782
+ *P. neoaphidis*	Left	1	0	0	1800
	Right	1	0	0	1800
	Left	1	0	0	1800
A. pisum*-damaged *V. faba					
+ *A. pisum*	Left	1	0	0	1800
	Right	1	0	0	1800
	Left	1	0	0	1620
+ *P. neoaphidis*	Left	1	0	0	1800
	Right	1	0	0	1800
	Left	1	0	0	1800

5.3.4 Petri dish bioassay to assess the effect of stage of infection by *Pandora neoaphidis* on the attack rate of naïve *Aphidius ervi*

There were no significant differences in the mean attack rate on healthy aphids between experiments 1 and 2 (ANOVA: $F_{1, 11}= 0.04$ p= 0.853), with a mean number of 4.67 and 4.83 attacks respectively. There were also no significant differences in the mean time to the first attack between experiments 1 and 2 (ANOVA: $F_{1, 11}= 0.12$ p= 0.738), with a mean first attack after 77.0 and 67.5 seconds respectively. In subsequent analysis, the results of experiments 1 and 2 were analysed together.

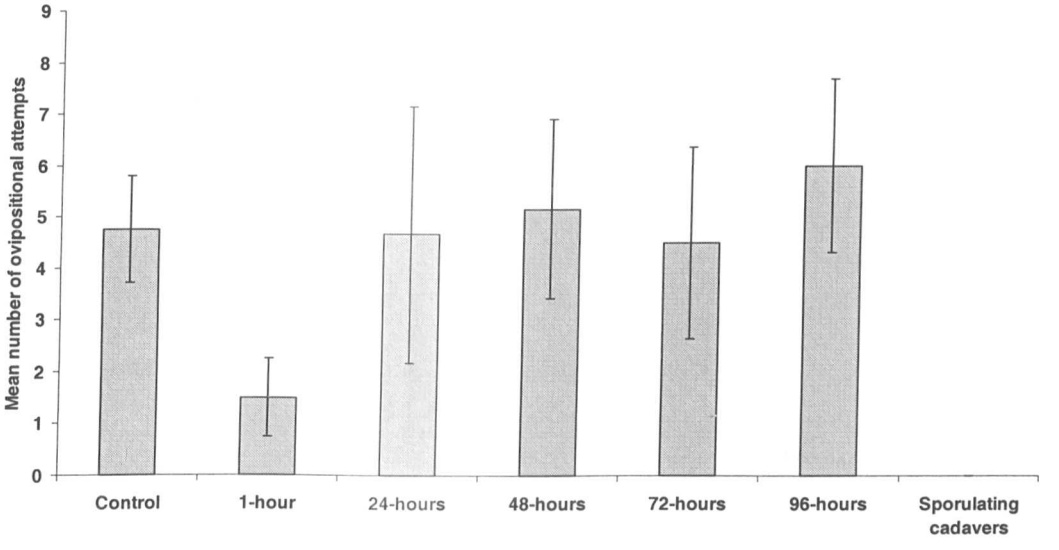

Figure 5.10 Mean number of ovipositional attempts made by naïve *A. ervi* on either healthy *Acyrthosiphon pisum*, *A. pisum* infected with *Pandora neoaphidis* for different periods of time or on *P. neoaphidis*-sporulating cadavers.

The mean attack rate of *A. ervi* was greatest on aphids infected for 96 hours and least on *P. neoaphidis*-sporulating cadavers. The mean number of attacks recorded was 3.92 per replicate. The greatest number of attacks, 16, was recorded in the 24-hour infected treatment. The minimum number of attacks, 0, occurred at least once in each of the seven treatments. *Aphidius ervi* was never observed attacking *P. neoaphidis*-sporulating cadavers and therefore this data set was removed from the remaining analysis.

The mean attack rate of *A. ervi* on uninfected, 1, 24, 48, 72 and 96 hour infected aphids did not differ significantly (ANOVA: $F_{5, 41}= 0.84$, p= 0.531), with there being a mean number of 4.48 attacks per replicate (Figure 5.10). For the replicates where an attack occurred, the time to the first attack on uninfected, 1, 24, 48, 72 and 96 hour infected aphids did not differ significantly (ANOVA: $F_{5, 32}= 0.21$, p= 0.958), with the mean time to first attack being after 77 seconds (Figure 5.10).

5.3.5 Entrainment of volatiles released by *Acyrthosiphon pisum* at different stages of *Pandora neoaphidis* infection

There were no qualitative differences in the volatiles entrained from either *P. neoaphidis*-sporulating cadavers or aphids infected with *P. neoaphidis* for 24, 48, 72 or 96 hours compared to those entrained from uninfected control aphids (Appendix 1 shows GC traces for each entrainment).

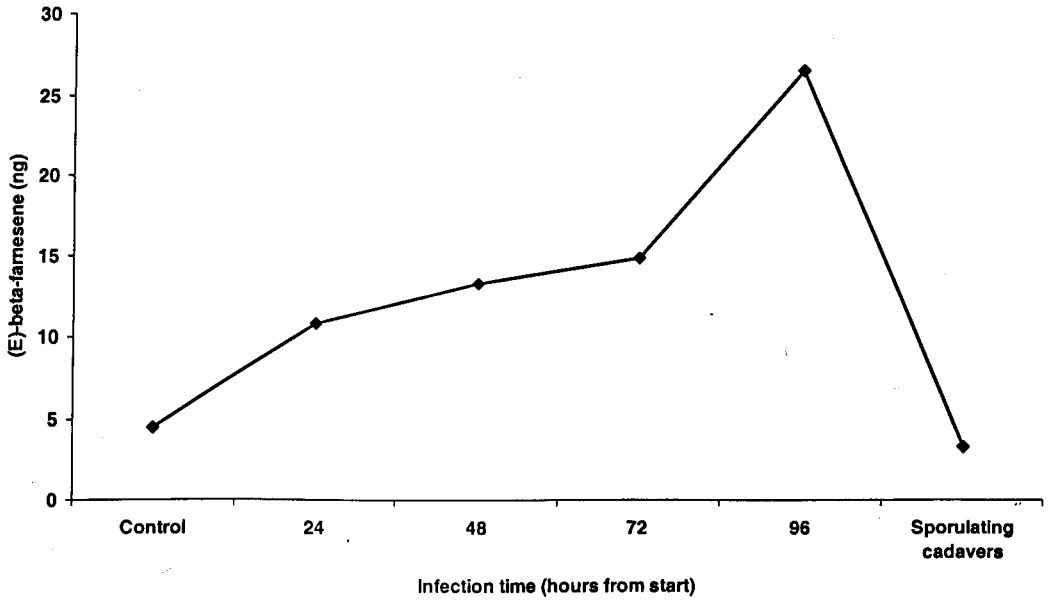

Figure 5.11 (*E*)-β-farnesene entrained from either healthy *Acyrthosiphon pisum* (control), *A. pisum* infected with *Pandora neoaphidis* for different periods of time (24-96h) or from *P. neoaphidis*-sporulating cadavers (=120h infection time).

The quantity of aphid alarm pheromone, (*E*)-β-farnesene, entrained from aphids infected with *P. neoaphidis* for 24, 48, 72 and 96 hours was greater than the amount entrained from uninfected control aphids, with 10.8, 13.3, 14.9, 26.6 and 4.47ng entrained respectively (Figure 5.11). Aphid alarm pheromone was entrained from sporulating cadavers at a level lower than that of the uninfected control aphids, with 3.32ng entrained (Figure 5.11).

5.4 DISCUSSION

These results indicate that *P. neoaphidis*-sporulating cadavers have no effect on the entry rate of either *A. ervi* or *C. septempunctata* into aphid colonies, no effect on their foraging behaviour and will only affect the ovipositional behaviour of *A. ervi* once sporulation has occurred.

These results also suggest that *C. septempunctata* does not use plant derived volatiles as a cue to locate prey. Previous studies into the response of *C. septempunctata* to volatile cues have conflicting results. For example, Schaller and Nentwig (2000) found no response of *C. septempunctata* to the volatiles released from 22 plant species, aphid honeydew or aphid odour in a Y-tube olfactometer. This is in contrast to the experiments done by Ninkovic *et al.* (2001) who found *C. septempunctata* was attracted to the volatiles released from barley plants infested with the aphid *R. padi*. A second example of a positive response of *C. septempunctata* to a volatile cue is from Al Abassi *et al.* (2000) who found that *C. septempunctata* were attracted to aphid alarm pheromone, (*E*)-β-farnesene. Although these results may be dependent on the plant species tested, the results may also be dependent on the olfactometer used. The results presented in this thesis and those from Schaller and Nentwig (2000) were obtained using a Y-tube olfactometer whereas Ninkovic *et al.* (2001) used a four-way-olfactometer and Al Abassi *et al* (2000) used a two-way olfactometer. In a Y-tube, the insect is required to detect and respond to a volatile cue by walking up the concentration gradient towards the odour source. In both a two-way and a four-way-olfactometer the insect is required to select between clean air and air containing the odour source but not make an orientated response along a concentration gradient. Therefore a Y-tube can be used to assess the attraction of an arthropod to an odour source whereas two and four way olfactometers can only assess the preference between odour sources and potentially detect arrestment, not attraction.

Intraguild interactions have been shown to occur between *C. septempunctata* and *P. neoaphidis* within the same aphid colony (Pell *et al.*, 1997; Roy, 1997). Intraguild predation of both living aphids infected with *P. neoaphidis* and *P. neoaphidis*-sporulating cadavers by *C. septempunctata* may reduce the reproductive potential of *P. neoaphidis* whereas increased transmission within and between aphid colonies would increase the reproductive potential of *P. neoaphidis* (Pell *et al.*,

1997; Roy, 1997; Roy *et al.*, 2001). However, these intraguild interactions were studied without first confirming that *C. septempunctata* would enter an aphid colony containing *P. neoaphidis*. The results presented here indicate that *C. septempunctata* would enter and forage in an aphid colony containing *P. neoaphidis* and, that the overall foraging behaviour of *C. septempunctata* is not modified in the presence of volatiles from damaged plants. *Coccinella septempunctata* spent longer searching for prey on plants containing sporulating cadavers when compared to the time spent foraging on plants infested with aphids. This may be a result of *C. septempunctata* spending less time feeding on plants containing sporulating cadavers, however, this was not confirmed statistically. It therefore appears that, as *C. septempunctata* would enter and forage in an aphid colony containing *P. neoaphidis*, that intraguild interactions may occur and that these interactions would not be modified by the presence of volatiles from damaged plants.

Previous studies have shown that during the process of host location, *A. ervi* used volatile cues released from *A. pisum*-damaged *V. faba* plants as a reliable cue to detect host aphids (Du *et al.*, 1998, Guerriei *et al.*, 1999). The results presented here support these findings, however, contrary to expectations, the presence of the fungus had no effect on *A. ervi*. *Pandora neoaphidis*-sporulating cadavers neither attracted nor repelled *A. ervi*. This may have been due to the fungus not releasing volatile compounds, the fungus releasing volatile compounds that were not recognised by the parasitoid or the fungus releasing volatile compounds that were recognised but not responded to by the parasitoid. From the result of the air entrainment experiment it appears that the parasitoid may not be able to detect the presence of the fungus due to the fungus not releasing any species-specific volatiles. Although living aphids infected with *P. neoaphidis* and *P. neoaphidis*-sporulating cadavers released (*E*)-β-farnesene, which has been shown to attract *A. ervi* and *C. septempunctata*, it is unlikely that the amount released during the process of fungal infection and sporulation is at a concentration great enough to have an effect on the foraging behaviour of *A. ervi* and *C. septempunctata* (Al Abassi *et al.*, 2000; Du *et al.*, 1998).

The presence of *P. neoaphidis*-sporulating cadavers did not affect the overall foraging behaviour of *C. septempunctata* or *A. ervi* on either undamaged or

damaged plants. This may have been a result of the experiment being done on a single plant. Therefore, the coccinellids and parasitoids were foraging on the only available plant. When given a choice of a plant containing sporulating cadavers and a second plant not containing sporulating cadavers, the coccinellids and parasitoids had the choice to preferentially forage on the plant not containing the sporulating cadavers. However, as the dual choice foraging behaviour experiments showed, *A. ervi* did not leave the plant containing the sporulating cadavers to forage on a second plant which did not contain sporulating cadavers. It therefore appears that the foraging behaviour observed during the single plant foraging behaviour experiment was representative of the natural behaviour of the coccinellids and parasitoids and was not an artefact of the experimental design.

Unlike *C. septempunctata*, *A. ervi* spent longer searching for host aphids on damaged plants. This may increase the potential of the parasitoid coming in to contact with both healthy aphids and sporulating cadavers. The presence of foraging parasitoids can increase the transmission of entomopathogenic fungus through increased disturbance and subsequent movement of the host (Fuentes-Contreras *et al.*, 1998). For example, the presence of the parasitoid *D. semiclausam* increases the movement of *P. xylostella* resulting in increased transmission of the entomopathogenic fungus *Z. radicans* (Furlong and Pell, 1996). Potentially the increased time spent searching by *A. ervi* on damaged plants compared to undamaged plants may increase the transmission of *P. neoaphidis* through increased aphid movement. This is studied in greater detail in chapter 6.

When foraging on both undamaged and damaged plants containing sporulating cadavers, *A. ervi* were observed crawling over the sporulating cadavers and examining them with their antennae. During this process conidia could become attached to the parasitoid and be vectored to a second previously uninfected aphid colony. Unlike *C. septempunctata* which has been shown to vector *P. neoaphidis* between aphid colonies, there is no evidence which indicates that parasitoids can act as vectors (Roy *et* al., 2001). If parasitoids can act as vectors, the increased search time on damaged plants may result in an increased vectoring efficiency. The ability of *A. ervi* to act as a vector is briefly studied in chapter 6.

Aphidius ervi uses both visual and chemical cues during the final stages of host detection and acceptance, including: aphid colour, cornicle secretion and contact kairomones, not all of which have to be present for an ovipositional attempt to occur (see section 1.1.2.3). These cues may explain why *A. ervi* attacked living aphids infected with *P. neoaphidis* for up to 96-hours but did not attack *P. neoaphidis*-sporulating cadavers. It takes approximately 108 hours from the initial penetration of a conidium through the cuticle of an aphid until death occurs (Brobyn and Wilding, 1977). However, it is not until the final stages of infection by the fungus that muscles and major organs are consumed and not until immediately prior to death that mummification begins and the aphid appears visually different (Brobyn and Wilding, 1977). Unlike healthy aphids which are green, *P. neoaphidis*-sporulating cadavers have a yellow/ brown colouration and contain no cornicle secretion. As the process of sporulation ruptures the aphids cuticle it is also unlikely that contact kairomones are present. It is therefore possible that *A. ervi* is unaware of the presence of the fungus within an aphid during the early stages of infection as the aphid's colour, cornicle secretions and contact kairomones may all be unaltered. Further evidence to suggest that *A. ervi* cannot detect the presence of the fungus until sporulation comes from the air entrainment experiment where the volatiles released from aphids infected with *P. neoaphidis* did not appear to differ qualitatively to those released from uninfected control aphids. It therefore seems likely that *A. ervi* does not attack *P. neoaphidis* sporulating cadavers as it is receiving no cues that indicate it has found a suitable host. This response is unaffected by the presence of host-aphid induced volatiles used by *A. ervi* to detect host-aphid colonies. These results are in contrast to those of Brobyn *et al.* (1988) who found that *A. rhopalosiphi* had a 'weakly developed' ability to detect *P. neoaphidis*, and hypothesised that this may be due to fungal infection having an effect on kairomone production (see section 1.3.1.3). These conflicting findings may be a result of *A. ervi* and *A. rhopalosiphi* having different abilities to detect the fungus or, differences in the physiological effects of fungal infection on the host aphid.

Although these results do not statistically support the findings by Pope *et al.* (2002) who found that the attack rate of *A. ervi* was lower on aphids infected with *P. neoaphidis* for 24 hours compared to healthy control aphids and aphids infected for

48 hours, a similar observation can be made. In the present experiment, the attack rate on 1-hour infected aphids was considerably less than on healthy, 24, 48, 72 and 96-hour infected aphids. Pope *et al.* (2002) suggest that parasitoids may use conidia on a plant to detect fungal infection. As with their bioassay, our aphids were not showered with the fungal conidia on the leaf where the bioassay took place. However, immediately after showering an aphid with *P. neoaphidis* conidia, the number of conidia on the aphid's cuticle is approximately 4 times greater than on aphids showered 24 hours previously (unpublished data). It is therefore possible that the decreased attack rate on aphids showered 1-hour previously compared to the other treatments is a result of the parasitoid detecting the conidia.

Alternatively, the life history of the parasitoid may determine whether it oviposits in an aphid infected with *P. neoaphidis*. When assessing the response of *A. ervi* to the presence of *C. septempunctata*, Nakashima and Senoo (2003) found that *A. ervi* with ovipositional experience foraged for a shorter time than *A. ervi* with no ovipositional experience in patches where adult and larval *C. septempunctata* had foraged previously. This finding supported previous theoretical and empirical studies which indicated that a decreased egg load increases the selectivity of foraging arthropods (Minkenberg, Tatar and Rosenheim, 1992). In the experiments by Pope *et al.* (2002) the parasitoids had previous ovipositional experience. These parasitoids could have had a decreased egg load and this may have increased their selectivity, resulting in avoidance of aphids infected with *P. neoaphidis*. However, the parasitoids used in the attack rate experiment described here had no prior ovipositional experience and may therefore have been less selective over suitable hosts.

The absence of *P. neoaphidis* during the early developmental stages of the parasitoid may explain its lack of ability to detect the fungus. Parasitoids show a preference to aphid species on which they have been reared (Cameron *et al.*, 1984). Parasitoids also show a preference for the plant species on which they were reared and, during emergence, parasitoids obtain cues relating to their life history from their mummy case in a process called emergence conditioning (Storeck *et al.*, 2000). The parasitoids used in these experiments were reared on *V. faba* plants infested with healthy *A. pisum* and had never been allowed to condition to the

presence of the fungus. The lack of response of *A. ervi* to *P. neoaphidis* in our bioassays may have been a result of the fungus not being present at any other stage in the parasitoid's development.

The amount of alarm pheromone entrained from the aphids increased with increased infection time. This may be a result of the aphids responding to infection by releasing small amounts of alarm pheromone. However, small amounts of alarm pheromone were also entrained from sporulating cadavers. It is thought that aphids do not produce alarm pheromone through a *de novo* pathway but involve sequestation of either (*E*)-β-farnesene or its immediate unstable precursor (Roy *et al.* submitted). As the cuticle of the aphid is ruptured during sporulation, alarm pheromone may be released from the sink. As previously stated, *A. ervi* was observed examining sporulating cadavers but never attempting to oviposit within a cadaver. This may be a result of the aphid alarm pheromone that is released from the sporulating cadavers attracting the parasitoid, but not being used as a primary cue for host detection. As (*E*)-β-farnesene is widespread among different aphid species, *A. ervi* would not be expected to use it as a primary cue during the process of host selection (Dixon, 1998).

For *A. ervi* to successfully out-compete *P. neoaphidis*, oviposition is required to take place approximately five days prior to inoculation with *P. neoaphidis* (Powell *et al.*, 1986). It is clear from these results that *A. ervi* will attempt to oviposit in an aphid that is infected with *P. neoaphidis* and, therefore, the parasitoid will be out-competed by the fungus. However, our attack rate bioassay assessed differences in the number of ovipositional attempts and did not assess whether the parasitoid deposited an egg in the aphid. The ovipositors of hymenopteran parasitoids have been shown to contain sensilla which are used to assess the internal environment of a potential host (Consoli, Kitajima and Parra, 1999). Therefore, it may be during this final stage of host assessment that *A. ervi* detects the presence of *P. neoaphidis* and avoids co-inhabiting the same host.

It cannot be assumed that the parasitoid would be competitively excluded by the pathogen and, potentially, interference between the parasitoid and pathogen e.g.

overlapping of attack time, may increase their effectiveness at controlling aphid populations. This is studied in more detail in chapter 6.

CHAPTER 6: FUNGUS-INSECT INTRAGUILD INTERACTIONS - PART 2: DO *COCCINELLA SEPTEMPUNCTATA* AND *APHIDIUS ERVI* INCREASE THE TRANSMISSION OF *PANDORA NEOAPHIDIS*?

6.1 INTRODUCTION

The success rate of insect introductions to control pest species is not high. In a review by Kindlmann and Dixon (2001), it was found that of the 419 species of coccinellids introduced as biological control agents, only 14 were successful, and, of the 1317 species of parasitoids introduced, only 97 were successful. Top down control by parasitoids is the 'essence' of classical biological control. However, it is thought that the low success rate of parasitoid introductions may be due to an over estimation of the top down force applied by a single species (Hunter, 2001; Mills, 2001). By using multiple species, the reliance on top down control by a single species is reduced.

Successful examples of using multiple species for biological control of aphid pests include using both *A. ervi* and *C. carnea* together to control the aphid *M. euphorbiae* on glasshouse tomato and, the use of *A. colemani* early in the season and then *H. convergens* later in the season to control field populations of *A. gossypii* (Bellows and Fisher, 1999). In some cases pests can be controlled by using more than two natural enemy species. For example, Frazer *et al.* (1981) found that the numbers of the pea-aphid, *A. pisum*, in alfalfa crops were reduced five-fold if a complex of predators including spiders, aphidiid parasites, *Anystis* mites and *Heterosoma* species were present. At present there is no consensus as to whether multiple natural enemy species will control a pest better than a single natural enemy species, however, it is likely to vary with the system and species involved (Chang, 1996).

In some cases intraguild interactions that occur between two species may reduce their effectiveness as biocontrol agents. For example, the parasitoid *E. formosa* can successfully control the glasshouse whitefly, *T. vaporariorum*, on tomato crops, but on cucumber crops, which are more susceptible to whiteflies, it cannot control the

pest (Fransen and van Lenteren, 1993). *Trialeurodes vaporariorum* can be successfully controlled on cucumber crops using both *E. formosa* and the fungus *A. aleyrodis* (Fransen and van Lenteren, 1993). As both *E. formosa* and *A. aleyrodis* utilise the host's body to complete their lifecycles and the parasitoid can only detect the presence of the fungus seven days after fungal infection, antagonistic intraguild interactions occur between the two species. Therefore, for the parasitoid and fungus to be used as biocontrol agents against *T. vaporariorum*, the fungus has to be released seven day prior to the release of the parasitoids.

Using multiple species for biological control therefore requires detailed information on the interactions between all of the species involved. Using multiple species to control a pest will only be successful if antagonistic intraguild interactions do not occur between the control agents or can be avoided through management. Intraguild interactions are not restricted to closely related species and can occur between species from different kingdoms (Hochberg and Lawton, 1990). Inter-kingdom competition occurs between many taxa, including parasitoids and fungus, and coccinellids and fungus (Fuentes-Contreras *et al.*, 1998; Furlong and Pell, 1996, 2000; Pell *et al.*, 1997; Roy and Pell, 2000). Both direct (fungus-parasitoid/ predator) and indirect (fungus-prey-parasitoid/ predator) intraguild interactions may occur between an arthropod and a fungal pathogen within the same guild and this may alter their effectiveness as multispecies control agents (Brodeur and Rosenheim, 2000; Brooks, 1993). For example, both the parasitoid *D. semiclausam* and the entomopathogenic fungus *Z. radicans* are natural enemies of the diamondback moth, *P. xylostella*, however, *D. semiclausam* is also susceptible to *Z. radicans* (Furlong and Pell 1996; Furlong *et al.*, 1995; Pell *et al.*, 1993). In this case the fungus has a direct effect on the parasitoid. It was also found that *Z. radicans* infection levels were larger in treatments where *D. semiclausam* was present. This is thought to be a result of *D. semiclausam* increasing the movement of *P. xylostella* larvae which increases the potential of contact with infective conidia (see section 1.3.1.2). Here the parasitoid is indirectly interacting with the fungus.

Intraguild interactions may not always have a negative effect on one or more of the guild members and may benefit the species involved. For example, *C. septempunctata* is an intraguild predator of *P. neoaphidis* and will have a direct

effect on the population size of the fungus through the consumption of both living *P. neoaphidis*-infected aphids and *P. neoaphidis*-infected sporulating cadavers (Roy and Pell, 2000; Pell *et al.*, 1997; Roy, 1997). Consumption of a cadaver will prevent sporulation whilst partial consumption decreases sporulation (Roy and Pell, 2000). However, foraging of *C. septempunctata* increased the population size of the fungus through both the disturbance of aphid (and therefore the frequency of which they encountered conidia) and through passively vectoring conidia to previously uninfected aphid colonies (Pell *et al.*, 1997; Roy, 1997). Experiments have shown that vectoring of *P. neoaphidis* conidia by *C. septempunctata* caused a 10% infection rate in an aphid population (Roy, 1997; Roy *et al.*, 2001).

The outcomes of direct and indirect intraguild interactions between arthropods and entomopathogenic fungi will directly affect both the population size of the natural enemies and their prey. However, the majority of studies of entomopathogenic fungus-arthropod intraguild interactions were carried out at a small spatial scale e.g. Petri dishes or on single plants (Fuentes-Contreras *et al.*, 1998; Furlong and Pell, 1996; Pell *et al.*, 1997; Roy 1997; Roy *et al.*, 1998; Roy *et al.*, 2001). This chapter examines the interactions between *P. neoaphidis, A. ervi* and *C. septempunctata* at the population scale and assesses the effects of intraguild interactions on both the aphid population and the populations of the individual natural enemy species themselves. More specifically:

- The intraguild interactions between *P. neoaphidis* and either *C. septempunctata* or *A. ervi* at the population scale
- The intraguild interactions amongst *P. neoaphidis*, *C. septempunctata* and *A. ervi* at the population scale
- The effect of previous infection of *A. pisum* by *P. neoaphidis* on subsequent intraguild interactions with *A. ervi* at the population scale
- The movement of parasitoids into aphid colonies and the intraguild interactions between *P. neoaphidis* and *A. ervi* at the population scale within a polytunnel

6.2 MATERIALS AND METHODS

6.2.1 Cage experiment to assess the effect of arthropod natural enemies on the transmission of *Pandora neoaphidis*

The cages used in these experiments were made from Perspex (0.5m^2 x 1m high) and contained a Perspex plant-pot frame (0.45m^2, 85mm tall) (Figure 6.1a). The frame was designed to support nine 85mm diameter plant-pots (Figure 6.1b) and allowed insects to walk between the plants. Nine 15-day-old bean plants, the soil at the bases of which was covered with a piece of filter paper (90mm diameter), were placed in the frame (Figure 6.1a). The matting on the floor of the cage was kept wet at all times to provide water for the plants and to create a relative humidity greater than 90%. Unless specified, four replicate cages were prepared in total. The cages were maintained within a controlled environment room (18°C, 16L:8D).

In experiments where the plants were transferred to a Perspex simulator cage (1.5m long x 1m wide x 1m high), individual pots were placed in water traps to prevent the movement of aphids between plants within and among treatments. The abiotic conditions in the simulator cage (relative humidity less than 40%) ensured that aphids previously infected with *P. neoaphidis* would form dried cadavers and that further transmission would not occur.

6.2.1.1 Coccinella septempunctata

Twenty four hours prior to the start of the experiment, 12 adult *C. septempunctata* (mixed sex) were removed from the rearing cage and starved in two batches of six (as described in section 2.2.3). Eighteen hours prior to the start of the experiment, 12 water-agar discs, each supporting five sporulating cadavers, were prepared as described in section 2.3.1.1.

The cages were prepared as described in section 6.2.1. Thirty eight-day-old *A. pisum* (4th instar) were placed at the bases of the eight peripheral plants in each of the four treatment cages. The central plant remained uninfested. The cages were then left for 3 hours to provide time for the aphids to colonise the plant and begin

feeding. After 3 hours the natural enemies were added to the cages to provide either: *A. pisum* only (control), *A. pisum* plus *P. neoaphidis*, *A. pisum* plus *C. septempunctata* or, *A. pisum* plus *P. neoaphidis* plus *C. septempunctata.* Treatments containing *P. neoaphidis* were produced by placing six water-agar discs supporting cadavers in random positions on the leaves of the central plant. Treatments containing *C. septempunctata* were produced by releasing six coccinellids (mixed sex) onto the central plant.

The cages were maintained for eight days within a controlled environment room (18°C, 16L:8D). After eight days, the number of living aphids and *P. neoaphidis*-sporulating cadavers was assessed on each plant. The experiment was repeated four times in total. A Latin Square design was used to ensure all four treatments had been carried out in each cage once.

Before analysis the data were transformed using a log(n+1) transformation. ANOVA was used to assess whether *C. septempunctata*, *P. neoaphidis* or an interaction between *C. septempunctata* and *P. neoaphidis* affected the number of living aphids and *P. neoaphidis*-sporulating cadavers recovered from each of the eight periphery plants. Mean values indicate numbers recovered per plant.

6.2.1.2 Aphidius ervi

Eighteen hours prior to the start of the experiment, twelve *P. neoaphidis*-agar discs, each supporting five sporulating cadavers, were prepared as described in section 2.3.1.1.

The experimental cages were prepared as described in section 6.2.1. Ten eight-day-old *A. pisum* were placed at the bases of the eight peripheral plants in each of the four treatment cages. The central plant remained uninfested. The cages were then left for 3 hours to provide time for the aphids to colonise the plant and start feeding. After 3 hours the natural enemies were added to the cages to provide either: *A. pisum* only (control), *A. pisum* plus *P. neoaphidis*, *A. pisum* plus *A. ervi* or, *A. pisum* plus *P. neoaphidis* plus *A. ervi.* Treatments containing *P. neoaphidis* were produced by placing six water-agar discs supporting cadavers in random positions

on the leaves of the central plant. Treatments containing *A. ervi* were produced by releasing three female and three male parasitoids near to the central plant.

The cages were maintained for 8 days within a controlled environment room (18°C, 16L:8D). After eight days the number of *P. neoaphidis*-sporulating cadavers on each plant was assessed. The plants were then placed in a Perspex simulator cage in the controlled environment room and maintained for a further 8 days. The number of *P. neoaphidis*-sporulating cadavers and *A. ervi* mummies were then assessed. The experiment was repeated four times. A Latin Square design was used to ensure all four treatments had been carried out in each cage once.

Before analysis the data were transformed using a log(n+1) transformation. ANOVA was used to assess whether *A. ervi*, *P. neoaphidis* or an interaction between *A. ervi* and *P. neoaphidis* affected the number of *P. neoaphidis*-sporulating cadavers and *A. ervi* mummies recovered from each of the eight peripheral plants. A two sample t-test was used to assess whether the there was a difference in the number of *P. neoaphidis*-sporulating cadavers recovered after 8 and 16 days. Mean values indicate numbers recovered per plant.

6.2.1.3 Coccinella septempunctata *plus* Aphidius ervi

Twenty four hours prior to the start of the experiment, six adult *C. septempunctata* (mixed sex) were removed from the rearing cage and starved (as described in section 2.2.3). Eighteen hours prior to the start of the experiment, twelve water-agar discs, each supporting five sporulating cadavers, were prepared as described in section 2.3.1.1.

The experimental cages were prepared as described in section 6.2.1. Ten eight-day-old *A. pisum* were placed at the bases of the eight peripheral plants in each of the four treatment cages. The central plant remained uninfested. The cages were then left for 3 hours to provide time for the aphids to colonise the plant and start feeding. After 3 hours the natural enemies were added to the cages to provide either: *A. pisum* plus *P. neoaphidis*, *A. pisum* plus *A. ervi*, *A. pisum* plus *C. septempunctata* or, *A. pisum* plus *P. neoaphidis* plus *A. ervi* plus *C. septempunctata*. Treatments

containing *P. neoaphidis* were produced by placing six water-agar discs supporting cadavers in random positions on the leaves of the central plant. Treatments containing *A. ervi* were produced by releasing two female parasitoids and one male parasitoid near to the central plant. Treatments containing *C. septempunctata* were produced by releasing three coccinellids (mixed sex) onto the central plant.

The cages were maintained for 8 days within a controlled environment room (18°C, 16L:8D). After eight days the number of living aphids and *P. neoaphidis*-sporulating cadavers was assessed on plants 2, 4, 6 and 8 (Figure 6.1b). The remaining plants were maintained for a further 8 days within Perspex simulator cages (1.5m long x 1m wide x 1m high) in the controlled environment room. After this time the number of *P. neoaphidis*-sporulating cadavers and *A. ervi* mummies was assessed. The experiment was repeated with the number of living aphids and *P. neoaphidis*-sporulating cadavers on plants 1, 3, 5 and 7 being assessed after 8 days and the remaining plants being assessed after 16 days. The experiment was repeated four times in total. A Latin Square design was used to ensure all four treatments had been carried out in each cage once.

Before analysis the data were transformed using a log(n+1) transformation. ANOVA was used to assess whether *A. ervi*, *C. septempunctata*, *P. neoaphidis* or an interaction between *A. ervi*, *C. septempunctata* and *P. neoaphidis* affected the number of living aphids, *P. neoaphidis*-sporulating cadavers and *A. ervi* mummies recovered from the eight peripheral plants. A two sample t-test was used to assess whether there was a difference in the number of *P. neoaphidis*-sporulating cadavers recovered after 8 and 16 days. Mean values indicate numbers recovered per plant.

6.2.2 Cage experiment to assess the effect of previous inoculation by *Pandora neoaphidis* on the reproductive success of *Aphidius ervi*

Seventy-two hours prior to the start of the experiment, 45 five-day-old *A. pisum* (3rd instar) were inoculated with *P. neoaphidis* (as described in section 2.3.1). This was repeated 24 hours prior to the start of the experiment with a further 45 seven-day-old *A. pisum* (3rd/ 4th instar).

The experimental cages were prepared as described in section 6.2.1. Ten eight-day-old uninoculated *A. pisum* were added to each plant in the first treatment cage, five healthy plus five 24 hour infected *A. pisum* were added to each plant in the second treatment cage and five uninoculated plus five 72 hours infected *A. pisum* were added to each plant in the third age. Aphids were placed at the bases of the plants and left for 3 hours. This provided time for the aphids to colonise the plant and begin feeding. Three female and three male *A. ervi* were then released near to the central plant in each cage.

The cages were maintained for 8 days within a controlled environment room (18°C, 16L:8D). After eight days the number of living aphids and *P. neoaphidis*-sporulating cadavers was assessed on plants 2, 4, 6 and 8 (Figure 6.1b). The remaining plants were maintained for a further 8 days in a Perspex simulator cage within the controlled environment room. After 8 days the number of *P. neoaphidis*-sporulating cadavers and *A. ervi* mummies was assessed. The experiment was repeated three times in total. A Latin Square design was used to ensure all three treatments had been carried out in each cage once.

Before analysis the data were transformed using a log(n+1) transformation and ANOVA used to assess whether the was an effect of previous fungal infection on the number of aphids, *P. neoaphidis*-sporulating cadavers and *A. ervi* mummies recovered.

Figure 6.1a Perspex cage with Perspex frame (plan view) containing nine 15-day-old *Vicia faba* plants.

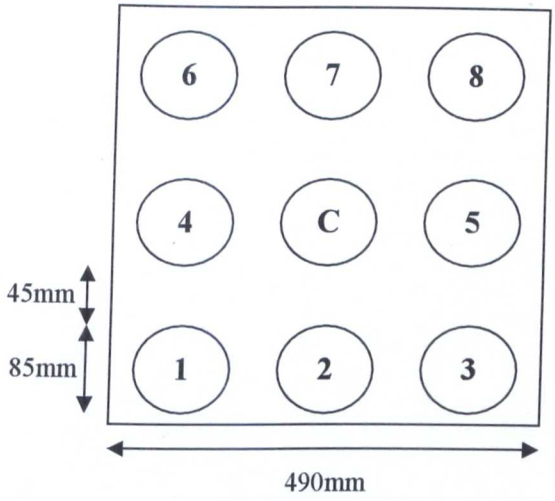

Figure 6.1b Schematic diagram of Perspex frame showing dimensions and plant layout (C= Central plant, 1-8= Periphery plants).

6.2.3 Polytunnel experiment to assess *Aphidius ervi-Pandora neoaphidis* interactions over a single parasitoid generation

The polytunnel used in this experiment was situated in the grounds of Rothamsted Research and was positioned in a North to South orientation. The polytunnel was made from a steel tube semi-circular frame (5.5mm wide x 3m high) covered in clear polythene (Figure 6.2.). The light cycle and temperature within the polytunnel were dependent on external abiotic conditions. A bank of four fans drew air in

through the south-facing end of the polytunnel and out through the north-facing end (flow rate of approximately 54 cm/s).

Eighteen hours prior to the start of the experiment, twenty-four water-agar discs, each supporting five *P. neoaphidis*-sporulating cadavers, were prepared as described in section 2.3.1.1. Two hours prior to the start of the experiment, forty female *A. ervi* were removed from the rearing cage and transferred to the releasing chamber (clear plastic cylindrical container (30cm diameter x 50cm tall) with a ventilated lid), in order to acclimatise.

The experimental design within the polytunnel is shown in Figure 6.2. The patches contained nine plant-pots (130mm diameter) each containing three 15-day-old bean plants. The plant-pots stood in seed trays (155mm x 210mm x 45 mm) containing water. This created a water trap to stop the movement of aphids within and between patches. The bean plants were planted in a triangle formation approximately 80mm apart. Thirty eight-day-old *A. pisum* (4^{th} instar) were placed on the soil between the three bean plants in each pot and left for 2 hours. This provided time for the aphids to colonise the plant and begin feeding. Two *P. neoaphidis*-agar discs were placed in random positions on the leaves of all three plants in pot 2, 4, 6, and 8 in the left-hand-patch (6 discs per pot, 24 discs per patch) (Figure 6.2). The lid of the parasitoid releasing chamber was then removed. After two days the plants were covered with a perforated bread-bag (Cryovac Supermicro, 305mm x 460mm) that was held in place with an elastic band. The plants were then transferred to a controlled environment room (18°C, 16L:8D).

The number of *P. neoaphidis*-sporulating cadavers on each plant was recorded four days after being transferred to the controlled environment room and the number of *A. ervi* mummies and *P. neoaphidis* sporulating cadavers recorded again after a further 12 days. The experiment was repeated six times, with the patch containing the fungus being on the left-hand-side three times and the right-hand-side three times.

Before analysis the data were transformed using a log(n+1) transformation. ANOVA was used to assess whether the number of *A. ervi* mummies and *P.*

neoaphidis-sporulating cadavers recovered differed within or between the patches. A two sample t-test was used to assess whether there was a difference in the number of *P. neoaphidis*-sporulating cadavers recovered 4 and 16 days after transfer to controlled conditions.

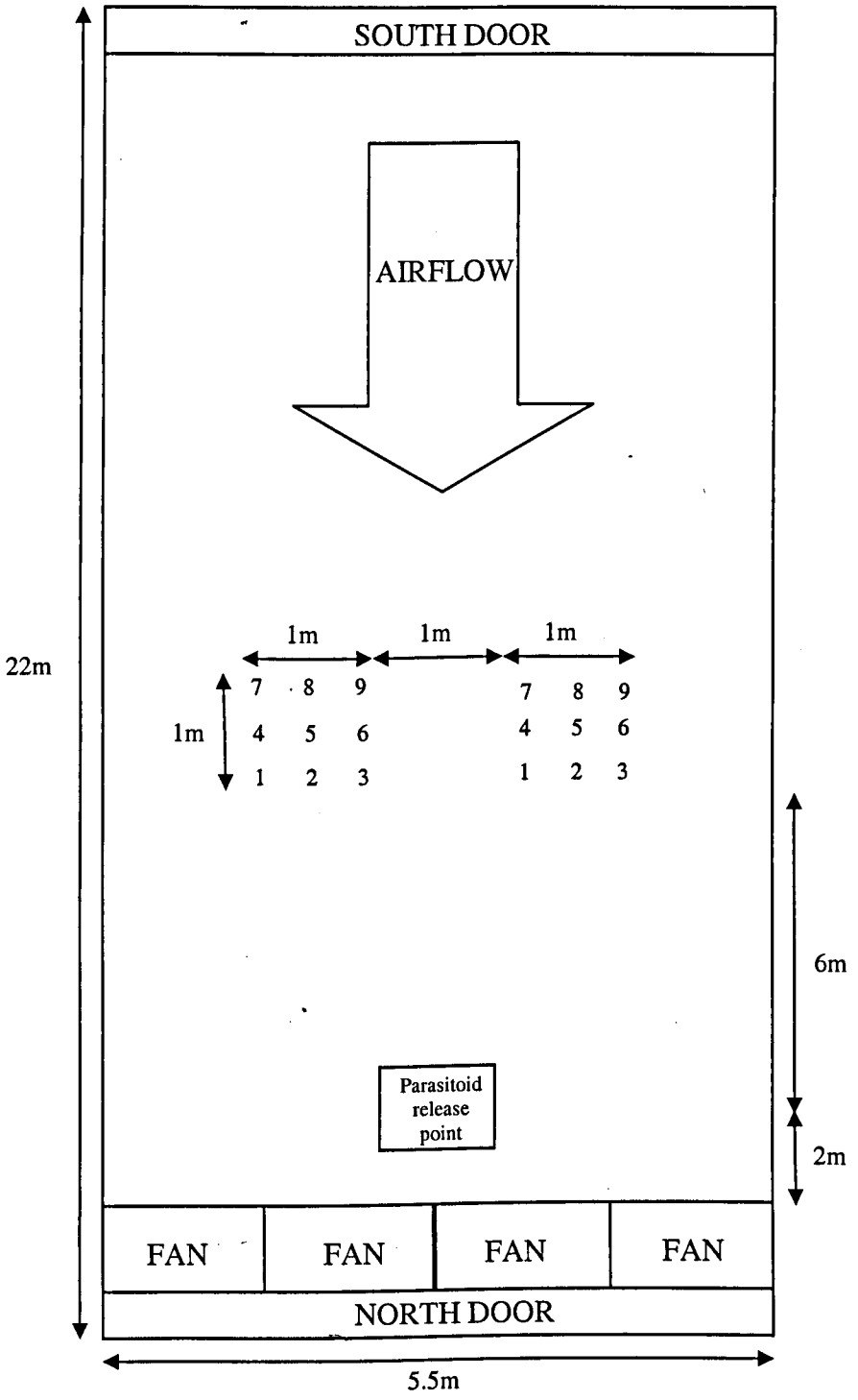

Figure 6.2 Polytunnel specifications and experimental design.

6.3 RESULTS

6.3.1 Cage experiment to assess the effect of arthropod natural enemies on the transmission of *Pandora neoaphidis*

6.3.1.1 Coccinella septempunctata

There were no significant effects of *P. neoaphidis* on the number of aphids recovered (ANOVA: $F_{1, 127}$= 1.52, p= 0.264), with a mean of 165 aphids recovered from treatments containing *P. neoaphidis* and 158 aphids from treatments not containing *P. neoaphidis* (Figure 6.3). There were significant effects of *C. septempunctata* on the number of aphids recovered (ANOVA: $F_{1, 127}$= 14.30, p= 0.009), with a mean of 104 aphids recovered from treatments containing *C. septempunctata* and 219 aphids from treatments not containing *C. septempunctata* (Figure 6.3). There were no significant interactions between *P. neoaphidis* and *C. septempunctata* that further affected the aphid population (ANOVA: $F_{1, 127}$= 0.81, p= 0.404).

There were significant effects of *C. septempunctata* on the number of sporulating cadavers recovered (ANOVA: $F_{1, 63}$= 34.01, p<0.001), with a mean of 1.13 sporulating cadavers recovered from the treatment containing both *P. neoaphidis* and *C. septempunctata* and 0.063 recovered from the treatment containing *P. neoaphidis* alone (Figure 6.4). Sporulating cadavers were found on a greater number of plants in the treatment containing *C. septempunctata* plus *P. neoaphidis* compared to the treatment containing *P. neoaphidis* alone, with 62.5% and 6.3% of plants found containing sporulating cadavers respectively.

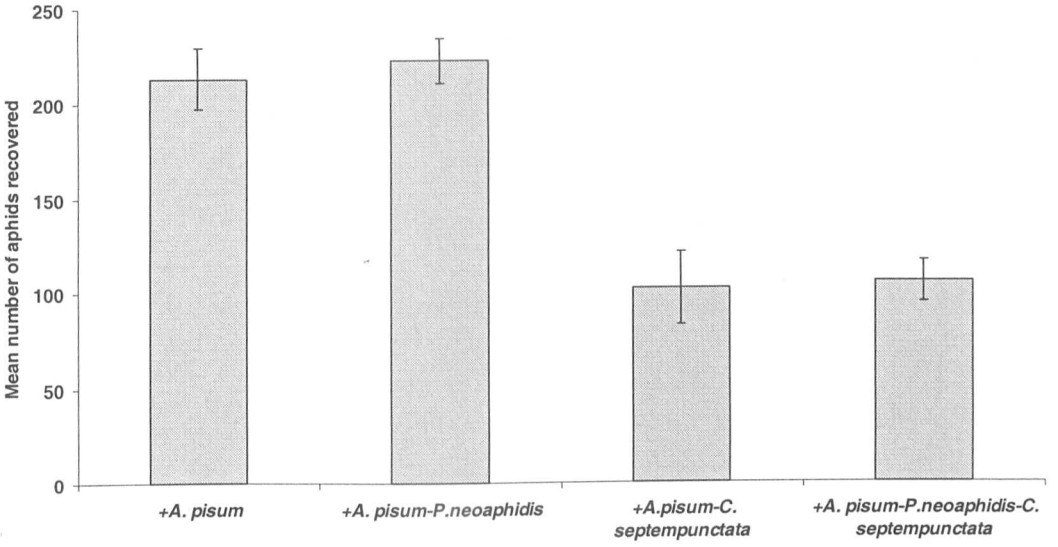

Figure 6.3 Mean number of *Acyrthosiphon pisum* recovered alive after 8 days from cages containing either no natural enemies, a single aphid natural enemy (*Pandora neoaphidis* or *Coccinella septempunctata*) or both natural enemies.

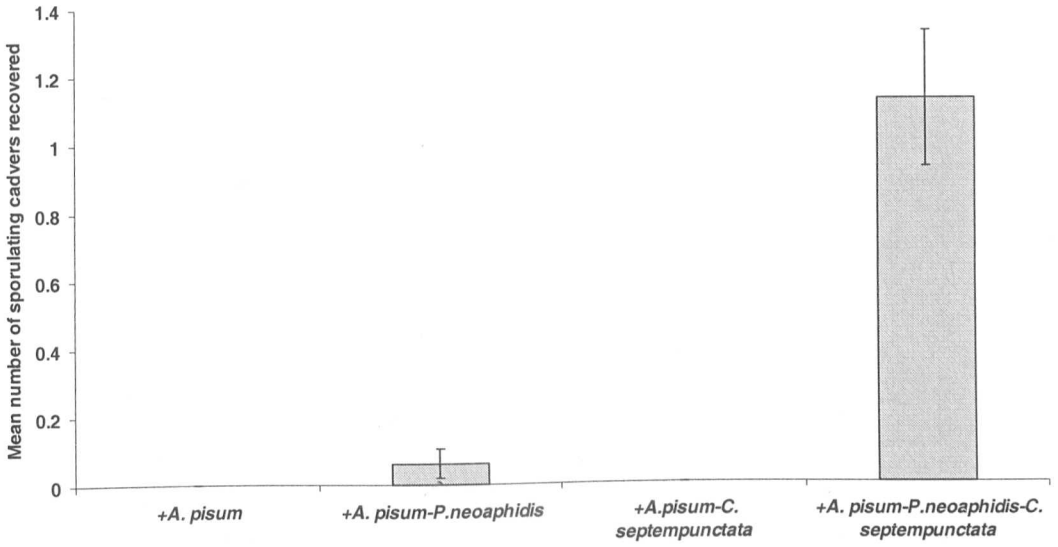

Figure 6.4 Mean number of *Pandora neoaphidis*-sporulating cadavers recovered after 8 days from cages containing either no aphid natural enemies, a single aphid natural enemy (*P. neoaphidis* or *Coccinella septempunctata*) or both natural enemies.

6.3.1.2 Aphidius ervi

There were significant effects of *P. neoaphidis* on the number of mummies recovered after 16 days (ANOVA: $F_{1, 63}$= 4.38, p= 0.041), with a mean of 11.1

mummies recovered from the treatment containing both *P. neoaphidis* and *A. ervi* and 17.4 recovered from the treatment containing *A. ervi* alone (Figure 6.5).

There were significant effects of *A. ervi* on the number of sporulating cadavers recovered after 8 days (ANOVA: $F_{1, 63}= 21.56$, $p<0.001$), with a mean of 4.41 sporulating cadavers recovered from the treatment containing both *P. neoaphidis* and *A. ervi* and 0.75 recovered from the treatment containing *P. neoaphidis* alone (Figure 6.5). There were significant effects of *A. ervi* on the number of sporulating cadavers recovered after 16 days (ANOVA: $F_{1, 63}= 34.82$, $p<0.001$), with a mean of 10.6 sporulating cadavers recovered from the treatment containing both *P. neoaphidis* and *A. ervi* and 0.313 recovered from the treatment containing *P. neoaphidis* alone (Figure 6.5).

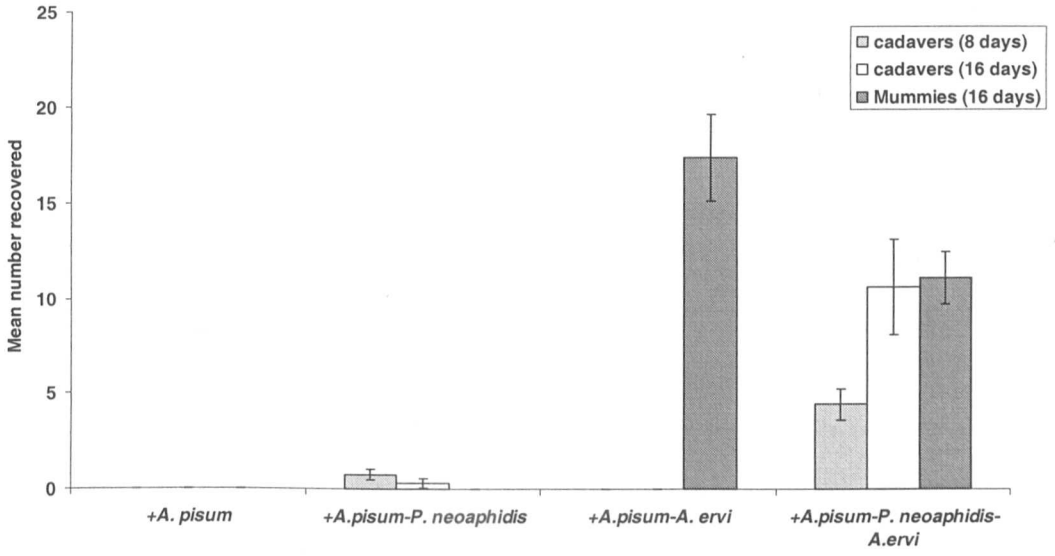

Figure 6.5 Mean number of *Aphidius ervi* mummies recovered after 16 days and, *Pandora neoaphidis*-sporulating cadavers recovered after 8 and 16 days from cages containing either no aphid natural enemies, a single natural enemy (*P. neoaphidis* or *A. ervi*) or both natural enemies.

After 8 days, sporulating cadavers were found on a greater number of plants in the treatment containing *A. ervi* plus *P. neoaphidis* compared to the treatment containing *P. neoaphidis* alone, with 68.8% and 31.3% of plants found containing sporulating cadavers respectively. After 16 days, sporulating cadavers were found on a greater number of plants in the treatment containing *A. ervi* plus *P. neoaphidis* compared to the treatment containing *P. neoaphidis* alone, with 62.5% and 6.3% of plants found containing sporulating cadavers respectively.

There were no significant differences in the number of sporulating cadavers recovered after 8 and 16 days from the treatment containing *P. neoaphidis* alone (T-test: $t_{114.80}$= -0.15, p= 0.879), with a mean of 0.75 sporulating cadavers recovered after 8 days and 0.313 recovered after 16 days (Figure 6.5). There were no significant differences in the number of sporulating cadavers recovered after 8 and 16 days from the treatment containing both *P. neoaphidis* and *A. ervi* (t-test: $t_{54.96}$= -0.96, p= 0.341), with a mean of 4.41 sporulating cadavers recovered after 8 days and 10.6 recovered after 16 days (Figure 6.5).

6.3.1.3 Coccinella septempunctata *plus* Aphidius ervi

There were significant differences in the number of aphids recovered from the four treatments after 8 days (ANOVA: $F_{3, 63}$= 27.13, p< 0.001), with a mean of 208, 58.4, 72.7, and 3.13 aphids recovered from the *P. neoaphidis*, *C. septempunctata*, *A. ervi* and, *P. neoaphidis* plus *C. septempunctata* plus *A. ervi* treatments respectively (Figure 6.6).

There were significant differences in the number of mummies recovered after 16 days (ANOVA: $F_{1, 31}$= 56.63, p< 0.001), with a mean of 0.188 mummies recovered from the treatment containing *P. neoaphidis* plus *C. septempunctata* plus *A. ervi* and 6.56 recovered from the treatment containing *A. ervi* alone (Figure 6.7).

There were no significant effects of *C. septempunctata* plus *A. ervi* on the number of sporulating cadavers recovered after 8 days (ANOVA: $F_{1, 31}$= 0.07, p= 0.800), with a mean of 0.125 sporulating cadavers recovered from the treatment containing *C. septempunctata* plus *A. ervi* and 0.188 recovered from the treatment containing *P. neoaphidis* alone (Figure 6.7). There were no significant effects of *C. septempunctata* plus *A. ervi* on the number of sporulating cadavers recovered after 16 days (ANOVA: $F_{1, 31}$= 3.30, p= 0.080), with a mean of 19.3 sporulating cadavers recovered from the treatment containing *C. septempunctata* plus *A. ervi* and 2.0 recovered from the treatment containing *P. neoaphidis* alone (Figure 6.7).

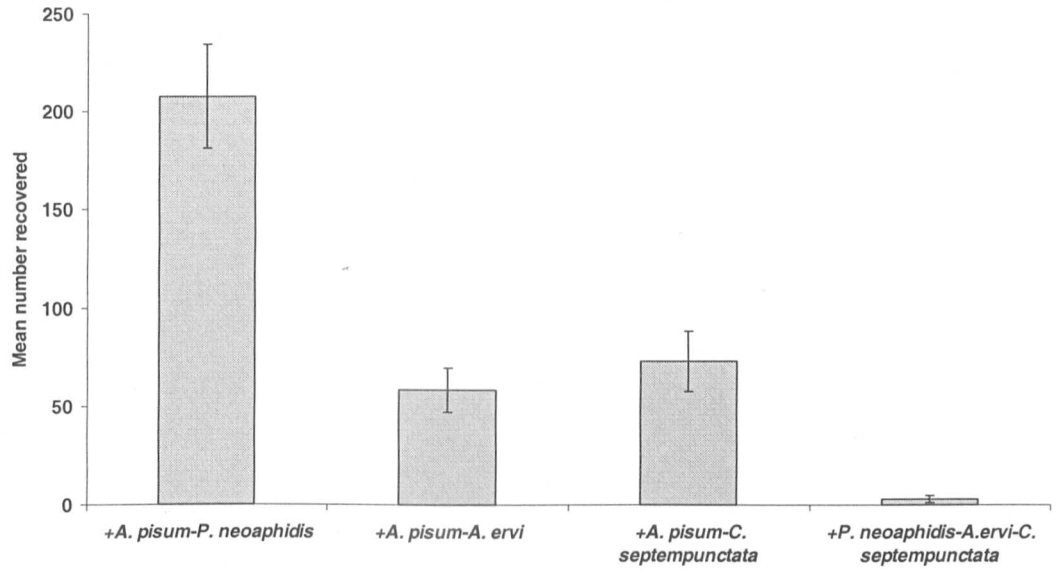

Figure 6.6 Mean number of *Acyrthosiphon pisum* recovered after 8 days from cages containing either a single aphid natural enemy (*Pandora neoaphidis*, *Aphidius ervi* or *Coccinella septempunctata*) or all three aphid natural enemies.

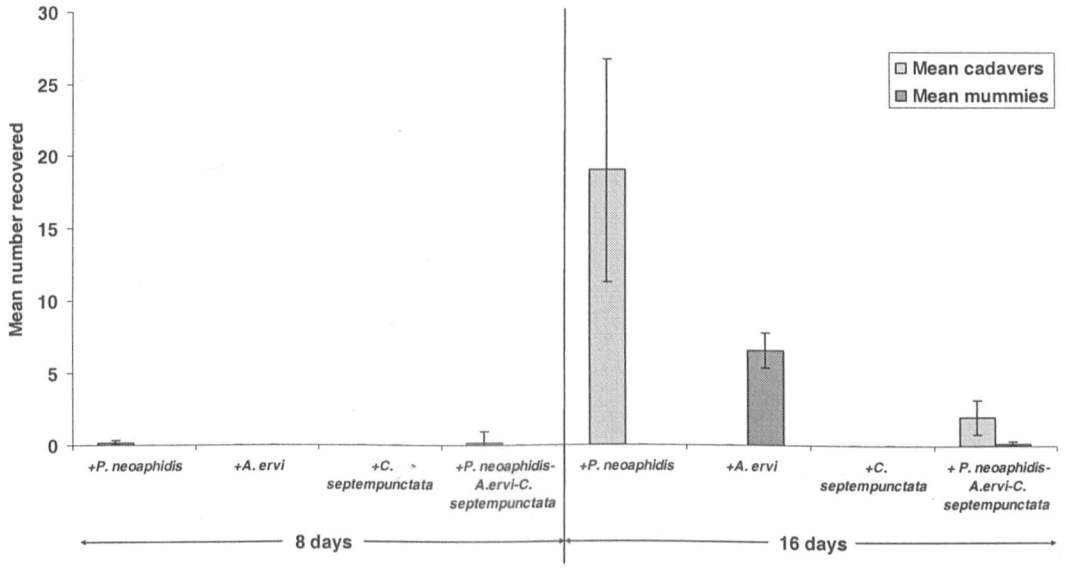

Figure 6.7 Mean number of *Aphidius ervi* mummies and *Pandora neoaphidis*-sporulating cadavers recovered after 8 and 16 days from cages containing either a single aphid natural enemy (*P. neoaphidis*, *A. ervi* or *Coccinella septempunctata*) or all three aphid natural enemies.

There were significant differences in the number of cadavers recovered after 8 and 16 days from the treatment containing *P. neoaphidis* alone (t-test: $t_{15.79}$= -2.66, p= 0.017), with a mean of 0.188 sporulating cadavers recovered after 8 days and 19.3

recovered after 16 days (Figure 6.7). There were no significant differences in the number of cadavers recovered after 8 and 16 days from the treatment containing *P. neoaphidis* plus *C. septempunctata* plus *A. ervi* (t-test: $t_{16..95}$= -1.61, p= 0.125), with a mean of 0.125 sporulating cadavers recovered after 8 days and 2.0 recovered after 16 days (Figure 6.7).

6.3.2 Cage experiment to assess the effect of previous inoculation by *Pandora neoaphidis* on the reproductive success of *Aphidius ervi*

There were significant differences in the number of aphids recovered from the three treatments (ANOVA: $F_{2, 35}$= 41.18, p<0.001). The number of aphids recovered decreased as infection time of the initial aphids increased, with 95.8, 7.42 and 2.5 aphids recovered from the uninfected, 24 and 72 hour previously infected treatments respectively (Figure 6.8).

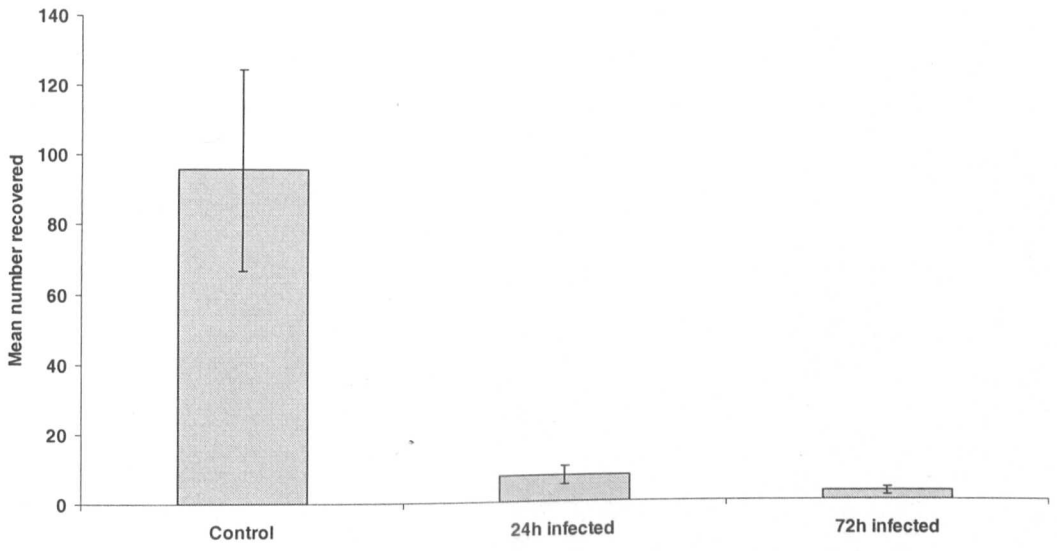

Figure 6.8 Mean number of *Acyrthosiphon pisum* recovered after 8 days from cages containing *Aphidius ervi* plus either uninfected *Acyrthosiphon pisum* or, *A. pisum* previously infected with *Pandora neoaphidis* for 24 or 72 hours.

There were significant differences in the number of mummies recovered from the three treatments (ANOVA: $F_{2, 44}$= 36.41, p<0.001), with 10.1, 0.13 and 0.67 mummies recovered from the uninfected, 24 and 72 hour previously infected treatments respectively (Figure 6.9).

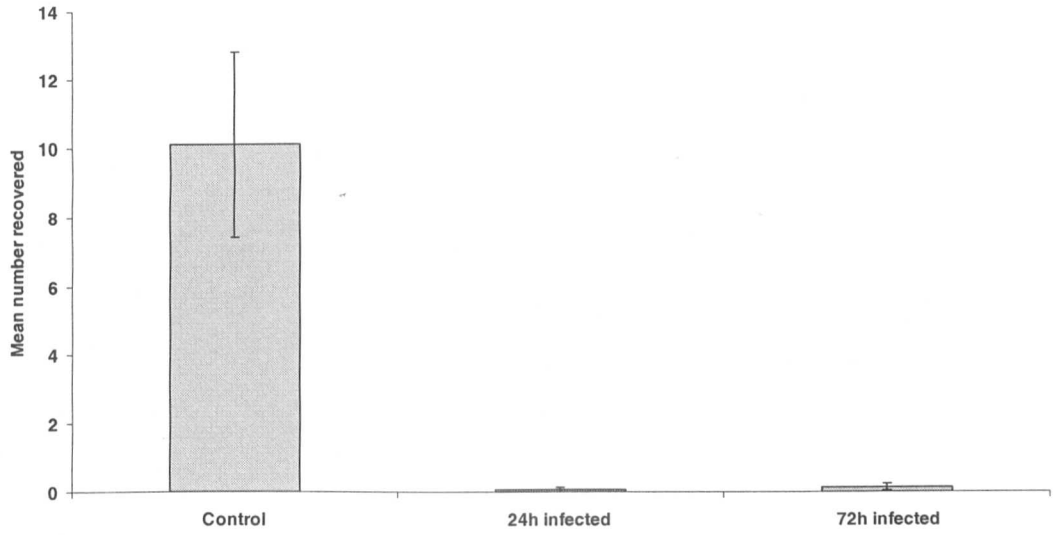

Figure 6.9 Mean number of *Aphidius ervi* mummies recovered after 16 days from cages containing either uninfected *Acyrthosiphon pisum* or, *A. pisum* previously infected with *Pandora neoaphidis* for either 24 or 72 hours.

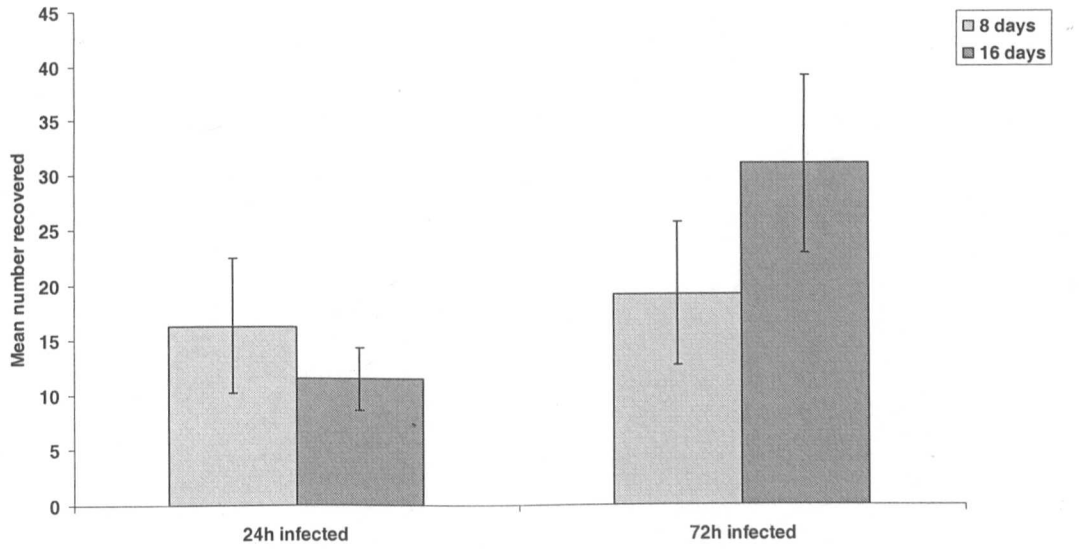

Figure 6.10 Mean number of *Pandora neoaphidis*-sporulating cadavers recovered after 8 and 16 days from cages containing *Aphidius ervi* plus *Acyrthosiphon pisum* previously infected with *Pandora neoaphidis* for either 24 or 72 hours.

After 8 days there were no significant differences in the number of sporulating cadavers recovered from the treatments containing aphids previously infected for 24 and 72 hours (ANOVA: $F_{1, 23}$= 0.07, p= 0.797), with 16.3 and 19.25 sporulating cadavers recovered respectively (Figure 6.10).

After 16 days there were no significant differences in the number of sporulating cadavers recovered from the treatments containing aphids initially infected for 24 and 72 hours (ANOVA: $F_{1, 27}$= 3.43, p= 0.076), with 11.5 and 31 sporulating cadavers recovered respectively (Figure 6.10).

6.3.3 Polytunnel experiment to assess *Aphidius ervi-Pandora neoaphidis* interactions over a single parasitoid generation

There were no significant differences in the number of mummies recovered from the patch initially containing sporulating cadavers and the control patch (ANOVA: $F_{1, 107}$= 1.28, p= 0.320), with a mean of 8.33 and 12.0 mummies recovered from each patch respectively (Figure 6.11). Within the treatment patch, there were no significant differences in the number of mummies recovered from individual pots initially containing sporulating cadavers and pots not supplemented with sporulating cadavers (ANOVA: $F_{1, 53}$= 0.46, p= 0.501), with a mean 0.75 and 1.06 mummies recovered from each treatment respectively (Figure 6.12).

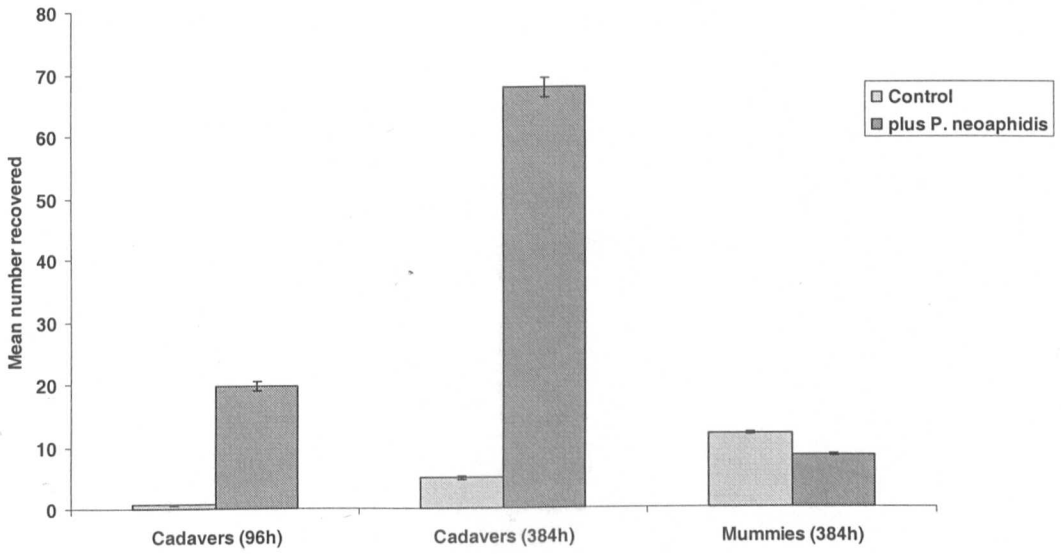

Figure 6.11 Mean number of *Aphidius ervi* mummies recovered after 16 days and *Pandora neoaphidis*-sporulating cadavers recovered after 4 and 16 days from either the control patch or the patch containing *P. neoaphidis*-sporulating cadavers within the polytunnel.

After four days there were no significant differences in the number of cadavers recovered from the patch initially containing sporulating cadavers and the control patch (ANOVA: $F_{1, 107}$= 4.94, p= 0.090), with a mean of 19.7 and 0.67 sporulating cadavers recovered from each patch respectively (Figure 6.11). Within the treatment patch, there were significant differences in the number of sporulating cadavers recovered from individual pots initially containing sporulating cadavers and pots not supplemented with sporulating cadavers (ANOVA: $F_{1, 53}$= 14.64, p<0.001), with a mean of 4.21 and 0.57 sporulating cadavers from each treatment respectively (Figure 6.13).

After 16 days there were significant differences in the number of cadavers recovered from the patch initially containing sporulating cadavers and the control patch (ANOVA: $F_{1, 107}$= 15.66, p= 0.017), with a mean of 68.2 and 5.0 sporulating cadavers recovered from each patch respectively (Figure 6.11). Within the treatment patch, there were significant differences in the number of sporulating cadavers recovered from individual pots initially containing sporulating cadavers and pots not supplemented with sporulating cadavers (ANOVA: $F_{1, 53}$= 7.37, p= 0.009), with a mean of 12.1 and 3.93 sporulating cadavers recovered from each treatment respectively (Figure 6.13).

There were significant differences in the number of sporulating cadavers recovered from the control patch after 4 and 16 days (t-test: $t_{177.04}$= -2.90, p= 0.004), with a mean of 0.67 and 5.0 sporulating cadavers recovered respectively (Figure 6.11). There were significant differences in the number of sporulating cadavers recovered from the patch initially containing sporulating cadavers after 4 and 16 days (t-test: $t_{93.26}$= -3.00, p= 0.004), with a mean of 19.7 and 68.2 sporulating cadavers recovered respectively (Figure 6.11).

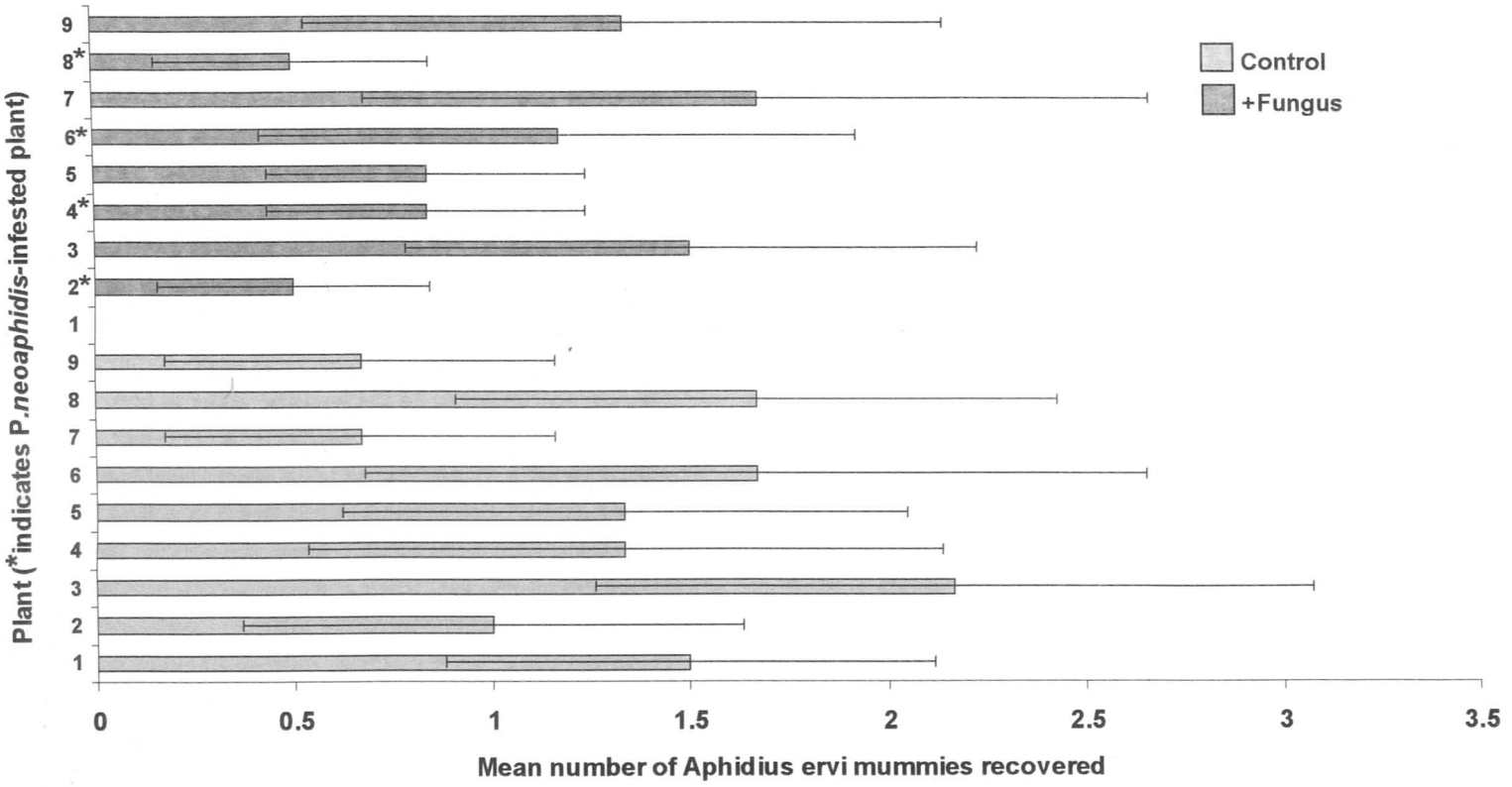

Figure 6.12 Mean number of *Aphidius ervi* mummies recovered after 16 days from each plant within either the control patch or the patch containing *P. neoaphidis*-sporulating cadavers within the polytunnel. *indicates specific pots within a patch initially containing *P. neoaphidis*-sporulating cadavers.

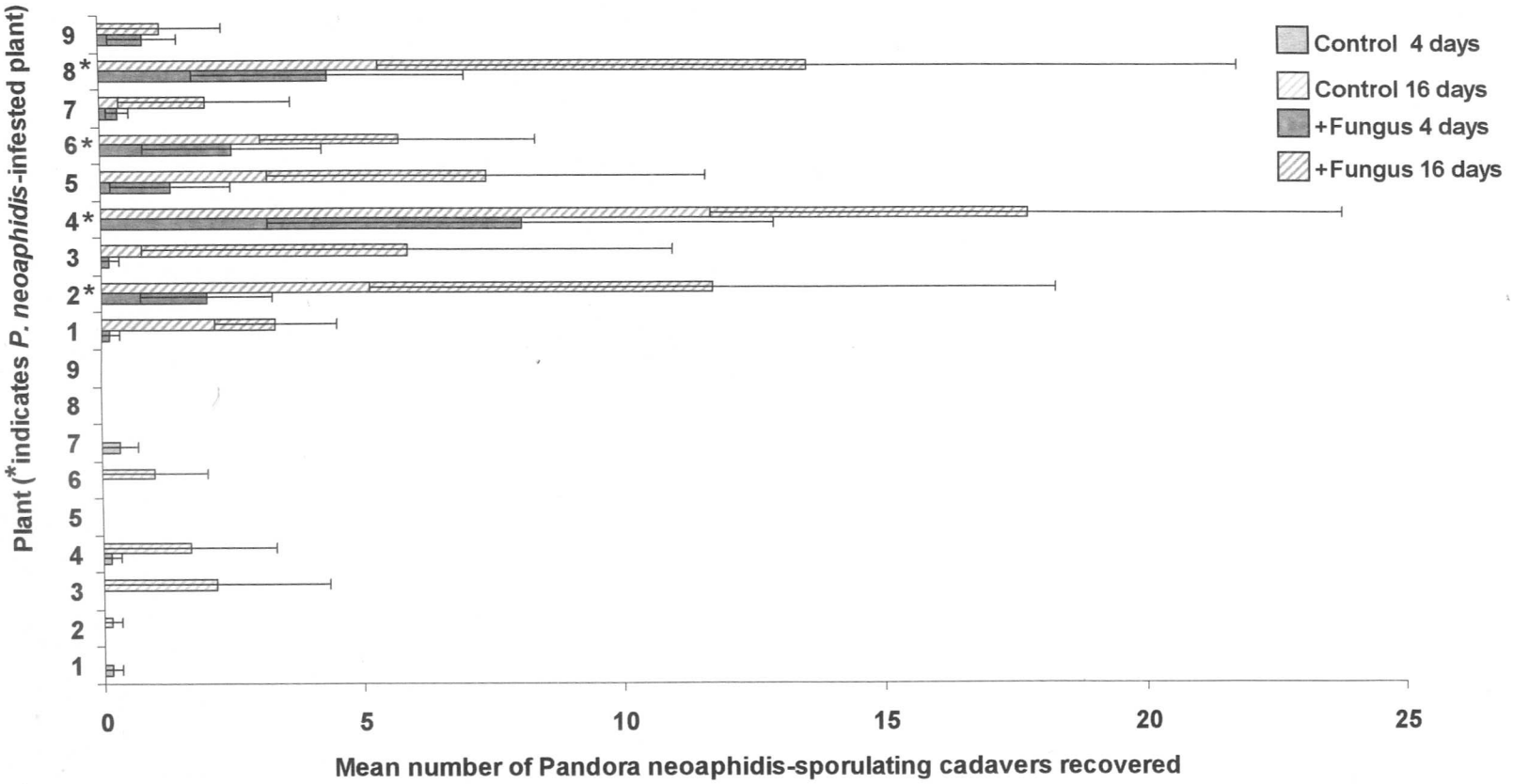

Figure 6.13 Mean number of *Pandora neoaphidis*-sporulating cadavers recovered after 4 and 16 days from each plant within either the control patch or the patch containing *P. neoaphidis*-sporulating cadavers within the polytunnel. *indicates specific pots within a patch initially containing *P. neoaphidis*-sporulating cadavers.

6.4 DISCUSSION

Pandora neoaphidis is an aphid specific fungal pathogen and has been recorded causing epizootics in field populations of aphids (Feng and Johnson, 1991; Pickering and Gutierrez, 1991; Pell *et al.*, 2001) (see section 1.1.2.1). However, the results of the '*Coccinella septempunctata*' cage experiment indicated no significant effects of *P. neoaphidis* on the population size of *A. pisum*. As the cages were maintained at 18°C and at relative humidity greater than 90%, the abiotic conditions were optimal for infection to take place (Glare and Milner, 1991). Infection can only occur once the fungus has become attached to a suitable host, therefore, the low infection observed may have been a result of a low encounter rate between *P. neoaphidis* conidia and *A. pisum*. In the absence of foraging arthropod competitors and host-aphids previously infected with *A. pisum*, dispersal of *P. neoaphidis* conidia from the central aphid-uninfested plant could only occur through the movement of conidia on air currents or by the attachment of conidia to foraging aphids. The cages used in these experiments were closed systems in which there was little movement of air. Therefore, transmission of *P. neoaphidis* was primarily dependent on foraging *A. pisum* moving from a peripheral plant to the central plant and making contact with conidia that had been discharged onto the leaf surface. Although the results in section 4.3 indicate that *A. pisum* will colonise a bean plant containing *P. neoaphidis* and that fungal infection will take place, this will only occur if an aphid has become dislodged from its host plant and is searching for a new plant. As the peripheral bean plants were infested with only 30 *A. pisum*, both the plants and aphids would have been relatively healthy. Movement of *A. pisum* from these plants would, therefore, be low and this may have limited the transmission of *P. neoaphidis*.

Foraging by both *C. septempunctata* and *A. ervi* reduced the aphid population by approximately 50%. Unlike predation by *C. septempunctata*, which removes the aphid from the system, following oviposition by *A. ervi* the aphid remains in the system until mummification occurs (approximately 10 days after oviposition). It was, therefore, surprising that after 8 days the number of *A. pisum* recovered from cages containing *A. ervi* was similar to the number recovered from cages containing *C. septempunctata*. This suggests that the reduced aphid population in the presence

of *A. ervi* is as a result of aphid death following disturbance by the foraging parasitoids and raises the question of whether the reduced aphid population in the presence of *C. septempunctata* is due to predation, disturbance or a combination of the two. There have been several studies on the response of *A. pisum* to foraging parasitoids. The effect of foraging by the parasitoid *Monoctonus paulensis* (Ashmead) on the behaviour of *A. pisum* was assessed by Chau and Mackauer (1997). *Acyrthosiphon pisum* respond to the presence of *M. paulensis* by dropping from the feeding site. Before parasitoid attack, fourth instar *A. pisum* were six times more likely to drop than first instars and sixteen times more likely to drop following a parasitoid attack. Separate studies using *A. pisum* and *A. ervi* have indicated that the dropping response is a function of internal stress (e.g. starved/ not starved), with aphids using behaviours such as 'kicking' when internal stress is high and 'dropping' when internal stress is low (Villagra *et al.*, 2002). Although dropping from a plant reduces the risk of parasitisation, it increases the mortality risk from: predation from ground dwelling arthropods (e.g. carabid beetles), an increased potential of contacting fungal pathogens both on the soil or the plant or, an increased chance of starvation if the aphid is unable to recolonise a plant.

Using multiple natural enemies (*P. neoaphidis* plus *C. septempunctata* plus *A. ervi*) reduced the aphid population to a level below that using the individual natural enemy species alone and, in many cases, the aphids were excluded from the bean plants entirely. Multiple natural enemies may interact synergistically, additively or antagonistically, and this interaction will be reflected in the size of the pest population (Roy and Pell, 2000). Although it cannot be assessed statistically, the total aphid mortality when using multiple natural enemies was approximately the sum of using each natural enemy species alone. Therefore, it appears that there was an additive interaction between *P. neoaphidis*, *C. septempunctata* and *A. ervi*. However, it should be noted that this observation was over an 8 day period, which is less than one generation of *A. ervi*. Therefore the effects of negative intraguild interactions that may occur between the aphid natural enemy species, for example, predation of parasitized *A. pisum* by *C. septempunctata*, would not have affected this data set but may have an effect over a longer time course.

These results support previous findings which show that the presence of foraging arthropods increase the abundance of entomopathogenic fungi (Fuentres-Contreras *et al.*, 1998; Furlong and Pell, 1996; Pell *et al.*, 1997; Roy, 1997; Roy *et al.*, 1998). Although the presence of foraging *C. septempunctata* and *A. ervi* were found to increase both the abundance and distribution of *P. neoaphidis*-sporulating cadavers, there were no significant interactions between *P. neoaphidis* and either *C. septempunctata* or *A. ervi* that further reduced the aphid population. Therefore the increased transmission of *P. neoaphidis* in the presence of either *C. septempunctata* or *A. ervi* cannot be regarded as being a synergistic interaction. However, it should be noted that these results were obtained over a single generation of the fungus. Over several generations of the fungus the small increase in *P. neoaphidis* in the presence of *C. septempunctata* and *A. ervi* may result in an epizootic, and this may indicate a synergistic interaction occurring between the natural enemy species. Evidence to support this comes from the results of section 6.3.1.3 (*C. septempunctata* plus *A. ervi* transmission) where it was found that the number of *P. neoaphidis*-sporulating cadavers was significantly greater after 16 days compared to 8 days.

The increased number of sporulating cadavers recovered in the presence of *C. septempunctata* and *A. ervi* may have been as a result of increased transmission or vectoring of conidia. Transmission occurs on the plant on which the host died and sporulation took place. The presence of a foraging coccinellid or parasitoid may increase transmission through either; increasing the distribution of the conidia over the plant (which would result in a larger 'infected area') or, increasing the movement of aphids (which would result in increased contact with conidia). In contrast, vectoring only occurs if conidia are transported by a 'third party' to a second plant. Unlike *C. septempunctata*, which has been shown to vector *P. neoaphidis* from aphid colonies containing *P. neoaphidis*-sporulating cadavers to a second aphid colony, there is no evidence of parasitoids acting as vectors of fungal conidia (Roy, 1997; Roy *et al.*, 2001). As described above, the presence of a foraging parasitoid will increase the likelihood of *A. pisum* dropping from a plant. Once an aphid has dropped from its host plant it may recolonise the same plant or move to a different plant. Movement to an alternative plant will increase the potential of *A. pisum* coming in to contact with *P. neoaphidis* conidia on the central

plant, and this may result in an increased transmission of *P. neoaphidis*. Therefore, it is likely that the increased number of sporulating cadavers recovered in the presence of *A. ervi* is a result of increased transmission. However, as the potential of *A. ervi* to vector *P. neoaphidis* was not assessed, it is not possible to stake categorically whether the increased number of *P. neoaphidis* in the presence of foraging *A. ervi* and *C. septempunctata* was as a result of transmission, vectoring or both mechanisms of dispersal.

The results of section 6.3.1.2 (*A. ervi* transmission) show that the presence of *P. neoaphidis* significantly reduced the number of *A. ervi* mummies recovered. This decrease in *A. ervi* mummies is likely to be a result of *P. neoaphidis* out-competing *A. ervi* for aphid hosts. The outcome of competition between aphid natural enemies will be largely dependent on the dominance of one natural enemy species over another and can be modelled using an interference term (Ψ). If both competitors have an equal share of the co-inhabited host $\Psi = 0.5$, with values less than 0.5 indicating an advantage to the parasitoid and values greater than 0.5 indicating an advantage to the pathogen (Hochberg *et al.*, 1990). The competitive outcome between *P. neoaphidis* and the parasitoid *A. rhopalosiphi* when co-inhabiting the rose-grain aphid *M. dirhodum* was assessed by Powell *et al.* (1986). If fungal infection occurred within four days of parasitisation the fungus out-competed the parasitoid ($\Psi > 0.5$), however, if fungal infection occurred five or more days after parasitisation, the parasitoid out-competed the fungus ($\Psi < 0.5$). An antagonistic interaction therefore occurs between the parasitoid and the fungus where dominance is determined by the relative timing of attacks. If competition is occurring between *A. ervi* and *P. neoaphidis*, it would be expected that the reproductive success of *A. ervi* would decrease as the competitive advantage of *P. neoaphidis* increases. This was assessed using *A. pisum* that were inoculated with *P. neoaphidis* for either 24 or 72 hours prior to the start of the experiment. *Pandora neoaphidis* was, in effect, given a head start. It was found that the number of mummies recovered from treatments containing aphids previously infected with *P. neoaphidis* was significantly less than from the cage containing uninfected aphids, and in some cases *A. ervi* had been competitively excluded. In contrast, the success of *P. neoaphidis* was greater in the treatment containing aphids previously infected for 72 hours compared to 24 hours. Therefore, the greater the competitive advantage of *P.*

neoaphidis the greater the reproductive success. To summarise, in a closed system, intraguild competition was found to occur between *A. ervi* and *P. neoaphidis*, the outcome of which was largely affected by the relative timing of fungal infection/ oviposition.

The presence of multiple species from the same guild may ultimately have a negative effect on *P. neoaphidis*. Although the abundance of *P. neoaphidis* was found to increase in the presence of either *A. ervi* or *C. septempunctata*, this was not found in the presence of both *A. ervi* and *C. septempunctata* together. This result may be due to multiple natural enemies reducing the aphid population to a threshold below which transmission could occur or, due to negative intraguild interactions occurring between *P. neoaphidis*, *A. ervi* and *C. septempunctata*. The results show that fewer sporulating cadavers and *A. ervi* mummies were recovered from the treatment containing *C. septempunctata* plus *P. neoaphidis* plus *A. ervi* compared to the number recovered from treatments containing *P. neoaphidis* and *A. ervi* only. Although the decrease in the population size of *A. ervi* could be as a result of competition with *P. neoaphidis* and vice-versa, it is likely that the decreased *A. ervi* and *P. neoaphidis* populations are as a result of intraguild predation by *C. septempunctata* (see sections 1.3.1.1 and 1.3.1.2). As *C. septempunctata* can have a direct effect on the parasitoid and fungus populations through consumption of infected aphids, but the fungus and parasitoid can have no effect on the coccinellid, asymmetric competition is occurring (Lawton and Hassell, 1981). Although over a short time period the use of multiple species has a greater effect on the aphid population than using each species individually, over several generations asymmetric intraguild predation by *C. septempunctata* may result in competitive exclusion of *A. ervi* and *P. neoaphidis*. Potentially competitive exclusion of *A. ervi* and *P. neoaphidis* by *C. septempunctata* followed by *C. septempunctata* leaving the system may result in a resurgence in the aphid population.

The results described in Chapter 5 indicated that foraging *A. ervi* would enter and forage in aphid colonies containing *P. neoaphidis*. However, these experiments were done at the laboratory scale and lacked any resemblance to field conditions. Facilities such as the 'Ecotron' (at Silwood Park) and the polytunnel (at Rothamsted Research) provide biologically realistic 'bridges' between laboratory scale and field

scale experiments (Lawton, 1996). Using the polytunnel the results described in Chapter 5 and those from the cage transmission experiments were assessed under larger, more realistic conditions and were found to support the results obtained in the laboratory. As there were no significant differences in the number of *A. ervi* mummies recovered from the control patch and the patch containing *P. neoaphidis*-sporulating cadavers, it appears that *A. ervi* would enter and forage in an aphid patch containing *P. neoaphidis*. There were also no significant differences in the number of mummies recovered from pots within a patch containing sporulating cadavers and those pots that did not contain the fungus. Therefore, when given a choice of alternative plants to forage upon, *A. ervi* will forage equally on plants containing *P. neoaphidis* and those not containing the fungus. Section 5.4 discusses at length why *A. ervi* may not avoid entering and foraging in aphid colonies containing the intraguild competitor *P. neoaphidis*.

Unlike the results of section 6.3.1.2 (*A. ervi* transmission) where the presence of *P. neoaphidis* significantly reduced the population size of *A. ervi*, the results of the polytunnel experiment provide no indirect evidence which indicates that *P. neoaphidis* has a negative effect on the population size of *A. ervi*. After 16 days, significantly more *P. neoaphidis*-sporulating cadavers were recovered from the patch initially containing *P. neoaphidis* compared to the number recovered from the control patch. If *P. neoaphidis* does have a negative effect on *A. ervi*, fewer mummies would have been expected to be recovered from the patch initially containing the fungus, however, this was not found. The different conclusions drawn from section 6.3.1.2 (*A. ervi* transmission) and the 'polytunnel experiment' may be due to differences in abiotic conditions affecting the interactions that occur between *A. ervi* and *P. neoaphidis*. For example, as stated previously the abiotic conditions used during the cage experiments were optimal for fungal infection whereas in the polytunnel both the temperature and humidity fluctuated with the diurnal cycle (Day≈ 34°C, 20% R.H.; Night≈ 8°C, 90% R.H.). Survival of *P. neoaphidis* conidia is affected by humidity, and a high relative humidity is required for conidia to germinate (Brobyn, Wilding and Clark, 1987) (see section 1.1.2.1). Whereas in the cage experiments germination could occur throughout the day, in the polytunnel the abiotic conditions were suitable for germination for approximately six hours in the evening. This may have resulted in a decreased

number of conidia germinating and therefore a reduction in the number of aphids becoming infected with *P. neoaphidis*. Once germination had taken place, the development time of the fungus may have also been affected by temperature fluctuations within the polytunnel. The time taken by *P. neoaphidis* to successfully infect a host has been shown to increase as temperature decreases (Wilding, 1970) (see section 1.1.2.1). The abiotic conditions experienced by *P. neoaphidis* within the polytunnel may therefore have a negative effect on the fitness of the fungus and this may have reduced its competitive advantage over *A. ervi*.

Unlike the 'cage transmission experiment' the plant pots used in the polytunnel experiment were placed in water traps to prevent the movement of aphids between patches and pots within patches. Movement of *P. neoaphidis* conidia from pots initially containing sporulating cadavers to fungus-uninfested pots was therefore dependent on either movement of conidia on air currents or vectoring by *A. ervi*. The fans in the polytunnel drew air unidirectionally over the patches. Conidia that had been ejected from sporulating cadavers may therefore have been transported in the air-flow and may have been deposited amongst plants within the same patch. Contrary to what was expected, after both 4 and 16 days *P. neoaphidis*-sporulating cadavers were recovered from both patches. There are two explanation of how this may have occurred. Potentially the air flow in the polytunnel may have contained turbulence resulting in a multidirectional air-flow and the movement of conidia between patches. Alternatively, the conidia may have been vectored by foraging *A. ervi*. As stated earlier there is no published evidence of parasitoids acting as vectors of entomopathogenic fungus. However, as both these results and those from the '*A. ervi*' cage transmission experiment indicate that *A. ervi* may act as a vector of *P. neoaphidis*, small scale laboratory experiments similar to those used to show the ability of *C. septempunctata* to vector *P. neoaphidis* conidia are now needed to further assess whether or not *A. ervi* can act as a vector of *P. neoaphidis* (Roy, 1997; Roy *et al.*, 2001).

CHAPTER 7: GENERAL DISCUSSION

7.1 IS *PANDORA NEOAPHIDIS* A PLANT BODYGUARD?

At present there is little evidence to suggest that entomopathogenic fungi use plant derived volatiles as synomones. The results presented in this study indicate a direct effect of plant damage by aphids on two factors that may influence the reproductive success of *P. neoaphidis*, the size of conidia and the proportion of conidia germinating (Chapter 3). The increase in conidial size and proportion of conidia germinating may have been a result of either aphid-induced plant volatiles or changes in the abiotic microclimate of the plant. If the increase in conidial size or the proportion of germinating conidia was a direct response of the fungus to the aphid-induced volatiles, and had resulted in an increase in the proportion of aphids becoming successfully infected with the fungus, the aphid-induced plant volatiles would be acting as synomones. However, the infectivity of *P. neoaphidis* towards aphids feeding on previously damaged plants was not significantly different to that of aphids feeding on undamaged plants (Chapter 4). This indicates that aphid-induced bean plant volatiles did not act as synomones.

The experiments described here were carried out using a single plant-aphid system under abiotic conditions that were optimal for the fungus. Under sub-optimal conditions, the direct effects observed may have resulted in an increase in the reproductive success of the fungus. Alternatively, volatiles released from different plant-aphid complexes may have a direct positive effect on the reproductive success of *P. neoaphidis*. The aphid-plant system used in these experiments was selected as there is both chemical and behavioural evidence that suggests *A. pisum*-infested bean plants release volatiles that attract *A. ervi* and, therefore, act as synomones (see section 1.3.2.2). However, a variety of Brassica and cereal plants that are infested with aphids have been shown to release volatiles that elicit a greater response from foraging parasitoids than those released from undamaged plants (personal communication with Prof. W. Powell, Rothamsted Research; Reed *et al.*, 1995). Further experiments using alternative plant-aphid systems under conditions that are both optimal and sub-optimal for the development of the fungus are

required to assess comprehensively whether the development of *P. neoaphidis* is manipulated by plants infested with host-aphids and, therefore, enhance its potential as a bodyguard.

7.2 TRANSMISSION OF *PANDORA NEOAPHIDIS*

Transmission of *P. neoaphidis* to foraging *A. pisum* increased with increased aphid disturbance. For example, transmission was greater during plant colonisation than during *in situ* feeding and in the presence of foraging predators and parasitoids (Chapters 4 & 6). This is likely to be a result of an increased encounter rate between the aphid and the fungus. Therefore, factors that either directly or indirectly affect the movement of an aphid are likely to result in increased transmission. Although plant condition did not have a direct effect on the transmission of *P. neoaphidis*, it had a direct effect on foraging behaviour of *A. ervi* and may, therefore, have an indirect effect on the transmission of *P. neoaphidis* (Chapters 4 & 5). For example, *A. ervi* elicits a greater response to bean plants previously infested with *A. pisum* compared to previously uninfested plants and will spend longer searching for hosts on previously damaged plants (Chapter 5). An increased search time could result in greater aphid disturbance and, in turn, an increased encounter rate between *A. pisum* and *P. neoaphidis*. However, disturbance of *A. pisum* increases the aphid's mortality risk and this may result in a decrease in transmission (Villagra *et al.*, 2002). Further experiments are required to assess the net effect of volatiles from damaged plants on the transmission of *P. neoaphidis*.

7.3 RESPONSE OF ARTHROPODS TO *PANDORA NEOAPHIDIS*

Insect herbivores are under selective pressure to conceal their presence from natural enemies, which are themselves under selective pressure to detect their prey. This may result in a co-evolutionary arms. As *P. neoaphidis* is both an aphid natural enemy and a sub-optimal prey item for foraging coccinellids, it may be under opposing selective pressures; to conceal its presence (from herbivores) and to disclose its presence (to foraging aphidophagous predators). *Acyrthosiphon pisum*, *A. ervi* and *C. septempunctata* all entered and foraged on bean plants containing *P.*

neoaphidis-sporulating cadavers, indicating they either could not detect the presence of the fungus or that the fungus did not affect their behaviour (Chapters 4 & 5). The results of the air entrainment experiment suggested that *P. neoaphidis*-infected *A. pisum* did not release fungus-specific volatiles, and indicated that the presence of *P. neoaphidis* may have been undetected by foraging arthropods. *Aphidius ervi* has been shown to use volatile cues to avoid aphid colonies containing intraguild predators. For example, cues released from foraging adult and larval *C. septempunctata* are used by *A. ervi* to determine the presence of the predator, which it then avoids (Nakashima and Senoo, 2003). If *P. neoaphidis* releases cues that disclose its presence, *A. ervi* would have been expected to avoid aphid colonies containing the fungus. *Pandora neoaphidis* therefore appears to have evolved to conceal its presence, and for this to occur, the fitness gain received through not repelling foraging aphids should out-weigh the potential fitness cost of intraguild predation.

7.4 USE OF *PANDORA NEOAPHIDIS* AS A MULTI-SPECIES BIOCONTROL AGENT

If *P. neoaphidis* is to be used as part of a multi-species biological control programme against aphids, detailed knowledge of its interactions with the other guild members is required. Although previous studies have assessed the competitive outcome of these intraguild interactions at a small spatial scale, they have not assessed the effects on the population size of both the pest and natural enemy species (Roy *et al.*, 1998, Roy and Pell, 2000; Pell *et al.*, 1997; Powell *et al.*, 1986). The results presented here show that *C. septempunctata* and *A. ervi* would enter and forage in aphid colonies containing *P. neoaphidis* (Chapter 5). Once entered, *C. septempunctata* increased the transmission of *P. neoaphidis* and, although intraguild predation may have taken place, the net effect of the interaction was an increase in the population size of the fungus (Chapter 6). Although this interaction did not further reduce the aphid population, over a longer time period, the two natural enemies may interact synergistically and a significant reduction in the aphid population may be found. As with *C. septempunctata*, the presence of *A. ervi* increased the transmission of *P. neoaphidis*. However, the presence of the fungus had a negative effect on the population size of the parasitoid (Chapter 6).

Although the negative intraguild interaction between the parasitoid and the fungus did not affect their ability to control the aphid population, over several generations of the parasitoid, competition with *P. neoaphidis* may result in competitive exclusion of the parasitoid. However, the results of the polytunnel experiment indicate that *P. neoaphidis* does not have an effect on the population size of *A. ervi*. The interaction between *P. neoaphidis* and *A. ervi* therefore requires further study.

Whilst intraguild interactions may have a negative effect on one or more of the guild members, their impact on the herbivore population may still be greater than using a single species. For example, the cotton aphid, *A. gossypii*, is preyed upon by the coccinellid *H. convergens* and is parasitised by *L. testaceipes* (Colfer and Rosenheim, 2001). Although *H. convergens* predated over 98% of *A. gossypii* parasitised by *L. testaceipes*, the greatest suppression of aphid populations came from using both the parasitoid and the predator together (Colfer and Rosenheim, 2001). However, in a recent review of the success rates of biological control programmes using single and multiple species, establishment of control was greatest when a single control agent was used (Denoth, Frid and Myers, 2002). It was suggested that this may have been due to negative interactions occurring between control agents. In over 50% of cases where multiple species had been successful at controlling the pest, a single control agent was responsible for the majority of control. Using multiple control species is thought, in many cases, to increase the chances of selecting a single correct control species (Denoth *et al.*, 2002). The success of a control species is dependent on both biotic and abiotic factors. Climatic conditions can regulate the efficiency of introduced species and, therefore, application of more than one species will also increase the probability of selecting a control species suitable for the abiotic conditions throughout a field season. Although these results indicate that *P. neoaphidis* and *C. septempunctata* appear to be compatible as multi-species biological control agents against aphids (whereas *A. ervi* and *P. neoaphidis* do not appear as compatible species), further work is required to assess the outcome of intraguild interactions between *P. neoaphidis* and *C. septempunctata* over several generations of the pest species, and to confirm that both control agents have a significant effect on the reduction of the pest species.

7.5 EXPLOITATION OF *PANDORA NEOAPHIDIS*-ARTHROPOD INTERACTIONS

As *C. septempunctata* is not repelled by *P. neoaphidis* and acts as a vector of the fungus, it would be suitable for use as part of an assisted-autodissemination scheme (Chapter 5 & Roy, 1997). Autodissemination is the "dispersal of a pathogen to members of its own population" (Vega *et al.*, 2000). Assisted-autodissemination involves the use of auto-inoculators, such as pheromone traps, to promote dispersal (Vega *et al.*, 2000). There have been several successful studies using auto-inoculators containing pheromone lures for the autodissemination of entomopathogenic fungi (Dowd and Vega, 2003; Furlong *et al.*, 1995; Hartfield *et al.*, 2001; Tsutsumi *et al.*, 2003). For example, the diamond back moth *P. xylostella* was attracted to traps containing both live females and synthetic sex pheromone, where it subsequently became inoculated with *Z. radicans* (Furlong *et al.*, 1995). After habituation to the pheromone the moth left the trap and became a vector of the fungus. The damson-hop aphid *Phorodon humuli* (Schrank) is attracted to traps containing *P. humuli* sex pheromone ((1R,S,4Ar, 7s, 7As)-nepetalactol). Once the trap was entered, the aphids were inoculated with *L. longisporum* (=Vertalec) (Hartfield *et al.*, 2001). These aphids then became infected with the fungus and contained enough inoculum to initiate colonies of the fungus on sterile agar plates. This indicated the potential for transmission to previously un-infected aphid colonies. However, there is less evidence of assisted-autodissemination via attraction and dissemination by a non-target arthropod, i.e. a vector. Honeybees, *Apis mellifera* (L.), that are inoculated with the entomopathogenic fungus *M. anisopliae* as they leave the hive, have been shown to vector the fungus to winter and spring rape, *B. napus*, resulting in a significant increase in the percentage of pollen beetles, *Meligethes aeneus* (Fabricius) becoming infected with the fungus (Butt *et al.*, 1998). However, for *C. septempunctata* to be a vector in the assisted-autodissemination of *P. neoaphidis*, an odour source that is highly attractive to foraging *C. septempunctata* is required to lure the coccinellids into a dissemination chamber. At present there are no known volatile cues that would fulfil this role and, as described in section 1.3.2.2, there is no conclusive evidence of whether or not *C. septempunctata* uses volatile cues as attractants.

Studying the outcome of *P. neoaphidis* introductions into field populations is hindered by the ubiquity of the fungus. *Pandora neoaphidis* is a common fungal pathogen of aphids in temperate regions, including the United Kingdom (Glare and Milner, 1991). Therefore, when sampling field populations of aphids for the presence of introduced isolates of *P. neoaphidis*, infections as a result of the introduced isolate need to be differentiated from infections as a result of naturally occurring isolates. Both biochemical and molecular techniques can be used to trace isolates of entomopathogenic fungi. Dowd and Vega (2003) used an assisted-autodissemination device to attract and inoculate the dusky sap beetle, *Carpophilus lugubris* (Murray) with *B. bassiana*, which was subsequently vectored to over-wintering populations of the beetle. Recovered isolates of *B. bassiana* were then compared with the released strain using isoelectric focusing, and the movement of the introduced isolate traced. As isoelectric focusing requires a large quantity of high quality fungus, samples recovered from the field may not always be in a condition suitable for analysis (Loxdale, H. personal communication). Molecular techniques such as PCR are under development as robust and reliable tools to differentiate between different species and isolates of entomopathogenic fungi (Coates, Hellmich and Lewis, 2002; Enkerli *et al.*, 2001; Rehner and Buckley, 2003). It is now possible to detect *P. neoaphidis* infection within aphids using PCR primers that are *P. neoaphidis* specific (Tymon, Shah and Pell, 2004). Although isolates of *P. neoaphidis* can be split into clusters, at present it is not possible to distinguish between individual isolates and to reliably track individual isolates of *P. neoaphidis* (Pell, J. K. personal communication). Therefore, the development of isolate specific PCR primers is required before experiments can be done to assess the interactions between *P. neoaphidis*, *C. septempunctata* and *A. ervi* at the field scale.

7.6 SUMMARY

Summary of experimental work done to address the objectives listed in section 1.4:

1. Although statistically significant functional interactions occur between *P. neoaphidis* and the host plant (increased conidial size and proportion of

germinating conidia), these results do not appear to be biologically significant.

2. *Pandora neoaphidis* had no effect on the attractiveness of bean plants to *Acyrthosiphon pisum*. Transmission occurred between *P. neoaphidis* and *A. pisum*; this was however, not affected by plant condition. Transmission was greater during plant colonisation than during *in situ* feeding indicating a positive association between aphid movement and fungal transmission.

3. The presence of *P. neoaphidis* had no effect on the entry rate of either *C. septempunctata* or *A. ervi* into *A. pisum* colonies nor did it have an effect on their foraging behaviour once the colony had been entered. *Aphidius ervi* attempted to oviposit in *P. neoaphidis*-infected aphids suggesting that it could not detect the presence of the fungus until sporulation had occurred.

4. *Coccinella septempunctata* and *Aphidius ervi* increased both the abundance and distribution of *P. neoaphidis* within an aphid colony. Although the increase in transmission had no apparent direct or indirect effects on *C. septempunctata*, increased transmission of *P. neoaphidis* was associated with a decrease in the population size of *A. ervi*. Movement of *P. neoaphidis* between patches within the polytunnel suggests that *A. ervi* may be able to vector *P. neoaphidis* between aphid colonies.

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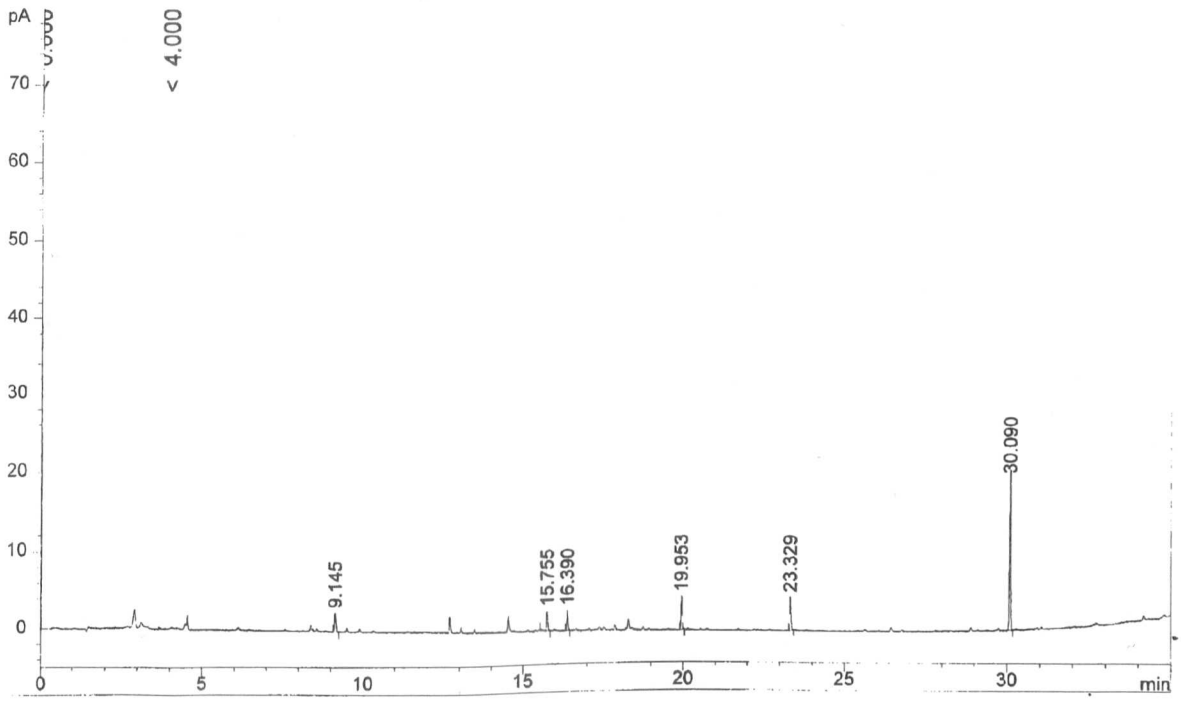
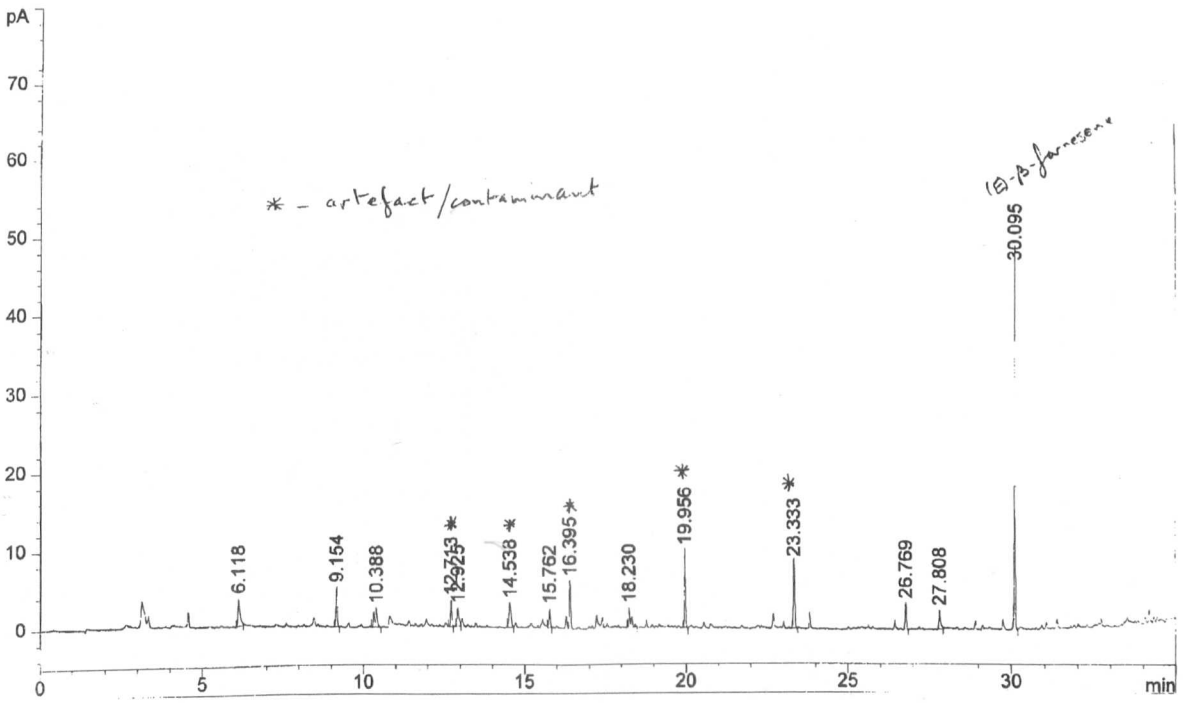
A1. Gas Chromatography traces

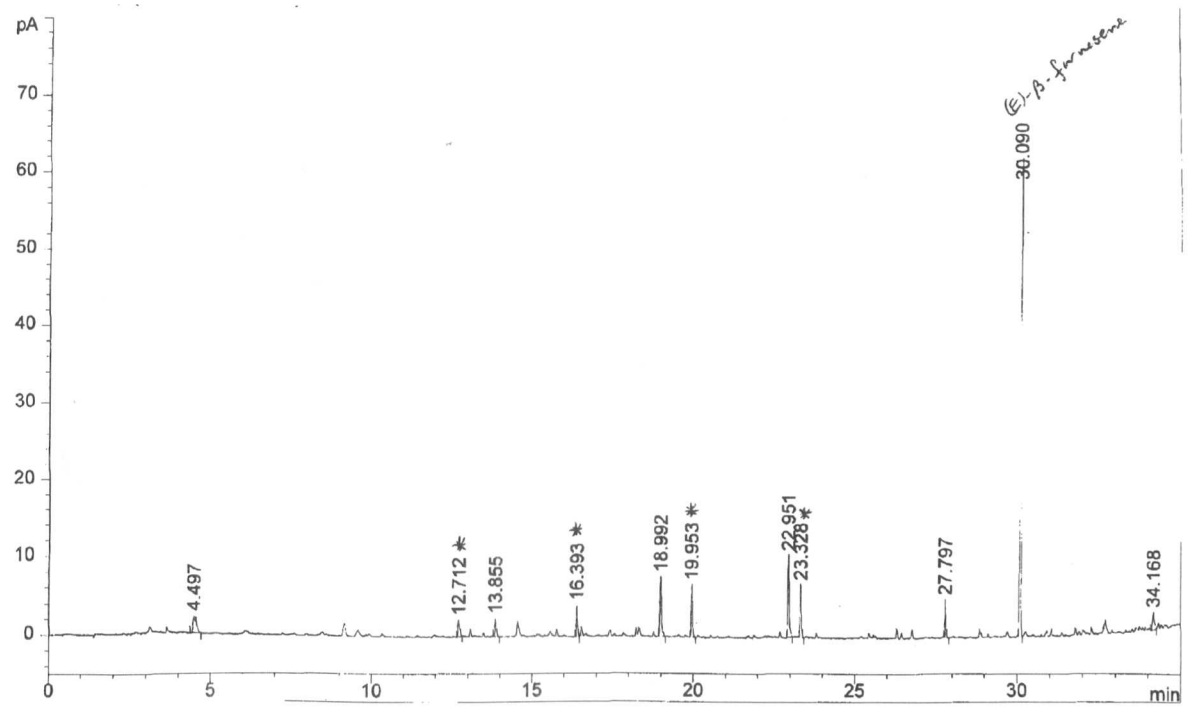
a) Uninfected aphids



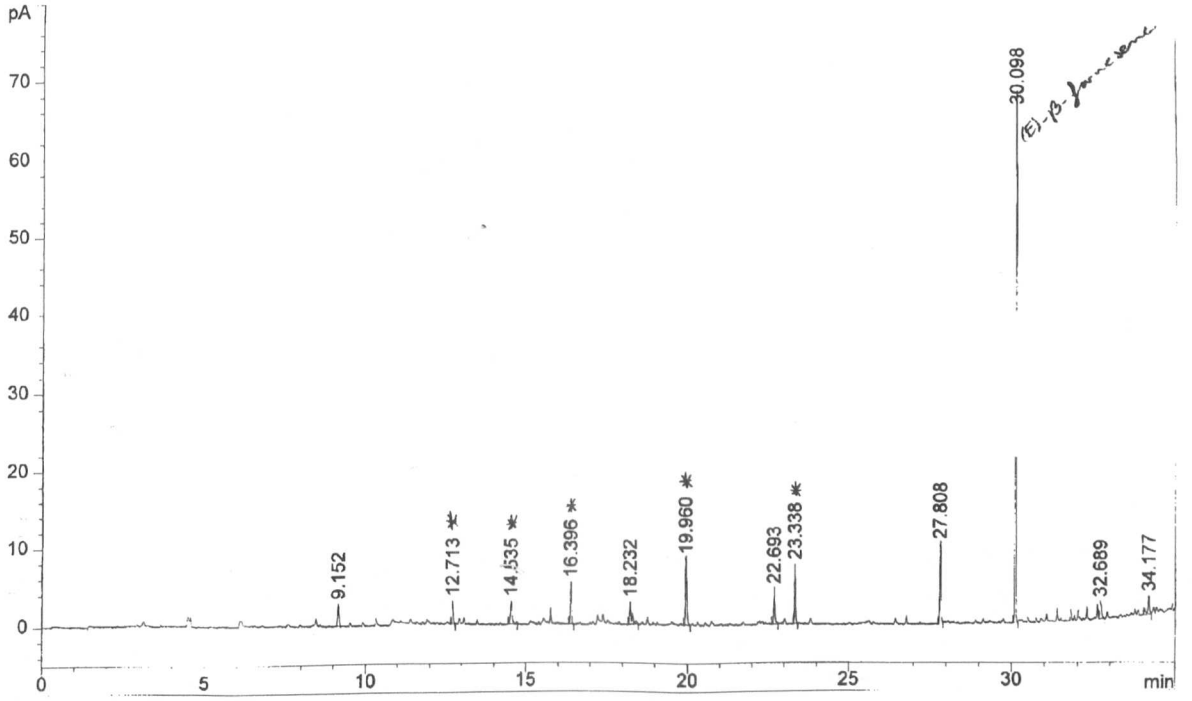
b) Aphids inoculated with *P. neoaphidis* for 24 hours



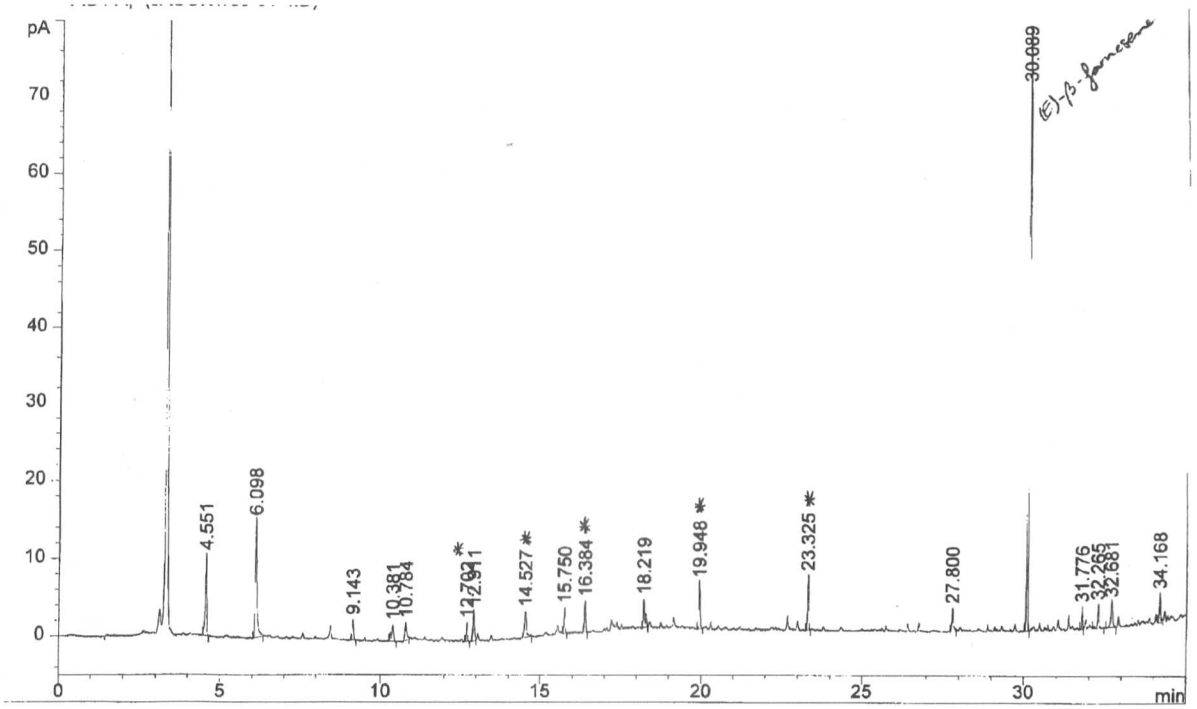
c) Aphids inoculated with *P. neoaphidis* for 48 hours



d) Aphids inoculated with *P. neoaphidis* for 72 hours



e) Aphids inoculated with *P. neoaphidis* for 96 hours



f) *Pandora neoaphidis*-sporulating cadavers