Relative toxicity of insecticides to crucifer pests and their natural enemies: interaction of insecticide and insect behaviours

Farida Anjum

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Department of Life Sciences

Silwood Park Campus

Imperial College London

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Dedication

Dedicated

To

My Parents, Husband, Teachers

Kids

Brothers and Sisters

Declaration

The work presented in this thesis is entirely my own and has not been submitted anywhere else.

Signed.....Farida Anjum

Certified.....Professor Denis J. Wright
(Supervisor)

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Abstract

Pesticides remain a necessary component of many agricultural systems and used judiciously they can play an important role in Integrated Pest Management (IPM) programmes. The aim of the present study was to investigate factors influencing the differential toxicity of insecticides against a cosmopolitan insect pest of crucifer crops, the diamondback moth, *Plutella xylostella*, and its respective hymenopteran parasitoid, *Cotesia vestalis.* Such knowledge can help in the effective use of insecticides with biological control agents in IPM. Three insecticides regarded as being compatible with some natural enemies (abamectin, spinosad, indoxacarb) and a compound generally regarded as harmful to natural enemies (lambda-cyhalothrin) were examined. Similar tests were also carried out with the peach potato aphid *Myzus persicae* and its parasitoid *Aphidius colemani* due to the loss of the Cotesia vestalis culture. A comparative measure of the intrinsic toxicity of fresh deposits (Day 0) of insecticides on Chinese cabbage was determined for both pest and parasitoid species. Lambda-cyhalothrin and abamectin were the most toxic compounds against both pests and their parasitoids, while indoxacarb and spinosad were less toxic. Residual bioassays were conducted using sprayed plants maintained under glasshouse conditions for 0-28 days after insecticide application. Results indicated lambda-cyhalothrin was the most persistent compound and abamectin and spinosad the least persistent. A leaf wax stripping technique was used with bioassays to compare the distribution of insecticide residues between the epicuticular wax layer and underlying leaf tissues. Wax removal significantly reduced the toxicity of all insecticides. No-choice and choice behavioural assays were conducted for both parasitoid species with leaf discs treated with LC_5 and LC_{50} levels of insecticides. Both parasitoids tended to avoid insecticide-treated leaves, giving preference to untreated leaves or the arena. Emergence of adult parasitoids from cocoons/mummies on insecticide-treated leaves was not significantly different from untreated controls. The results are discussed in terms of the bioavailability of insecticides to phytophagous and non-phytophagous insect species.

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CHAPTER 1: Introduction

1.1 Background to present study

Pesticides are used in agricultural cropping systems primarily to regulate arthropods, weeds and fungi (Cloyd, 2012). The widespread and intensive use of conventional pesticides, particularly insecticides, presents major toxicity risks to the target pest's natural enemies as well as to the environment in general (Johnson and Tabashnik, 1991). Overuse of pesticides can result in many ecological problems including, resistance, secondary pest outbreaks and pest resurgence due to a reduction in natural enemies (DeBach, 1974; Cloyd, 2012). Systemic insecticides, which are usually applied in the early stages of plant growth, are often persistent within plants and can also have a harmful effect on some other beneficial insects, notably pollinators (Johnson et al., 2009). The detrimental impact of pesticides on beneficial arthropods, including natural enemies, has been of great concern for many years and has been the subject of a large number of studies (see Croft, 1990; Haynes, 1998; Thompson, 2003). In Europe, North America and many other parts of the world, the testing of a novel pesticide for potential harmful effects against beneficial organisms is now a compulsory step for registration (WHO, 2013).

In Integrated Pest Management (IPM) emphasis is given to the contribution of indigenous natural enemies to control pest population density (Hajek, 2004), with pesticides used as a last resort according to economic thresholds (Europeon Commission, 2014). The real challenge for IPM is to minimise the use of pesticides, particularly insecticides by maximising the role of natural control measures and cultural practices. This can sometimes be

achieved through conservation measures but may also require the release of indigenous or non-indigenous natural enemies (Johnson and Tabashnik, 1999; Furlong and Zalucki, 2010).

Maximising the efficacy of pesticides as part of IPM programmes requires a thorough understanding of how such compounds interact with natural enemies. This requires assessing the effects of pesticides on the behaviour, physiology and population dynamics of natural enemies, at all stages of their life cycle (Desneux et al., 2006).

High value cash crops for industrial use (e.g. cotton) or human consumption (e.g. vegetables) have been areas where problems associated with overuse of pesticides have been particularly manifest (Zhao et al., 2002). One such example is control of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) on *brassica* crops (Sayyed et al., 2004; Badii et al., 2013; Furlong et al., 2013).

Brassica and other crucifer crops have long been used for their medicinal values as well as for a food source (Amoako et al., 2012), possessing, for example, anti-inflammatory and anti-cancer activity (Allen and Allen, 2009) and are important crops worldwide.

Plutella xylostella is the most important pest of *brassica* and other crucifer crops in many regions of the world, particularly in the tropics and sub-tropics, and has become particularly difficult to manage because of its ability to develop resistance rapidly to pesticides (Li et al*.,* 2007; Santos et al., 2011; Furlong et al., 2013). This ability is in part due to its relatively short life cycle (c. 14-28 days depending on temperature), high fecundity and to its being a *brassica* specialist (Talekar and Shelton, 1993; Furlong et al., 2013). *Plutella*

xylostella has the ability to migrate between and reproduce in different climatic zones and with the cultivation of crucifer crops worldwide has become cosmopolitan in its distribution (Chapman et al., 2002).

Overuse of insecticides against *P. xylostella* has resulted in damaging effects on its natural enemies, including *Cotesia vestalis* (Haseeb and Amano, 2002). *Cotesia vestalis* Kurdjumov (syn. *C. plutellae*) (Hymenoptera: Braconidae) is a key solitary larval endoparasitoid of *P. xylostella* (Haseeb et al., 2004) and parasitizes all four larval instars of *P. xylostella*, preferring the second and third instars (Shi et al., 2001). It has been observed that some insecticides (e.g. the pyrethroid, fenvalerate) are much more active against adults of *C. vestalis* compared with their effects on larvae of *P. xylostella* (Yuanxi et al., 2002). Contact toxicity of some insecticides can be relatively low to the cocoon stage of *C. vestalis* but moderately to extremely toxic to the adult stage (Haseeb and Amano, 2002).

The peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) is a cosmopolitan and major pest of many crops with over 400 known plant hosts, including *brassicas* (Kim et al., 2005). It damages the plant directly by feeding or indirectly as a virus vector (Askarianzadeh et al., 2013). As in the case of *P. xylostella*, overuse of insecticides against *M. persicae* can cause destruction of beneficial insects (Haskell and McEwen, 1998). Parasitoids, mainly Aphididae, are key natural enemies of *M. persicae* (Blumel, 2004).

Aphidius colemani (Hymenoptera: Braconidae) is an oligophagus endoparasitoid of *M. persicae* and about 40 other aphid species (Tomanovic et al., 2012), which originated in South Asia but now is widely distributed

(Starý, 1975; Kavallieratos et al., 2010; Tomanovic et al., 2012). *Aphidius colemani* is commercially available in Europe, including the UK (Goh et al., 2001; Boivin et al., 2012).

1.2 Insecticides

Currently, many insecticides with a relatively narrow spectrum of activity are marketed against specific groups of insect pests (Koul and Cuperus, 2007). These compounds, the so-called 'selective insecticides', can be far less harmful to beneficial insects, including natural enemies (Furlong et al., 2013). Such selective insecticides that are used widely against *P. xylostella* include indoxacarb, methoxyfenozide, emamectin benzoate (Cardwell et al., 2005), abamectin (Shi et al., 2004) lambda-cyhalothrin, *Bacillus thuringiensis* and spinosad (Haseeb et al., 2004; Biondi et al., 2013). Selective insecticides intensively used to control *M. persicae* include, pirimicarb, carbaryl, methamedophos (Scott and Zane, 1980), clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, thiamethoxam (Tomizawa and Casida, 2005), lambda-cyhalothrin (Desneux et al., 2003) and a combination of *δ*-endotoxin from *Bacillus thuringiensis* with abamectin, a new type of biocide has also been reported to be effective against *M. persicae* (Liu et al., 2003). With the availability of a large number of commercial insecticides, the present study was undertaken to compare toxicity of lambda-cyhalothrin (generally regarded as toxic to natural enemies) with abamectin, indoxacarb and spinosad (selective against natural enemies).

1.3 Aims and objectives of present study

The present study aims to investigate the factors influencing the differential toxicity of a range of insecticides against *P. xylostella*, *M. persicae and* their respective parasitoids, *C. vestalis* and *A. colemani.* Such knowledge can help inform the effective use of insecticides with biological control in IPM systems.

Two crucifer – insect – parasitoid systems were chosen for the present study for a number of reasons: 1) they are convenient laboratory systems that can also be studied easily under field conditions; 2) crucifers are valuable crops worldwide that are often subjected to excessive chemical pesticide treatments; 3) crucifers have dominant cosmopolitan insect pest species, *P. xylostella* and *M. persicae* which have well-studied natural enemies.

The objectives of the present study are:

1. To obtain a comparative measure of the intrinsic toxicity of fresh deposits (Day 0) of various insecticides on Chinese cabbage leaf discs against parasitoids and pests under optimal exposure conditions for the pesticide.
2. To use the above assay system to determine how the toxicity of insecticide residues on sprayed plants changes with age of pesticide deposit against both parasitoid (contact activity) and pests (contact and stomach activity).
3. To investigate the hypoyhesis that surface wax on plant leaves is the major barrier for the transport of chemicals, and greatly influences the uptake and availability of insecticide that has been applied to leaf surfaces by the insects. From the data (from 2 above), determine the impact of

stripping the leaf wax from leaf surfaces on the mortality of the parasitoid and pest insect.

4. To test the hypothesis that currently used insecticides are harmful both directly and indirectly to beneficial insects and the implications for possible management of pest populations of cabbage crops, the sub-lethal effect of insecticide residues on *Cotesia vestalis* and *Aphidius colemani*, the parasitoids of *Plutella xylostella* and *Myzus persicae* respectively was investigated.

CHAPTER 2: Literature Review

2.1 Overview

With increasing human population, demand for food crops has also increased (Douglas, 2009). Chemical pesticides continue to play an important role in increasing crop production (Bennett et al., 2013), and most farmers throughout the world use them. At the same time, over-reliance on chemical pesticides with their associated risks (below) has led to the development of pest and disease management practices that do not rely on pesticides alone. According to the International Code of Conduct on Pesticide Management (WHO, 2013): "*Pesticides play an important role in plant protection but in many cases, overuse and other inappropriate use has actually exacerbated pest problems through destructive effect on natural control mechanisms and development of pesticide resistance*". Risks related to pesticides include toxicological effects, particularly to growers, crop residue problems, environmental impact, such as killing of natural enemies and other non-target organisms, and the development of resistance in pest populations (Matthews et al., 2014).

One of the most valuable groups of food crops worldwide are members of the Brassicaceae, particularly the genus *Brassica*. Cabbages and other *brassicas* have long been used for medicinal as well as food purposes (Amoako, 2012). One of the constraints to production of *Brassica* crops is heavy infestation by insects, including *P. xylostella*, the cabbagehead caterpillar, *Crocidolomia binotalis* L., flea beetles, *Phyllotreta* spp., the

common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae) and various aphid species (Buchholz and Nauen, 2002).

The most important cosmopolitan insect pests of *Brassica* crops are *P. xylostella* (Sarfraz and Keddie, 2005; Furlong et al., 2013; Liu et al., 2014) and aphids, in particular the cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae) and the generalist aphid species, *M. persicae* (Baker, 1994; Cole, 1997). To control these pests insecticides are often still required where brassicas are grown as cash crops. Frequent applications over a prolonged period have resulted not only in the development of resistance in *P. xylostella* and *M. persicae* but also destruction of their natural enemies, including parasitoids (Van Toor et al., 2009; Furlong et al., 2013; Liu et al., 2014).

Many species of hymenopteran parasitoids have been reported to attack *P. xylostella* (Furlong et al., 2013), and among these larval parasitoids are regarded as the most effective as biological control agents (Bopape et al., 2014). Many hymenopteran parasitoids of aphids have been identified; almost all are endoparasitoid belonging to the Aphidiinae (Foster et al., 2007), a group with 60 genera and sub genera and over 400 described species (Stary, 1975).

2.2 Pesticides, pests and risk

A pesticide is any agent that can be used to prevent, mitigate or to destroy pests (US EPA, 2013). Pesticides can include both chemical pesticides and microbial agents (ACP, 2012). An estimated loss of 14% in crop yield is estimated to be caused by insect pests annually, compared with

a 13% loss due to plant pathogens, and a 13% loss due to weeds (Pimentel 2009). The global population is expected to rise further by 2050 (Figure 2.1; UN DESA, 2004), increasing world food demand (FAO, 2002). This may result in a corresponding increase in the use of pesticides (Figure 2.2; Matthews et al., 2014) (Tilman et al., 2001), although with more emphasis on IPM, the use of pesticides should be considered as a last resort.

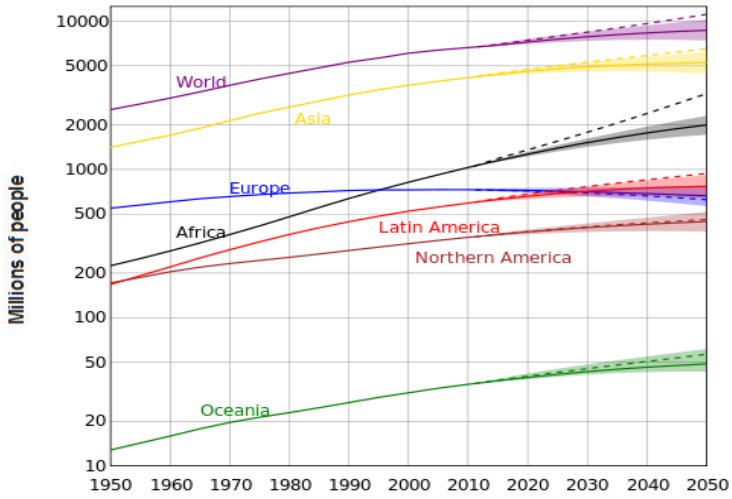


Figure 2.1 World and different continents estimated population levels between 1950 and 2050.
(http://en.wikipedia.org/wiki/Projections_of_population_growth).

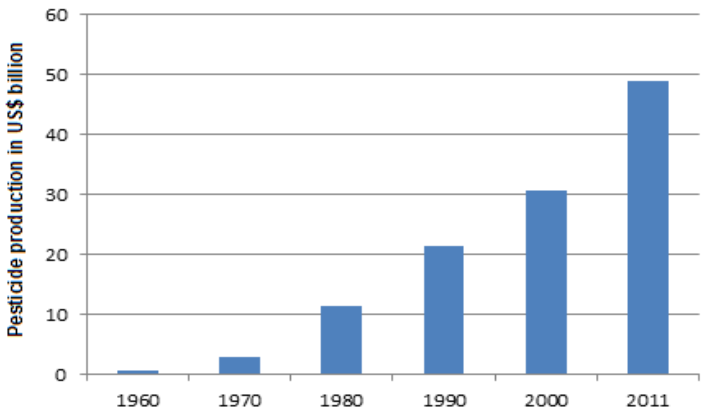


Figure 2.2 Global increase in pesticide use in $ billion.

Pesticide usage has increased in many tropical countries, where there has been less control of their use in a hot climate where farmers often do not have protective clothing. Under these conditions, there have been more people adversely affected by exposure to the more toxic pesticides (WHO, 2013). One major problem is that the amount of applied pesticides reaching the targeted pest is usually low compared with the amount contaminating the environment; leading, for example, to side effects on non-target organisms, including beneficial insects. If pesticides are not applied accurately, there can also be unacceptable levels of pesticide residues in crop produce (Matthews, 2006). Residues of persistent organic pesticides, e.g. DDT accumulate in food webs and ultimately cause negative impacts in both terrestrial and aquatic ecosystems (Hamilton and Crossley, 2004).

2.3 *Brassica* vegetables

Vegetable crops cover 1.1% of agriculture land worldwide (FAOSTAT, 2011) and can be particularly valuable sources of income and nutrition. Members of the *brassica* group of vegetables (Brassicaceae) are cultivated throughout the world. China tops the world rankings for production of cabbages and other *brassicas*, with a total of 31,750,000 Metric Tonnes in 2011 (FAOSTAT, 2011).

Brassica crops such as cabbage, kale, broccoli, cauliflower and bok choy are good sources of vitamins and minerals, and serve as cash crops in many part of the world, especially among lower income groups (Manyangarirwa et al., 2006). Other benefits linked with consumption of brassicas include a reported significant inverse relationship between *Brassica*

vegetable intake and lung cancer risk among smokers and between consumption of *brassicas* and prostate cancer (Tang et al., 2010). Brassicas have also been reported to have beneficial effects on the thyroid gland (Fowke et al., 2001).

2.4 Pests of *brassica* vegetables

Brassica crops are attacked by a variety of insect pests (Amoako, 2012*),* including various **Hemiptera** species, e.g. *Bagrada hilaris* (Hemiptera: Pentatomidae) native to Africa, India, Pakistan and other Asian countries and now been introduced to various western countries including south-western deserts of the United States of America (Reed et al., 2013), *Brevicoryne brassicae* (Hemiptera: Aphididae) originated in Europe but is worldwide distributed and has been mainly reported in Canada, The Netherlands, South Africa, India and China (Gill et al., 2013)*, Myzus persicae* (Hemiptera: Aphididae) originated in Asia and is worldwide distributed (Zhang et al., 2008) briefly discusses in section 2.9, **Diptera** e.g. agromyzid leafminer species found throughout the world including Europeon countries such as Germany, Belgium and also been recorded in the United States of America (Spencer, 1973), and **Lepidoptera** species, e.g. *Pieris brassicae* (Lepidoptera: Pieridae) available throughout Europe, north Africa, Asia and migrated to the Great Britain from the continents , with a chance to spread throughout Australia and New Zealand (Moore, 2010), *Hellula undalis* (Lepidoptera: Crambidae) is a major pest in the lowland Malaysia and is distributed in the tropical region including their presence in temperate regions where *brassica* crops are grown (Sivapragasa and Chua, 1997), *Crocidolomia binotalis* (Lepidoptera:

Crambidae) is distributed in highland tropics of the world, principally in Australia, India, Indonesia and Thailand (Othman, 1982), *Trichoplusia ni* (Lepidoptera: Noctuidae) found in the southern Palaearctic ecozone, parts of Africa, Europe and all over North America, *Spodoptera litura* (Lepidoptera: Noctuidae) abundant in the tropical and temperate Asia, Australia and the Pacific Islands (Feakin 1973) *Pieris rapae* (Lepidoptera: Pieridae) widely distributed across Europe, Asia, and North Africa (Scudder, 1987) and *P. xylostella* distributed in tropics and sub-tropics (Wei et al., 2013) brief detail is given below in section 2.5.

The high market value of many Brassica crops worldwide has led to an excessive application of pesticide sprays, particularly against *P. xylostella*, leading to problems of resistance, pest resurgence and toxic residues (Talekar and Shelton, 1993; Verkerk and Wright, 1997; Gelernter and Lomer, 2000; Furlong et al., 2013; Liu et al., 2014).

2.5 Plutella xylostella

Plutella xylostella was recorded for the first time in 1746 (Harcourt, 1962). It is a highly destructive pest of *Brassica* vegetables, Its host plants include a wide range of *Brassica* vegetables, such as mustard, kale, broccoli and particularly cabbages (Sarfraz et al., 2006; Sharma et al., 2007). The development of *P. xylostella* is highly dependent upon temperature (Bahar et al., 2014). The development of each stage can be increase with a decrease in temperature (Bahar et al., 2012). There can be more than 20 generations per annum in the tropical lowlands of Southeast Asia (Talekar and Shelton, 1993).

The *Plutella xylostella* life cycle consists of egg, larva, pupa and adult (Faithpraise et al., 2014). Adult females lay 11-118 eggs over about a 10-day period from shortly after mating (Talekar and Shelton, 1993). The eggs are oval and flattened (0.44 x 0.26 mm), and are laid either singly or in small clusters along the veins of both the upper and lower sides of leaves (Ho, 1965). Egg hatch, mainly influenced by temperature, occurs in 5-6 days but this depends on temperature (Talekar and Shelton, 1993).

There are four larval instars; their duration is influenced by temperature (Lu et al., 1988). During the summer season in Canada the duration of development from 1^{st} to 4^{th} instar larva lasts for 4, 4, 5, and 5.6 days respectively (Harcourt, 1954), with the fourth larval instar c. 1 cm in length. The first larval instar is a leafminer, the remaining three larval instars feeding on the surface of the leaf (Branco et al., 2001).

Pupae are c. 8 mm in length, yellowish in colour and are wrapped in a loose silken cocoon; the pupal stage lasts for 5-15 days, depending upon the temperature (Bahar et al., 2014).

The adult moths have a wingspan of c. 15 mm and body length of c. 6 mm. Their fore wings are brownish-grey with fine dark speckles. The posterior margin of the wings has a creamy coloured stripe with a wavy edge, which can form a light coloured diamond shape - the basis for the common name of this species. With a temperature from 4^0C to 37^0C adult moths live on average for about 17 to 4 days, respectively (Bahar et al., 2014).

The total developmental time from egg to pupae is 14-28 days, depending on temperature, with an increase in population size of up to 60-fold from generation to generation (De Bortoli et al., 2011).

Plutella xylostella is generally thought to have originated in Asia Minor and migrated worldwide with the introduction of *brassica* crops (Figures 2.3; 2.4, Carter, 1984; Shelton et al., 2001). The moths are weak flyers but are readily carried by prevailing winds and are thus highly dispersive. *Plutella xylostella* has continuous generations in the tropics and sub-tropics (Wei et al., 2013) but in the more southerly and northerly parts of its range (e.g. Argentina, Scandinavia, Northern Japan and Canada), it cannot over-winter (Dosdall et al., 2001). In these areas, pest outbreaks are thought to be due to long distance migration (Chapman et al., 2002). Whether *P. xylostella* overwinters in some more temperate regions of the world, such as South-eastern Australia remains controversial (Gu et al., 2010).

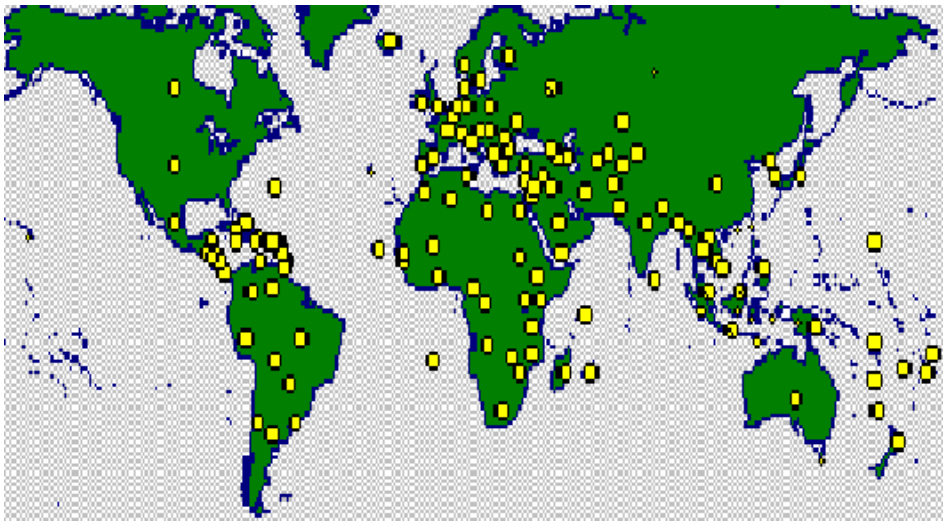


Figure 2.3 Distribution of *Plutella xylostella* worldwide http://www.dfid.gov.uk/r4d/PDF/Outputs/CABI/CabbageManualPart1.pdf.

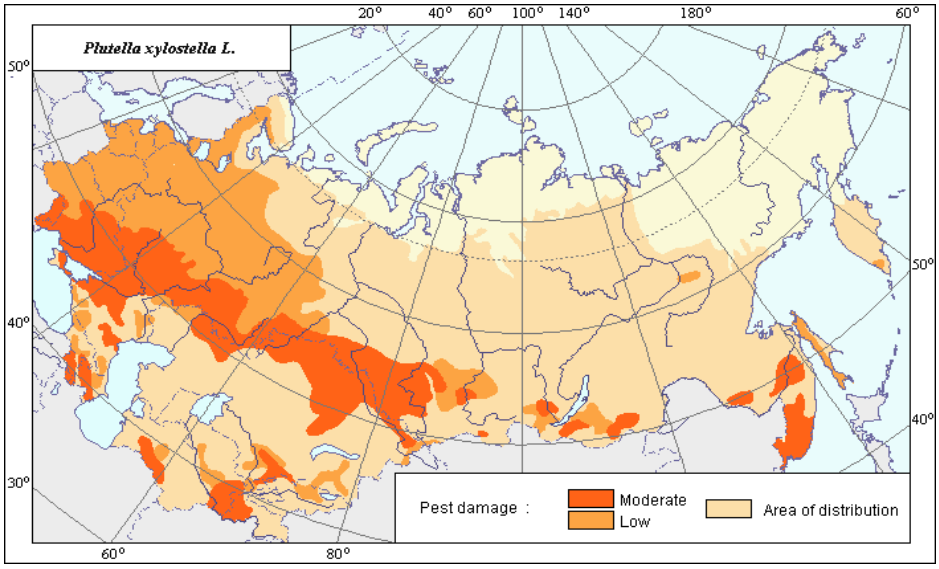


Figure 2.4 Distribution and damage by *Plutella xylostella* in Palearctic zone [after Ovsyannikova et al., 2004]

2.6 Chemical control of *Plutella xylostella*

Some of the insecticides that have been used in Asian countries like Pakistan to control *P. xylostella* on cabbage, include abamectin, acephate, chlorfluazuron, cyfluthrin, cypermethrin, lambda-cyhalothrin and spinosad (Abro et al., 2013) and in Malaysia some of the insecticides marketed to control *P. xylostella* on Brassica crops include acephate, methamidophos, bendiocarb, *Bacillus thuringiensis* (*Bt*), diflubenzuron, spinosad, indoxacarb and fipronil (Mohamad et al., 1979; Verkerk and Wright, 1997) .

In Brassica crops, loss of marketable crop is caused by the larval stages of *P. xylostella*, which results in not only loss of quality of the crop but also quantity and cosmetic value (Talekar and Hu, 1996). Economic loss can be up to 100% (Calderon and Hare, 1986). The ability of *P. xylostella* to develop resistance to a wide variety of insecticides is one of the major factors

contributing to the economic importance of this pest species (Chapman et al., 2002, 2003; Furlong et al., 2013).

From 1947 to the present day most farmers have relied on chemical sprays to control *P. xylostellla* on cash crops (Sibanda et al., 2000; Johnson et al., 2009; Furlong et al., 2013; Liu et al., 2014). The cost of pesticides used against *P. xylostella* alone has been estimated at US $1 billion annually with an estimated combined management cost, including yield loss, at US $4-5 billion (Furlong et al., 2013). The situation is particularly serious in parts of Asia and in Africa, where, due to lack of knowledge, farmers often rely on insecticides which are old, broad spectrum, highly toxic to natural enemies and the environment (Rauf et al., 2004; Badenes-Perez and Shelton, 2006).

Another alarming fact is that most of the small scale farmers (survey report in Zimbabwe, 1997) apply insecticides to the brassica crops using a knapsack or compression sprayer with little or no training, having no protective clothing on. They usually use high volumes of water to dilute the insecticide and add a surfactant/adjuvant to improve wetting of the difficult–to-wet leaf surfaces. When the dilute spray has apparently had little effect (possibly due to poor underleaf coverage), they spray sometimes every 2-3 days. It is the frequent applications over several weeks that has led to rapid selection of resistance pests and killed the natural enemies (Sibanda et al., 2000)

Prolonged and intensive use of insecticides has led to the development of resistance in populations of *P. xylostella* in the tropics and sub-tropics. *Plutella xylostella* was the first insect pest of crops to show resistance against DDT (Ankersmit, 1953) and the first insect pest to show field-based resistance

to *Bacillus thuringiensis* (*Bt*) - based insecticidal sprays (Furlong et al., 2013; Bopape et al., 2014).

Farmers often make the problem of resistance worse by increasing the dose and frequency of chemical applications as insect control declines (Furlong et al., 2013). These activities have resulted in reports of widespread resistance in *P. xylostella* to almost all major groups of pesticides (Furlong et al., 2013; Gong et al., 2013), including pyrethroids (Liu et al., 2000), organophosphates (Xia et al., 2008), insect growth regulators (Ismail and Wright, 1991), *Bacillius thuringensis* (Raymond et al., 2013), and many newer types of insecticides (Sarfraz and Keddie, 2005; Ridland and Endersby, 2011). The situation has often become worse due to the migratory ability of *P. xylostella* (Wang et al., 2005; Li et al., 2006; Saw et al., 2006).

With the development of resistance in populations of *P. xylostella*, farmers have often resorted to adding new products to existing ones, applying mixtures of compounds, where resistance may already exist, a practice which often appears to have made the situation worse, particularly when selective products, less damaging to natural enemies, are mixed with broader-spectrum compounds (Furlong et al., 2013).

The high level of selection pressure through overuse of insecticides on *brassica* vegetables, combined with the short generation time and high fecundity of *P. xylostella* are major factors in the development of resistance in *P. xylostella* (Furlong et al., 2013).

Various attempts to introduce parasitoid natural enemies of *P. xylostella* have been reported but most have been compromised by the continued use of non-selective insecticides (Verkerk and Wright, 1997;

Furlong et al., 2013). The negative effects of many insecticides have been widely reported, including the risk to human health, the environment and the killing of natural enemies (Haseeb et al., 2004; Bommarco et al., 2011; Liu et al., 2014) and this has led to attempts worldwide to develop sustainable IPM programmes against *P. xylostella*, including reduced use of insecticides, combined with other strategies, including biological control (Sarfraz et al., 2006; Goulart et al., 2012; Furlong et al., 2013). Hymenopteran parasitoids are known to be particularly effective in reducing damage by *P. xylostella* (Kfir and Thomas, 2001).

2.7 Biological control of *Plutella xylostella*

Biological control is the beneficial action of predators, parasites, pathogens, and competitors in controlling pests and their damage (Flint and Dreistadt, 1999).

The role of natural enemies in controlling pests is an important one, especially where insecticides have failed (Talekar and Yang, 1991; Talekar and Shelton, 1993). In cases where naturally occurring biological control is not enough to maintain pest populations below an economic threshold level for crop damage, the manipulation of biological control by farmers and growers can enhance pest management.

There are three basic tactics for biological control: Classical; Conservation and Enhancement; and Augmentation (Figure 2.5). The introduction of a non-indigenous natural enemy of an introduced pest species is referred to as Classical biological control (Van Driesche and Bellows, 1996). Conservation and enhancement, which is the most frequently used

tactic in IPM is defined as actions to preserve and increase indigenous natural enemies by environmental manipulation (Debach, 1964; Ehler, 1998). Augmentation is defined as the process which aims to increase the population size of indigenous natural enemies either by propagation and release or by environmental manipulation (DeBach, 1964).

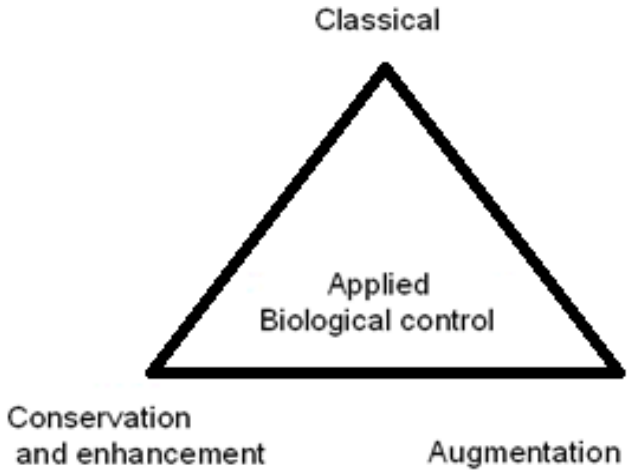


Figure 2.5 Three tactics for using natural enemies (after Flint and Dreistadt, 1999)

The use of biological agents against *P. xylostella* has been widely reported (e.g. Kfir, 1997; Sarfraz and Keddie, 2005; Macharia et al., 2005; Furlong et al., 2013). The first attempts at classical biological control of *P. xylostella* were in New Zealand with the introduction of *Diadegma semiclausum* (Hellen) (Hymenoptera: Ichneumonidae), a solitary larval endoparasitoid, from the UK (Hardy, 1938). During 1999, in the South Atlantic Ocean with the introduction of *C.vestalis* and the pupal parasitoid, *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae) successful control of *P. xylostella* been accomplished (Kfir, 2005). During 1984 successful control of

P. xylostella in Southeast Asia (Indonesia) with introduction of *Diadegma eucerophaga Horstman* (Hymenoptera: Ichneumonidae) had been reported (Sastrosiswojo and Sastrodihardjo, 1986). Failure to biological control is often correlated to the pesticide use in the vicinity, such as in South Africa *P. xylostella* control in past been contributed to the natural occurring biological control agents such as *C. vestalis* (Kfir, 1997) with a later outbreak of *P. xylostella,* as a result of indiscriminate use of insecticides and resistance against pyrethroids, organophosphates and carbamates (Waladde and Leutle, 2001).

Over 130 parasitoid species are known to parasitise *P. xylostella* (Sarfraz et al., 2005). The parasitoid species reported to give the most effective control being within the ichneumonid genus, *Diadegma*; the braconid genera, *Microplitis* and *Cotesia*; and the eulophid genus, *Oomyzus* (Sarfraz et al., 2006).

To control *P. xylostella* using an egg parasitoid requires frequent releases of large numbers of the parasitoids (Talekar and Shelton, 1993) and they are not always host specific (Mason and Huber, 2002). Larval parasitoids are reported to give much better control against *P. xylostella* (Furlong et al., 2013).

One of the key larval endoparasitoids of *P. xylostella*, *C. vestalis* can reach very high levels of parasitism (Verkerk and Wright, 1997). When used together, *C. vestalis* and *Diadegma* spp. were reported to result in 90% parasitism of *P. xylostella* in Ethiopia (Ayalew and Ogol, 2006). Sometimes biological control can be effective by itself for control of insect pests but more usually several methods must combined in an IPM programme for optimal

pest control (Flint and Dreistadt, 1999). Combining biological and chemical strategies can, however, prove difficult, as more non-selective/broad spectrum insecticides such as organophosphates, carbamates and pyrethroids can kill both the pest and its natural enemies (Furlong and Wright, 1993; Xu et al., 2001; Furlong et al., 2013).

2.8 *Cotesia vestalis*

Cotesia vestalis is an important endolarval parasitoid of *P. xylostella* (Haseeb and Amano, 2002; Rincon et al., 2006). It is believed to have originated in Europe and has, to at least some extent, spread with its host *P. xylostella* (Talekar and Shelton, 1998), including to China (Liu et al., 2000), Pakistan (Mushtaq and Muhyuddin, 1987) and India (Joshi and Sharma, 1974) – but see also below. In most parts of the world *C. verstalis* is more abundant in lowlands and compared with some other parasitoids, such as *Diadegma* spp. is relatively heat tolerant (Verkerk and Wright, 1997). In South Africa it is abundant in both lowland and highland regions (Kfir, 1997; Waladde et al., 2001).

Cotesia vestalis is a solitary endoparasitoid of *P. xylostella* (one individual develops per host) (Sarfraz and keddie, 2005). *C. vestalis* development is effected by photoperiod and temperature (Ahmed et al., 2007). At 25 ± 1ºC and 60-65% RH egg maturation occurs in two days. *C. vestalis* has three larval instars, the first two instars molt inside their host body and the third emerge out to spin a cocoon. Under the same conditions 1st, 2nd and 3rd instars takes 2, 5 and 1 day to develop respectively (Yu et al., 2008).

As a kinobiont parasitoid, *C. vestalis* allows the host to continue to develop although the host is eventually killed prior to parasitoid emergence; all four larval instars of *P. xylostella* can be parasitized, but the second and third instar is the preferred host for parasitism by the female parasitoid *C. vestalis* (Liu et al., 2014), Superparasitism, the nutritional status of the host after parasitism, and the extent of resistance to insecticide affect the biology of *C. vestalis* (Li et al., 2007).

Field and laboratory evidence suggests that *C. vestalis* is less sensitive to some insecticides compared with other parasitoids of *P. xylostella*, particularly *Diadegma* spp. (Furlong and Wright, 1994; Verkerk and Wright, 1997; Shi et al., 2004). For example, while some insecticides show moderate to high toxicity against *C. vestalis* (Haseeb and Amano, 2002; Liu et al., 2014; Bopape et al., 2014), the avermectin, abamectin which shows high toxicity towards *Oomyzus sokolowskii* (Hymenoptera: Eulophidae) (a larval–pupal endoparasitoid of *P. xylostella)*, was found to show very little toxicity to *C. vestalis* (Shi et al., 2004). According to Liu et al. (2000), *Cotesia* and *Plutella* can exhibit the same mechanism of resistance to pyrethroid insecticides and reports of field resistance against pyrethroids been reported for *C. vestalis* in South Africa (Smith and Villet, 2004).

As mentioned above, there is evidence of long distance migration of *P. xylostella* (Xing et al., 2013) but this does not apply to its parasitoids. This could be one reason for the lack of effective control of *P. xylostella* by parasitoids, particularly in regions where *P. xylostella* cannot overwinter. A more generally applicable reason is the continued use of broad-spectrum insecticides that are harmful for non-target organisms (Stapel et al., 2000).

There is, however, evidence that the introduction of parasitoids can reduce damage by *P. xylostella* (Talekar and Shelton, 1993; Kfir and Thomas, 2001). Generally, parasitoids are host specific and parasitize the host in a density dependent manner (Annecke and Moran, 1982), and when exploited successfully this can be an effective way to also reduce the frequency of pesticide applications, leading to reduced environmental damage (Talekar and Yang, 1991).

It is possible, therefore, to use parasitoids within IPM systems for control of *P. xylostella* if selective insecticides are used and spray applications are applied as required rather than by 'calendar spraying' (Stapel el al., 2000; Desneux et al., 2007; Furlong et al., 2013). The real challenge is to use chemicals at a level that will be effective against the targeted pest with minimal effects on biological control agents. Over the past few decades, a number of relatively selective compounds have been introduced, including indoxacarb, abamectin and spinosad (Furlong et al., 2013). The present investigation was undertaken to evaluate the basis of selectivity of action of such compounds against *C. vestalis*.

2.9 *Myzus persicae*

Myzus persicae has long been known as an important pest of vegetable crops (Heathcote, 1962) and is found throughout the world (Peccoud et al., 2010), having originated in Asia (Zhang et al., 2008). It is a primary aphid pest of peach (Kuroli and Lantos, 2006). Its secondary host plants include potato and over a 100 crop and ornamental plants (Baker, 1994; Van Emden and Harrington, 2007). Adult *M. persicae* range in size from

1.5 to 3.5 mm (Borror et al., 1976). Single species of the aphid show polymorphism, and can found with different colour forms and shapes and can be winged (alate) or wingless (apterous) (Blackman and Eastop 2000).

Myzus persicae has a complex life cycle (Figure 2.6) (Van Emden et al., 1969), which varies in different parts of the world (Blackman, 1974). In temperate regions, *M. persicae* reproduces by parthenogenesis (incomplete cycle or anholocycle) in some countries the spring and summer months and sexually (complete cycle or holocycle) in the autumn, where the aphid overwinter as eggs (Van Emden et al., 1969). Occurrence of sexual reproduction can vary greatly with temperature with no sexual reproduction at 20-25^{0}C (MacGillivray and Anderson, 1964; Blackman, 1972). Parthenogenesis can also occur throughout the winter months if conditions are relatively mild (Blackman, 1972). There are three distinct stages in the life cycle of *M. persicae*: egg, nymph and adult; showing incomplete metamorphosis to adults (Minks and Harrewijn, 1987). There can be 20 or more generations per year in moderate climates (Blackman and Eastop, 2000). *Myzus persicae* eggs are shiny and black, nymphs are green, with black antenna and legs, and are similar to wingless adults (Broadbent, 1949).

The syringe-like mouthparts (stylet) of aphids allow them to penetrate deep into plant tissues and suck phloem sap (Godfrey, 1997; Campbell, 2014). *Myzus persicae* is known for its ability to act as a virus vector, and virus transmission is a major factor in plant damage plant due to this aphid species (Radcliffe and Ragsdale, 2002; Ansteada et al., 2004). For example, *M. persicae* is a vector of PLRV (potato leaf roll virus), which is transmitted in a persistent manner (Virus once acquired by a vector remains associated

throughout their life) (Blackman and Eastop, 2000; Ansteada et al., 2004; Andret-Link and Fuchs, 2005).

Nymphs as well as adults of *M. persicae* are capable of transmitting viruses but the adults, being mobile, are far more important as virus vectors (Flanders et al., 1991).

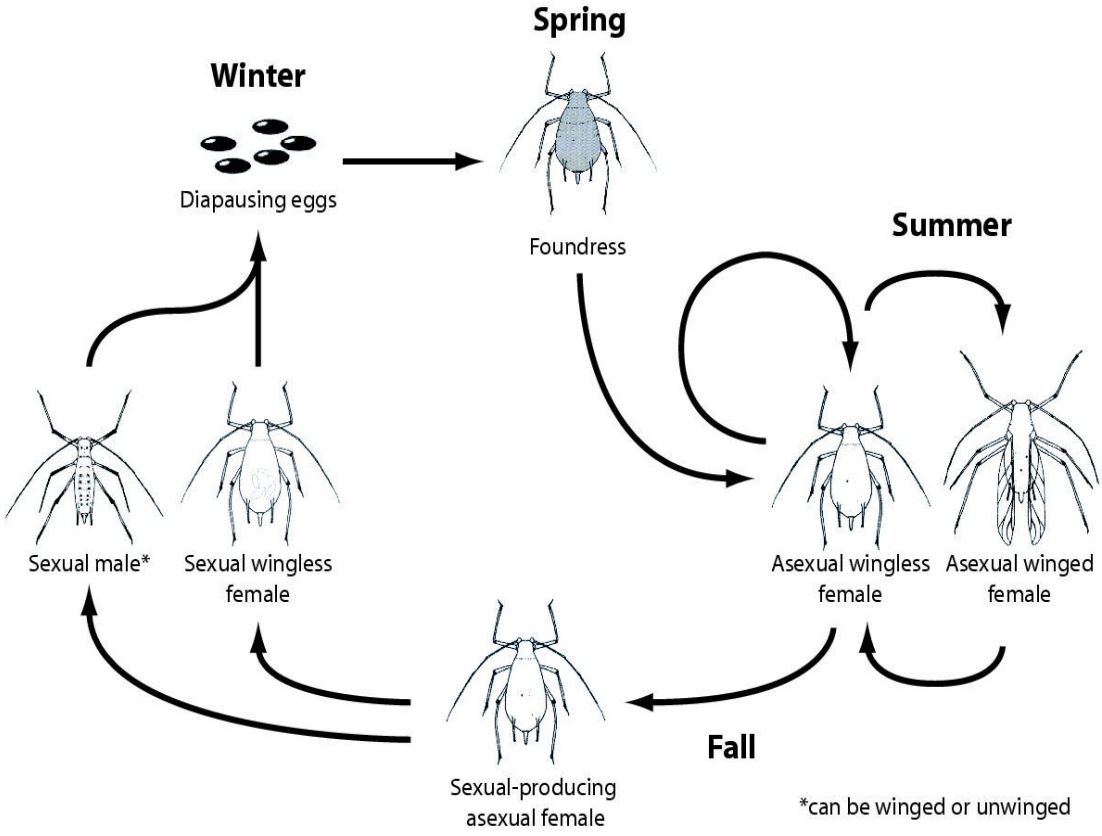


Figure 2.6 Life cycle of *Myzus persicae*. https://theaphidroom.files.wordpress.com/2012/02/aphid-life-cycle.jpg. (28/11/2014).

Myzus persicae is economically very important and on soybean alone is estimated to cause $1billion of crop and input losses in the U.S.A. per annum (Radcliffe and Ragsdale, 2002).

Most of the methods used to control aphids rely on chemical insecticides (Robert, 1992) and the ability of *M. persicae* to develop resistance to insecticides makes this species particularly challenging to control (Foster et al., 2007).

Aphid-specific parasitoids can be used as part of an IPM system (Chambers et al., 1986; Milne, 1999; Van Driesche and Bellows, 1996) but further information on the lethal and sublethal effect is required to evaluate their compatibility with insecticides (Desneux et al., 2007).

2.10 Biological control of *Myzus persicae* using *Aphidius colemani*

The parasitoid, *A. colemani* is known to be particularly effective against *M. persicae* (Van Steenis, 1993). It originated in India (Frank, 2010) and is available commercially in many countries worldwide (Bengochea et al., 2012). It has been used to control *M. persicae* in greenhouses in particular but has also been used on cereal crops (Van Steenis, 1995; Malais and Ravensberg, 2003). *A. colemani* is a solitary, endolarval, kinobiont parasitoid, whose development is closely related to that of its host (Kalule and Wright, 2005).

The life cycle of *A. colemani* starts when the female oviposits in an aphid's body, the eggs then hatch and the resulting larva passes through three larval stages. After the host aphid has died, parasitoid pupation starts and the aphid turns into a mummy. Then there are the prepupal and pupal stages, followed by emergence of the adult wasp out of the mummy (Longley, 1999). One life cycle takes about 9-17 days at 20-21^{0}C (Hagvar and Hofsvang, 1991).

Reliance on broad-spectrum pesticides resulted in lethal effects on this biological control agent (Roubos et al., 2014) as a result, a high population growth of secondary pests occurs (Longley et al., 1997). Even If not killing natural enemies, pesticides may result in sublethal effects (Haynes, 1998; Bengochea et al., 2012). Parasitoids are exposed to the pesticides directly through spray droplets or to the residues during foraging (Longley and Jepson, 1996), ingestion during feeding as honeydew droplets (Longley and Stark, 1996) or within the host during development (Suss, 1983; Longley, 1999). Known sublethal effects include impaired host finding ability (Elzen, 1989; Desneux et al., 2004) and disrupted foraging patterns (Longley and Jepson, 1996). The failure of biological control agents to achieve full control of pests in most cases means that insecticides will remain an integral part in IPM (Bengochea et al., 2012). There is a need, therefore, to ensure that compounds that are relatively harmless to natural enemies are used wherever possible, or that the doses applied are relatively harmless.

CHAPTER 3: General Materials and Methods

3.1 Plants

Chinese cabbage (*Brassica chinensis* var pekinensis cv Wonk Bok) (E.W. King and Co., Ltd, Kelvendon, Colchester, U.K.) were grown in a greenhouse at 25 ± 1 ^{0}C with supplementary lighting (16-h photoperiod). Seeds were sown in individual pots (13 cm dia.) using John Innes No 2 compost (Fargro Ltd, Littlehampton, UK) having N:P:K @ 10.2%: 6.4%: 16.2%. Seeds were sown weekly to have a continuous supply of plants of the same age throughout the experiments. Plants were watered every two days. No chemical sprays or other chemical treatments were used during plant production. To maintain the culture of insects, and to carry out experiments, six to eight week-old potted plants were used.

3.2 Insects

3.2.1 *Plutella xylostella*

A *Plutella xylostella* culture (Lab-UK) was obtained from a laboratory strain, kept at Silwood Park, Imperial College London. Insects were reared in screen cages (42 cm x 43 cm x 55 cm) in a controlled temperature (CT) room at 25 ± 1 ^{0}C, with a relative humidity (RH) of 70 ± 5 % and a 16-h photoperiod. Adults were provided with 20% (w/v) honey solution as a source of food. Chinese cabbage plants were provided for oviposition and larvae were reared on the plants, which were replaced as required.

3.2.2 *Cotesia vestalis*

Cotesia vestalis were initially obtained from a culture at Silwood Park (Staley et al., 2011). For some later work, following loss of the original culture, a second population of *C. vestalis* was obtained from Fiji. *Cotesia vestalis* were reared on *P. xylostella* by exposing a mixed population of larval stages to newly emerged male and female parasitoids in a screen cage. Parasitoid cultures were reared in screen cages (42 cm x 43 cm x 55 cm) in a controlled temperature (CT) room at 25 ± 1 ^{0}C, with a relative humidity (RH) of 70 ± 5 % and a 16-h photoperiod (the same conditions as *P. xylostella*) in a separate CT room from the main *P. xylostella* culture. Adult parasitoids were provided with a 20% honey solution as a source of food.

3.2.3 *Myzus persicae*

A *Myzus persicae* culture was obtained from Koppert Ltd (Haverhill, Suffolk, U.K.) and maintained on six to eight week-old Chinese cabbage under CT room conditions (Section 3.2.1).

3.2.4 *Aphidius colemani*

Aphidius colemani mummies were obtained from Koppert Ltd as required. On arrival, the parasitoid mummies were kept in a collecting cage (42 cm x 43 cm x 55 cm) under CT room conditions (Section 3.2.1) until adult *A. colemani* emerged. The adults were fed with 20% honey solution.

3.3 Chemicals

Four formulated insecticide products, as emulsifiable concentrates (EC), suspension concentrates (SC) or Granules (G), were assessed during this study: lambda-cyhalothrin (Karate®, 5% w/v active ingredient (a.i.) EC, Syngenta, U.K.), indoxacarb (Steward®, 30% w/v a.i. G, Dupont de Nemours, France), spinosad (Tracer®, 48% w/v a.i. SC, Dow AgroSciences, U.K.) and abamectin (Vertimec® 1.8% w/v a.i. EC, Syngenta, U.K.). Compounds were stored at 5°C. Test solutions were freshly prepared in 50 ppm Triton X-100 (Sigma Aldridge, U.K.) as a surfactant for each set of experiments. Use of a surfactant was based on the fact that cabbage leaves are hard to wet, due to their waxy nature, so to have a complete coverage there is need of an additional wetting agent (Zhang et al., 2006). HPLC grade chloroform (BDH Ltd, UK) was used for wax extraction from leaves.

3.4 Removal of wax from pesticide treated and untreated leaves

Epicuticular wax was removed from Chinese cabbage leaf discs using the method of McDonald et al., (1993). Leaf discs were immersed in 50 ml of chloroform in a glass beaker for 30 s. Treated leaf discs were then placed in a fume hood for 2 h to allow for complete evaporation of chloroform.

3.5 Bioassays

3.5.1 Leaf-dip bioassay for *Plutella xylostella* larvae

3.5.1.1 With leaf wax experiment

All experiments were conducted at a temperature of 25 ± 1^0C. Leaf disc bioassays for *P. xylostella* 3rd instar (L3) larvae were conducted on

Chinese cabbage leaves (Sayyed et al., 2000). Leaf discs (4.8 cm dia.) were immersed for 10 s in test solutions and were then placed on a corrugated aluminium foil rack and left at ambient temperature (25^0C) for 1 h to dry. The following dose ranges (based on a series of preliminary experiments) were used for each compound: lambda-cyhalothrin (0.001 to 1 ppm); abamectin (0.001 to 0.10 ppm); indoxacarb (0.003 to 1 ppm) and spinosad (0.005 to 1 ppm). Control leaf discs were dipped in a 50 ppm Triton X-100 solution.

Leaf discs were then transferred to 5 cm diameter, deep plastic Petri dishes containing a single 4.8 cm dia. filter paper (Whatman No. 1) moistened with distilled water. Three L3 *P. xylostella* were introduced per Petri dish with 10 replicates per treatment including the control (n=30 larvae per treatment). Mortality for each pesticide was assessed after 24 h and 120 h. In a separate set of treatments, *P. xylostella* larvae were transferred to untreated leaf discs, after 24h exposure to treated leaves, and mortality was recorded at 120 h following initial exposure to pesticide.

3.5.1.2 Without leaf wax experiment

Bioassays followed the same procedure as described in Section 3.5.1.1 except after drying leaf discs, insecticide-treated leaves were subjected to wax stripping (Section 3.4). Triton X-100 (50 ppm) was used as the control. The dose ranges (based on a series of preliminary experiments) used were: lambda-cyhalothin (1 to 4 ppm); abamectin (0.1 to 1 ppm); indoxacarb (3 to 6 ppm) and spinosad (4 to 7 ppm). Leaf discs were transferred to 5 cm diameter Petri dishes containing moistened filter paper.

Three L3 *P. xylostella* were introduced into each Petri dish (n=30). Insect mortality was recorded after 24 h exposure.

3.5.2 Residual bioassay on foliage for *Plutella xylostella* larvae

3.5.2.1 With leaf wax experiment

Plants (4 plants per treatment) were sprayed with: 0.001, 0.1 and 1.5 ppm lambda-cyhalothrin, 0.001, 0.1 and 1.0 ppm abamectin, 1.0, 2.0 and 3.5 ppm indoxacarb and 0.001, 0.1 and 1.0 ppm spinosad respectively (doses were selected based on a series of preliminary experiments) using a Mardrive linear track sprayer fitted with laser jet nozzle (Marine Engineering Ltd., U.K) at 3.5 bar [350 kPa] at a rate equivalent to 500 l ha^{-1}. Triton X-100 (50 ppm) was used as the control. Sprayed plants were allowed to dry in a glasshouse (25 ± 5ºC) for 2 h prior to use (day 0 treatment). Treated plants were maintained in the glasshouse and bioassays conducted for 0, 7, 14 and 28 days. Leaves from treated plants were randomly selected and leaf discs cut for bioassay. Bioassays were conducted as described in Section 3.5.1.1. Mortality was recorded after 120 h.

3.5.2.2 With and without leaf wax experiment

Plants were sprayed with: 2.05, 0.05, 4.6 and 5.0 ppm lambda-cyhalothrin, abamectin, indoxacarb and spinosad respectively (doses based on LC50 values of without leaf dip bioassay experiment). Triton X-100 (50 ppm) was used as the control. The same bioassay procedure was used for wax-stripped leaves (Section 3.5.2.1), except half of the leaf discs were

subjected to wax stripping. Three insects per treatment were assessed, with ten replicates (n=30). Mortality was recorded after 24 h exposure.

3.5.3 Leaf-dip bioassay for adult *Cotesia vestalis*

3.5.3.1 With leaf wax experiment

Cotesia vestalis adults (male and female 1-7 day-old) were exposed to treated leaf discs (Section 3.5.1.1). For *C. vestalis*, the lids of the 5 cm dia. Petri dishes were modified with small a hole in the centre to introduce three adult parasitoids (mixed sexes) per dish, which was then covered with tape. The doses of insecticides (based on preliminary experiments) used were lambda-cyhalothrin (2 to 10 ppm); abamectin (8 to 18 ppm); indoxacarb (8 to 14 ppm) and spinosad (8 to 18 ppm). Control leaf discs were dipped in 50 ppm Triton X-100 solution. Mortality was recorded after 24 h and 120 h. In a second experiment, insects were exposed to pesticide residues for 24 h and then transferred to untreated leaf discs in Petri dishes with 20% honey as a food source for a further 96 h. Each treatment was replicated 10 times (n=30).

3.5.3.2 Without leaf wax experiment

The same bioassay procedure was used for wax-stripped leaves (Section 3.5.1.2). Mortality was recorded after 24 h exposure. The following dose ranges (based on preliminary experiments) were used: lambda-cyhalothrin (70 to 100 ppm); abamectin (100 to 140 ppm); indoxacarb (130 to 160 ppm); spinosad (90 to 120 ppm). Control leaf discs were treated with a 50 ppm Triton X-100 solution.

3.5.4 Residual bioassay on foliage for adult *Cotesia vestalis*

Plants were sprayed at rates (based on preliminary experiments) of 10, 20 and 30 ppm lambda-cyhalothrin, 10, 30 and 50 ppm abamectin, 24, 36 and 48 ppm indoxacarb and 39, 52 and 65 ppm spinosad as described above (Section 3.5.2.1). Triton X-100 (50 ppm) was used as the control. Three adult *C. vestalis* (1-7 day-old) were introduced into each of 10 Petri dishes (n=30) and after 24 h exposure, insects were transferred to untreated leaf discs and provided with 20% honey solution. Mortality was recorded after 120 h.

3.5.5 Leaf-dip bioassay for adult *Myzus persicae*

3.5.5.1 With leaf wax experiment

Myzus persicae were bioassayed on leaf discs treated with insecticides as described above (Section 3.5.1.1) with three adult apterous *M. persicae* per Petri dish. Each treatment was replicated 10 times (n=30). Mortality was recorded after 24 h and 120 h exposure. In a second experiment, insects were transferred to untreated leaves after 24 h exposure and mortality recorded after 120 h. Doses used were (based on preliminary experiments) lambda-cyhalothrin (1.5 to 3.5 ppm); abamectin (2.5 to 4.5 ppm); indoxacarb (12 to 20 ppm); spinosad (20 to 40 ppm). Control leaf discs were treated with Triton X-100.

3.5.5.2 Without leaf wax experiment

The same bioassay procedure was used as for wax-stripped leaves (Section 3.5.1.2). Mortality was recorded after 24 h exposure. Doses (based on preliminary experiments) used were lambda-cyhalothrin (150 to 200 ppm);

abamectin (150 to 200 ppm); indoxacarb (200 to 250 ppm); spinosad (250 to 300 ppm). Control leaf discs were treated with Triton X-100.

3.5.6 Residual bioassay on foliage for adult *Myzus persicae*

Four Chinese cabbage plants were sprayed at rates (based on preliminary experiments) of 5.0, 9.0 and 12 ppm lambda-cyhalothrin, 4.0, 7.0 and 10 ppm abamectin, 20, 35 and 45 ppm indoxacarb, and 33, 45 and 60 ppm spinosad. Triton X-100 (50 ppm) was used as the control. For each bioassay day (0, 7, 14, 28 days) leaves were randomly selected from each plant for leaf disc bioassays (Section 3.5.2.1). Mortality was recorded after 120 h.

3.5.7 Leaf-dip bioassay for adult *Aphidius colemani*

3.5.7.1 With leaf wax experiment

The same method was used as for *C. vestalis* (Section 3.5.3.1). Doses (based on preliminary experiments) used were lambda-cyhalothrin (1.5 to 6.5 ppm); abamectin (2 to 6 ppm); indoxacarb (14 to 22 ppm) and spinosad (20 to 35 ppm). Control leaf discs were treated with Triton X-100. Three adult *A. colemani* (1 day-old) were used per Petri dish (n=30).

3.5.7.2 Without leaf wax experiment

The same bioassay procedure was used for wax-stripped leaves (Section 3.5.3.2). Mortality was recorded after 24 h exposure. Doses used (based on preliminary experiments) were lambda-cyhalothrin (80 to 120 ppm);

abamectin (80 to 120 ppm); indoxacarb (250 to 300 ppm); spinosad (250 to 300 ppm). Triton X-100 (50 ppm) was used as the control

3.5.8 Residual bioassay on foliage for adult *Aphidius colemani*

3.5.8.1 With leaf wax experiment

Four Chinese cabbage plants were sprayed (doses based on preliminary experiments) with 3.0, 10 and 15 ppm lambda-cyhalothrin; 10, 15 and 20 ppm abamectin; 10, 30 and 45 ppm indoxacarb; 30, 45 and 60 ppm spinosad (Section 3.5.2.1). Triton X-100 (50 ppm) was used as the control. For each bioassay day (0, 7, 14, 28 days) leaves were randomly selected from each plant for leaf disc bioassays, as for *C. vestalis* (Section 3.5.4.1). Mortality was recorded after 120 h.

3.5.8.2 With and without leaf wax experiment

Plants were sprayed (doses based on LC_{50} values in without wax leaf dip bioassay experiment) with 90, 85, 279 and 294 ppm lambda-cyhalothrin, abamectin, indoxacarb and spinosad, respectively (Section 3.5.2.2). Half of the leaf disks were subjected to wax removal. Triton X-100 (50 ppm) was used as the control. Mortality was recorded after 24 h.

3.6 Sub-lethal effects of insecticide residues on parasitoids

3.6.1 Time spent on treated leaves (no choice experiment)

Chinese cabbage leaf discs were dipped in pesticide solutions using the estimated LC_{50} and LC_5 concentrations (24 h exposure + 96 h on untreated leaf discs) for each compound and placed in Petri dishes as

described above (Section 3.5.3.1). Control leaf discs were dipped in a 50 ppm Triton X-100 solution.

A single adult male or female parasitoid one-day-old (*C. vestalis* or *A. colemani*) was introduced into each Petri dish, with 12 replicates per treatment (n=12). Two hours after parasitoid release into the experimental arena, each parasitoid was observed for 10 minutes and the time spent in seconds by each parasitoid on the leaf surface or elsewhere in the arena was recorded.

3.6.2 Time spent on treated leaves or untreated leaves (choice experiment)

The same procedure was followed as in Section 3.6.1 except each Petri dish contained two hemi-spheres of leaf discs (treated and untreated) with a small space (c. 2 mm) between the two halves. Control leaf discs were dipped in a 50ppm Triton X-100 solution. The time spent by parasitoids on treated and untreated leaf hemispheres and on the surrounding arena was recorded. Each treatment was replicated 12 times.

3.6.3 Percent emergence of parasitoids

Chinese cabbage leaf discs were treated with the estimated LC_{50} and LC_5 concentrations of each insecticide (Section 3.6.1). Control leaf discs were dipped in a 50ppm Triton X-100 solution.

Three one-day-old cocoons of *C. vestalis* or three mummies of *A. colemani* were placed in each Petri dish with 10 replicates per treatment

(n=30). The percentage emergence of adult parasitoids was recorded daily for 7 days.

3.7 Statistical analysis

All analyses were conducted using R version 3.0.2 (R Development Core Team, 2013). Estimated LC_{50} values with 95% fiducial limits (FL) and the slope ± S.E. of the dose-response curve were obtained using generalized linear models (GLIM) with binomial errors. Due to non-compatibility with GLIM, Abbott's formula (1925) was not used for correction of mortality. Instead, mortality was corrected by subtracting the number of dead insects in controls from the total number of insects per insecticide treatment (Crawley, 2007). Pairwise comparisons of LC_{50} values were significant at the 1% level if their respective 95 % FL's did not overlap (Crawley, 2007).

Residual bioassay data at different spray rates were analysed using GLIM with quasibinomial errors to correct for overdispersion, and were subjected to analysis of variance (ANOVA) with significance at the 1% level (Crawley, 2007).

Behavioural data were analysed using linear model. To compare differences in time spent on the leaf surface, treated and untreated leaf halves and the arena using LC_5 and LC_{50} doses, data were subjected to ANOVA with a significance difference at the 1% level.

Effects of LC_5 and LC_{50} doses of insecticides on the emergence of adult parasitoids were evaluated using GLIM with binomial errors. Data were subjected to ANOVA with a significant different at 1% level. Chi test used to compare means.

CHAPTER 4: Intrinsic and residual toxicity of insecticides against *Plutella xylostella* and *Cotesia vestalis*

4.1 Introduction

Pesticides used appropriately enhance agriculture production but their misuse has led to human health problems (El-Wakeil et al., 2013) and fatalities especially in developing countries due to lack of knowledge/ training on spraying techniques, inadequate clothing and extensive use of insecticides (Pimentel, 2005; Soomro et al., 2008). Spray applications have also had lethal and sublethal effects on non-target organisms (Akobundu, 1987; Cloyd, 2012), and over use has led to target species becoming resistant to many insecticides (Kilin et al., 1981; Hirai, 1993; Johnson and Tabashnik, 1999). Pest outbreaks can occur following the harmful effects that many pesticides, particularly broad-spectrum insecticides, have on natural enemies, leading to pest resurgence (Way and Heong, 1994; Tanaka, 2000). The detrimental impact of pesticides on beneficial arthropods, including natural enemies, has been of great concern for many years, and has been the subject of a large number of studies (for example, see Haynes, 1988; Croft, 1990; Thompson, 2003).

Plutella xylostella, the most important pest of brassica crops worldwide (Section 2.4), has become particularly difficult to manage because of its ability to develop resistance to a wide variety of pesticides (Li et al., 2007; Furlong et al., 2013, Liu et al., 2014). The overuse of insecticides against *P. xylostella*

has also resulted in damaging effects on its natural enemies, particularly key parasitoids such as the larval endoparasitoid species, *C. vestalis* (Haseeb and Amano, 2002).

Today, many insecticides with a relatively narrow range of activity are marketed against specific groups of insect pests; these compounds can be far less harmful to the beneficial insects, including natural enemies such as *C. vestalis* (Cardwell et al., 2005).

Abamectin, indoxacarb and spinosad are insecticides generally regarded as reduced risk or compatible with natural enemies (Zhao et al., 2006; Liu and Zhang, 2012) and lambda-cyhalothrin is regarded as harmful to the natural enemies (Tillman and Mulrooney, 2000).

Lambda-cyhalothrin is a pyrethroid insecticide (Henry et al., 2005), having a wide range of insecticidal activity against Lepidoptera, Diptera, Coleoptera, Hemiptera and mites. Lambda-cyhalothrin has a harmful effect on natural enemies due to both lethal and sublethal doses (Desneux et al., 2003). Lambda-cyhalothrin is more persistent in nature and has both contact and ingestion activity (Seenivasan and Muraleedharan, 2009).

Abamectin is a fermented product of a soil bacterium *Streptomyces avermitilis,* which is regarded as effective against mites, leafminers and thrips (Lasota and Dybas, 1990). Abamectin is less persistent with both contact and ingestion activity and has a low toxicity to natural enemies (Lasota and Dybas, 1991).

Indoxacarb is an oxadiazine insecticide, which is moderately persistent and is effective against lepidopteran, coleopteran and sucking insect pests,

having both contact and ingestion activity. It has a reduced risk to natural enemies (McCann et al., 2001; Liu et al., 2002; Prasanna et al., 2008).

Spinosad is a broad-spectrum insecticide used against a variety of insects including lepidopterans (Liu and Zhang, 2012) and is derived from soil bacterial species *Saccharopolyspora spinosa* (Crouse and Sparks, 1998). It has both contact and ingestion activity and is regarded as less toxic to natural enemies and is less persistent in nature (Sparks et al., 2001).

The objective of the present study was to compare the intrinsic (baseline) and residual toxicity of four insecticides (lambda-cyhalothrin, abamectin, indoxacarb and spinosad) on Chinese cabbage against *P. xylostella* and *C. vestalis*.

4.2 Materials and Methods

4.2.1 Plants

Chinese cabbage plants were maintained in a greenhouse at 25±1 ^{0}C with supplementary lighting (16-h photoperiod). Seeds were sown in individual pots (13 cm diameter) (Section 3.1).

4.2.2 Insect cultures

Plutella xylostella and *Cotesia vestalis* were reared as described in Section 3.2. All bioassays were conducted in a CT room at 25 ± 1 ^{0}C with 70 ± 5 % RH (Section 3.2.2).

4.2.3 Chemicals

Formulated products of lambda-cyhalothrin, abamectin, indoxacarb and spinosad were tested (Section 3.3).

4.2.4 Leaf dip bioassays with *Plutella xylostella* larvae and *Cotesia vestalis* adults

Leaf dip bioassays were conducted with L3 *P. xylostella* (Section 3.5.1.1) and *C. vestalis* adults (Section 3.5.3.1) on Chinese cabbage leaves. Mortality for each pesticide was assessed after 24 h (A) and 120 h (B). In a separate experiment, *P. xylostella* larvae and *C. vestalis* adults were transferred to untreated leaf discs, after 24 h exposure to treated leaves, and mortality was recorded at 120 h (C) following initial exposure to pesticide.

4.2.5 Residual bioassays with *Plutella xylostella* larvae and adult *Cotesia vestalis* following spray application of insecticides on Chinese cabbage

Plants were sprayed with pesticides (Sections 3.5.2.1 and 3.5.4.) and bioassays conducted with plants from 0 – 28 days from pesticide application.

4.2.6 Statistical analysis

Data analysis was conducted using R version 3.0.2 (R Development Core Team, 2013) (Section 3.7).

4.3 Results

4.3.1 Leaf dip bioassays with *Plutella xylostella* larvae

Abamectin had markedly lower LC_{50} values compared with lambda-cyhalothrin, spinosad and indoxacarb, although abamectin was only significantly more toxic compared with indoxacarb and spinosad after 120 h exposure (B) or 24 h exposure + 96 h (C) (Table 4.1; B and C).

For any insecticide tested, following exposure to pesticide for 24 h, the mortality rate of *P. xylostella* larvae did not markedly increase over the next 96 h while the larvae were on an untreated leaf surface (Table 4.1; A v C). Exposure to pesticide for 120 h compared with 24 h increased the mortality rate of *P. xylostella* larvae for abamectin and indoxacarb when mortality was assessed immediately after exposure (Table 4.1; A v B; statistical comparisons were not conducted due to pseudo-replication). For all insecticides, there was no significant difference when mortality was assessed after 120 h exposure compared with the mortality assessed after 24 h exposure + 96 h on untreated leaves (Table 4.1; B v C).

Table 4.1 Intrinsic toxicity of insecticides on Chinese cabbage leaves against *Plutella xylostella* larvae after: A) 24 h exposure to insecticide; B) 120 h exposure; C) 24 h exposure + 96 h on untreated leaves.

	A		B		C	
Insecticide	LC_{50} [ppm] (95% FL)*	Slope ± SE [log $_{10}$ (dose)]	LC_{50} [ppm] (95% FL)*	Slope ± SE [log $_{10}$ (dose)]	LC_{50} [ppm] (95% FL)*	Slope ± SE [log $_{10}$ (dose)]
lambda-cyhalothrin	0.3 (0.1-1.1)[a]	0.4 ± 0.1	0.03 (0.01-0.3)[a,b]	0.3 ± 0.1	0.1 (0.04-0.53)[a,b]	0.3 ± 0.2
abamectin	0.02 (0.01-1.2)[a]	0.3 ± 0.2	0.002 (0.001-0.01)[b]	0.5 ± 0.2	0.01 (0.008-0.2)[b]	0.1 ± 0.1
indoxacarb	0.8 (0.4-1.6)[a]	0.8 ± 0.1	0.3 (0.1-0.4)[a]	1.1 ± 0.2	0.4 (0.2-0.7)[a]	0.7 ± 0.2
spinosad	1.1 (0.1-10.9)[a]	0.2 ± 0.1	0.4 (0.1-1.3)[a]	0.3 ± 0.1	0.7 (0.2-2.8)[a]	0.3 ± 0.1

*Values within columns sharing a common letter have non-overlapping 95% FLs and are not significantly different at $p<0.01$.

4.3.2 Residual activity of insecticides against *Plutella xylostella* larvae following spray application on Chinese cabbage leaves

Residual activity (toxicity) for lambda-cyhalothrin and abamectin was greater over a 0-28 day and a 0-7 day period, respectively, compared with indoxacarb and spinosad (Table 4.2). In each case, as expected, residual activity for each compound was positively correlated with the concentration applied (Figures 4.1 – 4.4).

There was no significant decline in residual activity for lambda-cyhalothrin at any of the concentrations tested up to 14 days after application ($F=0.95$, $df=6, 40$, $p=0.47$); by day 28 residual activity was significantly reduced compared with day 0 (Table 4.2, Figure 4.1; $F= 181.98$, $t=9.37$, $df=46$, $p=0.001$).

The residual activity of abamectin had significantly decreased 14 days after treatment (Table 4.1, Figure 4.2; $F=26.28$, $t=6.9$, $d.f= 25$, $p<0.001$).

The residual activity of indoxacarb appeared to remain relatively stable up to 14 days after treatment but all detectable residual activity was lost 28 days after treatment ($F=5.61$, $d.f=4, 28$, $p<0.01$; Table 4.2, Figure 4.3).

Spinosad showed a significant decrease in residual activity at 7 days after treatment ($t=5.28$, $d.f=34$, $p<0.01$), with little detectable residual activity remaining 14 days after treatment ($F=84.12$, $t=11.98$, $d.f=34$, $p<0.001$; Figure 4.4).

Table 4.2 Residual toxicity (LC_{50}) of insecticide deposits on Chinese cabbage against *Plutella xylostella* larvae.

Days after application	Insecticide	LC_{50} [ppm] (95% FL)	Slope ± SE
0	lambda-cyhalothrin	0.04 (0.01-0.1)	0.4 ± 0.07
7	lambda-cyhalothrin	0.08 (0.03-0.2)	0.4 ± 0.08
14	lambda-cyhalothrin	0.09 (0.02-0.2)	0.4 ± 0.07
28	lambda-cyhalothrin	0.3 (0.1-1.1)	0.3 ± 0.09
0	abamectin	0.006 (0.002-0.02)	0.3 ± 0.07
7	abamectin	0.03 (0.01-0.1)	0.3 ± 0.07
14	abamectin	<5% mortality (1 ppm)	
0	indoxacarb	0.6 (0.04-0.1)	1.0 ± 0.2
7	indoxacarb	1.2 (0.8-71.7)	1.4 ± 0.3
14	indoxacarb	2.04 (1.6-2.7)	1.8 ± 0.5
28	indoxacarb	no mortality	
0	spinosad	1.7 (1.5-1.9)	3.2 ± 0.6
7	spinosad	3.2 (2.7-3.9)	3.7 ± 0.9
14	spinosad	<5% mortality (3.5ppm)	

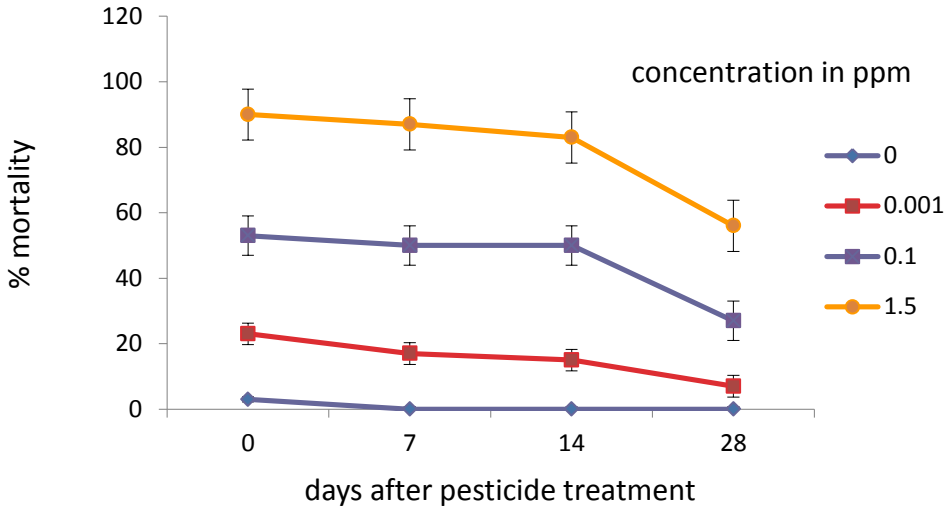


Figure 4.1 Residual activity (% mortality ± S.E) of lambda-cyhalothrin on Chinese cabbage against *Plutella xylostella* larvae following spray application.

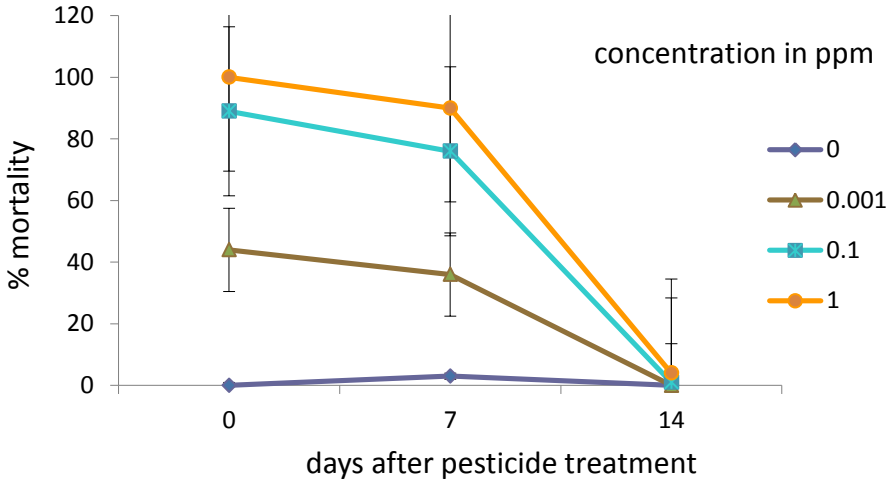


Figure 4.2 Residual activity (% mortality ± S.E.) of abamectin on Chinese cabbage against *Plutella xylostella* larvae following spray application.

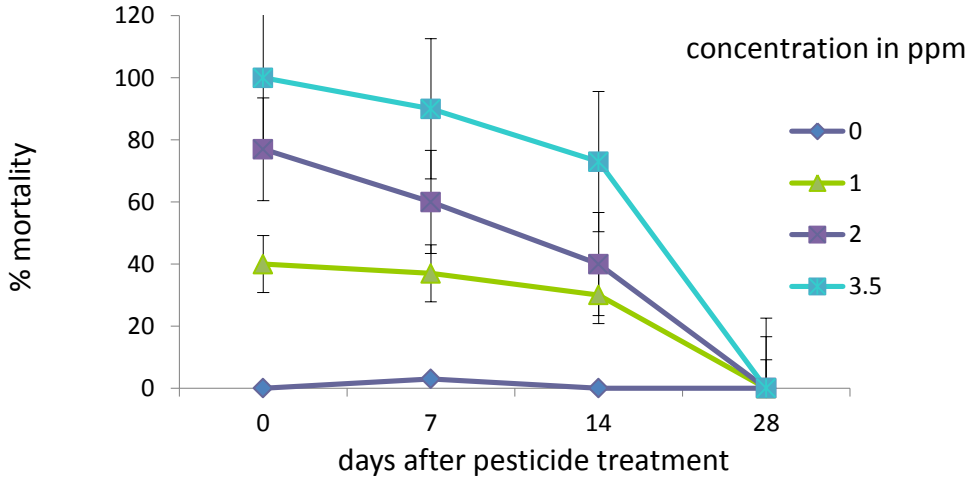


Figure 4.3 Residual activity (% mortality ± S.E.) of indoxacarb on Chinese cabbage against *Plutella xylostella* larvae following spray application.

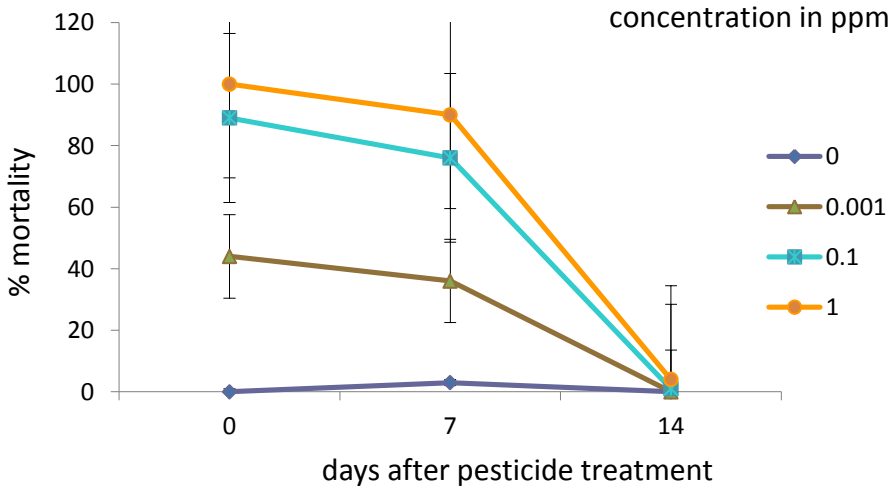


Figure 4.4 Residual activity (% mortality ± S.E.) of spinosad on Chinese cabbage against *Plutella xylostella* larvae following spray application.

4.3.3 Leaf dip bioassays with *Cotesia vestalis* adults

Lambda-cyhalothrin was significantly more toxic compared with indoxacarb and spinosad (Table 4.3; A, B and C), while abamectin did not differ significantly in toxicity compared with all other insecticides except after 120 h exposure, when it was significantly more toxic compared with spinosad (Table 4.3; A, B and C).

Following exposure to pesticide for 24 h, the mortality rate of *C. vestalis* adults did not increase significantly over the next 96 h on untreated leaf surfaces for any insecticide tested (Table 4.3; A v C). For all insecticides except abamectin, there was significantly greater mortality after 120 h exposure compared with when mortality was assessed after 24 h exposure + 96 h on untreated leaves (Table 4.3; B v C).

Table 4.3 Intrinsic toxicity of insecticides on Chinese cabbage against *Cotesia vestalis* adults after: A) 24 h exposure to insecticide; B) 120 h exposure; C) 24 h exposure + 96 h on untreated leaves.

	A		B		C	
Insecticide	LC_{50} (ppm) (95% FL)*	Slope ± SE [$\log_{10}$ (dose)]	LC_{50} (ppm) (95% FL)*	Slope ± SE [$\log_{10}$ (dose)]	LC_{50} (ppm) (95% FL)*	Slope ± SE [$\log_{10}$ (dose)]
lambda-cyhalothrin	8.5 (6.4-11.0)[a]	1.1 ± 0.3	2.9 (2.2-4.0)[c]	1.8 ± 0.4	7.9 (6.1-10.3)[a]	1.2 ± 0.3
abamectin	10.5 (7.3-13.3)[a,b]	1.1 ± 0.3	4.9 (3.8-6.3)[c,d]	1.7 ± 0.3	9.3 (5.2-16.7)[a,b]	0.6 ± 0.1
Indoxacarb	13.4 (12.7-14.2)[b]	7.2 ± 1.2	7.2 (6.1-8.7)[d,e]	4.8 ± 1.4	12.2 (11.6-12.8)[b]	9.1 ± 1.4
spinosad	14.7 (12.1-15.3)[b]	2.8 ± 0.6	8.5 (7.3-9.9)[e]	3.5 ± 0.7	13.1 (12.0-14.3)[b]	3.9 ± 0.7

*Values within columns sharing a common letter are non-overlapping at 95% FL and are not significantly different at $p<0.01$

4.3.4 Residual activity of insecticides against adult *Cotesia vestalis*

For all insecticides tested, residual activity was positively correlated with the concentration applied (Figures 4.5-4.8). At day 0, the residual toxicity of lambda-cyhalothrin was significantly greater compared with the other insecticides tested (Table 4.4).

The residual activity (toxicity) for lambda-cyhalothrin and indoxacarb was found to be more persistent over 0-14 days compared with the other insecticides tested, although there was a significant reduction in toxicity of lambda-cyhalothrin between 14 and 28 days after application (Table 4.4; $F=14.22$, $t=14.49$, $d.f=24,27$, $p<0.001$).

The residual activity of spinosad did not change significantly up to 7 days after application (Table 4.4; $F=0.2$, $t=1.2$, $d.f=0,24$ $p=0.1$) but had declined to a very low level after 14 days.

The residual activity of abamectin decreased significantly 7 days after application (Table 4.4, $F=42.12$, $t=8$, $d.f=20,23$, $p<0.001$) and there was no detectable toxicity after 14 days.

Table 4.4 Toxicity of insecticides at different days after treatment against adults *Cotesia vestalis.*

Days after application	Insecticide	LC_{50} [ppm] (95% FL)	Slope ± SE
0	lambda- cyhalothrin	14.7 (12.6-17.1)	2.8 ± 0.5
7	lambda- cyhalothrin	26.2 (20.9-32.7)	1.9 ± 0.5
14	lambda- cyhalothrin	38.3 (25.9-56.6)	2.1 ± 0.6
28	lambda-cyhalothrin	<10% mortality (30 ppm)	
0	abamectin	28.0 (22.4-35.0)	1.6 ± 0.4
7	abamectin	103 (40.7-258.1)	1.5 ± 0.6
14	abamectin	no mortality	
0	indoxacarb	33.3 (27.6-40.0)	2.4 ± 0.8
7	indoxacarb	45.2 (35.9-56.9)	2.5 ± 0.9
14	indoxacarb	65.0 (41.6-101.4)	3.1 ± 1.3
28	indoxacarb	no mortality	
0	spinosad	46.8 (41.13-53.3)	3.6 ± 1.1
7	spinosad	62.8 (52.84-74.8)	3.4 ± 1.2
14	spinosad	< 5% mortality (65 ppm)	

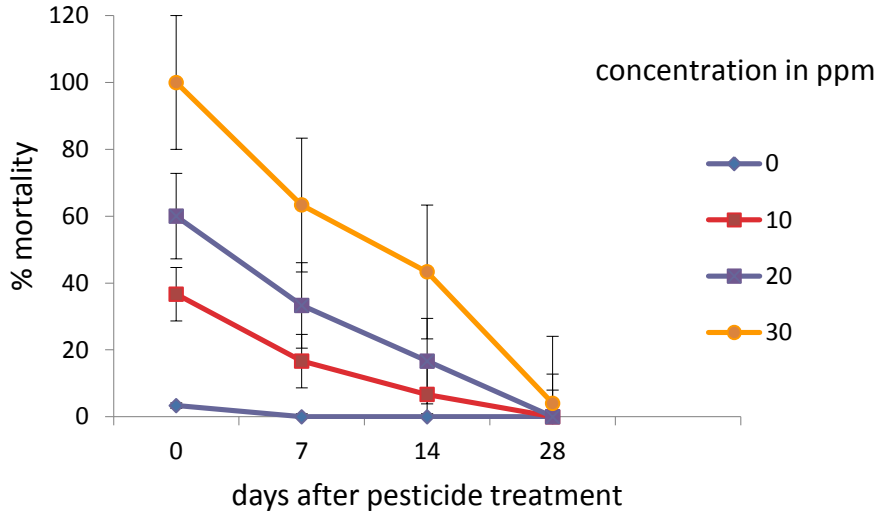


Figure 4.5 Residual activity (% mortality ± S.E) of lambda-cyhalothrin on Chinese cabbage against *Cotesia vestalis* adults following spray application.

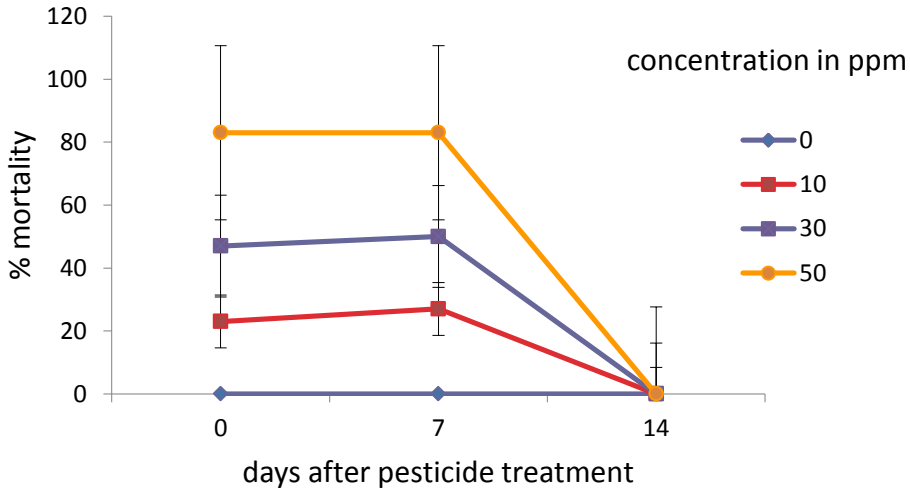


Figure 4.6 Residual activity (% mortality ± S.E) of abamectin on Chinese cabbage against *Cotesia vestalis* adults following spray application.

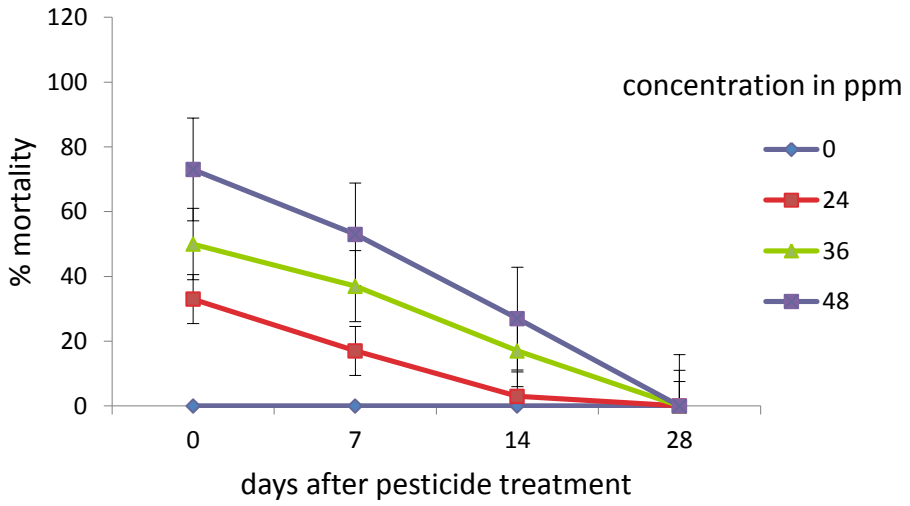


Figure 4.7 Residual activity (% mortality ± S.E) of indoxacarb on Chinese cabbage against *Cotesia vestalis* adults following spray application.

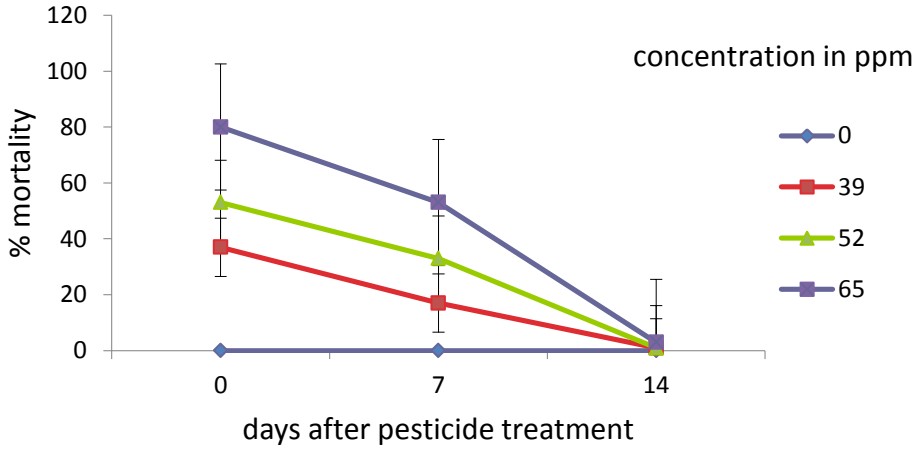


Figure 4.8 Residual activity (% mortality ± S.E) of spinosad on Chinese cabbage against *Cotesia vestalis* adults following spray application.

4.4 Discussion

Jepson et al. (1990) observed that it is difficult to predict the side effects of insecticides due to the many interacting factors involved. The present results suggest that in the case of abamectin very marked differences in intrinsic toxicity between the target pest and natural enemy may be a key factor in the relative selectivity of this compound (Figure 4.9). Shi et al. (2004) also reported a visible difference in avermectin toxicity to *P. xylostella* and *C. vestalis*.

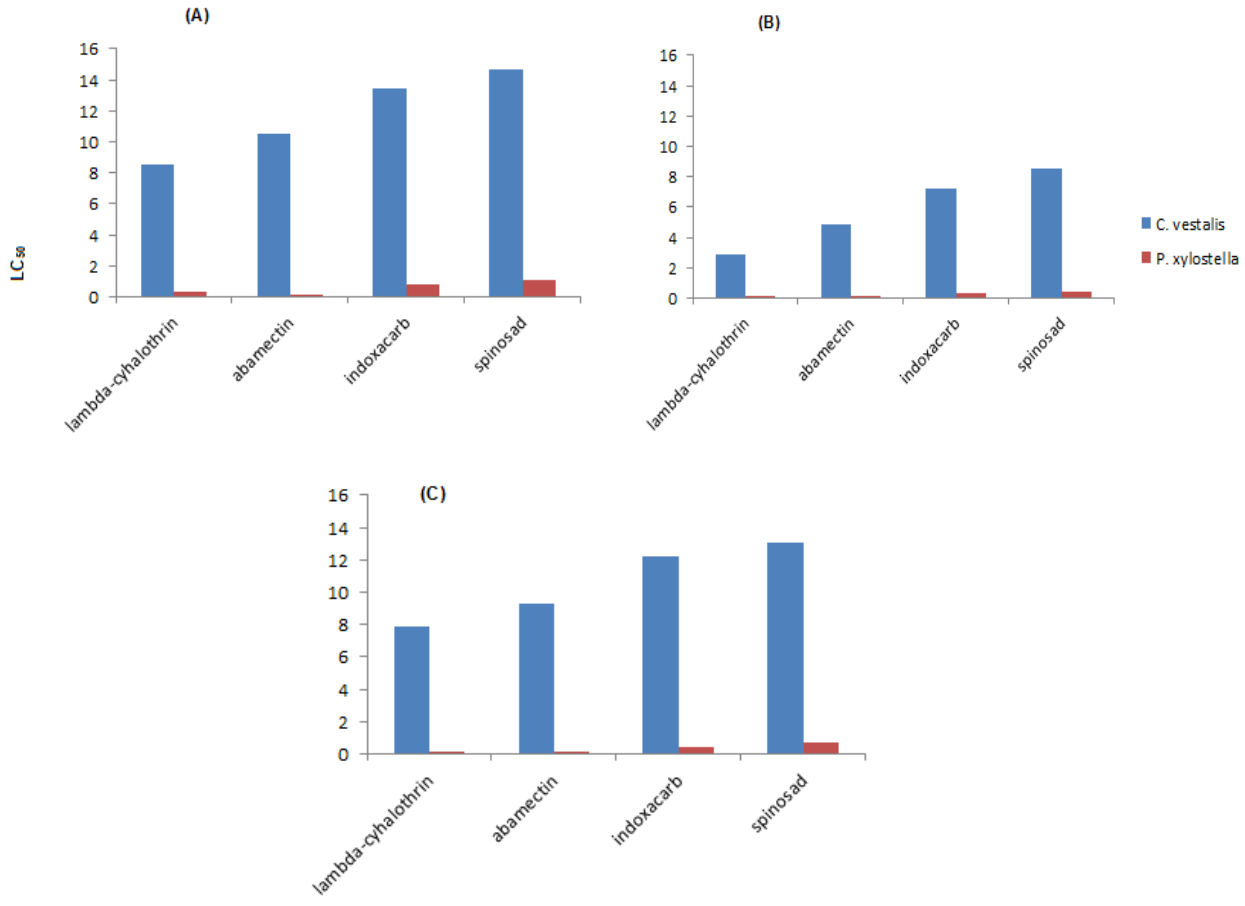


Figure 4.9 Comparing LC_{50} values of four insecticides against *P. xylostella* larvae and *C. vestalis* adults. (A) 24 h exposure to insecticide; (B) 120 h exposure; (C) 24 h exposure + 96 h on untreated leaves.

In the present study, marked differences in the intrinsic toxicity of insecticides to *P. xylostella* larvae were observed in leaf-dip bioassays, with abamectin an order of magnitude more toxic than lambda-cyhalothrin and two orders of magnitude more toxic than indoxacarb and spinosad. These differences in toxicity are in agreement with a previous study on *P. xylostella* (Sayyed and Wright, 2006). Abamectin has been reported to be highly active against *P. xylostella* populations in various studies (e.g. Arora et al., 2003; Eziah et al., 2008).

In contrast, there was relatively little difference in the intrinsic toxicity of the above insecticides to *C. vestalis* adults, with lambda-cyhalothrin and abamectin showing similar toxicity. Lambda-cyhalothrin was significantly more toxic compared with indoxacarb and spinosad but the difference in toxicity was only 2.5 – 3 fold; the same order of toxicity has been reported for these compounds by Haseeb et al., (2004).

All the compounds tested were less toxic to *C. vestalis* compared with *P. xylostella*. Such differences are likely to be due in part to differences in the rate of uptake of pesticides by these two species, with the non-phytophagous species *C. vestalis* taking up pesticide primarily through the cuticle by contact action rather than by contact and feeding as in the case of *P. xylostella* larvae. The rapid adsorption of pesticide residues into the leaf will also reduce the amount available for contact uptake (e.g. Iqbal et al., 1996; Kaspi and Parrella, 2005).

When the LC_{50} ratio of each compound was calculated [LC_{50} for *Cotesia* / LC_{50} for *Plutella*], abamectin showed a toxicity ratio of c. 2450, compared with c. 96 for lambda-cyhalothrin, c. 21 for spinosad and c. 24 for indoxacarb (Table 4.5; B). This suggests differential toxicity may be one factor responsible for the reported

compatibility of abamectin under field conditions with parasitoids of *P. xylostella* (Verkerk and Wright, 1997). However, the pyrethroid, lambda-cyhalothrin, which is reported to be harmful to natural enemies (Section 2) also had a high toxicity ratio, while indoxacarb and spinosad, which are reported to be relatively compatible with natural enemies, had by far the lowest toxicity ratios. Clearly, other major factors are involved in determining selectivity for such compounds such as sub lethal effects on natural enemies and their adverse effect on the environment and to humans (US EPA, 2002: Bahlai et al., 2010).

Table 4.5 Relative toxicity of insecticides to *C. vestalis* adults and *P. xylostella* larvae. (A) 24 h exposure to insecticide; (B) 120 h exposure; (C) 24 h exposure + 96 h on untreated leaves.

Insectcicide	Relative toxicity (LC_{50} *C. vestalis* /LC_{50} *P. xylostella*)		
	(A)	(B)	(C)
lambda-cyhalothrin	28.3	96	79
abamectin	525	2450	930
indoxacarb	16.8	24	30.5
spinosad	13.4	21.3	18.7

The residual toxicity experiments showed a clear difference between lambda-cyhalothrin and indoxacarb, which retained significant residual toxicity against *P. xylostella* and *C. vestalis* for at least 14 days, compared with the less persistent compounds, abamectin and spinosad. These results are in agreement with the work of Xu et al. (2004), who reported that indoxacarb and lambda-cyhalothrin were more persistent on foliage than spinosad. The relative lack of persistence of abamectin

and spinosad on foliage has been reported in various other studies (Hassan et al., 1998; Haseeb and Amano, 2002; Veire et al., 2002; Kamel et al., 2007; Sharma et al., 2007; Soudamini et al., 2009).

The rate of adsorption of pesticide into the leaf may be a major factor in limiting the exposure of non-phytophagous insects to residual deposits of insecticides. Reduced bioavailability of pesticide residues to non-phytophagous insects could be assumed to be greater for systemic or translaminar compounds (e.g. abamectin), due to their adsorption into the epicuticular wax layer and into the underlying leaf tissues, compared with non-systemic compounds, such as pyrethroids, which would remain in the epicuticular wax layer (Iqbal et al., 1996; Kaspi and Parrella, 2005). Pesticide residues that remain on the leaf surface may also be vulnerable to more rapid loss due, for example, to greater exposure to photochemical degradation (Connell et al., 1989). In the next section, the distribution of toxic pesticide residues between the epicuticular wax layer and underlying leaf tissues is examined using insect bioassays.

CHAPTER 5: Effect of leaf surface wax on intrinsic and residual toxicity of insecticides against *Plutella xylostella* larvae and intrinsic activity against adult *Cotesia vestalis*

5.1 Introduction

The surface of the plants is covered by a supporting layer called cuticle (Samuels et al., 2008), composed of two main parts, an insoluble cuticular membrane and soluble waxes, cutin and polyester polymers (Kolattukudy, 1996; Stark and Tian, 2006). Wax is a complex mixture of lipids (Koch et al., 2004), usually comprising long chain hydrocarbons, such as esters, ketones, alkanes, primary alcohols and other derived compounds (Shepherd and Griffiths, 2006), and is a solid partially crystalline aggregate at room temperature (Reynhardt, 1997). Waxes are either embedded into the leaf polymeric matrix, called intracellular wax, or deposited at the leaf surface, called epicuticular wax (Jetter and Schaffer, 2001). Epicuticular wax is the outer most part of the plants and is a barrier between the plant and its surroundings, protecting the plant against environmental stresses (Figure 5.1; Schönher, 1982; Riederer and Schreiber, 1995; Beittenmiller, 1996).

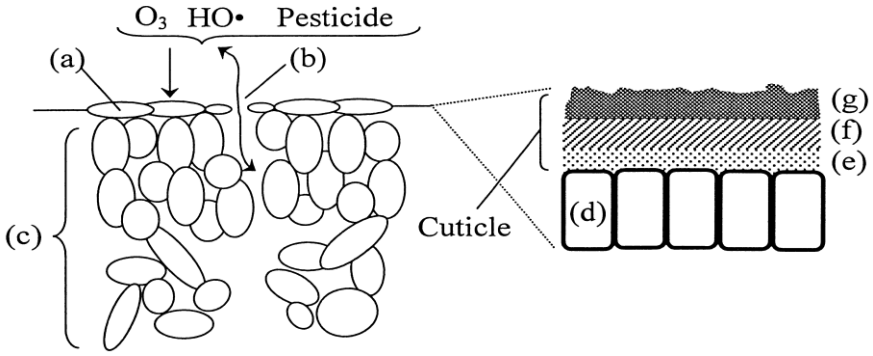


Figure 5.1 Transverse view of the typical surface structure of plant foliage: (a,d) epidermal cell, (b) stoma, (c) mesophyll, (e) pectin, (f) cutin and embedded waxes, (g) epicuticular waxes (Katagi, 2004).

De Bary in 1871, using light microscopy, was the first person to describe leaf waxes calling them ‘kristalloids’ (Koch et al., 2004). The crystalline structure of most plant surface waxes has now been described (Reynhardt, 1997; Meusel et al., 1994, 1999, 2000).

Briefly, plant surface wax is a hydrophobic structure that can be extracted in organic solvents such as chloroform or hexane (Buschhaus and Jetter, 2011). The proportion of major wax components varies between and within the plant species (Beittenmiller, 1996). The same plant may vary in its wax components from part to part, tissue to tissue or organ to organ, depending on genetic and environmental factors, maturation and growth (Beittenmiller, 1996; Angioni et al., 2004). Commonly paraffins are found as wax in leaves of peas and cabbages (Holloway and Jeffree, 2005).

Plant wax morphology and chemistry play an important role in plant-insect interactions (Takahashi et al., 1990), with the outer most boundary of all organs, the plant cuticle, acting as the principal barrier between the plant and its

environment (Bernard and Joubes, 2013). The cuticle plays a vital role in the plant stress response and adaptation and is also involved in plant development. The biochemistry and thickness of the plant cuticle varies between species and in the same species at different developmental times (Jenks et al., 1995; Buschhaus and Jetter, 2011). Plant cuticles comprise of two lipophilic parts, the cutin and the epicuticular wax (Samuels et al., 2008). The latter plays an important role in plant protection against UV radiation and conservation of light for photosynthesis (Holmes and Keiller, 2002) and is a key factor in restricting water loss (Riederer and Schreiber, 2001).

The permeability of the plant cuticle to water and non-ionic compounds (pesticides, herbicides, xenobiotics) has been of particular interest, and is directly proportional to the mobility and solubility of these molecules (Schreiber, 2005). The permeability of plants to water and organic compounds, including pesticides, increased on removal of surface waxes by 10- to 1000-fold (Schönher, 1976; Schönher and Baur, 1994).

The penetration and persistence of foliar pesticide sprays is influenced by various leaf characteristics, including cuticle composition (epicuticular wax, cutin and pectin), leaf angle and the number of stomata (McWhorter, 1985; Wanamarta and Penner, 1989; Riederer and Schreiber, 1995; Kirkwood, 1999; Chowdhury et al., 2005; Riccio et al., 2006) as well as pesticide characteristics, such as formulation, lipophilicity and concentration of active ingredient (Riederer and Schönher, 1988; Bukovac et al., 1990; Baur et al., 1996; Baur et al., 1997). The removal of epicuticular wax has been shown to increase the absorption of pesticide (Kirkwood et al., 1982).

Pesticide persistence in plants is influenced by various factors, including formulation, volatility, solubility, the method of application; and environmental factors, notably temperature, humidity and wind (Willis and McDowell, 1987; Navarro and Navarro, 2007; Haydock et al., 2012; Matthews et al., 2014); and plant characteristics, such as species and life stage (Edward, 1975).

There have been various studies on the effects of plant wax on insect behaviour, including oviposition, feeding, mobility and attachment (Bodnaryk, 1992; Eigenbrode and Espelie, 1995; Juniper, 1995; Eigenbrode, 1996; Brennan et al., 2001; Gorb and Gorb, 2002; Gaume et al., 2002). Less attention has been given to the effect of the epicuticular wax layer on the bioavailability of pesticides to target and non-target insects (Chowdhury et al., 2005). In the present study, the interaction between plant surface wax and efficacy of foliar pesticide applications against *P. xylostella* larvae and *C. vestalis* adults was examined. Due to the loss of the culture, residual experiments could not be conducted with *C. vestalis*.

5.2 Materials and Methods

5.2.1 Plants

Chinese cabbage plants were produced as described in Section 3.1.

5.2.2 Insects

Plutella xylostella and *C. vestalis* cultures were maintained as described in Sections 3.2.1 and 3.2.2.

5.2.3 Chemicals

Test solutions of insecticide formulations (lambda-cyhalothrin 5% EC, Indoxacarb 30% G, Spinosad 48% SC and abamectin 1.8%EC) were prepared in 50 ppm Triton X-100 as described in Section 3.3. HPLC grade chloroform was used for extracting leaf wax (Section 3.3).

5.2.4 Leaf-dip bioassays

Leaf-dip application of insecticides to Chinese cabbage leaf discs (4.8 cm) was as described in Section 3.5.1.1 Bioassays with *P. xylostella* larvae and *C. vestalis* adults were conducted as described in Sections 3.5.1.2 and 3.5.3.2.

5.2.5 Wax extraction

Wax from the leaf discs was extracted with chloroform as described in Section 3.4.

5.2.6 Residual activity on sprayed Chinese cabbage plants

Plants were sprayed with the estimated LC_{50} dose for each insecticide described in section 2.5.2.1 and bioassays conducted with *P. xylostella* larvae as described in Section 3.5.2.2.

5.2.7 Statistical analysis

Data for intrinsic toxicity and pesticide residual activity were analysed in R as described in Section 3.7.

5.3 Results

5.3.1 Leaf surface wax and intrinsic toxicity of pesticides on Chinese cabbage leaves (leaf dip application) to *Plutella xylostella* larvae

Removal of epicuticular wax from freshly-treated (day 0) leaf discs appeared to reduce the residual toxicity (LC_{50}) of each insecticide against *P. xylostella* larvae (2.5 to 7-fold) although none of the differences were significant at the 1% level (Table 5.1).

Table 5.1 Effect of removal of epicuticular wax on the intrinsic toxicity of insecticides on Chinese cabbage leaf discs to *Plutella xylostella* larvae (24 h bioassay).

Insecticide	With wax		Without wax	
	LC_{50} [ppm]* (95% FL)	Slope ± SE	LC_{50} [ppm]* (95% FL)	Slope ± SE
lambda-cyhalothrin	0.3 (0.09-1.1) a,b	0.4 ± 0.07	2.1 (0.03-3.1) a,b	0.1 ± 0.1
abamectin	0.02 (0.05-1.2) a,b	0.3 ± 0.07	0.05 (0.04-0.1) b	3. 5 ± 1.3
indoxacarb	0.8 (0.4-1.6) a	0.8 ± 0.06	4.6 (0.07-27.4) a,b	0.2 ± 0.1
spinosad	1.1 (0.1-10.9) a,b	0.2 ± 0.05	5.0 (0.01-12.6) a,b	0.1 ± 0.1

*Values sharing a common letter have non-overlapping 95% FL and are not significantly different at p>0.01.

5.3.2 Leaf surface wax and intrinsic toxicity of pesticides on Chinese cabbage leaves (leaf dip application) to *Cotesia vestalis* adults

Removal of epicuticular wax from freshly-treated (day 0) leaf discs significantly ($p<0.01$) and consistently reduced (c. 10-fold) the residual toxicity (LC_{50}) of each insecticide against *C. vestalis* adults (Table 5.3). Lambda-cyhalothrin was significantly ($p<0.01$) more toxic than indoxacarb and spinosad on leaves with wax and significantly ($p<0.01$) more toxic than abamectin and indoxacarb on leaves without wax (Table 5.2).

Table 5.2 Effect of removal of epicuticular wax on the intrinsic toxicity of insecticides on Chinese cabbage leaf discs to *Cotesia vestalis* adults (24 h bioassay).

Insecticide	With wax		Without wax	
	LC_{50} [ppm]* (95% FL)	Slope ± SE	LC_{50} [ppm]* (95% FL)	Slope ± SE
lambda-cyhalothrin	8.5 (6.4-11.0) a	1.1 ± 0.3	80.0 (68.3-93.7) c	4.5 ± 2.5
abamectin	10.5 (7.3-13.3) a,b	1.1 ± 0.3	118 (104.4-132.5) d,e	6.9 ± 3.7
indoxacarb	13.4 (12.7-14.2) b	7.2 ± 1.2	138 (126.7-150.2) e	9.3 ± 4.3
spinosad	14.7 (12.1-15.3) b	2.8 ± 0.6	98.4 (89.2-108.4) c,d	8.1 ± 3.3

*Values sharing a common letter have non-overlapping 95% FL and are not significantly different at p>0.01

5.3.3 Leaf surface wax and residual activity of pesticides on Chinese cabbage leaves (spray application) to *Plutella xylostella* larvae

Removal of epicuticular wax significantly reduced the residual activity for each insecticide at different times after application (Table 5.3; F=54.23, d.f=30,31, p<0.001). The greatest reduction in residual toxicity with wax removal occurred at day 0 for each compound.

Table 5.3 Effect of removal of epicuticular wax on the residual toxicity of insecticides on Chinese cabbage to *Plutella xylostella* larvae (24 h bioassay).

Leaf surfaces	Days after treatment	% mortality ± SE			
		lambda-cyhalothrin (2.05ppm)	indoxacarb (4.6ppm)	spinosad (5ppm)	abamectin (0.05ppm)
With wax	0	100	100	100	100
	7	100	63 ± 0.2	43 ± 0.1	30 ± 0.3
	14	60 ± 0.2	27 ± 0.2	10 ± 0.2	10 ± 0.6
Without wax	0	17 ± 0.7	20 ± 0.8	23 ± 0.7	17 ± 0.8
	7	13 ± 0.8	10 ± 0.9	0	7 ± 0.9
	14	10 ± 0.8	0	0	0

5.4 Discussion

The present study found that removal of epicuticular wax reduced by about 11-fold the toxicity of fresh, leaf-dip insecticide deposits on Chinese cabbage for all compounds against adult *C. vestalis* (Table 5.4). This suggests that for recently applied insecticide, most of the active compound remains available within the leaf wax layer for insect contact activity, irrespective of whether the compound applied is known to have translamInar activity (e.g. abamectin) or not (lambda-cyhalothrin) (Iqbal et al., 1996; Kaspi and Parrella, 2005).

Table 5.4 Reduction in toxicity of insecticides after removal of wax against *C. vestalis* adults.

insecticide	(LC_{50} *C. vestalis* without wax/LC_{50} *C. vestalis* with wax)
lambda-cyhalothrin	9.4
abamectin	11.2
indoxacarb	10.3
spinosad	6.7

The same trend was found for leaf-dip bioassays with *P. xylostella* larvae (Table 5.5) but the very high variances observed for the 'without wax' treatment precluded any significant differences between treatments. The greater variances found in the 'without wax' treatment for *P. xylostella* larvae may be due to differences in the rate and depth of feeding on leaf tissues of individual insect larvae, where insecticide pick-up is by feeding as well as contact, compared with lower differences in contact pick up by individual adult parasitoids. Other potential sources of variance

include differences in the amount and composition of epicuticular waxes between different leaves and plants and variation in the efficiency of the wax removal method.

Table 5.5 Reduction in toxicity of insecticides after removal of wax against *P. xylostella* larvae.

insecticide	(LC_{50} *P. xylostella* without wax/LC_{50} *P. xylostella* with wax)
lambda-cyhalothrin	7.0
abamectin	2.5
indoxacarb	5.8
spinosad	4.5

For fresh deposits of insecticide on intact leaves (with wax), the relative toxicity or 'selectivity' (LC_{50} parasitoid/LC_{50} pest) (Table 5.6) was very similar for lambda-cyhalothrin (c. 30-fold), indoxacarb and spinosad (c.15-fold) when compared with abamectin (c. 500-fold). This difference can be accounted for by the much greater intrinsic activity of abamectin compared with the other compounds against *P. xylostella* larvae.

Table 5.6 Relative toxicity of insecticides to *C. vestalis* adults and *P. xylostella* larvae.

insectcicide	Relative toxicity (LC_{50} *C. vestalis* /LC_{50} *P. xylostella*) with intact leaf
lambda-cyhalothrin	28.3
abamectin	525
indoxacarb	16.8
spinosad	13.4

As discussed in the previous chapter (Section 4.4), bioassays with *P. xylostella* larvae indicated that lambda-cyhalothrin and indoxacarb were more persistent than abamectin and spinosad following spray application to Chinese cabbage plants. In the current chapter, using single doses of each insecticide, bioassays with *P. xylostella* on leaves with wax indicated that lambda-cyhalothrin, and to a lesser degree indoxacarb, was more persistent than abamectin and spinosad. When the wax layer was removed prior to bioassay, freshly sprayed deposits of each insecticide (day 0) showed a similar pattern to the leaf-dip experiments, in this case reducing mortality from 100% to c. 20%. The effect of wax removal from leaves 7 and 14 days after spray application on insect mortality indicated that a considerable amount of the total toxic residues still present in the leaf were retained in the surface wax layer. In the case of spinosad, removal of epicuticular wax after 7 days completely removed detectable insecticidal activity; removal of epicuticular wax after 14 days had the same effect for indoxacarb and abamectin.

The finding that all the compounds tested retained a considerable amount of their insecticidal activity in the epicuticular wax layer over a period of weeks is of particular interest. The present work was conducted with plants maintained under relatively controlled glasshouse conditions. For plants under field conditions, where more variable conditions will occur, including temperature, rainfall, wind and ultra violet radiation, pesticide deposits in the epicuticular wax layer would be more vulnerable than those under glass to the process of physical and chemical degradation.

CHAPTER 6: Intrinsic and residual toxicity of insecticides against *Myzus persicae* and *Aphidius colemani*

6.1 Introduction

Myzus persicae infests a wide range of plants in almost 40 different families and has a cosmopolitan distribution (Blackman and Eastop, 1984; Foster et al., 2007; Section 2.9). In addition to causing direct damage to plants through feeding, *M. persicae* is an important vector of plant viruses (Unruh and Willett, 2008). Chemical control remains the principal method of controlling *M. persicae* on field crops and overuse of insecticides has resulted in widespread resistance in this species (Barber et al., 1999, Foster et al., 2007).

Aphidius colemani is a key parasitoid of *M. persicae* (Van Steenis, 1993; Jones et al., 2003), which is available commercially for control of aphids on protected crops (Section 2.10).

In this chapter, studies on the intrinsic and residual toxicity of insecticides on *M. persicae* and *A. colemani* in order to compare the selectivity observed with *P. xylostella* and *C. vestalis* are described (Chapter 4) in a second brassica pest-parasitoid system. Such studies should help define the importance of factors such as insect behaviour in determining insecticide selectivity.

6.2 Materials and Methods

6.2.1 Plants

Chinese cabbage plants were grown in John Innes No. 2 compost in a greenhouse under controlled environment conditions (Section 3.1).

6.2.2 Insect cultures

A *M. persicae* culture was obtained from Koppert (Section 3.2.3). *Aphidius colemani* were provided by Koppert (Section 3.2.4).

6.2.3 Chemicals

Formulated products of lambda-cyhalothrin, abamectin, indoxacarb and spinosad were used (Section 3.3).

6.2.4 Leaf-dip bioassay with *Myzus persicae* and *Aphidius colemani* adults

Leaf-dip bioassays were conducted as described in Sections 3.5.5.1 and 3.5.7.1.

6.2.5 Residual bioassays with *Myzus persicae* and *Aphidius colemani* adults following spray application of insecticides on Chinese cabbage leaves

Chinese cabbage plants were sprayed with insecticides and kept in a greenhouse under controlled environment conditions (Section 3.5.2.1). Residual bioassays with *M. persicae* and *A. colemani* were as described in Sections 3.5.6 and 3.5.8.1.

6.2.6 Statistical analysis

Analyses were conducted using R version 3.0.2 (R Development Core Team, 2013). Estimated LC_{50} values with 95% fiducial limits (FL) and the slope ± S.E. of the dose-response curve were obtained using generalized linear models (GLIM) with binomial errors. Pairwise comparisons of LC_{50} values were significant at the 1% level if their respective 95 % FL's did not overlap (Crawley, 2007). Residual bioassay data at different spray rates were analysed using GLIM with quasibinomial errors to correct for overdispersion, and were subjected to analysis of variance (ANOVA) with significance at the 1% level (Crawley, 2007).

6.3 Results

6.3.1 Leaf dip bioassay with *Myzus persicae* adults

Lambda-cyhalothrin was markedly more toxic ($p<0.01$) than indoxacarb or spinosad under all exposure conditions but was only significantly ($p<0.01$) more toxic than abamectin after 120 h exposure (Table 6.1). Abamectin was significantly

($p<0.01$) more toxic than indoxacarb or spinosad under all exposure conditions. Spinosad was significantly ($p<0.01$) less toxic than the other compounds except compared with indoxacarb after 24 h exposure plus 96 h on untreated leaves. Increased exposure time (120 h) significantly ($p<0.01$) increased the mortality of *M. persicae* for lambda–cyhalothrin compared to 24 h exposure plus 96 h on untreated leaves (Table 6.1, B v C). Increased exposure time had no apparent effect on the *M. persicae* for the other compounds with the exception of indoxacarb (Table 6.1, B v C).

Table 6.1 Intrinsic toxicity of insecticides on Chinese cabbage leaves against *Myzus persicae* adults after: A) 24 h exposure to insecticide; B) 120 h exposure; C) 24 h exposure + 96 h on untreated leaves.

	A		B		C	
Insecticide	LC_{50} [ppm]* (95% FL)	Slope ± SE	LC_{50} [ppm]* 95% FL)	Slope ± SE	LC_{50} [ppm]* (95% FL)	Slope ± SE
lambda-cyhalothrin	4.1 (2.6-6.3)[a]	1.6 ± 0.6	1.7 (1.5-1.9)[c]	5.2 ± 0.9	3.5 (3.0-4.5)[a]	1.8 ± 0.7
abamectin	5.5 (1.4-7.1)[a]	3.1 ± 1.1	3.8 (3.5-4.0)[a]	2.5 ± 0.5	4.4 (3.7-5.4)[a]	3.9 ± 1.1
indoxacarb	18.1 (15.6-19.6)[b]	3.3 ± 1.0	13.5 (12.7-14.4)[d]	7.5 ± 1.3	16.4 (13.5-20.0)[b]	3.1 ± 1.0
spinosad	24.5 (22.1-27.4)[b]	3.9 ± 0.8	21.7 (19.5-24.0)[b]	5.2 ± 1.0	23.1 (18.9-28.1)[b]	3.3 ± 0.9

*Values within columns sharing a common letter have non-overlapping 95% FLs and are not significantly different at $p<0.01$.

6.3.2 Residual activity of insecticides against *Myzus persicae* adults following spray application on Chinese cabbage leaves

All four compounds showed a significant ($p<0.01$) decrease in residual activity with time (Table 6.2). Residual activity for each compound was positively correlated with the spray concentration applied (Figures 6.1–6.4). Lambda-cyhalothrin showed the greatest persistence in activity, although activity decreased significantly ($F=13.5$, $d.f=8,14$ $p<0.001$) 28 days after spray application (Table 6.2; Figure 6.1). Indoxacarb showed a significant ($F=11.0$, $d.f=8,14$, $p<0.001$) decline in residual activity after day 14; little residual activity remained after 28 days (Table 6.2; Figure 6.3). Both abamectin ($F=7.5$, $d.f=8,14$, $p<0.001$) and spinosad ($F=9.2$, $d.f=8,14$, $p<0.001$) showed a significant reduction in residual activity after 7 days (Table 6.2; Figures 6.2 and 6.4).

Table 6.2 Residual toxicity (LC_{50}) of insecticide deposits on Chinese cabbage against *Myzus persicae* adults.

Days after application	Insecticides	LC_{50} [ppm] (95% FL)	Slope ± SE
0	lambda-cyhalothrin	4.01 (3.3-4.9)	2.6 ± 0.6
7	lambda-cyhalothrin	8.1 (5.7-11.4)	1.4 ± 0.5
14	lambda-cyhalothrin	23.6 (4.3-130.6)	0.9 ± 0.6
28	lambda-cyhalothrin	39.9 (13.5-117.3)	0.3 ± 0.3
0	abamectin	5.9 (5.2-6.8)	3.2 ± 0.7
7	abamectin	25.5 (4.8-133.7)	2.5 ± 1.3
14	abamectin	<8% mortality (at maximum applied dose of 10 ppm)	
0	indoxacarb	23.4 (21.3-25.7)	5.0 ± 1.1
7	indoxacarb	42.9 (31.6-58.3)	10.3 ± 6.1
14	indoxacarb	56.1 (26.3-119.3)	2.4 ± 1.4
28	indoxacarb	<6% mortality (at maximum dose of 45 ppm)	
0	spinosad	35.7 (32.4-39.5)	5.1 ± 1.6
7	spinosad	97.8 (41.9-226.3)	1.7 ± 0.9
14	spinosad	<15% mortality (at maximum dose of 60ppm)	

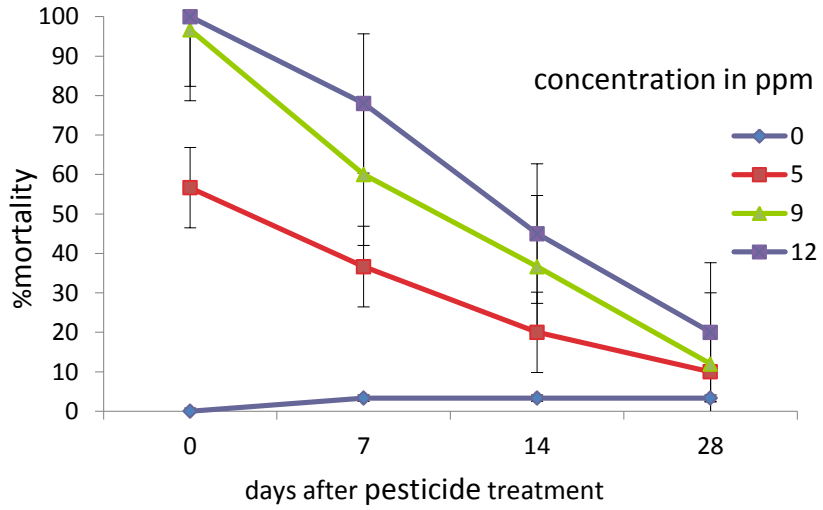


Figure 6.1 Residual activity (% mortality ± S.E) of lambda-cyhalothrin on Chinese cabbage against *Myzus persicae* adults following spray application.

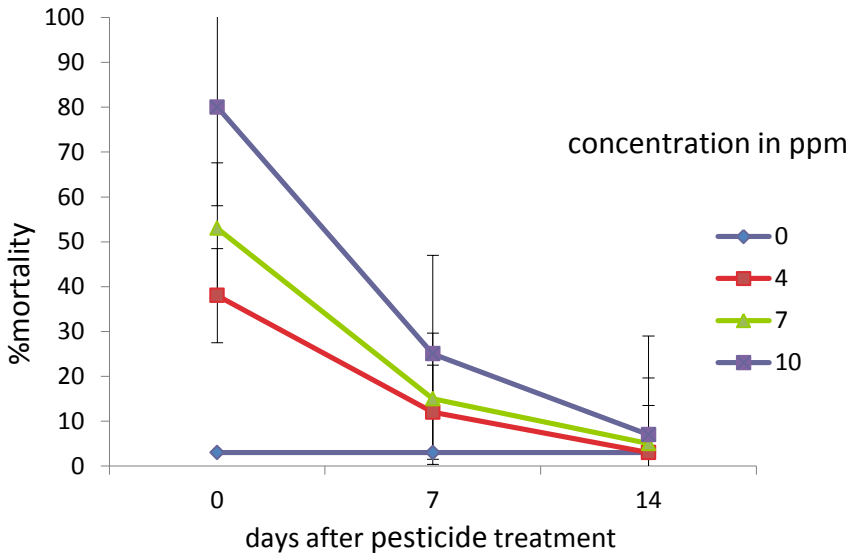


Figure 6.2 Residual activity (% mortality ± S.E.) of abamectin on Chinese cabbage against *Myzus persicae* adults following spray application.

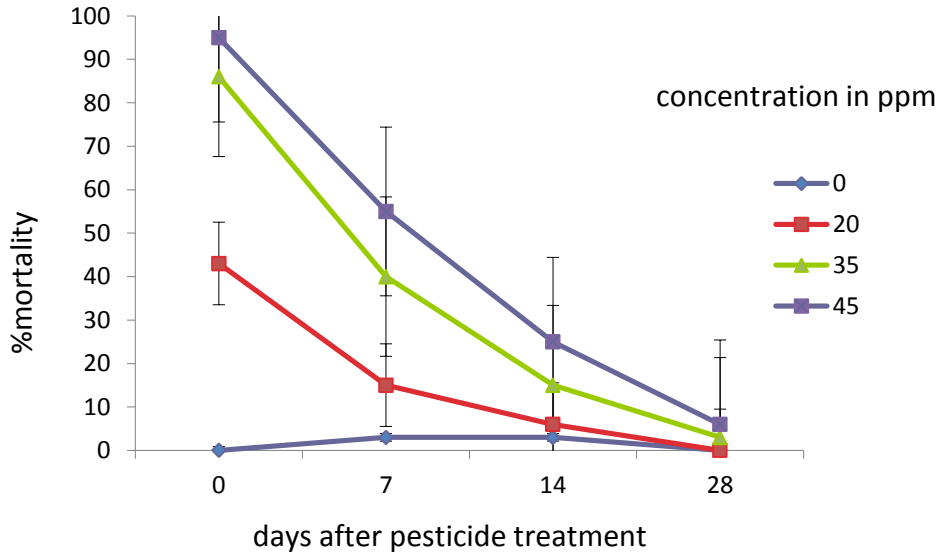


Figure 6.3 Figure 4.3 Residual activity (% mortality ± S.E.) of indoxacarb on Chinese cabbage against *Myzus persicae* adults following spray application.

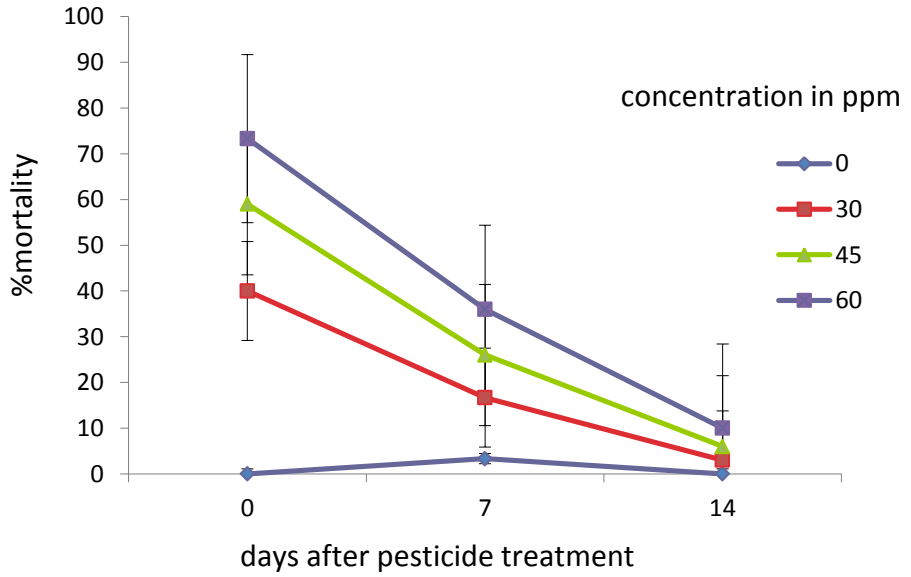


Figure 6.4 Residual activity (% mortality ± S.E.) of spinosad on Chinese cabbage against *Myzus persicae* adults following spray application.

6.3.3 Leaf dip bioassay with *Aphidius colemani* adults

Lambda-cyhalothrin was markedly more toxic ($p<0.01$) than indoxacarb or spinosad under all exposure conditions but was only significantly ($p<0.01$) more toxic than abamectin at 120 h exposure (Table 6.3). Abamectin was significantly ($p<0.01$) more toxic than indoxacarb or spinosad. Spinosad was significantly ($p<0.01$) less toxic than the other compounds. Increased exposure time (120 h) significantly ($p<0.01$) increased mortality of *A. colemani* for lambda–cyhalothrin and indoxacarb compared 24 h exposure plus 96 h on untreated leaves. Increased exposure time had no apparent effect on the mortality of *A. colemani* for abamectin or spinosad (Table 6.3).

Table 6.3 Intrinsic toxicity of insecticides on Chinese cabbage against *Aphidius colemani* adults after: A) 24 h exposure to insecticide; B) 120 h exposure; C) 24 h exposure + 96 h on untreated leaves.

	A		B		C	
Insecticide	LC_{50} [ppm])*		LC_{50} [ppm]*		LC_{50} [ppm]*	
	(95% FL	Slope ± SE	(95% FL)	Slope ± SE	(95% FL)	Slope ± SE
lambda-cyhalothrin	4.9 (2.7-8.8)[a]	1.7 ± 0.7	2.5 (2.1-4.2)[d]	2.2 ± 0.6	3.9 (3.5-4.5)[a]	3.1 ± 0.5
abamectin	6.6 (4.8-9.3)[a]	1.9 ± 0.6	3.9 (3.2-4.8)[a]	1.5 ± 0.6	4.2 (3.7-4.9)[a]	2.5 ± 0.5
indoxacarb	19.6 (18.5-20.7)[b]	7.5 ± 1.4	16.4 (15.8-17.2)[b]	9.9 ± 1.9	18.5 (17.6-19.4)[b]	7.7 ± 1.3
spinosad	27.1 (23.1-29.5)[c]	4.7 ± 1.3	22.2 (17.5-28.1)[c]	1.9 ± 0.6	25.3 (24.3-27.2)[c]	8.5 ± 1.4

*Values within columns sharing a common letter are non-overlapping at 95% FL and are not significantly different at $p<0.01$.

6.3.4 Residual activity of insecticides against adults *Aphidius colemani*

All four compounds showed a significant ($p<0.01$) decrease in residual activity with time (Table 6.4). Residual activity for each compound was positively correlated with the spray concentration applied (Figures 6.5–8.8). Lambda-cyhalothrin showed the greatest persistence in activity, although activity decreased significantly ($F=7.95$, $d.f=4,8$ $p<0.01$) 28 days after spray application (Table 6.4; Figure 6.5). Indoxacarb showed a significant ($F=5.6$, $d.f=4,8$ $p<0.01$) decline in residual activity after day 14; little residual activity remained after 28 days (Table 6.4; Figure 6.7). Both abamectin ($F=4.8$, $d.f=4,8$, $p<0.01$) and spinosad ($F=3.7$, $d.f=4$, $p<0.01$) showed a significant reduction in residual activity after 7 days (Table 6.4; Figures 6.6 and 6.8).

Table 6.4 Toxicity of insecticides at different days after treatment against *Aphidius colemani* adults.

Days after application	Insecticides	LC_{50} [ppm] (95% FL)	Slope ± SE
0	lambda-cyhalothrin	5.5 (3.94-7.8)	3.6 ± 1.6
7	lambda-cyhalothrin	7.01 (2.1-23.8)	0.8 ± 0.6
14	lambda-cyhalothrin	30.3 (5.1-180.5)	1.1 ± 0.9
28	lambda-cyhalothrin	48.4(33.3-70.2)	1.3 ± 0.5
0	abamectin	7.9 (3.10-12.02)	3.8 ± 1.4
7	abamectin	19.5 (9.5-40.4)	1.3 ± 0.8
14	abamectin	<10% mortality (at maximum dose of 20 ppm)	
0	Indoxacarb	29.1 (24.8-34.2)	4.7 ± 1.6
7	Indoxacarb	44.7 (30.5-65.5)	1.4 ± 0.8
14	Indoxacarb	66.8(45.5-73.5)	2.1 ± 0.8
28	indoxacarb	<5% mortality (at maximum dose of 45 ppm)	
0	spinosad	37.2 (33.3-41.5)	4.2 ± 0.9
7	spinosad	94(33.9-262.01)	2.5 ± 1.6
14	spinosad	<10% mortality (at maximum dose of 60 ppm)	

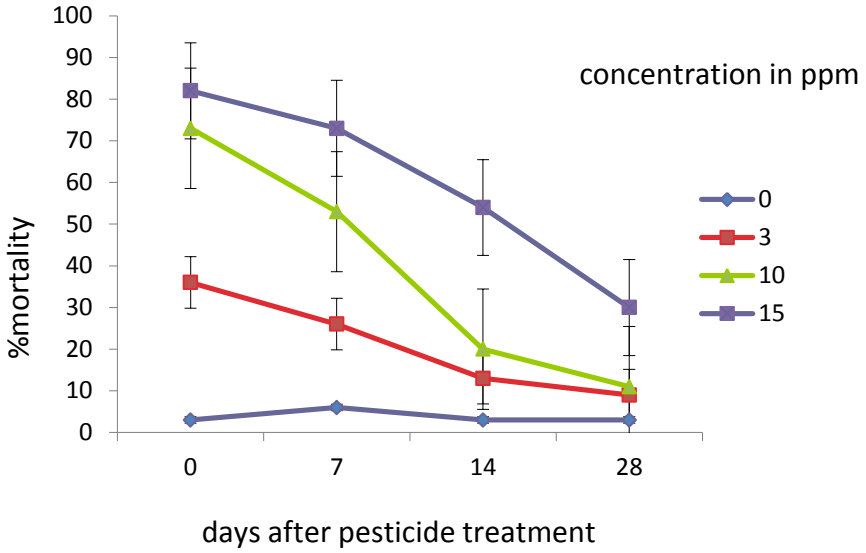


Figure 6.5 Residual activity (% mortality ± S.E) of lambda-cyhalothrin on Chinese cabbage against *Aphidius colemani* adults following spray application.

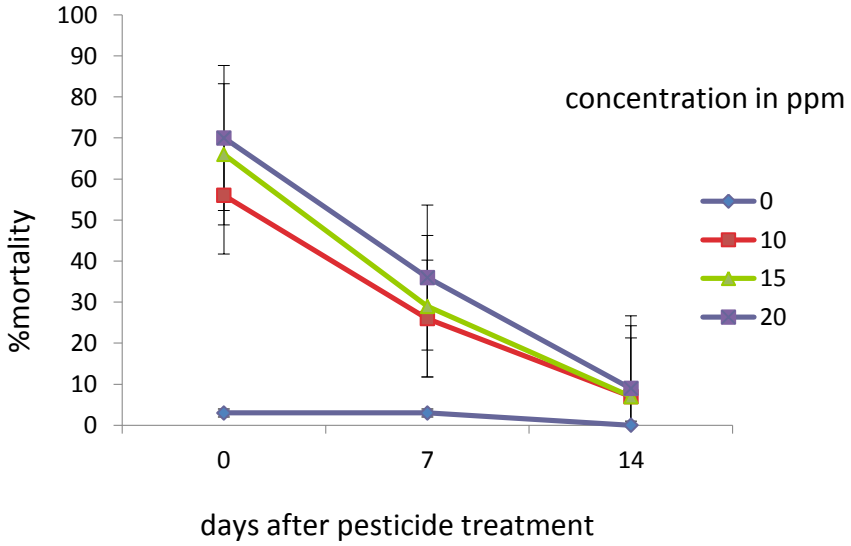


Figure 6.6 Residual activity (% mortality ± S.E) of abamectin on Chinese cabbage against *Aphidius colemani* adults following spray application.

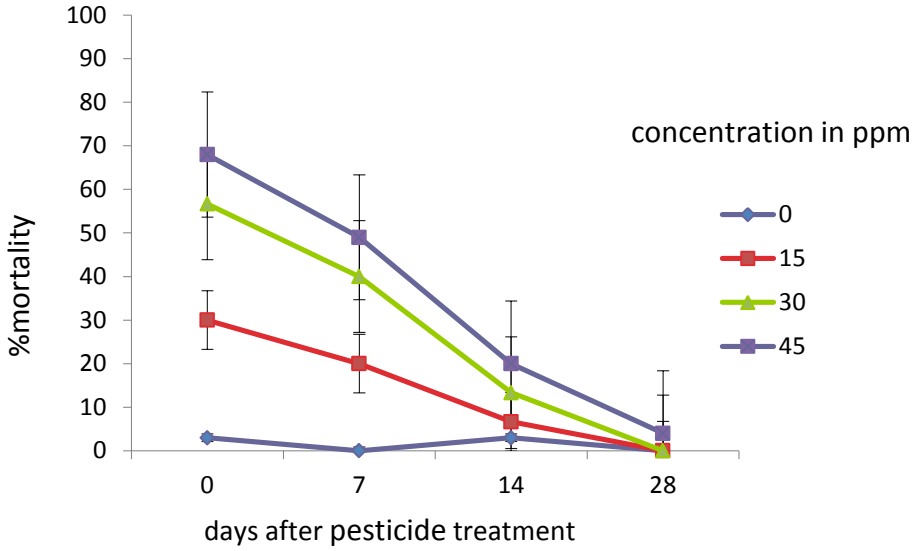


Figure 6.7 Residual activity (% mortality ± S.E) of indoxacarb on Chinese cabbage against *Aphidius colemani* adults following spray application.

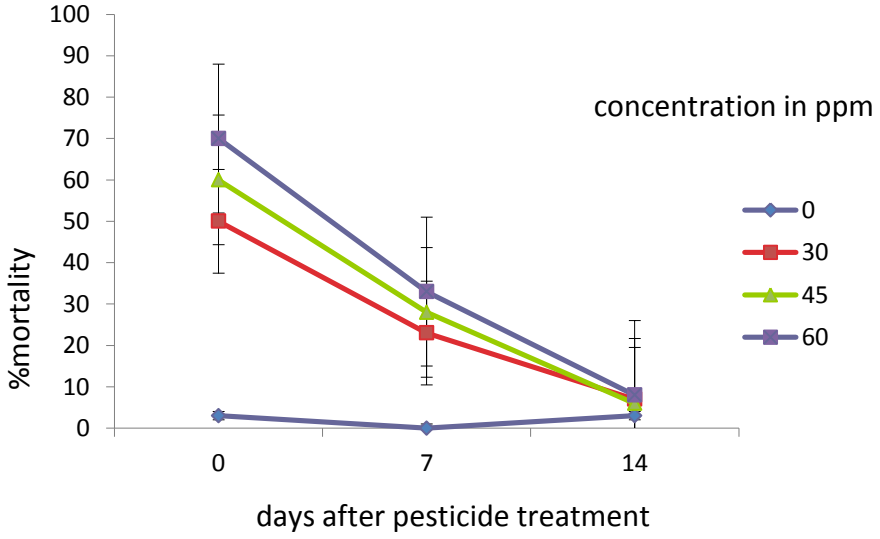


Figure 6.8 Residual activity (% mortality ± S.E) of spinosad on Chinese cabbage against *Aphidius colemani* adults following spray application.

6.4 Discussion

In contrast to *P. xylostella* larvae and *C. vestalis* adults, where there was a marked difference in the intrinsic activity of the insecticides tested between the pest and the parasitoid (Chapter 4), there was no apparent difference in intrinsic toxicity of these compounds to *M. persicae* adults and *A. colemani* adults (Figure 6.9). This difference in response between the two brassica pest-parasitoid systems was due largely to the much lower toxicity of each compound against *M. persicae* adults compared with *P. xylostella* larvae.

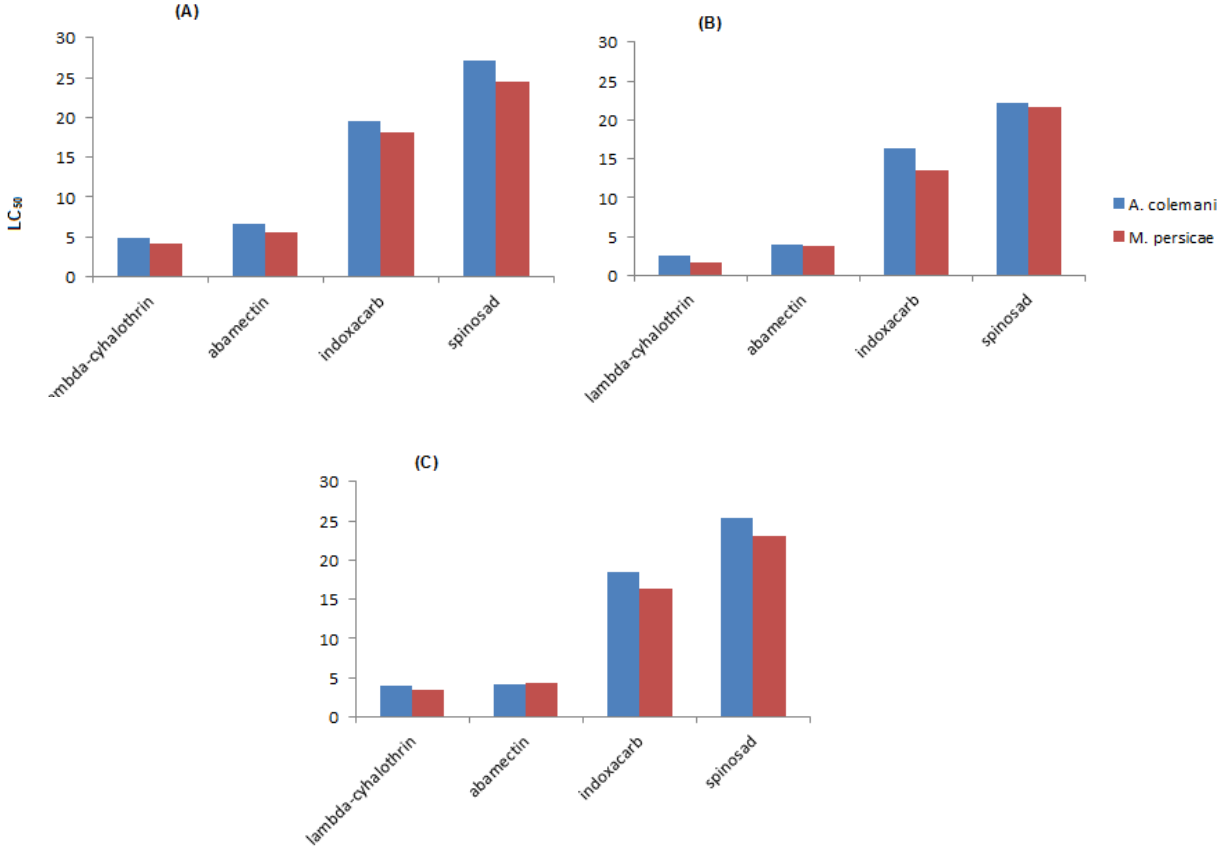


Figure 6.9 Comparing LC_{50} values of four insecticides against *M. persicae* and *A. colemani* adults. (A) 24 h exposure to insecticide; (B) 120 h exposure; (C) 24 h exposure + 96 h on untreated leaves.

While the toxicity for each compound was relatively similar for *C. vestalis* and *A. colemani*, the data did suggest that indoxacarb and spinosad were about 3-fold less toxic against the latter parasitoid species.

The impact of insecticides on non-target organisms is influenced by the routes of exposure (Longley and Stark, 1996), pesticide persistence (Wennergren and Stark, 2000) and dose (Desneux et al., 2004). The differences in relative toxicity found for each of the insecticides tested against the two pest species in the present study most likely reflects differences in the activity of the insects on foliage and in particular their feeding behaviour, which would influence the rate of uptake of toxicant by contact action and by ingestion, respectively. Differences in the toxicity of insecticides related to differences in insect feeding behaviour have been reported by several authors (Shi et al., 2004; Sarfraz and Keddie, 2005). Interacting effects due to differences in target site activity between the aphid and parasitoid species examined in the present study are also likely.

Of the insecticides tested, lambda-cyhalothrin is reported to be active against a wide range of insects, including aphids, lepidopteran and coleopteran species (Jutsum et al., 1984, Vandekerkhove and Clercq, 2004), its activity is greater by ingestion rather than by contact action (Vandekerkhove and Clercq, 2004). Abamectin is very active against phytophagous mite species, dipteran leafminers and various lepidopteran species, including *P. xylostella*, and is also reported to have activity against aphids, thrips and whiteflies (Dybas, 1989; Dehghani et al., 2012). Indoxacarb and spinosad are primarily marketed against lepidopteran pests and are not reported to be particularly active against aphid species (Nauen and Bretschneider, 2002; Grafton-Cardwell et al., 2005).

The relatively similar levels of intrinsic activity of each insecticide against the two parasitoid species (Figure 6.10) might suggest that uptake through contact activity was relatively similar for the two species, although, as above, interacting effects of differences in target site activity between species could be involved. Predators show more variable responses to pesticides than the parasitoids (Theiling and Croft, 1988), which may reflect differences in rates of prey consumption as well as differences in contact activity on foliage.

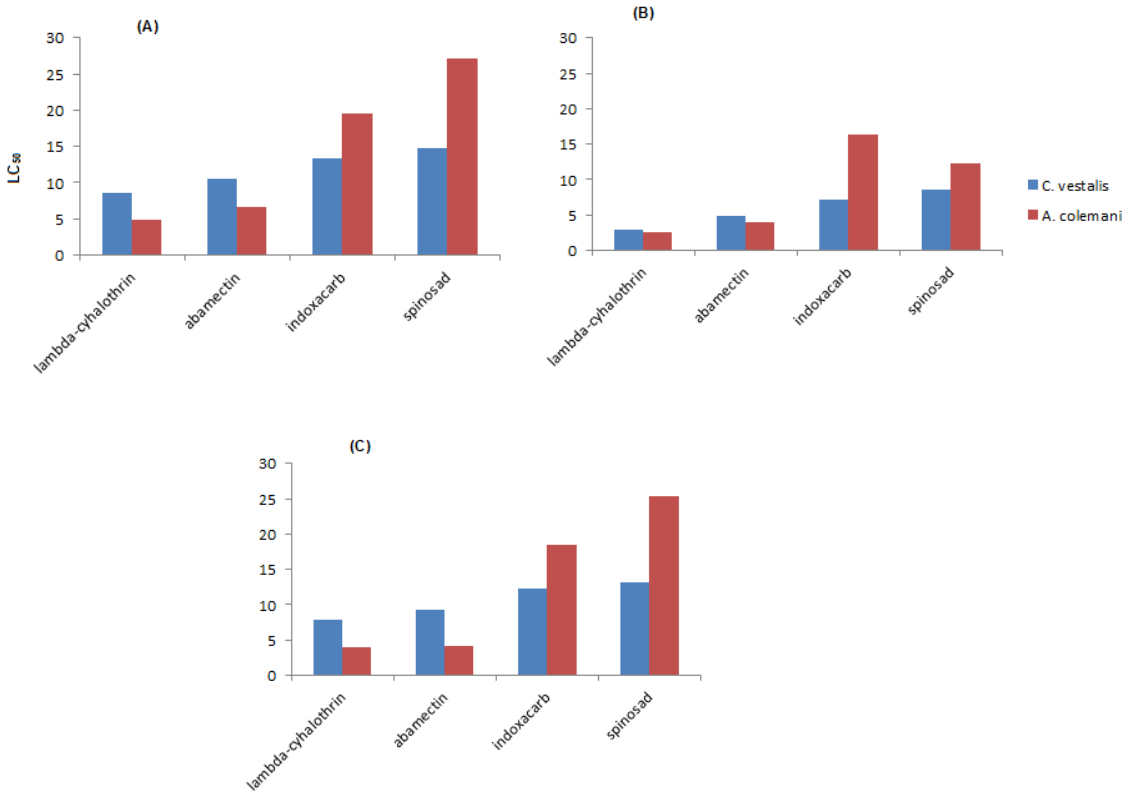


Figure 6.10 Comparing LC_{50} values of four insecticides against *C. vestalis* and *A. colemani* adults. (A) 24 h exposure to insecticide; (B) 120 h exposure; (C) 24 h exposure + 96 h on untreated leaves.

In the next section (Chapter 7), the distribution of toxic pesticide residues between the epicuticular wax layer and underlying leaf tissues is examined using insect bioassays to determine whether the pattern of activity found in wax stripping experiments with *P. xylostella* and *C. vestalis* (Chapter 5) also applies to a comparison between a phloem-feeder (*M. persicae*) and another parasitoid species (*A. colemani*).

The persistence of residual toxicity was found to be the same against both *M. persicae* and *A. colemani*, and followed the same pattern as in experiments with *P. xylostella* and *C. vestalis* (Chapter 4). These findings are, as discussed previously (Section 4.4), in agreement with previous studies on these compounds. The greater persistence of lambda-cyhalothrin on foliage makes it a potentially greater threat to the environment and non-target organisms, including natural enemies, compared with the less persistent compounds examined particularly abamectin and spinosad (Starner, 2007; Wali et al., 2007).

CHAPTER 7: Effect of leaf surface wax on Intrinsic and residual toxicity of insecticides against *Aphidius colemani* and *Myzus persicae*

7.1 Introduction

In this Chapter, similar studies to those conducted with *P. xylostella* and *C. vestalis* (Chapter 5) are described. The primary aim was to examine further the potential influence of feeding behaviour of *M. persicae* compared with *P. xylostella* larvae on the intrinsic insecticide toxicity as the insecticides partition in the epicuticular wax layer and underlying leaf tissues. Residual bioassays with *A. colemani*, with and without leaf wax, were also conducted.

7.2 Materials and Methods

7.2.1 Plants

Chinese cabbage plants were produced as described in Section 3.1.

7.2.2 Insects

Myzus persicae and *A. colemani* adults were obtained as described in Sections 3.2.3 and 3.2.4.

7.2.3 Chemicals

Test solutions of lambda-cyhalothrin, indoxacarb, spinosad and abamectin were prepared as described in Sections 3.3 and 5.2.3. HPLC grade chloroform was used for wax extraction (Section 3.3).

7.2.4 Leaf dip bioassays

Leaf-dip application of insecticides to Chinese cabbage leaf discs (4.8 cm) was as described in Section 3.5.1.1. Half of the treated leaf discs were subjected to wax removal (Section 3.4). Leaf discs without pesticide, were used as controls. Bioassays with *M. persicae* and *A. colemani* adults were set up as described in Sections 3.5.5.2 and 3.5.7.2. Three adult *M. persicae* or *A. colemani* were introduced to each arena. Mortality was recorded after 24 h exposure to each treatment including the control. Each treatment was replicated 10 times (n=30).

7.2.5 Wax extraction

Wax from the leaf discs was extracted with chloroform as described in Section 3.4.

7.2.6 Residual activity on sprayed Chinese cabbage plants

Chinese cabbage plants were sprayed with insecticides and kept in a greenhouse under controlled environment conditions (Section 3.5.2.1). Half of the treated leaf discs were subjected to wax removal (Section 3.4). Leaf discs without

pesticide, with and without wax removed were used as controls. Residual bioassays with *A. colemani* were as described in Section 3.5.8.2. Three adult *A. colemani* were introduced to each arena. Mortality was recorded after 24 h exposure to each treatment including the control. Each treatment was replicated 10 times (n=30).

7.2.7 Statistical analysis

Data for intrinsic and residual toxicities were analysed using R, version 3.0.2 (R Development Core Team, 2013) (Sections 3.7 and 6.2.6).

7.3 Results

7.3.1 Leaf surface wax and intrinsic toxicity of pesticides on Chinese cabbage leaves (leaf dip application) to *Myzus persicae* adults

Removal of epicuticular wax from freshly-treated (day 0) leaf discs markedly reduced ($p<0.01$) the residual toxicity (LC_{50}) of each insecticide against adult *M. persicae* (10 to 27-fold; Table 7.1). Removal of epicuticular wax also reduced the difference in relative toxicity between the compounds tested, with lambda-cyhalothrin and abamectin significantly ($p<0.01$) more toxic (4.5- to 6-fold) than indoxacarb and spinosad on intact leaves but only apparently 2-fold more toxic (not significant, $p>0.01$) on leaves without wax (Table 7.1).

Table 7.1 Effect of removal of epicuticular wax on the intrinsic toxicity of insecticides on Chinese cabbage leaf discs to adults of *Myzus persicae* (24 h bioassay).

Insecticide	With wax		Without wax	
	LC_{50} [ppm]* (95% FL)	Slope ± SE	LC_{50} [ppm]* (95% FL)	Slope ± SE
lambda-cyhalothrin	4.1 (2.6-6.3) a	1.6 ± 0.6	111 (106-156) d	3.3 ± 1.9
abamectin	5.5 (1.4-7.1) a	3.1 ± 1.1	135 (114-161) d	6.7 ± 2.8
indoxacarb	18.1 (15.6-19.6) b	3.3 ± 1.0	229 (131-398) d	2.0 ± 1.8
spinosad	24.5 (22.1-27.4) c	3.9 ± 0.8	249 (154-401) d	3.2 ± 2.2

*Values sharing a common letter have non-overlapping 95% FL and are not significantly different at p>0.01.

7.3.2 Leaf surface wax and intrinsic toxicity of pesticides on Chinese cabbage leaves (leaf dip application) against *Aphidius colemani* adults

Removal of epicuticular wax from freshly-treated (day 0) leaf discs markedly reduced ($p<0.01$) and consistently reduced the residual toxicity (LC_{50}) of each insecticide against *A. coelmani* adults (11- to 18-fold; Table 7.2). Removal of epicuticular wax had less effect on the relative toxicity of the different compounds tested, with lambda-cyhalothrin and abamectin significantly ($p<0.01$) more toxic than indoxacarb and spinosad on intact leaves (3- to 5.5-fold) and on leaves without wax (3- to 3.5-fold; Table 7.2).

Table 7.2 Effect of removal of epicuticular wax on the intrinsic toxicity of insecticides on Chinese cabbage leaf discs to adults of *Aphidius colemani* (24 h bioassay).

Insecticide	With wax		Without wax	
	LC_{50} [ppm]* (95% FL)	Slope ± SE	LC_{50} [ppm]* 95% FL	Slope ± SE
lambda-cyhalothrin	4.9 (2.7-8.8) a	1.7 ± 0.7	89.8 (67.23-120) d	1.5 ± 0.5
abamectin	6.6 (4.8-9.3) a	1.9 ± 0.6	85.3 (71.2-102) d	2.8 ± 0.6
indoxacarb	19.6 (18.5-20.7) b	7.5 ± 1.4	279 (165-471) e	1.1 ± 0.5
spinosad	27.1 (23.1-29.5) c	4.7 ± 1.3	294 (216-399) f	1.4 ± 0.8

*Values sharing a common letter have non-overlapping 95% FL and are not significantly different at p>0.01.

7.3.3 Leaf surface wax and residual toxicity of pesticides on Chinese cabbage leaves (spray application) to *Aphidius colemani* adults

The residual activity of the compounds tested against *A. colemani* adults declined from day 0 to day 14 after spray application (Table 7.3; F= 48.32, d.f= 82,72, p<0.001). Removal of epicuticular wax markedly reduced the residual activity for each compound, with the greatest reduction in toxicity with wax removal occurring at day 0 (Table 7.3).

Lambda-cyhalothin showed the greatest residual toxicity at day 7 and day 14 leaves compared with the other compounds tested; removal of wax significantly reducing its toxicity at day 0 (F=3.9, d.f=116,149, p<0.001) and day 7 (F=5.7, d.f=116, 149, p<0.001), with little toxicity remaining after 14 days (Table 7.3; F=0, d.f= 116, 149, p<0.001).

Indoxacarb appeared to show greater levels of residual activity compared with spinosad and abamectin at day 7 and day 14 after spray application (Table 7.3); removal of wax significantly reduced its toxicity at day 0 (F=3.5, d.f=116, 119, p<0.001), day 7 (F=0, d.f=116, p<0.001) and day 14 (F =0, d.f=116, 119, p<0.001).

On intact leaves, abamectin and spinosad had little remaining residual toxicity left at day 14 (Table 7.3). For abamectin and spinosad, removal of leaf wax significantly reduced residual toxicity at day 0 (F=22.31, d.f=116, 119, p<0.001 and F=4.11, d.f=116, 119, p<0.001, respectively) and day 7 (F=0, d.f=116,119 P<0.001 and F=2.9, d.f=116,119, p<0.05, respectively), with no detectable residual activity for either compound at day 14 (Table 7.3).

Table 7.3 Effect of removal of epicuticular wax on the residual toxicity of insecticides on Chinese cabbage to adult *Aphidius colemani* (24 h bioassay).

Leaf surfaces	Days after treatment	% mortality ± SE			
		lambda-cyhalothrin (90ppm)	abamectin (85ppm)	indoxacarb (279ppm)	spinosad (294ppm)
With wax	0	100	100	100	100
	7	100	45 ± 0.3	77 ± 0.2	59 ± 0.3
	14	75 ± 0.2	10 ± 0.6	38 ± 0.3	12 ± 0.6
Without wax	0	28 ± 0.5	22 ± 0.6	24 ± 0.6	24 ± 0.6
	7	19 ± 0.4	12 ± 0.6	14 ± 0.5	10 ± 0.6
	14	12 ± 0.5	No mortality	3 ± 0.8	No mortality

7.4 Discussion

The present study found that removal of epicuticular wax reduced the toxicity of fresh, leaf-dip insecticide deposits by 11- to 18-fold on Chinese cabbage against *A. colemani* adults (Table 7.4), similar findings to those obtained for *C. vestalis* (Chapter 5). This again suggests that for recently applied insecticide, most of the active compound remains available within the leaf wax layer for insect contact activity, irrespective of whether the compound applied is known to have translaminar activity (Section 5.4 and below).

Table 7.4 Reduction in toxicity of insecticides after removal of wax against *A. colemani* adults.

insecticide	(LC_{50} *A. colemani* without wax/LC_{50} *A. colemani* with wax)
lambda-cyhalothrin	18.3
abamectin	12.9
indoxacarb	14.2
spinosad	10.8

The same trend was found for leaf-dip bioassays with adult *M. persicae* with particularly marked reductions in the residual toxicity for lambda-cyhalothrin (c. 27-fold) and abamectin (c. 25-fold) following wax removal (Table 7.5). In contrast to similar experiments with *P. xylostella* (Chapter 5), the experimental variances observed for the 'without wax' treatments for *M. persicae* were similar to those observed for intact leaves. This suggests that the greater variances observed with *P. xylostella* larvae are more likely to be related to greater variance in feeding activity and behaviour (e.g. leaf area versus depth of tissue eaten) in the latter species, than to differences in epicuticular waxes between different leaves and plants and variation in the efficiency of the wax removal method (Section 5.4).

Table 7.5 Reduction in toxicity of insecticides after removal of wax against *M. persicae* adults.

insecticide	(LC_{50} *M. persicae* without wax/LC_{50} *M. persicae* with wax)
lambda-cyhalothrin	27
abamectin	24.5
indoxacarb	12.7
spinosad	10.2

For fresh deposits of insecticide on intact leaves, the relative toxicity or 'selectivity' (LC_{50} parasitoid/LC_{50} pest) was very similar for all compounds (Table 7.6), with no significant differences involved in the toxicity of each particular compound against pest or parasitoid. This is in contrast to the much greater selectivity of these compounds against *P. xylostella* compared with *C. vestalis*, with abamectin showing 500-fold selectivity (Section 5.3). This difference can be accounted for by the much greater intrinsic activity of all compounds, and in particular abamectin, against *P. xylostella* (Section 5.3) compared with *M. persicae.* In contrast, the intrinsic toxicity of each compound was similar against the two parasitoid species tested (Sections 5.3 and 7.3).

Table 7.6 Relative toxicity of insecticides to *C. vestalis* adults and *P. xylostella* larvae.

insectcicide	Relative toxicity (LC_{50} *A. colemani* /LC_{50} *M. persicae*) with intact leaf
lambda-cyhalothrin	1.2
abamectin	1.2
indoxacarb	1.1
spinosad	1.1

As discussed in previous chapters (Sections 4.4, 5.4 and 6.4), bioassays with *P. xylostella*, *C. vestalis* and *M. persicae* have all indicated that lambda-cyhalothrin and indoxacarb are more persistent than abamectin and spinosad following spray

application to Chinese cabbage plants. Similar results were obtained in the present experiment with *A. colemani*.

The leaf wax experiments with *A. colemani* showed a marked reduction in residual activity at day 0 for all compounds following wax removal, indicating that a considerable proportion of insecticidal activity was in the epicuticular wax at this point and available for contact pick up by the parasitoid. In Section 5.4, it was noted that this appeared to be irrespective of whether the compound applied had significant translamlnar activity (e.g. abamectin) or was relatively immobile (lambda-cyhalothrin) (Iqbal et al., 1996; Kaspi and Parrella, 2005).

Wax stripping at day 7 gave a greater reduction in residual toxicity for lambda-cyhalothrin and indoxacarb compared with abamectin and spinosad. This might suggest that a greater proportion of abamectin and spinosad had been absorbed into the leaf tissues below the wax layer compared with the other two compounds but it may also reflect a relatively greater degradation rate for abamectin and spinosad in the surface wax layer (Bull et al., 1984; Thompson et al., 2000).

CHAPTER 8: Sub-lethal effects of insecticide residues on parasitoids

8.1 Introduction

Biological control agents, including parasitoids, conserve Agro ecosystems by providing protection against pest populations, for example in British Columbia, synergistic suppression of winter moth (*Operophtera brumata*) was achieved by introduction of parasitoids (*Cyzenis albicans* and *Agrypon jlaveolatum*) and rove beetle predators that were already present (Roland and Embree, 1995; Gardiner et al., 2011).

Parasitoids represented mainly in two major orders of the Insecta, the Hymenoptera (including 45 families) and the Diptera (particularly the family Tachinidae) (Wajnberg and Colazza, 2013). Before release of a non-indigenous parasitoid species into the environment for biological control (Section 2.7), a detailed risk analysis is required, including host specificity tests and off-target effects (DAFF, 2013).

Agrochemicals, particularly insecticides, can present a major negative influence on the effectiveness of parasitoids in controlling pest populations (Stavrinides and Mills, 2009). Usually, the effects of these chemicals on parasitoids are measured in terms of mortality (He et al., 2013) but in the outside world sub-lethal effects can also be important (Desneux et al., 2007).

Sub-lethal effects of pesticides are defined as “effects, either physiological or behavioural, on individuals that survive exposure to a pesticide (the pesticide dose/concentration can be sub-lethal or lethal)” (Desneux et al., 2007).

Initially registration of pesticides for compatibility in IPM was based on acute toxicity (mortality) on biological control agents (Candolfi et al., 2000). With greater awareness of their importance, researchers have now concentrated more on the sub-lethal effects that a pesticide can have on biological control agents (Desneux et al., 2004; Desneux et al., 2007; Biondi et al., 2012a; Wrinn et al., 2012).

Sub-lethal effects of pesticides can be divided into two major categories: a) physiology and b) behavioural (Desneux et al., 2007; Biondi et al., 2013). Most studies to date on sub-lethal effects of pesticides have been on reproduction (Bengochea et al., 2013), longevity (Planes et al., 2013), orientation (Campos et al., 2011) and feeding (He et al., 2013).

The primary aim of the present study was to evaluate the effect of insecticide residues on leaf surfaces on the time spent on leaves by *C. vestalis* and *A. colemani* in no-choice and choice arenas.

8.2 Materials and Methods

8.2.1 Plants

Chinese cabbage plants were grown in John Innes No. 2 compost in a greenhouse under controlled environment conditions (Section 3.1).

8.2.2 Insect cultures

Maintenance of *Cotesia vestalis* and *A. colemani* was as described previously (Section 3.2).

8.2.3 Chemicals

Formulated products of lambda-cyhalothrin, abamectin, indoxacarb and spinosad were used (Section 3.3).

8.2.4 Insecticide application to leaf discs

Leaves were randomly selected from Chinese cabbage plants, and 4.8 cm dia. leaf discs dipped in insecticide solution and bioassays set up as described previously (Section 3.5). Doses selected for experiments on sub-lethal effects were based on the LC_{50} and LC_5 values for each insecticide against each parasitoid species (Table 8.1). The LC_{50} dose was used to examine the behavioural abilities of parasitoids that survived this high dose, while LC_5 dose was used as a sublethal dose to evaluate the response of parasitoids to this lower dose. Control leaf discs were treated with Triton X-100 solution.

Table 8.1 LC_{50} and LC_5 doses used to evaluate the sub-lethal effects on parasitoids.

	Cotesia vestalis		*Aphidius colemani*	
	LC_5 dose (ppm)	LC_{50} dose (ppm)	LC_5 dose (ppm)	LC_{50} dose (ppm)
Lambda-cyhalothrin	0.8	7.9	1.4	3.9
Abamectin	0.08	9.3	1.3	4.2
Indoxacarb	8.8	12.2	12.6	18.5
Spinosad	5.7	13.1	15.4	25.3

8.2.5 Time spent on treated leaves (no choice experiment)

A single one-day-old adult male or female parasitoid (*C. vestalis* or *A. colemani*) was introduced to the test arena (5 cm dia. Petri dish) containing either a treated or a control leaf disc. Observations were made for 10 minutes per insect (Section 3.6.1). Each treatment was replicated 12 times.

8.2.6 Time spent on treated or untreated leaf hemispheres (choice experiment)

Leaf discs were cut into two halves (hemispheres), one half treated with insecticides and the other remained untreated. The two halves were then placed in a Petri dish arena, an adult parasitoid was introduced and observations made as described in the no-choice experiment (Section 8.2.5). Leaves without any pesticide solution were cut into two halves and placed in a Petri dish as a control treatment.

8.2.7 Percent emergence of parasitoids

Three one-day-old *C. vestalis* cocoons or mummies of *A. colemani* were introduced to a Petri dish with an insecticide treated leaf disc. Each treatment was replicated 10 times (n=30). Control leaf discs were dipped in a 50ppm Triton X-100 solution. Adult emergence was recorded after 7 days. This study was performed keeping in mind that there might be some insecticide residues remaining on the foliage which may indirectly affect adult emergence out of their respective cocoons or mummies.

8.2.8 Statistical analysis

Behavioural data for time spent (seconds) on leaf surfaces (choice, no choice) and on the rest of the arena was subjected to ANOVA using R (Section 3.7). Percentage emergence data was analysed using GLIM with binomial errors. Data were subjected to ANOVA; Chi square tests were used to compare means (Section 3.7).

8.3 Results

8.3.1 Time spent on treated leaves (no choice experiment)

8.3.1.1 *Cotesia vestalis*

Cotesia vestalis spent significantly more time on control leaves compared with leaves treated with insecticides (Figure 8.1: $F=171$, d.f=18, $p<0.001$). *C. vesatlis* were significantly repelled by leaves treated with the LC_{50} dose and spent

more time on the arena when compared to leaves treated with the LC_5 dose for all insecticides ($F=157$, $d.f=18$, $p<0.001$).

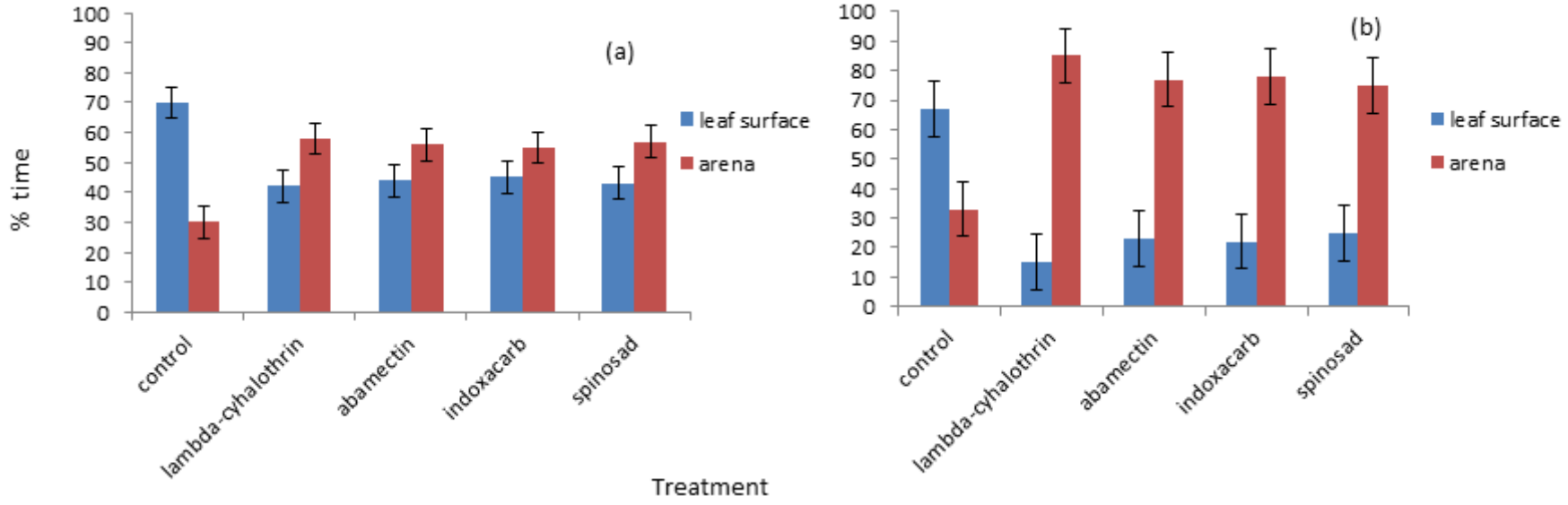


Figure 8.1 Mean time ± SE (%) spent by adult *C. vestalis* on a leaf surface or Petri dish arena in a no-choice experiment with Chinese cabbage leaves pre-treated with the LC_5 (a) or LC_{50} (b) dose of insecticide.

8.3.1.2 *Aphidius colemani*

In both LC_5 and LC_{50} treatments *A. colemani* spent significantly more time on the leaf surface in the control treatment compared with the insecticide treatments (LC_{50} or LC_5) (Figure 8.2; F=5.8, d.f=18, $p<0.001$). *A. colemani* spend significantly less time on LC_{50} treated leaves than LC_5 treated leaves (F=4, d.f=18, $p<0.001$).

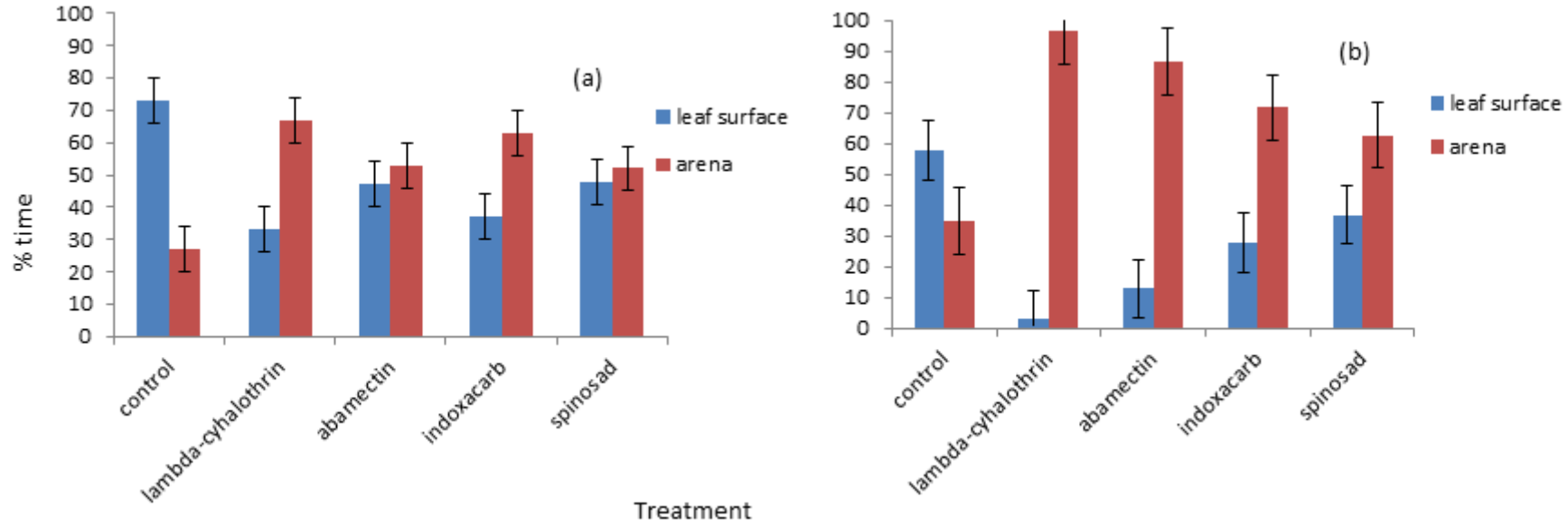


Figure 8.2 Mean time ± SE (%) spent by adult *A. colemani* on a leaf surface or Petri dish in a no-choice experiment with Chinese cabbage leaves pre-treated with the LC_5 (a) or LC_{50} (b) dose of insecticides.

8.3.2 Time spent on treated leaves or untreated leaf hemispheres (choice experiment)

8.3.2.1 *Cotesia vestalis*

Cotesia vestalis adults spent significantly less time on the treated leaf surface in all treatments compared to the control ($F=21$, d.f=25, $p<0.001$). Time spent by *C. vestalis* on the treated leaf was significantly less with the LC_{50} dose than the LC_5 dose ($F=15$, d.f= 24, $p<0.001$).

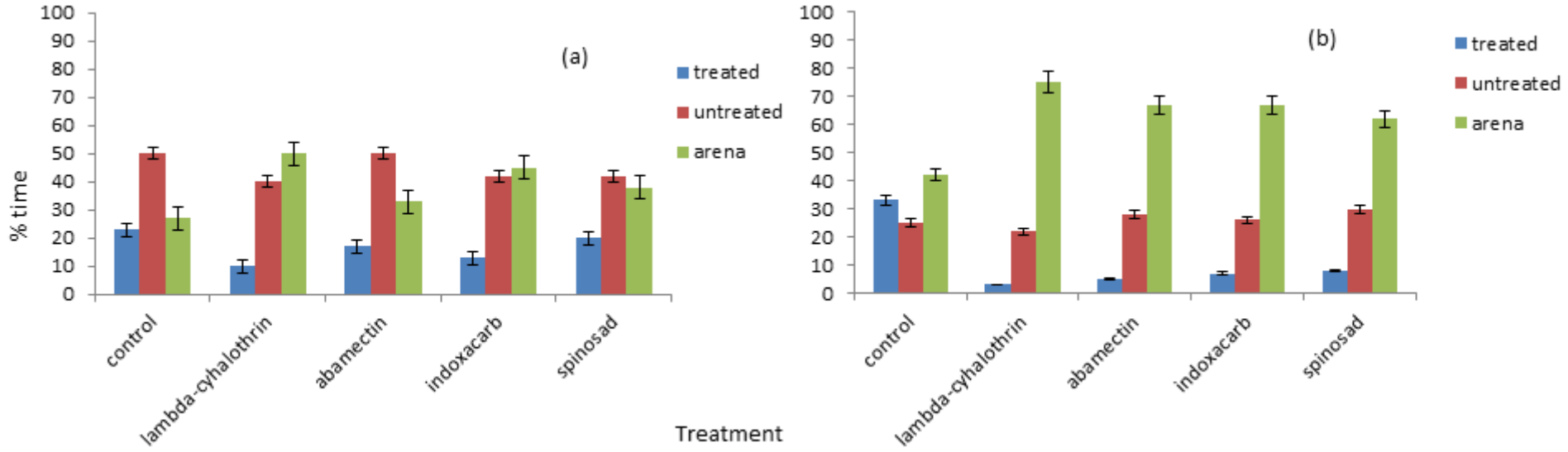


Figure 8.3 Mean time ± SE (%) spent by adult *C. vestalis* on a treated or untreated leaf surface or Petri dish arena in a choice experiment with Chinese cabbage leaves pre-treated with the LC_5 (a) or LC_{50} (b) dose of insecticides.

8.3.2.2 *Aphidius colemani*

When given a choice *A. colemani* spent significantly more time on a leaf surface in the control ($F=17$, d.f=28, $p<0.001$) compared with the insecticide-treated leaf surface. The relative amount of time spend by *A. colemani* on LC_{50} treated leaves was significantly lower than on the LC_5 treated leaves ($F=6.5$, d.f=27, $p<0.001$).

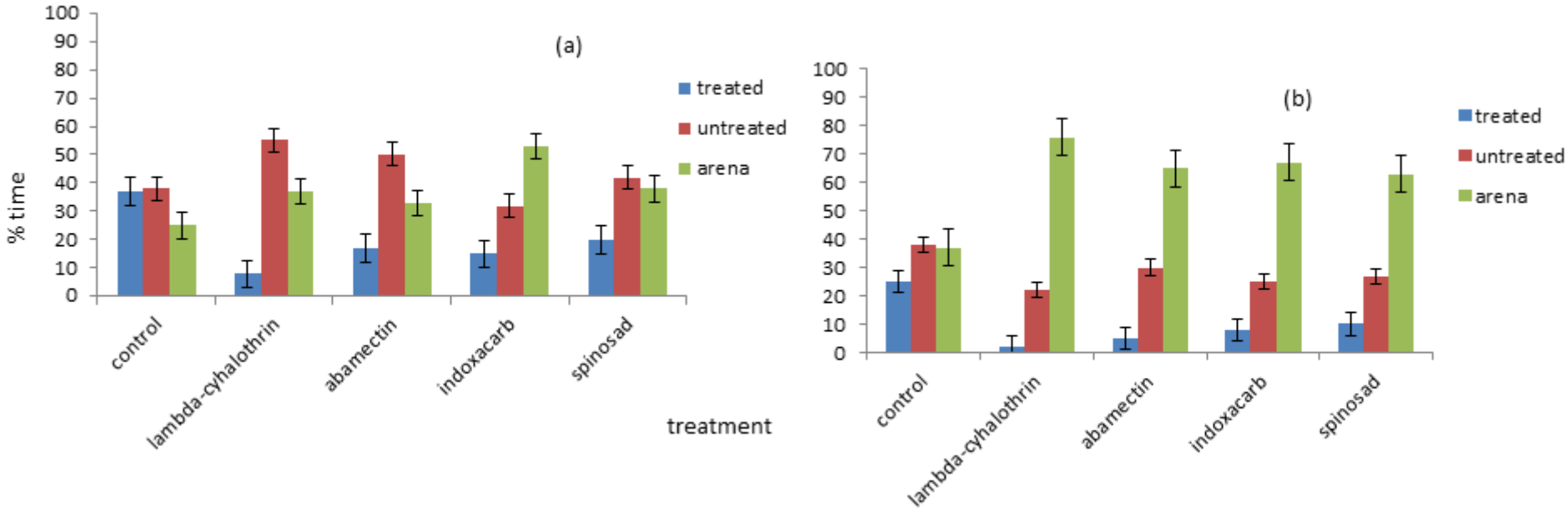


Figure 8.4 Mean time ± SE (%) spent by adult *A. colemani* on treated or untreated leaf surfaces or a Petri dish in a choice experiment with Chinese cabbage leaves pre-treated with the LC_5 (a) or LC_{50} (b) dose of insecticides.

8.3.3 Percent emergence of parasitoids

8.3.3.1 *Cotesia vestalis*

Emergence of *C. vestalis* in the insecticide treatments did not differ significantly from the control (Figure 8.5; z=1.5, d.f=6, p=0.2). There was no significant difference between the different insecticide treatments (z=0.4, d.f=7, p=0.65).

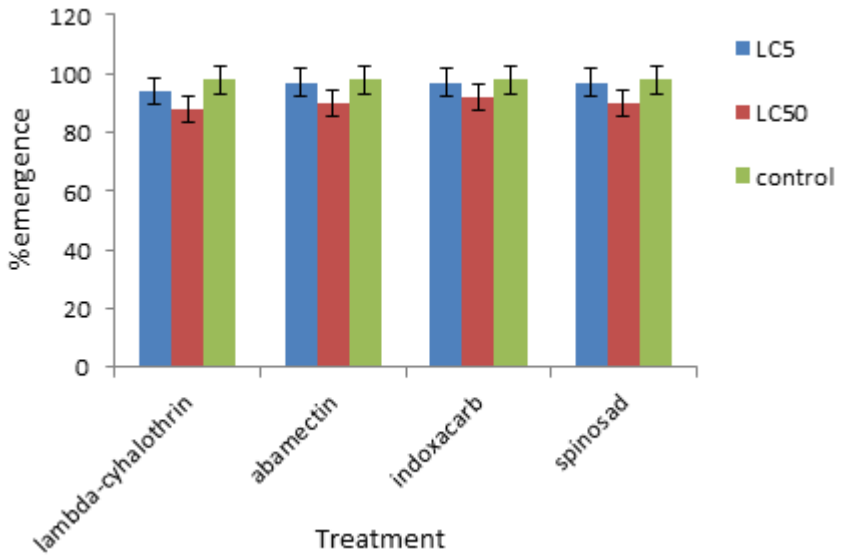


Figure 8.5 Effect of insecticide leaf residues on emergence (Mean ± SE) of *C. vestalis* adults from their cocoons.

8.3.3.2 *Aphidius colemani*

Emergence of *A. colemani* in the insecticide treatments did not differ significantly from the control (Figure 8.6; z=1.4, d.f=7, p=0.15). There was no significant difference between the different insecticide treatments (z=0.12, d.f=7, p=0.7).

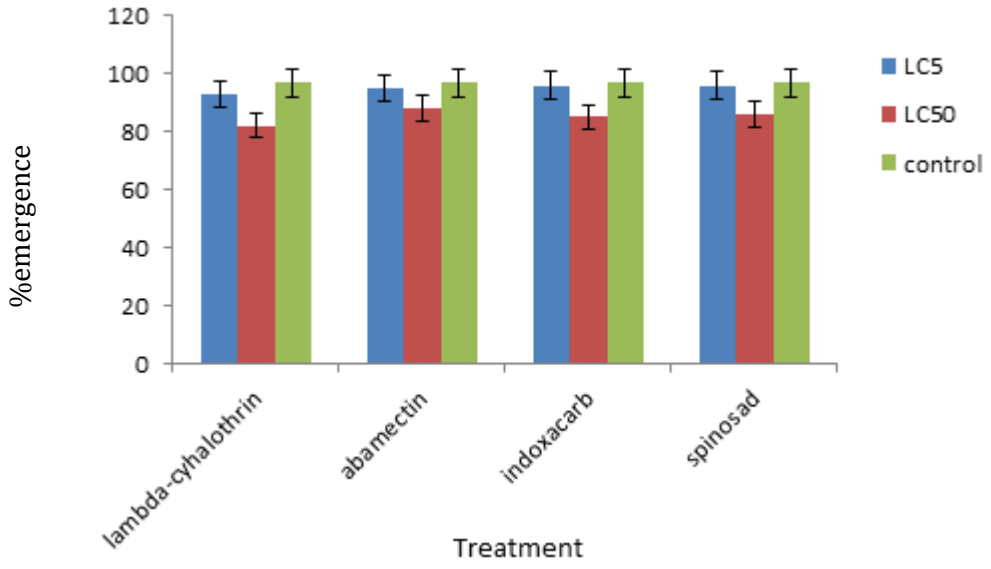


Figure 8.6 Effect of insecticide leaf residues on emergence (Mean ± SE) of *A. colemani* adults from their mummies.

8.4 Discussion

The no-choice experiments indicated that both parasitoid species tended to avoid the insecticide treated surfaces. All the insecticides tested are neuroactive, and behavioural effects through their interaction with peripheral and/or central parts of the insect nervous system would be expected (Raymond-Delpech et al., 2005; Casida and Durkin, 2013). Such effects have been widely documented in the applied entomology literature (Narahashi, 1996; Bass et al., 2014; Taillebois et al., 2014), particularly for pyrethroids (Narahashi, 1996). A topical practical example of this effect is the use of insecticide-treated bed nets (with the pyrethroid, permethrin) to help reduce transmission of malaria (Gimnig et al., 2003; Boulay et al., 2014; McCann et al., 2014). A positive relationship with dose was observed for the time spent on insecticide treated leaves by both parasitoids. A strong behavioural effect on female *Aphidius ervi* in response to a lethal dose was also observed by Desneux et al. (2004). Insect avoidance of insecticide-treated areas has been reported by numerous authors (Desneux et al., 2007), including avoidance of the pyrethroid, deltamethrin on treated plants by *Aphidius* spp. (Longley and Jepson, 1996) and by *Chrysoperla externa* and *Ceraeochrysa cubana* to malathion, permethrin and azadirachtin (Cordeiro et al., 2010); repellance of etofenprox-, methomyl- and malathion-treated areas by *C. vestalis* was reported by Kawazu et al. (2011).

The choice experiments showed that both parasitoid species preferred untreated to insecticide-treated leaves. If this effect is also found under field conditions it might be expected to reduce the impact of insecticides on such natural enemies. Whether such effects would also reduce the impact of parasitoids as natural enemies of pests is a more complex matter and would depend on a variety of factors including the distribution of spray deposits (Matthews et al., 2014). It could be postulated that parasitoid avoidance of insecticide sprayed areas might complement

insecticidal efficacy by focusing parasitoid attack on pests on unsprayed parts of the plant but there is no evidence from laboratory or field studies to suggest an overall additive or synergistic effect. Holland et al. (2000) have reported clustering of beneficial arthropods on unsprayed field areas in wheat crops, suggesting therefore avoidance of sprayed areas (Alyokhin et al., 2010). In contrast, no significant difference in the number of predatory beetles in dry residues of imidacloprid-treated and untreated areas were found for turf grasses by Kunkel et al. (2001).

In the present study, none of the insecticides tested affected the emergence of adult parasitoids. Xia et al. (2008) and Haseeb et al. (2004) also found that abamectin; various azadirachtin products, various *Bt* products, spinosad, indoxacarb and lambda-cyhalothrin had no significant effect on emergence of *C. vestalis* from their cocoons. While Bostanian et al. (2005) found no significant effect of abamectin on emergence of *A. colmani*. It is possible that the outer layers of parasitoid cocoons and mummies, which act as a protective shell against the environment (Danks, 2004), may provide also provide protection against insecticides. Hill et al. (1984) reported that the cocoons of alfalfa leafcutter bees (Hymenoptera: Megachilidae) provide protection against dichlorvos vapours.

CHAPTER 9: Summary, General Discussion, Conclusion and Future Work

9.1 Summary

Chapter 4 Intrinsic and residual toxicity of insecticides against *Plutella xylostella* and *Cotesia vestalis*

- Abamectin was the intrinsically the most toxic of the compounds tested against *P. xylostella* larvae, but it was not significantly different from lambda-cyhalothrin at all exposure times. Abamectin was significantly more toxic than indoxacarb and spinosad after 120 h exposure or 24 h exposure + 96 h,
- Lambda-cyhalothrin had the greatest residual activity against *P. xylostella* larvae, persisting for at least 28 days; indoxacarb showed intermediate persistence of up to at least 14 days, while abamectin and spinosad were the least persistent compounds, with negligible residual toxicity at 14 days.
- Lambda-cyhalothrin was more intrinsically toxic against adult *C. vestalis* compared with indoxacarb and spinosad, but was not significantly more toxic than abamectin; overall, the four compounds tested showed less difference in toxicity against *C. vestalis* (≤ 3-fold) than against *P. xylostella* larvae (up to c. 100-fold).
- The activity of lambda-cyhalothrin persisted for at least 28 days against *C. vestalis* adults; indoxacarb persisted for up to at least 14 days, while abamectin and spinosad were the least persistent compounds, with little residual toxicity at 14 days.

Chapter 5 Effect of leaf surface wax on intrinsic and residual toxicity of insecticides against *Plutella xylostella* larvae and intrinsic toxicity against adult *Cotesia vestalis*

- Removal of epicuticular wax from freshly-treated leaf discs appeared to reduce by 2.5 to 7-fold the residual toxicity of each insecticide tested against *P. xylostella* larvae, although none of the differences were significant.
- Removal of epicuticular wax from freshly-treated leaf discs significantly reduced the residual toxicity of each insecticide tested against adult *C. vestalis* by about 11-fold.
- Removal of epicuticular wax significantly reduced the residual activity against *P. xylostella* larvae with time for each insecticide; The greatest reduction in residual toxicity with wax removal occurring at day 0 for each compound.

Chapter 6 Intrinsic and residual toxicity of insecticides against *Myzus persicae* and *Aphidius colemani*

- Lambda-cyhalothrin was markedly more toxic against adult *M. persicae* when compared with indoxacarb or spinosad but was only significantly more toxic than abamectin after 120 h exposure. Abamectin was significantly more toxic than indoxacarb or spinosad. Spinosad was generally less toxic than the other compounds tested.
- Lambda-cyhalothrin showed the greatest persistence in activity against adult *M. persicae*, although residual activity decreased significantly 28 days after spray application. Indoxacarb showed a significant decline in residual activity after day 14 and little activity remained after 28 days. Abamectin and

spinosad showed a significant reduction in residual activity after 7 days, with little activity remaining after 14 days.

- Lambda-cyhalothrin was the most toxic insecticide against adult *A. colemani* compared with indoxacarb or spinosad during all observations but was only significantly more toxic than abamectin after 120 h exposure. Spinosad was significantly less toxic than the other compounds tested.
- Residual toxicity of all four insecticides against *A. colemani* suggested lambda-cyhalothrin to be the most persistent compound, although activity had decreased significantly by 28 days after spray application; indoxacarb was the second persistent insecticide with a significant decline in residual activity after 14 days, with little residual activity remaining after 28 days; abamectin and spinosad showed a less persistent nature, with little activity remaining after 14 days.

Chapter 7 Effect of leaf surface wax on intrinsic and residual toxicity of insecticides against *Aphidius colemani* and *Myzus persicae*

- Removal of epicuticular wax from freshly-treated leaf discs reduced the residual toxicity of insecticides against adult *M. persicae* by 10 to 27-fold.
- Removal of epicuticular wax from freshly-treated leaf discs significantly reduced the residual toxicity of insecticides against adult *A. colemani* by 11- to 18-fold.
- Removal of epicuticular wax markedly reduced the residual activity for each compound against adult *A. colemani*; the greatest reduction in toxicity occurring at day 0.

- Lambda-cyhalothin showed the greatest residual toxicity against *A. colemani* at day 7 and day 14 leaves; removal of wax significantly reducing its toxicity at day 0 and day 7, with little toxicity remaining after 14 days.
- Indoxacarb had greater apparent levels of residual activity compared with spinosad and abamectin at day 7 and day 14; removal of wax significantly reduced toxicity at day 0, day 7 and day 14.
- Abamectin and spinosad had little remaining residual activity at day 14; removal of leaf wax significantly reduced toxicity at day 0 and day 7, with no residual activity at day 14.

Chapter 8 Sub-lethal effects of insecticide residues on parasitoids

- In no-choice experiments, both *C. vestalis* and *A. colemani* spent significantly more time on untreated leaves in control arenas compared with arenas with insecticide-treated leaves.
- In choice experiments, both parasitoid species spent significantly more time on untreated compared with insecticide-treated leaf surface in the same arena.
- Repellency of the treated leaf surface to both parasitoids was significantly more for the LC_{50} than the LC_5 dose both? when given a choice or no choice.
- Insecticide treatments on leaf surfaces had no significant effect on the emergence of adult parasitoids when compared with emergence of adult parasitoids on untreated leaf surfaces.

9.2 General Discussion

9.2.1 Intrinsic and residual toxicity of insecticides on foliage to pests and parasitoids

In the *P. xylostella* - *C. vestalis* - brassica system used in the current work, abamectin was the most potent toxicant against the pest in contact-ingestion bioassays on insecticide-treated foliage, with the evidence suggesting that lambda-cyhalothrin was the second most potent compound. In the 120 h exposure bioassays, there was up to a 200-fold difference in toxicity (at LC_{50}) between the most toxic (abamectin) and least toxic compounds (indoxacarb and spinosad). In contrast, while lambda-cyhalothrin (reported as harmful to natural enemies) and abamectin (reported to be compatible with IPM) appeared to be the most toxic compounds against the parasitoid species, the differences in toxicity between the most and least toxic compounds tested were relatively small (c. 3-fold).

Other studies have also indicated lambda-cyhalothrin and abamectin are relatively harmfull to natural enemies (Sections 4.4 and 6.4; Studebaker and Kring, 2003), while reports on the effects of spinosad and indoxacarb on natural enemies are more mixed (Ruberson and Tillman, 1999; Studebaker and Kring, 1999; Haseeb et al., 2004; Biondi et al., 2012a; Roubos et al., 2014).

The present results suggest that differences in the intrinsic toxicity of these compounds against *C. vestalis* under laboratory conditions cannot account for the reported marked differences in their relative harmfulness against *C. vestalis* and other parasitoids under field conditions (e.g. Section 1.1; Furlong et al., 2013). When the relative toxicity ratio for each compound is calculated, however, abamectin stands apart, being far more toxic (2450-fold LC_{50} ratio) against *P. xylostella*

compared with *C. vestalis*; whereas the relative toxicity ratios for lambda-cyhalothrin (c. 930-fold) and, in particular for indoxacarb (c. 30-fold) and spinosad (c. 21-fold) are much lower. If the recommended field application rates for abamectin (Table 9.1) are complied with, the very large toxicity ratio found here may in part explain the reported compatibility of this compound with parasitoids of *P. xylostella* under field conditions (Verkerk and Wright, 1997; Furlong et al., 2013).

Table 9.1 Recommended field rate of insecticides for vegetable crops.

Field rate	Manufacturer	Active ingredient	Product
150-250 ml /ha	Syngenta, U.K.	lambda-cyhalothrin	Karate® 50g/l
300-450 ml / ha	Syngenta, U.K	abamectin	Vertimec® 18g/l
85 g/ha	DuPont, U.K	Indoxacarb	Steward®300g/kg
200 ml /ha	Dow AgroSciences, U.K	spinosad	Tracer®480g/l

Due to problems with the initial population of *C. vestalis* used in experiments, and the considerable delays experienced in obtaining a new population, a second pest-parasitoid system was included in the present study. To benefit from work already conducted with *C. vestalis* on Chinese cabbage (Section 4.3.1, 4.3.2, 4.3.3, 5.3.1, 5.3.2 and 5.3.3), an aphid-parasitoid system on Chinese cabbage was chosen using the same four insecticides. While not all of these compounds are used specifically against aphid pests, they have been widely applied on Chinese cabbage and other crucifer crops (Badii et al., 2013; Liu et al., 2013) and some do refer to aphids in the product label instructions (Section 6.4). More importantly, the use of the same four insecticides in both crucifer systems allowed direct comparison between

the two parasitoid species (Haseeb et al., 2004; Desneux et al., 2007; Kawazu et al., 2010; Stara et al., 2011; Liu et al., 2014).

The intrinsic residual toxicity for each insecticide against *A. colemani* was found to be very similar to the results obtained for *C. vestalis*. In contrast to the large intrinsic toxicity ratios observed with *P. xylostella* and *C. vestalis*, the toxicity ratio for each compound for *M. persicae* and *A. colemani* was effectively 1, a reflection of the much lower toxicity of all the compounds tested against *M. persicae* when compared with *P. xylostella*. The lack of any apparent differential toxicity for these compounds against pest and parasitoid highlights the need for caution in pesticide selection when several pest species are present. Such an apparent lack of differential selectivity may, however, be offset by other factors, such as persistence, and the distribution of toxic residues between the leaf surface, where they are available to pest and natural enemy, and underlying tissues, where they are not directly available to the natural enemy. Increased mortality was generally observed when *C. vestalis* were exposed to pesticide for 120 h compared with 24 h (Sections 4.3), increases in mortality with exposure time were only found for lambda-cyhalothrin with *A. colemani* (Section 6.3).

In the present study, differences in the persistence of insecticides on foliage, measured in terms of residual activity against pests and parasitoids gave consistent results, with lambda-cyhalothrin the most persistent compound tested, and abamectin and spinosad the least persistent. Xu et al. (2004) also reported lambda-cyhalothrin and indoxacarb were more persistent than spinosad, and similar findings have been reported by other authors (Haseeb and Amano, 2002; Sharma et al., 2007; Soudamini et al., 2009).

Various biotic and abiotic factors can influence the bioavailability and metabolism of pesticides on plants (Wright and Welbourn, 2002). Bioaccumulation of pesticides is largely dependent on their persistence and/or frequency of application, and is influenced by their relative lipophilicity (Hodgson, 2010) and absorption through the plant surface (Kirwood, 1999). In the present study, residual activity (persistence) experiments were conducted on insecticide-sprayed plants that were then maintained under protected and relatively controlled environmental conditions in a glasshouse. The plants used were also produced under glasshouse conditions. Further studies are required, therefore, under field conditions, where effects of ultra-violet radiation, temperature, wind and rain would be expected to reduce pesticide persistence at a faster rate than under glass, particularly on the leaf surface.

Removal of the epicuticular wax layer reduced the residual toxicity of all the insecticides tested for both pest and parasitoid species (Sections 5.3 and 7.3), which suggests that the wax layer retained a significant proportion of each compound (Riccio et al., 2006). However, the results for the pest species are more difficult to interpret, due in part to variation in feeding behaviour between lepidopteran larvae and aphids and difference in feeding activity between individual insects, and this may in part explain the larger variances found in the wax removal experiments with *P. xylostella* (Section 5.4). The results for the parasitoid species were more consistent, due in at least part to pesticide uptake by parasitoids being essentially by contact activity alone, and the results suggest that considerable toxic residues were present in the epicuticular wax layer for all compounds tested at day 0 and day 7, and for indoxacarb and, in particular for lambda-cyhalothrin, at day 14 (Sections 5.4 and 7.4). The results for lambda-cyhalothrin may reflect this compound's relative lack of mobility compared with the other compounds studied (Section 5.4; Leistra et al., 2004).

In interpreting wax removal data, there is the possibility that the extraction method may have also have removed some (lipophilic) pesticide residues from the underlying leaf tissues (Nigg et al.,1981). Further studies are required to elucidate this, and to study further the distribution of toxic pesticide residues between the outer wax and underlying leaf tissues with time.

9.2.2 Behavioural interactions with insecticide toxicity

The present study focussed on whether parasitoid species actively avoided Insecticide-treated leaf surfaces. The results indicated that both species avoided insecticide residues, either by spending more time on the arena in the no-choice experiments, or on untreated leaf segments / arena in the choice experiments. It was not surprising that the same effect was observed with each of the compounds tested since they are all neuroactive (Section 8.4). Both parasitoids tried to spend significantly less time on leaf surface treated with a lethal dose than on a leaf surface with a sublethal dose. If residues of insecticide that are low enough not to have any significant effect on the pest species can still disrupt parasitoid behaviour, the residual effects of relatively non-persistent insecticides on parasitism success may be greater than expected.

Severe effects of pesticides on the reproduction of heteropteran and phytoseiid predator species were reported by Biondi et al. (2012). The effect of carbamate and pyrethroid insecticides on flight response of *C. vestalis* was described by Kawazu et al. (2011). Adverse effect of IGRs (chlorfluazuron, flufenoxuron and teflubenzuron) on parasitism of *C. vestalis* has been reported by Haseeb and Amano (2002). Shi et al. (2004) reported that field doses of fipronil, avermectin, chlorfluazuron fenvalerate and methomy had no significant effect on adult emergence from their respective cocoons in *C. vestalis*. Sub-lethal effects of insecticides on *A. ervi* have

also been described by Desneux et al. (2004). Stara et al. (2011) reported a negative effect of Methoxyfenozide on the fecundity of *A. colemani*.

9.3 Conclusion and Future work

The application of synthetic insecticides causes many associated problems associated, such as pests becoming resistant, as well as lethal and sub lethal effects on natural enemies. Although new insecticides with a reduced risk to the environment, as well as to natural enemies, have been introduced, research is still needed to evaluate their impact on natural enemies. In IPM the real challenge is to combine both pesticide and natural control methods in a manner which produces significant benefit to the farmer by increasing yield and reducing pests without any risk to the environment. In this study an effort was made to identify the potential risks associated with insecticides used along with parasitoids in an IPM system.

In a *Plutella xylostella-Cotesia vestalis*-Chinese cabbage system, the clearest factors linked to 'selectivity' or 'non-selectivity' were found for abamectin and lambda-cyhalothrin, respectively due to their high level of relative toxicity to the parasitoid and the pest. In the *Myzus persicae-Aphidius colemani*-Chinese cabbage system, however, the relative toxicity was very minor. Which suggests that when selecting insecticides, a relative toxicity test to the pest and the natural enemies must be performed, before incorporating them into the IPM system.

For lambda-cyhalothrin, a key factor determining its relative harmfulness against parasitoids, appeared to be how persistent spray deposits remained on the leaf surface as uptake of the toxicant is by contact action, compared with the persistence of the other more 'selective' compounds tested, particularly abamectin

and spinosad. This suggests that in addition to relative toxicity, the persistence of the spray deposit is another factor that should be in mind while incorporating pesticides to the IPM system

In the case of abamectin, in addition to its relative lack of persistence against both parasitoids, a key factor determining the selectivity of abamectin under field conditions could be its relatively low rate of application (Table 9.1) compared with many other insecticides.

For conservation of the parasitoids and to have their maximum benefits of controlling pests, it is clearly important not to use insecticides that are sub-optimal for the target pest, as the lack of relative toxicity for any of the compounds tested against *M. persicae* and *A. colemani* indicated. It is therefore important to select a pesticide which is efficient to control pests and be selective to natural enemies, because natural enemies are crucial agents to maintain control of the pest population density.

When surface wax was removed from the insecticide-treated leaves during the current study, it greatly influenced the toxicity of all insecticides tested against all insects. Leaves with wax showed greater toxicity than those from which wax been removed. This suggests that penetration of insecticides is influenced by the presence of the wax layer. The wax layer in the same plant can differ in its contents from one part to another part of the plant, or even on the same part as a result of plant growth. The thicker the wax layer the more difficult it will be to have a pesticide coverage, which then requires additional additives to be added, and more pesticide will be required to be effective, which in turn could remain available over a longer period and have a greater impact in the environment and on the natural enemies.

Even if the natural enemies survive, pesticide exposure may still be affecting their behaviour (e.g. Wang et al., 2008) and the sub-lethal effects of pesticides should ideally be taken into account before they are incorporated in IPM management programmes. In practice, this suggests that neuroactive compounds should be avoided wherever possible although this may not be practical. The results of the behavioural assays during the present study suggested that all insecticides tested caused marked avoidance behaviour by both parasitoid species, though the response for high doses (LC_{50}) were much more pronounced as compared with the low doses (LC_5). If such avoidance effects are as marked under field conditions the overall effect is likely to depend on a variety of interacting factors, including the efficiency of spray application (coverage of crop) and whether unsprayed refugia are present. Contact toxicity to the cocoons or the mummies of the parasitoids was not significant.

There should be a proper training for farmers on application techniques, and they must be aware of the appropriate time for application of pesticide, and instead of following calendar spraying, careful observation and monitoring of crops must be done and an insecticide applied only when the pest population justifies it.

Future studies could include:

- Residual toxicity bioassay experiments (mortality and sub-lethal studies) to examine insecticide selectivity against pests and parasitoids under more realistic, semi-field (potted plants) and field conditions, and in a range of climatic conditions.

- To refine methods for removal of epicuticular wax that limits any potential co-extraction of pesticide from underyling leaf tissues.
- To conduct chemical analysis of pesticide residues in the leaf after wax removal and within the wax; using GCMS and HPLC-MS [methods that proved too expensive to use in the present study].
- To conduct field cage and open-field experiments using ‘selective’ compounds at a range of application rates, to determine whether relative toxicity ratios between key target pests and their parasitoids can be exploited, and if so whether they are robust enough for use in IPM systems.

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