

THE UNIVERSITY OF HULL

**FACTORS AFFECTING THE DISPERSAL OF THE  
INVASIVE LADYBIRDS *COCCINELLA*  
*SEPTEMPUNCTATA* AND *HARMONIA AXYRIDIS***

being a Thesis submitted for the Degree of

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by

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


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## Factors affecting the dispersal of the invasive ladybirds *Coccinella septempunctata* and *Harmonia axyridis*

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### General Abstract

Biological invasions can have deleterious impacts on native ecosystems, but also offer opportunities for studying evolution. A fundamental question in invasion biology is: what factors are important determining invasion success? Dispersal is essential to the establishment and persistence of populations in heterogeneous environments. This thesis investigated dispersal for its importance in invasions in two ladybirds, *Harmonia axyridis* (Pallas) and *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae). The effects of abiotic and biotic factors on ladybird aerial densities (AD) were investigated using Vertical Looking Radar, a tool which allows for detailed observations of high altitude coccinellid flight for the first time. Temperature was found to be the strongest predictor of AD, wind speed had a negative relationship with AD and aphids had no significant predictive effect. Comparison of *H. axyridis* distribution and topographical distribution of temperature and wind speed in the U.K. suggests that unfavourable meteorological conditions, for example over the Pennines and Cambrian Mountains, may act as a barrier to dispersal; slowing the spread of the *H. axyridis* in the U.K. To investigate characteristics of ladybird flight in controlled conditions, a novel *in vitro* flight test method was designed. This successful method was used to test the hypothesis that increased dispersal ability at range expansion boundaries will be selected for. No significant difference in flight time was found between native and invasive *H. axyridis* populations. An explanation may be the trade off between dispersal ability and fecundity at range expansion boundaries, which warrants further investigation. The results presented in this thesis add to our understanding of dispersal ability in coccinellids and could be used to help predict future spread of invasive ladybirds. Future work should investigate the potential trade off between dispersal ability and fecundity at range expansion boundaries and whether phenotypic plasticity in dispersal is a predisposition to invasion success.

# **Chapter 1.**

## **Factors affecting the dispersal of the invasive ladybirds *Coccinella septempunctata* and *Harmonia axyridis* :**

### **General introduction**

## General Introduction

“Invasive alien species” (IAS) can impose dramatic impacts on native ecosystems (Mooney & Cleland, 2001) and economies (Pimentel, 2001; Pimentel et al., 2004). IAS is a term that refers to the successful establishment of an “alien” species in a habitat outside of its native range. By establishment it is meant that this alien species is self sustaining in its non-native range (Colautti & MacIsaac, 2004). IAS can impact both on ecosystems (Mooney & Cleland, 2001) and economies (Pimentel, 2001; Pimentel et al., 2004) in their invasive range and are therefore important in evolution and conservation biology. One question often addressed in the study of IAS is, what are the factors that allow alien species to become invasive? Many life history traits have been put forward in answer of this question, one of which is dispersal; a trait which is known to be essential for the maintenance of genetic variation between sub-populations (Bowler & Benton, 2005; Peterson & Vieglais, 2001). Knowledge of the dispersal abilities and behaviours of IAS and how they evolve is therefore integral to understanding their ability to establish and persist in their invasive range (Travis et al., 2009).

In this thesis dispersal in invasions is investigated with the use of two invasive coccinellid study species (Coleoptera: Coccinellidae); the 7-spot ladybird, *Coccinella septempunctata* (L.) and the harlequin ladybird, *Harmonia axyridis* (Pallas). Both species have been widely introduced as biological control agents in the past and have since become established in many countries around the globe. *Coccinella septempunctata* is native in the U.K. and Europe and is invasive throughout North America and Canada (Hodek & Michaud, 2008). *Harmonia axyridis* has a large native range including Eastern Russia, eastern Kazakhstan, Siberia and Japan. *Harmonia axyridis* is now invasive in Europe (including the U.K.), North America, South America and in Egypt and South Africa (see Table 1. and Fig. 1 in Appendix 1).

This Chapter will first describe previous research surrounding invasive species, including the ways in which invasions are studied; secondly the importance of dispersal will be discussed both in general and in relation to invasions. Thirdly the study system used in this thesis; *C.*

*septempunctata* and *H. axyridis* will be introduced and lastly, the specific aims of the thesis will be outlined.

Biological invasions are a natural process and are considered a major driver of evolution in both native species and IAS (Hughes et al., 2007; Petit, 2004; Reznick & Ghalambor, 2001). However due to the human mediated transport of many species outside of their natural range, the rate and frequency of invasions has increased far beyond natural rates (Cohen & Carlton, 1998). These human mediated introductions are often accidental, for example, the transport of species on goods via routes of commerce is common (Clavero & García-Berthou, 2005; Cohen & Carlton, 1998). However many species have also been intentionally introduced in biological control programmes, many of which were employed before the implications of such introductions were realised (Simberloff & Stiling, 1996). Although only a fraction of these introduced species become established, the cumulative effect of many human mediated introductions over time has resulted in countless IAS across the globe (Mooney & Cleland, 2001). This has allowed species that were previously separated by dispersal barriers to come into contact with each other, which in many cases, has resulted in drastic changes in native communities and ecosystems (Fritts & Rodda, 1998; Gurevitch & Padilla, 2004; Wilcove et al., 1998).

IAS can impose dramatic evolutionary impacts on native species (Mooney & Cleland, 2001). Carroll & Dingle (1996) show evidence of evolution in response to invasion in the North American soapberry bug *Jadera haematoloma* (Herrich-Schäffer) (Hemiptera: Rhodopalidae) which has adapted its feeding apparatus to exploit an introduced tree species. Many of the evolutionary impacts of invasions however, are highly detrimental to native species, including competitive exclusion, niche displacement, introgression and predation (Hänfling et al., 2005; Huxel, 1999; Mooney & Cleland, 2001). Despite these, there is wide debate regarding whether or not invasive species are drivers of extinction. Gurevitch & Padilla (2004) call for a more critical analysis of the “anecdotal and speculative” evidence that invasive species cause

extinctions. Clavero & Garcia-Bethou (2005) replied to this with a reanalysis of the International Union for Conservation of Nature (IUCN) data base and found that in 34 out of 680 cases, invasions were the only cause of extinction. A similar debate occurred between Blackburn et al. (2004) and (Didham et al., 2005), the latter of whom highlighted the need to include a set of explanations when looking for causes of extinctions, including land use changes, climate change, disease and more alongside invasions.

It seems paradoxical however, that a species new to an environment and its inherent pressures can out-perform and displace native species, which have adapted to these pressures over long periods of time. Additionally, IAS will often colonise a new area in small numbers and therefore the genetic variation and thus adaptability within these “founder” populations will be limited. This can then be exacerbated by inbreeding and genetic drift (Kliber & Eckert, 2005). This phenomenon is called a founder effect and has been demonstrated in a number of IAS (Hawley et al., 2006; Kliber & Eckert, 2005; Lambrinos, 2004 and references therein).

Therefore in light of the challenges that face potential IAS, invasion biologists are understandably interested in the life history traits and evolutionary processes that are important for successful invasions?

One trait that has been put forward as increasing the potential for invasion success relates to the genetic structure of the source population from which the invasion stemmed. Invasive species must adapt to environments often very different from their native range, therefore it follows that success in these environments may require rapid adaptation to novel conditions (Kliber & Eckert, 2005). The adaptability of an introduced species is therefore integral to invasion success in these species (Lee et al., 2007). There is evidence to imply that invasion success may depend on the pre-existing genetic attributes of the source population on which natural selection can act (Lee, 2002). For example, the genetic potential for phenotypic plasticity could result in an increase in fitness under unfavourable conditions (Peacor et al., 2006) that may be encountered during invasion. Additive genetic variance (the combined

effect of a number of genes on a trait), and epistatic interactions have also shown to be important genetic attributes in adaptation in *J. haematoloma* (Peacor et al., 2006). Therefore these genetic attributes may predispose some species to invasion success.

Genetic adaptability is just one potential predictor for invasion success put forward, another is the enemy release hypothesis (ERH); the lack or scarcity of natural enemies in an introduced range, which leads to an increase in fitness of alien species (Colautti et al., 2004). However this area is complex; the fitness increase in an introduced species from the loss of a natural enemy is likely dependent on the initial relationship between them; if a high level of resistance to the natural enemy already existed in the native range of the introduced species, release from this enemy would not necessarily produce significant fitness increase (Colautti et al., 2004). However if the defence against an organism was costly in the native range, release from this enemy may allow for the redistribution of the resources used for defence for other life history traits, such as growth and reproduction. This could result in the evolution of increased competitive ability (EICA) which is a hypothesis proposed to explain the vigour observed many species in their introduced range in comparison to their native range (Blossey & Notzold, 1995).

To further complicate this area of invasion biology, a natural enemy might change its effect on a host between a native and an introduced range, i.e. the enemy inversion hypothesis (EIH). A good example of which is the use of the gall fly *Urophora affinis* (Frauenfeld) (Diptera: Tephritidae) in the biological control of the knapweed *Centaurea maculosa* (Lam.) (Centaureinae: Centaurea) (Blossey & Notzold, 1995). This introduction caused the native deer mouse *Peromyscus maniculatus* (Wagner) (Rodentia: Cricetidae) to alter its microhabitat selection in order to feed on the larvae of *U. affinis* present on *C. maculosa*. In doing so *P. maniculatus* inadvertently became a disperser of *C. maculosa* seeds, thus this natural enemy used as a biological control agent indirectly facilitated the dispersal of its intended control

species. Therefore the ability of ERH to improve the probability of invasion success is still a point of much debate (Blossey & Notzold, 1995).

Another key trait in species invasions is dispersal, which is of the highest importance to the persistence of a species in a heterogeneous landscape (Travis et al., 2009). Gene flow as a result of dispersal between otherwise isolated sub populations is essential for the prevention of genetic isolation and the inherent downfalls such as inbreeding depression that follow (Bowler & Benton, 2005). During invasions genetic isolation can be associated with aforementioned founder effects, therefore dispersal is likely to play an important role in reducing the severity of low genetic variation in founding populations (Rivas et al., 2004). Dispersal is also essential in foraging, reproduction and diapause in many species (Roff & Fairbairn, 2007), processes which will likely be under increased pressure in a non-native range.

Invasions by definition involve the expansion of species range boundaries, during which the importance of dispersal is thought to increase (Hughes et al., 2007; Travis et al., 2009). There are fitness advantages associated with being highly dispersive such as reduced kin competition and lower probability of inbreeding (Travis et al., 2009). These are likely to change with changing selective conditions however, McPeck & Holt (1992) show through modelling that selection always favours dispersal in spatially and temporally changing environments such as those encountered by IAS. However there can also be large costs associated with dispersal, for example highly dispersive individuals may experience more predation, or may move into unsuitable areas; in these cases high levels of dispersal would be selected against (Travis et al., 2009). These opposing selection pressures, which drive the evolution of dispersal strategies, will differ between core ranges and at range expansion boundaries. In addressing the question of how dispersal changes at range expansion boundaries, Travis et al. (2009) found that density dependent dispersal strategies altered to favour higher dispersal propensity at lower population densities. Also, Neubert & Caswel (2002) found that in models containing stratified dispersal, i.e. long and short flights; it was long distance flights that governed the speed of

invasion. However, as the authors highlight, modelling is prone to oversimplifying ecological variables, therefore the results of such studies may not truly reflect the role that dispersal plays in invasions.

Many of the studies assessing the evolution and importance of dispersal in invasions employ statistical modelling; this is likely due to the difficulties in observing and recording dispersal in many species. The dispersal of insects is notoriously difficult to study and field observations of fast and high altitude flights of many insect species have previously only been possible through ground level observations (as in Elliott, 2000; Osawa, 2000), indirect inferences made from ground-based light traps (Williams, 1948) or suction traps (Johnson & Taylor, 1955) or *in vitro* tests. However such observations are invaluable as flight is an integral part of insect ecology (Dixon et al., 1993).

The ability of insects to move around between often ephemeral foraging and ovipositioning sites is essential and has resulted in the adaptation of many flexible flight behaviours (Roff & Fairbairn, 2007). An extreme adaptation to this problem is phenotypic dimorphism, for example in aphids, the phenology (life stage) of which changes between individuals as a result of adaptations to environmental cues. Aphid alates are winged and are colonisers of new foraging habitats. Once a suitable host plant is found these alates produce apterous offspring which do not have wings. Instead these apterous individuals focus their natural resources on reproduction and rapidly produce many offspring. According to a number of environmental cues such as population density or host senescence apterous aphids will produce migrants whose job it is again to search for the next host (Dixon, 1985). Aphid populations are therefore, like their required foraging sites, ephemeral and so it follows that predators like *H. axyridis* & *C. septempunctata* must adapt strategies to track them.

*In vitro* methods have been a valuable tool in insect flight study, including the use of flight mills (Dingle, 1966; Miller et al., 2008; Rankin et al., 1994), fixed tethering experiments (Dingle, 1965) some of which using force-displacement transducers (Coelho, 1991) and take-off



experiments (Tourniaire et al., 2000). These have allowed for studies of environmental and ontological factors in flight behaviour in controlled conditions that would likely be impossible to perform in the field.

Modelling has proven a more recent solution for example using dispersal kernel and Integrodifference models, which have been used to the location of an individual as a probability distribution calculated from a given starting position (Goldwasser et al., 1994; Kot et al., 1996; Neubert & Caswel, 2002) and have highlighted the importance of dispersal in invasions. However, as previously mentioned, modelling is prone to oversimplifying ecological variables such as complexities in dispersal strategies (Neubert & Caswel, 2002) and also Allee effects (Kot et al., 1996). Thus there is a need for studies addressing the importance of dispersal in invasions using field observations.

Harmonic radar is a means through which insect activity at ground level can be observed. Harmonic radar uses diode transponders which re-emit a signal originally emitted from a ground based scanning station at a different (specific) frequency, allowing the tagged insect to be identified amongst environmental interference (O'Neal et al., 2004). Harmonic radar has a range of approximately 1km and produces accurate flight path measurements that can be superimposed onto landscape maps. Capaldi et al. (2000) and Riley et al. (2005) have used this approach to study foraging in bumblebees an extremely important part of insect ecology which is essential to their conservation and their role as pollinators. Harmonic radar has also provided direct observation of the Nobel-prize-winning hypothesis behind the honey bee "waggle dance" (Riley et al., 2005).

Vertical looking radar (VLR) is a tool which enables observations of high altitude insect flight which would otherwise be impossible. VLR was developed after it was noticed that insects produced radar backscatter signals at the time dubbed "air angels" (Schaefer, 1976). This was later capitalised upon by Schaefer in 1968, who developed the first special-purpose entomological radar (Schaefer, 1976). The invention of VLR revolutionised the way

entomologists viewed insect migration. For example, (Chapman et al., 2003) has since extrapolated that “*millions of metric tons of insects are aloft in Earth’s atmosphere at any one moment*”; a previously un-thought-of statistic.

Since its birth, VLR has advanced and now enables entomologists to observe a multitude of insect migration parameters including; numbers flying, insect size, shape and mass for insect identification, speed, heading, altitude and wing beat frequency. Not to mention allowing for the simultaneous detection of many insects at a range of altitudes between 150 – 1200m above ground level (AGL) (Chapman et al., 2003). Additionally the recent advances in computer technology have lead to the automation of VLR control and data analyses. This has allowed for long term studies of insect migration that were previously impractical (Chapman et al., 2003). VLR therefore provides an opportunity to study aspects of insect dispersal such as long distance migratory behaviour using wind currents, vertical distribution and insect layering, (Chapman et al., 2003) and temporal variation in insect dispersal. The monitoring of devastating locust swarms is an important example of how VLR technology can provide useful information for the management of pest species (Riley & Reynolds, 1997). The more recent VLR automation now allows for the long term monitoring of migrating insects for research into migratory behaviour. Chapman et al. (2002) supplemented VLR observations with light trap insect catches to show that a large migration of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) from Holland occurred in May 2000 and that this and others like it were responsible for the re-establishment of the species in the U.K. In a more recent study Chapman et al. (2010) used meteorological data in conjunction with that of VLR to backtrack noctuid moth flight paths; showing that the moths actively modify their flight headings to compensate for cross-wind-drift, thus maximising long distance migration efficiency. In this thesis, VLR will be used to examine the dispersal behaviour of two key coccinellid species. Although the dispersal of these species has received some attention in the past (Hodek & Honěk, 1996; Elliott et al., 2000; Osawa, 2000), the VLR provides a unique opportunity to study their high altitude dispersal behaviour for the first time.

## The study system

*Harmonia axyridis* is a highly voracious generalist, an individual can consume up to 370 aphids during larval development and up to 800 in a lifetime (Berkvens et al., 2009). For this reason they were viewed by agricultural pest controllers to be a perfect biological control agent for aphids (Koch, 2003; Tedders & Schaefer, 1994; Tourniaire et al., 2000) and have consequently been widely introduced across Europe, parts of Asia, North & South America and Egypt (see Appendix 1). The use of *H. axyridis* larvae as biological control agents on crops was at first hindered by their short development time; usually 3<sup>rd</sup> instar and 4<sup>th</sup> instar larvae were used which matured within approximately 2 weeks and dispersed due to low prey densities (Ferran et al., 1996). In order to overcome this problem Tourniaire et al. (2000) used selective breeding to exploit a naturally occurring wing muscle mutation; thus producing generations of *H. axyridis* that were unable to fly. This resulted in biological control agents that were essentially trapped in the agricultural patches on which they were used and so provided prolonged pest control. Previous to this however, introductions of flight-worthy *H. axyridis* were common and are considered an important factor in the establishment of this ladybird in much of its invasive range (Gordon, 1985; Koch et al., 2006).

*Coccinella septempunctata* has also been extensively used in the past as a biological control agent, being first introduced in North America in 1956 (Lucas et al., 2002). Like *H. axyridis* it was selected for its voracity and polyphagy, being recorded to feed on up to 97 different prey species (Lucas et al., 2002). This use as a biological control agent is identified as a cause of the wide present distribution of *C. septempunctata* throughout the Nearctic ecozone (Evans, 2000; Hodek & Michaud, 2008). Many governments no longer allow the use of coccinellids as biological control agents due to the number of non-target species impacts in their invasive range, for example through direct competition and intraguild predation (Brown et al., 2011; Hodek & Michaud, 2008; Hughes et al., 2007; Koch, 2003). The invasion of *C. septempunctata* has been identified as the cause of declines in many native aphidophagous coccinellids (Elliott

et al., 1996; Obyrcki et al., 2000). Similarly *H. axyridis* is also considered to be an important intra-guild predator among aphidophages, especially at the larval stage in which it has been shown to predate on the immature stages of other coccinellids in its U.K. invasive range (Hughes et al., 2007; Ware & Majerus, 2008; Brown et al., 2011). The impacts of *H. axyridis* and *C. septempunctata*, like many IAS, also extend to the economy, impacting fruit production in many countries as well as becoming a nuisance pest in households (Koch & Galvan, 2008).

Since its arrival in the U.K. *H. axyridis* has spread rapidly from the south east (Brown et al., 2008) at a calculated mean north westerly spread rate of 94.3km year<sup>-1</sup>. *Coccinella septempunctata* also exhibited rapid spread in its invasive range; after its first use in biological control in 1956 the species first became established in 1973 and by 1987 it was present widely across the U.S.A. This rapid spread is indicative of highly dispersive species, which is also noted by Hodek & Honěk (1996), therefore the high dispersal ability of *H. axyridis* and *C. septempunctata* is likely to have played an important role in their ongoing invasions.

It is generally accepted that coccinellids perform 4 main types of flight, classified according to the motivations behind them; trivial (or appetitive) flight, hectic trivial flight, migration to dormancy sites and non directional dispersal from dormancy sites (Hodek et al., 1993). However before the description of these flight types, it is prudent to clarify the language relating to flight used in this report, as ambiguity in the definitions used by authors in the past has caused confusion.

The term appetitive flight is used to describe short flights of as little as <2m (Elliott, 2000) in coccinellids, which are performed in search of prey and ovipositioning sites in a small area. The term migratory flight is used here to describe flight to dormancy sites in autumn for the purposes of overwintering. The important and defining feature of migratory flight is the insect's inability to respond to appetitive cues (e.g. volatiles present due to prey etc.) that would otherwise cause them to land (Chapman & Drake, 2010). Long distance flight is used to define flight that is not migratory (i.e. to dormancy sites) but that is longer than appetitive

flights. The reason for the differentiation between long distance flight and migratory flight is that there is no evidence as to whether or not coccinellids undergoing long distance flight are capable of responding to appetitive cues. As used by Chapman & Drake (2010) the term dispersal is used here to define flight that results in the spreading out of a population either within a current range (occupying previously un-occupied areas or resulting in mixing in those that were already occupied) or leading to expansion of that range. Therefore here, dispersal is used as an umbrella term to incorporate appetitive, long distance and migratory flight.

Appetitive flight, which is associated with foraging and ovipositioning behaviour, links intricately with aphid population dynamics. This type of dispersal is essential in *H. axyridis* and other aphidophagous coccinellids due to the ephemerality of their prey (Hodek & Honěk, 1996)

Hectic trivial flight is a form of long distance flight and is seen as an extension of appetitive flight; at times of low food abundance coccinellids are forced to perform prolonged flight in search of new foraging sites. It is during these irregularly long flights that coccinellids are thought to be carried to high altitudes on thermal currents and subsequently disperse on winds at these heights (Hodek & Michaud, 1993). Occasionally this phenomenon can occur across a large area simultaneously and results in large groups of dispersing coccinellids being passively carried together on wind currents which then deposit them all in the same place in the landscape (Hodek & Michaud, 1993).

Foraging and reproduction provide two powerful motivations for flight, another is diapause. Migration to dormancy sites is a behaviour which changes between coccinellid species. In the case of *C. septempunctata* not all individuals perform long distance migrations to overwintering sites; many perform short flights to nearby forest dormancy sites (Hodek et al 1993). This may also be the case in *H. axyridis*, which now commonly overwinter in manmade structures i.e. homes (Huelsman et al, 2002). In their native habitat *H. axyridis* would typically overwinter in the crevices of rocks and similar locations offering shelter (Riddick et al., 2000),

however in the U.K. and generally in more urban areas these environments are less common. It has previously been suggested that some coccinellids use odour of pheromones of other ladybirds to return to the same specific overwintering habitat i.e. a particular rock crevice, over consecutive seasons however, if this is true it is likely only at a small spatial scale (Majerus, 1994). The majority of evidence points to long distance migrations to dormancy sites being hypostatically driven, i.e. driven by visual cues (Riddick et al., 2000; Hodek et al., 1993; Koch, 2003), as they are often observed landing on prominent, light coloured landmarks. Dispersal from overwintering sites is non-directional, the main difference between this and migratory flight being that the return journey consists of short, almost exploratory flights to areas adjacent to the overwintering sites and upon encountering aphid prey *H. axyridis* will likely halt its dispersal. This is likely a behaviour born out of a necessity to replenish energy stores after dormancy periods (Honěk & Hodek, 1996). Despite the previous research surrounding coccinellid dispersal, there are still many aspects that remain unknown. Although it is stated that coccinellids use winds in order to facilitate long distance flight and migration (Honěk & Hodek, 1996), no direct observations of this have previously been possible. Furthermore, long distance flight is likely to be governed by many environmental factors, the effects of which are again unknown. In light of the invasions of these species and the importance of dispersal in adaptability at range boundaries, increasing knowledge within these research gaps could potentially be valuable to the understanding of dispersal's role in invasions and the in predicting future range expansions (Peterson & Vieglais, 2001).

## **Thesis aims**

In Chapter 2 of this thesis, VLR is used to investigate the dispersal of *H. axyridis* & *C. septempunctata* at altitudes above 150m; an aspect of their ecology that has previously been impossible to observe. These observations of flying ladybirds will be compared to meteorological factors and aphid population dynamics in order to look for and examine the relationships between them. This information will then be considered in relation to the *H.*

*axyridis* invasion in the U.K. Chapter 3 will examine a newly designed protocol for the testing of coccinellid flight *in vitro* and uses this method to compare flight abilities between native populations and individuals from the invasion range boundary in the U.K. Finally, Chapter 4 will discuss the conclusions of Chapters 2 & 3 in relation to the importance of dispersal in invasions and also the evolutionary implications of invasions on dispersal.

## **Chapter 2.**

# **Biotic and abiotic predictors of dispersal in ladybirds and implications for the spread of invasive species.**



## Abstract

Dispersal is essential to the establishment and persistence of populations in heterogeneous environments and so likely plays a key role in species invasions. The dispersal above 150m of two coccinellids; *Harmonia axyridis* (invasive in the U.K.) and *Coccinella septempunctata* was examined here using aerial density (AD) estimated using vertical looking radar (VLR) at three sites in England with the aim of identifying the main predictor of dispersal in these species. The effects of temperature and wind speed on aerial density were addressed using met office data from the U.K. The effects of local aphid abundance on aerial density were addressed using aphid abundance data from the Rothamsted insect survey suction trap network. Temperature was found to be the strongest predictor of aerial density and a significant negative relationship was found between ladybird AD and wind speed. No significant relationship was found between AD and local aphid abundance. The distribution of temperature and wind speed in relation to U.K. topography suggests that meteorological conditions that are unfavourable for flight over the Pennines (Yorkshire) and Cambrian Mountains (Wales) may act as a dispersal barrier; slowing the spread of the *H. axyridis* invasion in the U.K.

**Keywords:** Dispersal, trivial appetitive flight, migration, invasion, ladybird, temperature, wind speed, aphid, vertical looking radar.

## 2.1 Introduction

Dispersal is essential for the establishment, persistence and evolution of species (Olivieri et al., 1995) and can have a strong impact on the progress and success of invasions (Travis & Dytham, 2002; Hughes et al 2007). However the mechanisms behind the effect of dispersal on invasions are not yet well understood.

Insect dispersal is governed by both behavioural and physiological factors. The former relates to motivations for flight, e.g. the search for foraging, ovipositioning or overwintering sites. The latter refers to physical constraints imposed by the environment, namely meteorological conditions such as temperature, sunlight, humidity, wind speeds and rainfall. These factors are highly variable both spatially and temporally, and changes in them will affect aspects of the flight of different insect species in different ways. Knowledge of the specific dispersal characteristics of invasive species in relation to environmental factors could aid prediction of future range expansions. In this Chapter we examine the effects of some of these factors on the dispersal of two invasive ladybird species (Coleoptera: Coccinellidae), *Coccinella septempunctata* (L.) and *Harmonia axyridis* (Pallas), with the aim of adding to the limited present knowledge in this area.

*Coccinella septempunctata* and *H. axyridis* are key aphid predators and consequently have been widely used as biological control agents against aphid pests (Straub & Snyder, 2006; Koch, 2003). However, the traits that have made them desirable biological control agents such as polyphagy, voracity, multivoltinism and high dispersal capacity (Honěk & Hodek, 1996) may well be the traits that predispose them to invasive success (Hodek & Michaud, 2008; Hughes et al, 2007). Both *C. septempunctata* and *H. axyridis* are invasive in various parts of the world. *Coccinella septempunctata*, native to Eurasia, is now invasive in U.S.A. and Canada (Hodek & Michaud, 2008). *Harmonia axyridis*, which is native to Eastern Russia, Eastern Kazakhstan, Siberia, China, Korea and Japan, has an extensive invasive range; now present on almost every continent (see Appendix 1). The introduction of these species for biological control is

considered a major contributing factor to their invasion success and the resulting deleterious impacts in many countries outside of their natural range (Koch, 2003). However the importance of their dispersal ability in their invasion success has not previously been addressed. In fact, much of the current knowledge concerning the behaviours, abilities and motivations for dispersal of *C. septempunctata* and *H. axyridis* is derived only from broad observations of coccinellids in general.

Hodek & Honěk (1996) outline four main flight behaviours applicable to coccinellids; 1) trivial flight, (also known as appetitive flight) is used for foraging and searching for ovipositioning sites and comprises short flights (both in distance and duration). 2) Hectic trivial flight; an irregular elongation of trivial flight, which arises as a response to low prey density or overcrowding. 3) Long distance migration to dormancy sites. 4) Dispersal from dormancy sites. These classifications focus on the motivations for flight in coccinellids. Williams (1940) suggested that from a physiological perspective, the total activity of insects is determined by (activity due to temperature) x (their activity due to light) x (activity due to wind speed).

Of the environmental factors that can influence insect dispersal, three that are considered to be of major importance are addressed in this study: temperature, wind speed and local aphid abundance.

Taylor (1963) identified an important relationship between minimum and maximum temperature thresholds and flight of a number of insect species and further points out that, in a temperate climate, it is the minimum threshold that will be the most influential. The mechanisms of flight inhibition by low temperatures are concerned predominantly with flight muscle; in order to achieve flight, the energy output of an insect's flight muscle must exceed the minimum threshold required for take-off. At low temperatures wing muscles are unable achieve this (Stevenson & Josephson, 1990) and it is this that limits flight at low temperatures. Where these thresholds exist will determine when and where an insect can fly. There is also evidence to suggest that temperature can act as a cue for dispersal, for example, Heulsman et

al. (2002) found that *H. axyridis* begin their dispersal to dormancy sites on the first day over 18°C after a period of colder weather.

The use of winds can greatly increase the migratory capability of insects, as shown by Chapman et al. (2008) who found that moths can travel up to 650m per flight using air currents. Wind speeds increase with altitude and this has an effect on the altitudinal distributions of flying insects. Taylor, (1974) described a phenomenon called the flight boundary layer (FBL), which is the height at which wind speed exceeds the maximum self-powered flight speed of an insect. Above this height insect flight direction will be downwind. Wind speed has also been found to have a predictive effect on the take off propensity of some insect species (Briers et al., 2003). Wind currents are thought to be important in coccinellid flight; observations have been made of coccinellids using wind currents to travel along mountain gorges to overwintering sites (Hodek & Michaud, 1993 and references therein) however to our knowledge, no direct study of the effects of wind currents on *C. septempunctata* or *H. axyridis* has yet been performed.

Aphids (Hemiptera: Aphididae) are thought to be an important motivation for coccinellid flight; during summer months females search for areas of high aphid abundance as foraging and ovipositioning sites (Hodek & Honěk, 1996). Aphid populations are highly ephemeral (Dixon, 1985), therefore in order to exploit such a prey coccinellid ovipositioning must be timed in relation to the characteristics of aphid colonies to provide the best environment for the development of larvae (Hemphill et al., 2010). In turn, the dispersal of female coccinellids must have a high degree of plasticity to allow them to track such a prey. A major motivation behind male flight however is the location of females and in doing so, males locate suitable foraging sites (Evans, 2003). The increase in number of adult coccinellids in an area as a result of a decrease in emigration rates has often been observed where aphid prey is abundant (Osawa, 2000; Krivan, 2008; Obata, 1986). Aphids can therefore be considered an arrestant to dispersal (Hodek & Honěk, 1996).

The lack of information on dispersal of *H. axyridis* and *C. septempunctata* is likely due in part to difficulties in observing ladybird flight in real time. As a result, conclusions have previously been limited to assumptions inferred from ground level observations (Elliott et al., 2000; Johnson, 1969; Hodek & Michaud 1993 and references therein) and *in vitro* experiments (Rankin & Rankin, 1980; Solbreck, 1974). Technological advancement has allowed the study of insect migration to progress in recent years, namely with the invention of Vertical Looking Radar (VLR) (see Chapman et al., 2003 for a review of VLR technology). Using VLR, it is now possible to observe high altitude dispersal in real time. Here we combine the use of VLR data from three sites in England with U.K. meteorological office and aphid abundance data from one site in order to: 1) assess the temporal trends in the dispersal of *C. septempunctata* and *H. axyridis*, 2) examine the flight characteristics of these species, namely their vertical distribution during flight, their FBL, and the distance that they able to cover during dispersal, 3) identify the most important predictor of dispersal in these species specifically between temperature, wind speed and local aphid abundance, and 4) to examine the implications of the findings from the above on the invasion of *H. axyridis* in the U.K.

## **2.2 Methods**

### ***2.2.1 Vertical Looking Radar data***

The data used here was obtained from three VLR sites: at Rothamsted research in Harpenden, Hertfordshire; Malvern, Worcestershire and Chilbolton, Hampshire. The VLR emits a narrow vertical beam that allows for the detection of insects within 15 range gates (altitudinal bands) between 150 - 1200m above ground level (AGL) depending on insect mass. Insect detections are recorded for 5 minute periods with 15 minute intervals for 24 hours a day (see Chapman et al 2002, 2003) and data used in this study encompassed years 2000-2010.

Insects at altitudes below 150m AGL are not be detectable by the VLR as a result of the minimum interval between the time that the radar pulse is emitted and the time that the system is able to receive backscatter signals (Chapman et al., 2002). Above this height, the

maximum altitude of detection for an insect is related to its size; the minimum height of detection AGL for a 1mg target is 150m and being so small, insects of this size are only detectable in a portion of the first altitudinal range gate. However, larger insects can be detected at higher range gates therefore the potential sampling volume increases with insect mass. In order to allow analysis of VLR detections it was necessary to use the parameter “aerial density” (AD) which standardises all insect detections by their volume of potential detection (Chapman et al., 2003). We used AD as a proxy for estimating dispersal; however we note that this is a conservative estimate as it only accounts for flight above 150m. Because of their relatively large size, *H. axyridis* and *C. septempunctata* are detectable throughout the VLR height range. The VLR also gives readings of insect shape (length of body axes), mass, displacement speed and direction, orientation and the altitude of the range gate in which an insect is detected.

***Identifying C. septempunctata and H. axyridis in the VLR data.*** In order to discriminate between *C. septempunctata* and *H. axyridis* and the numerous other insect species detected by the VLR, two species-diagnostic characteristics were used: 1) mass and 2) shape (described below). Identification of species in the VLR data cannot be done with 100% confidence as it is impossible to physically catch insects detected by the VLR and match them with detection data. However certain characteristics of *H. axyridis* and *C. septempunctata* make it very likely that they make up the majority of insects detected. *Harmonia axyridis* and *C. septempunctata* are among the largest U.K. ladybirds, along with the striped ladybird (*Myzia oblongoguttata*) (L.), the cream streaked ladybird (*Harmonia quadripunctata*) (Pontippodan), the scarce 7-spot ladybird (*Coccinella magnifica*) (Redtenbacher) and the eyed ladybird (*Anatis ocellata*) (L.) (Coleoptera: Coccinellidae) (see Appendix 2). Of these larger coccinellids *H. axyridis* and *C. septempunctata* are by far the most numerous in the U.K. (data.nbn.org.uk; www.ladybird-survey.org; Hodek & Honěk, 1996). Additionally, relative to other insects and many other coccinellids, *H. axyridis* and *C. septempunctata* have a characteristically circular body shape, and therefore a low body axis ratio (Length:Width) as detected by the VLR. Therefore when

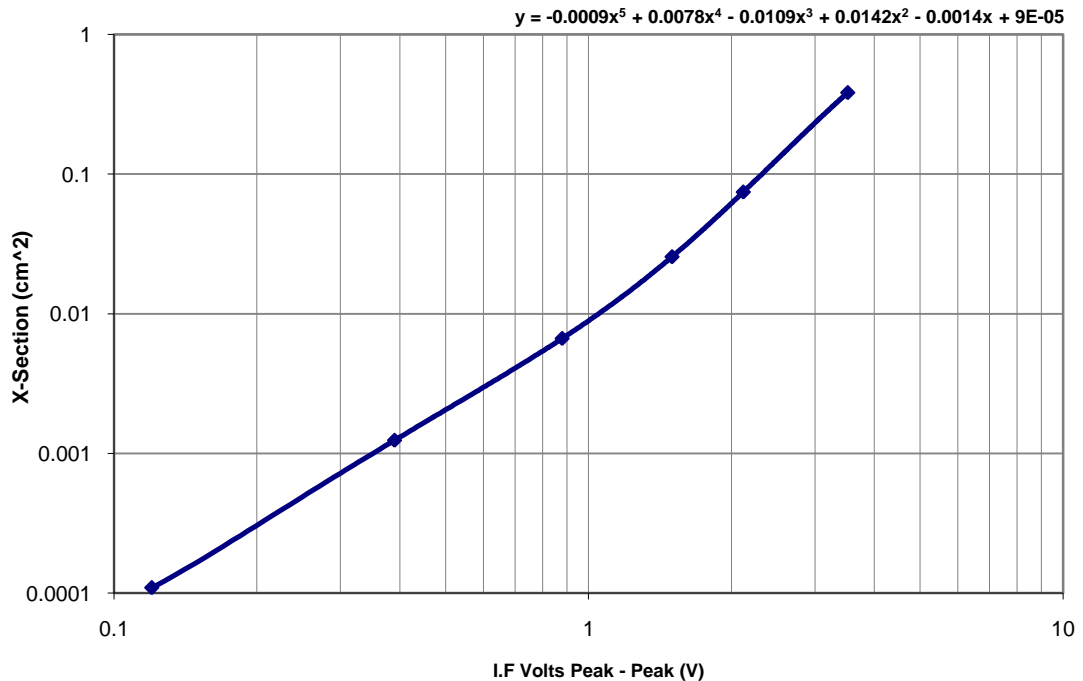
used together, the mass and body axis ratio are diagnostic characteristics for *C. septempunctata* and *H. axyridis*. Mass and body axis ratio ranges were used to extract detections from the total VLR database that can, with confidence be taken to include the vast majority, if not all of *C. septempunctata* and *H. axyridis* in the air (J. Chapman *pers. comm.*).

To provide mass data for *H. axyridis*, 20 individuals (10 male and 10 female), were selected from field collections from Hull, Humberside that represented as wide a size range as possible. Each sample was frozen live for approximately 10 minutes immediately prior to weighing and sexing. Mass measurements were previously made for 5 *C. septempunctata* individuals (J. Chapman *pers. comm.*), however the sex and origin of these samples is not known (Appendix 3.).

VLR produces data on the shape and orientation of detected insects by rotating the plane of beam polarisation around the central vertical axis of the radar. The amplitude of the returned signal is (for most insects in the UK) maximal when the beam's plane of polarisation is in line with the insects major body axis ( $\sigma_{xx}$ ) i.e. its length, and minimal when parallel to its minor axis, its width ( $\sigma_{yy}$ ) (Riley, 1985). By analysing the degree of modulation between the backscatter signals of the two body axes ( $\sigma_{xx} / \sigma_{yy}$ ) a ratio for the insect's shape can be calculated. However, VLR does not give actual measurements of insect dimensions; instead  $\sigma_{xx}$  and  $\sigma_{yy}$  are representative values. In order to correctly identify *H. axyridis* and *C. septempunctata* it was first necessary to produce estimates of how these species are "seen" by the VLR (Riley, 1985). It was not feasible to catch and measure insects that have flown through the VLR sample area, instead a laboratory-based transmission line rig was used to produce measurements that are indicative of those given by the VLR.

The same 20 samples used for mass measurements were used to produce a body axis range reflective of the natural population. Calibration measurements were first made of a selection of steel spheres of known size covering the possible range of the target species size. The

backscatter values for the spheres were plotted against diameter to produce a calibration curve (Fig. 2.1) used to translate backscatter signals of *H. axyridis* measured in the rig into measurements of the insect's actual cross section.



**Figure 2.1.** Calibration chart plotting backscatter values of 6 spheres (x) against their known cross sectional diameter (y). The associated polynomial regression equation was used to translate backscatter values for *H. axyridis* (indicative of those produced by the VLR) samples into actual cross sectional values.

*Harmonia axyridis* samples were placed into the transmission rig individually; samples were glued onto a 0.1mm nylon thread with the head pointing upwards so that the longest body axis (y) was in line with the plane of the radar beam. Twirling the thread on which the insects were suspended alternated the position of the insect, presenting dorsal, lateral or ventral aspects to the beam source. It was however easy to determine (and already known, Riley, 1985) that the backscatter was maximal when ventral aspect was presented, and minimal when the insect was positioned laterally. The line was weighted below the rig to reduce movement and once any motion had ceased 3 independent blind measurements of the maximum backscatter signal were taken from the ventral aspect for the longest body axis (y). Samples were then rotated 90° and the process was repeated for the shorter body axis (x).



The calibration measurements identified a range of backscatter values for *H. axyridis* and *C. septempunctata* (Appendix 3). These two ranges were then combined to give a range (Fig. 2.2a) that, when used to filter data from the VLR database, would identify the majority of *H. axyridis* and *C. septempunctata* from the insects detected by the VLR (Fig. 2.2b).

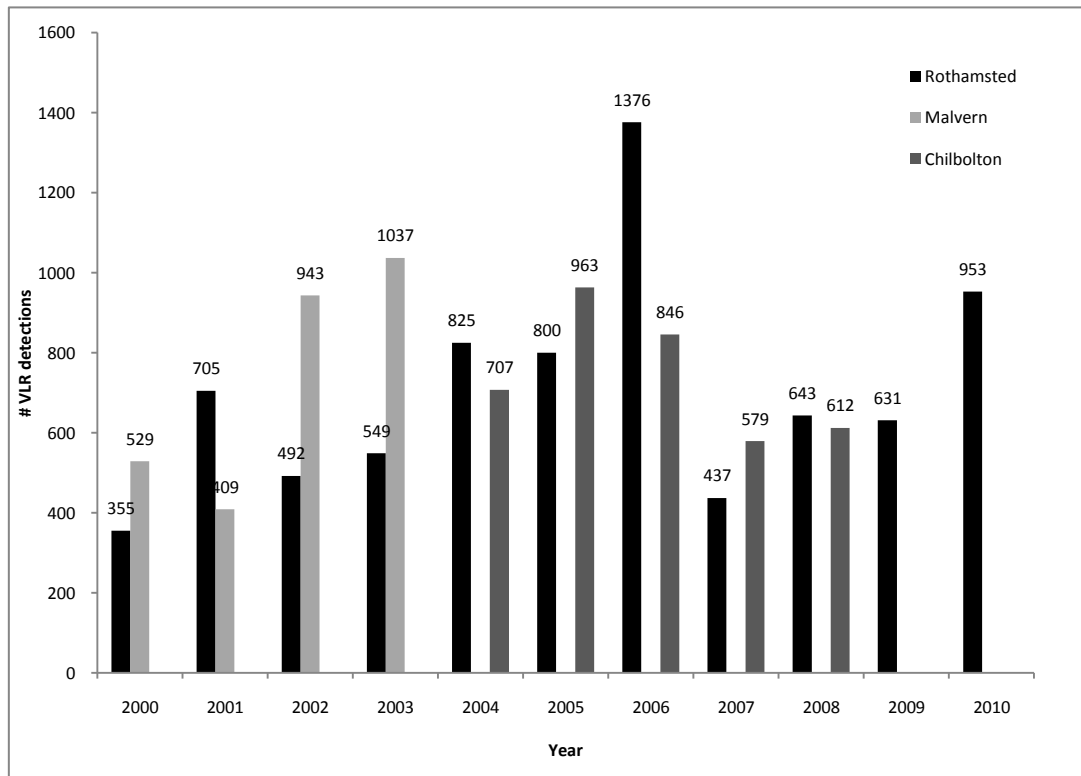
Previous studies (e.g. Taylor, 1974; Hodek & Honěk, 1996) suggest that migratory insects show increased flight activity during summer months. In light of this, the data used in the analyses of this study are from the months May – October (incl.). Detections that fell within the above ranges were extracted for hours 09:00 – 15:00 from May – October 2000 – 2010 (Table 2.2a) as coccinellids undergo diurnal flight in the warmest hours of the day within the warmest months of the year (Hodek & Honěk, 1996). This allowed for the temporal trends both during and between years to be analysed (see “Statistical analyses”).

**Flight classification and FBL.** In order to make biologically significant conclusions pertaining to ladybird flight it was necessary to identify the type of flight performed by insects detected in the VLR. This was done by estimating the FBL for *H. axyridis* and *C. septempunctata*. An assumption was made here that flight above the FBL is intentional for the purposes of wind-borne long distance dispersal in which insects are carried downwind by air currents faster than their own flight capabilities would allow (Taylor, 1974). This would therefore confer an increase in displacement distance. Below this height flight is directional and considered appetitive. It was also assumed that at heights above the FBL flight is oriented with wind direction. To calculate the FBL the air speed (flight speed discounting the effects of air currents) was estimated for *H. axyridis* and *C. septempunctata* by subtracting the wind speed at the time and altitude of detection from the insect’s displacement speed (air speed + wind speed). The approximate FBL calculated here is the altitude at which average wind speed exceeds average air speed.

a)

Filtering Criteria	Selected range
Time of Year	May – Oct
Time of Day	09:00 – 15:00
Altitude	All
Mass range (Mg)	25 – 42
$\sigma_{xx} / \sigma_{yy}$ Ratio range	1.5 – 4.1

b)



**Figure 2.2 a)** Data filtering criteria for extraction of *H. axyridis* and *C. septempunctata* from VLR database. **b)** Number of *H. axyridis*/*C. septempunctata* like detections identified from Rothamsted, Malvern and Chilbolton VLR sites for May – Oct of 2000-2010, 2000 – 2003 and 2004-2008 respectively. All data was taken from 09:00 – 15:00 of each day.

## 2.2.2 The role of abiotic and biotic factors in dispersal

**Meteorological data.** To assess the role that abiotic factors play in influencing dispersal of *H. axyridis*/*C. septempunctata* first temperature and then wind speeds were compared with VLR detections using time series analyses (see “Statistical analyses” below). The meteorological data used in the present study was produced by the U.K. Met office Unified Model (UM). The model uses weather measurements at given locations to extrapolate theoretical values at chosen coordinates between them (Wood et al., 2006). Outputs were extracted for each hour of each day encompassing the period for which VLR data was filtered (see Fig. 2.2.2a). Values at 15 altitudes were produced for each hour providing an altitudinal and temporal profile for temperature and wind speed at the location of the Rothamsted Research Centre. Met office data was only available for the Rothamsted site therefore only VLR data from this site were used in these analyses, however variation in AD for the filtering criteria used was not significant between sites ( $F = 0.104$ ,  $df = 2$ ,  $p = 0.903$ ) (Appendix 4.).

Unfortunately due to limitations of the VLR it was not possible to measure the number of *H. axyridis* like insects during times of heavy rain; the VLR detects insects when the radar beam is reflected by the water content of the insects body to create a backscatter signal; as a result of this, in times of heavy rain the radar detects rain drops in the sampling area. The VLR uses an iterative computing procedure run by the automated computer system that operates the VLR to identify insect detections from “non-biological particles”. This is done by comparing backscatter parameters, as detected by the VLR, with a previously produced “insect scattering model” (Chapman et al., 2002). With this method each insect detection is automatically given a coefficient which relates to the likelihood that it is indeed an insect. Detections that are assigned coefficients below 0.9 are excluded from further analyses and so not included in the data in this study. At times of rain this procedure fails to describe an adequate coefficient because of signal interference from rain drops and so many insect detections will not be registered. However, at these times insect flight is minimal (J. Chapman *pers. comm.* ) and so

it is assumed here and in other radar studies that the missing insect detections would have little effect on the overall analyses.

**Aphid data.** To examine the flight behaviour of *C. septempunctata* and *H. axyridis* in relation to local aphid abundance data from the “Aphid Bulletin” run by Rothamsted Insect Survey (RIS) ([www.rothamsted.bbsrc.ac.uk/insect-survey/](http://www.rothamsted.bbsrc.ac.uk/insect-survey/)) was used. This data comprises aphid abundances from a network of 16 suction traps (each 12.2m in height and sampling 0.75m<sup>3</sup> of air per second) across the U.K. (Macaulay et al., 1988). Aphids caught in the suction traps are identified to species where possible, if not, then to species group or genus. Data for all 23 aphid species recorded by the RIS (See appendix 5) from the “Rothamsted Tower” (at the same site as the Rothamsted VLR) were used to give monthly totals of aphids caught at this site from 2000-2010. The generalist nature of *C. septempunctata* and *H. axyridis* makes it very likely that many of these species are predated upon to some degree, also there is little direct evidence describing aphid species which either *C. septempunctata* or *H. axyridis* do not consume; therefore all 23 aphid species were included in the analyses (see Appendix 5).

### **2.2.3 Statistical analyses**

The VLR, meteorological and aphid catch data used here presents two problems when analysing for causal relationships between variables. The first is autocorrelation (or temporal pseudoreplication); the correlation between points within a data series that is constrained by time. For example, July is likely to be warm in all years and so the temperatures for July between years will correlate with each other. The second problem is covariation of explanatory variables, e.g. as temperature rises, wind speed falls. A correlative relationship with one may therefore result in a relationship with the other with no indication of which is the most important predictor. Two types of statistical analyses are therefore used here; time series analyses in “R” (R development core team, 2011). To control for autocorrelation, and linear and stepwise multiple regressions in order to control for covariation. However, neither method accounts for both problems.

To look for a relationship between the number of ladybirds flying and the local aphid abundance at that time AD and aphid time series were analysed using the STL function from the basic R package (stats); this is the Seasonal Decomposition of Time Series by Loess. This function was used to fit a model to the data to describe seasonal cycles across the years. Underlying trends in the data could then be examined after accounting for the seasonality (Crawley, 2007). Autocorrelation analyses were carried out for *H. axyridis*/*C. septempunctata* aerial densities, aphid catches, average temperature and average ground level wind speed time series. The impact of wind speed on AD was assessed with wind speed data at 10m AGL (level 0). Missing data for AD and Met office (May 2001, Oct 2007, 2008) were reconstructed using means from the same month in other years to fill the requirements of time series analyses.

Cross correlations were used to look for correlations indicative of causal relationships between time series for AD and Average temperature, average ground level wind speed and Aphid density. Linear regressions were used for pair wise comparisons to look for relationships between AD and each predictor.

In order to identify an optimal temperature range for flight in *H. axyridis* and *C. septempunctata*; a sub-sample of data was compiled matching ladybird detections with the temperature at the time and altitude of detection. A sample size of 700 detections was used as matching meteorological conditions with detections for a larger sample size would have been impractical. The total database was sampled so that detections used were spread evenly over the parameters: time of day, month and year to constitute an adequate representation of the variation within the larger data set.

A linear regression was performed on the above sub-sampled data to test for a relationship between the number of flying *H. axyridis* and *C. septempunctata* and the ground level wind speed at the time of detection. Days were selected where ground temperature was between 16-21°C in order to minimise any affect that lower and upper temperature thresholds as

described by Taylor (1974) may have had within this data. Linear regression was performed in order to confirm that no relationship between AD and temperature existed within this range. Ground level wind speeds were used in these analyses as a proxy for high altitude wind speeds; met office data showed a consistent positive linear relationship between altitude and wind speed (Appendix 6). Averages of the wind speed at ground level were then taken for each day and compared to the corresponding aerial density total for that day.

The cross correlation between temperature and ground level wind speeds was examined to address the possibility that any relationship between AD and wind speed might be driven by a strong relationship between AD and temperature and vice versa.

In order to identify the strongest predictor of ladybird dispersal, backwards stepwise multiple regression was used to identify minimal adequate regression models (IBM SPSS for windows release 19.0.0).

#### ***2.2.4 Implications for invasion***

The implications of the dispersal ability for the spread of invasion was first assessed by comparing range distribution data from The Harlequin Ladybird Survey ([www.harlequin-survey.org](http://www.harlequin-survey.org), Roy et al., 2006) with the distribution of temperature and wind speed across the U.K. from the Met Office ([www.metoffice.gov.uk/](http://www.metoffice.gov.uk/)).

The potential distance travelled per flight for detected ladybirds was estimated by multiplying the displacement speed given by the VLR, which was averaged for all detections in the month of July, 2006, by the average flight time of *H. axyridis*. Average flight time was calculated from observations of tethered flight experiments carried out *in vitro* (see Chapter 3 for full details). 40 *H. axyridis* individuals (20 from U.K., 20 from Japan) were tethered behind their pronotum with fine fishing line and suspended in a clear perspex cube. Tests were allowed to run for up to two hours and several test subjects flew for this amount of time. Each flight was recorded with a video camera and flight time per test was recorded. Average flight time was taken from

the uninterrupted flights (i.e. flight time before first pause in flight) of all individuals from the U.K. (see Chapter 3).

## 2.3 Results

### *2.3.1 Vertical looking radar data*

Filtering of the main VLR database produced a total of 7766 detections for the Rothamsted VLR between 2000 and 2010. 3273 detections were filtered for the Malvern VLR for years 2000-2003 only and 3707 for the Chilbolton VLR for 2004-2008 (Fig. 2.2b)

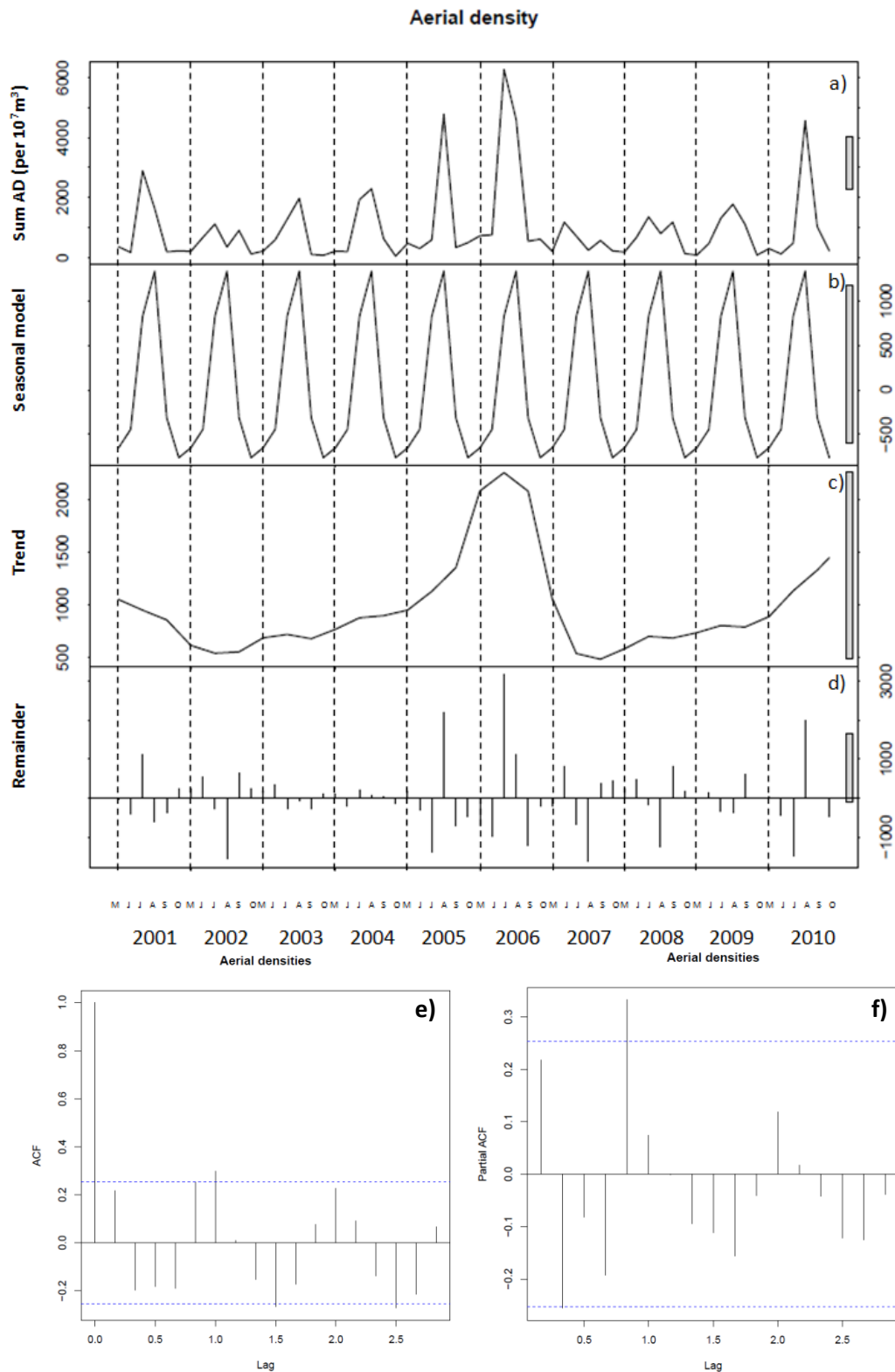
Time series analyses here were performed on Rothamsted data only and show that aerial densities exhibit annual cyclic behaviour, peaking, in the majority of years in July and August (Fig. 2.3a). This is supported by the decomposition of the time series which describes the seasonal nature of these cycles (Fig. 2.3b). The signal remaining after accounting for seasonality shows no increasing or decreasing trend during 2001 – 2010 but shows a large peak in 2006 and 2010, with low aerial densities in 2002 and 2007 (Fig. 2.3c).

Analysis of autocorrelation highlights a significant relationship at a lag of 1 year, indicating correlation between the same months across years. Significant negative autocorrelations are found at lags of 1.5 and 2.5 where high AD of midsummer months (June, July) are inversely correlated with the low AD for 3 months earlier in late spring (May) and of 3 months later in early Autumn (Sept) (Fig. 2.3e). No autocorrelation is shown between one month and the previous month suggesting that monthly AD averages are independent.

***Flight classification and FBL.*** The average air speed of detected ladybirds, calculated by subtracting wind speed from the velocity of VLR ladybird detections, was  $5.3\text{ms}^{-1}$  which equates to approximately 19km/h. Wind speeds can fluctuate at ground level according to the time of day (approx  $1\text{-}6\text{ms}^{-1}$  normally lowest in early morning and highest in mid-afternoon) and time of year (averaging between 3 for some midsummer months to approx 4.5 for spring and autumn), however on average the wind speed at 150m was  $6.3\text{ms}^{-1}$ . This exceeds the

maximum air speed of  $5.3 \text{ ms}^{-1}$ , thus the average FBL for detected ladybirds was lower than minimum detection altitude of the VLR.





**Figure 2.3** a) Time series for AD of *H. axyridis* & *C. septempunctata* over Rothamsted research centre for May – Oct, 2001-2008 inc. b) Seasonal decomposition model for time series. c) Overall annual trend after accounting for seasonal cycles. d) Remaining variation not accounted for by seasonal model or overall trend. Vertical grey bar represents relative scale of Y axes. e) Autocorrelation plot for AD to test for temporal pseudoreplication within the data. A lag unit of 1.0 equates to 1 year. As only 6 months (May-Oct) were used for each year the 6 peaks within each lag unit represent a lag of one month each. Peaks crossing the horizontal dashed blue lines represent significant autocorrelation at the 0.05 level. f) Partial auto-correlation for AD using the same plot properties as in e).

### ***2.3.3 Effects of abiotic and biotic factors on dispersal***

**Temperature.** Cross correlation of time series data for AD and temperature showed that there is a significant relationship between the two, with strong correlations between AD and temperature of the same month. The similar seasonal cycles in both time series result in the correlations between months that are 1 and 2 years apart. Inverse correlations exist for 3 and 15 month lags matching the low temperatures in spring and autumn (Fig. 2.4b).

Matching detections with corresponding temperatures in the sub-sampled data set shows that flight activity is essentially non-existent below temperatures of 6°C - 7°C, after which there is an increase in the number of ladybirds flying which peaks at 19°C and remains high at 17-20°C. AD then fall gradually as temperature continues to increase and no detections after 29°C are recorded (Fig. 2.5).

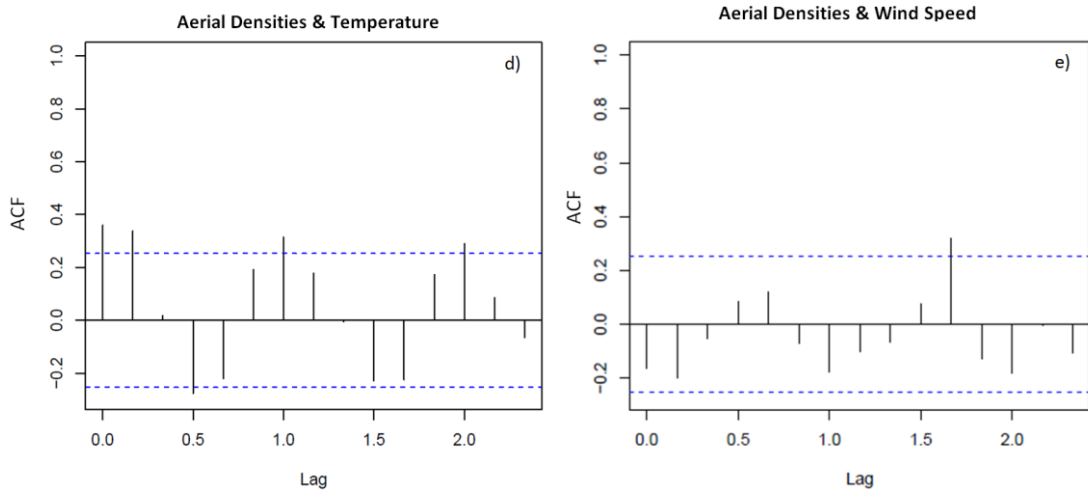
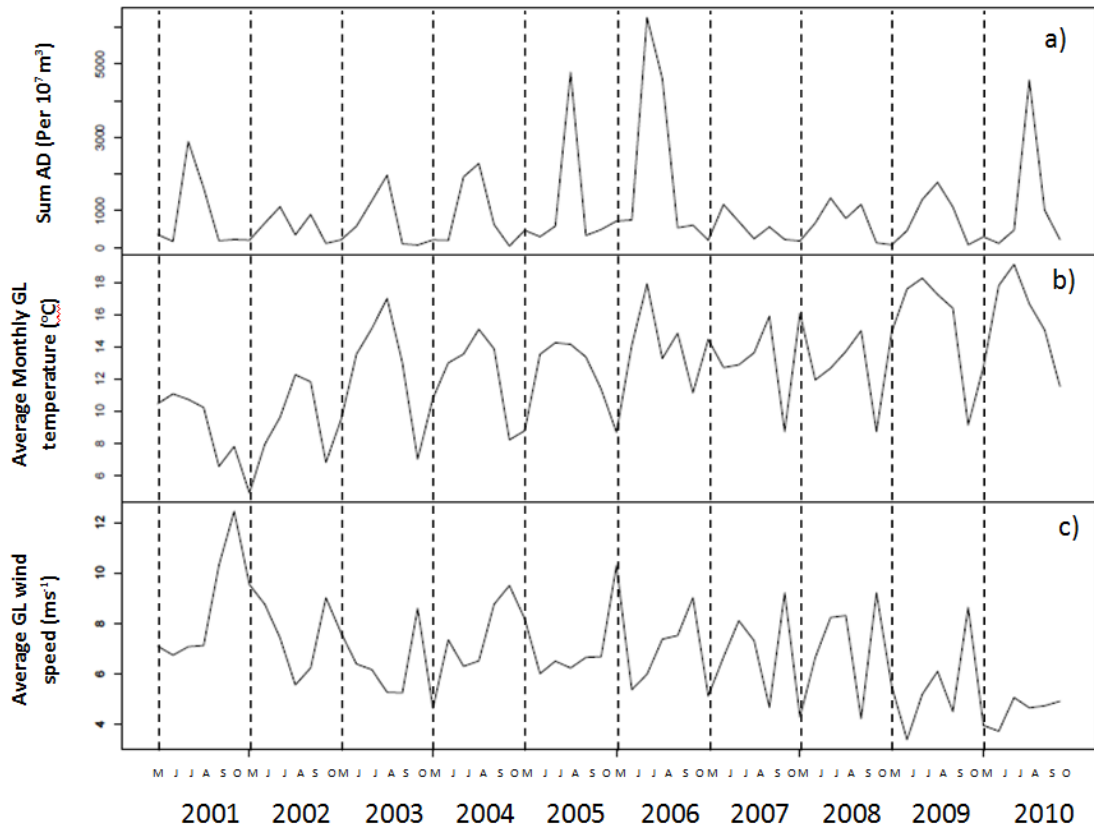
**Wind speed.** Cross correlation analyses of time series for AD and wind speed identified no significant relationship for AD and ground level wind speed for the same months (Fig. 2.4 e). However, linear regression for AD and wind speed at ground level from the sub-sampled data set show a significant negative relationship between aerial density and average ground level wind speeds at the time of detection ( $R^2 = 0.22$ ,  $n = 74$ ,  $p < 0.001$ ) (Fig. 2.6). No significant relationship was found between temperature and AD within this range ( $R^2 = 0.004$ ,  $n = 74$ ,  $p = 0.647$ ), power analyses showed that the sample size used was adequate to detect a relationship with an effect size 0.2 (minimum sample size required, = 60). The amount of variation in AD decreases as wind speed increases; AD do not reach above 50 for wind speeds exceeding  $5\text{ms}^{-1}$ . The cross correlation between temperature and ground level wind speeds showed no significant relationship, therefore temperature is not the driver of the relationship between AD and wind speed at ground level.

**Aphids.** The seasonal patterns in aphid suction trap catches show different cyclic patterns than those seen in ladybird flight (Fig. 2.7a). The seasonal decomposition model for the aphid time

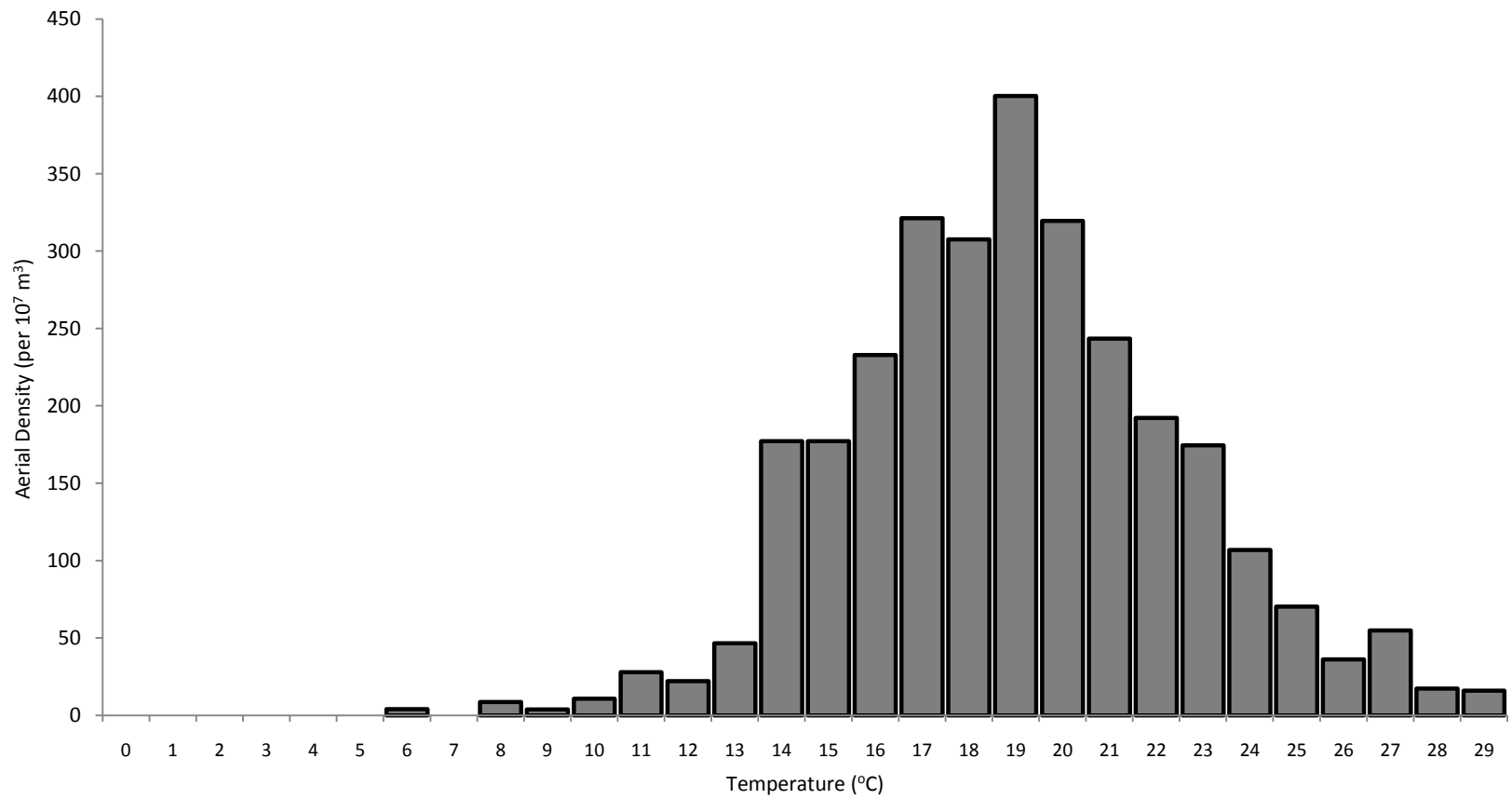
series data indicates the presence of 3 peaks in aphid abundance within most years the largest of which is in May, the second in July and one in October (Fig. 2.7b). The overall trend highlights the three years of relatively high aphid abundance larger peaks 2004, 2006 and 2010 and the decline in 2007 and 2008 also seen in AD and temperature.

Cross correlation showed a significant relationship between AD and the aphid catch numbers with a lag of 3 months (Fig. 2.7f). Regression analyses however showed no significant correlation between AD and the aphid suction trap catches ( $R^2 = 0.67$ ,  $n = 49$ ,  $p = 0.07$ )

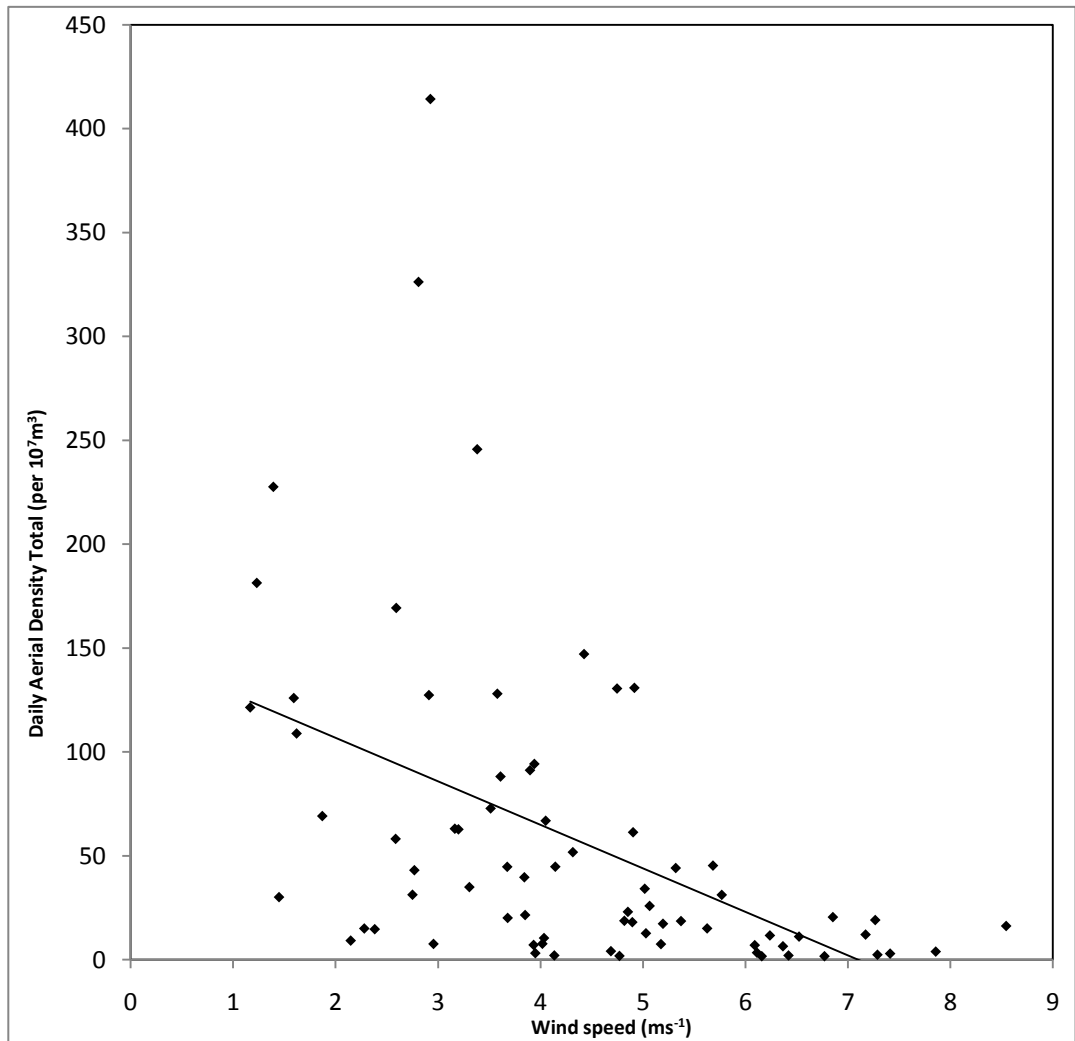
***The strongest predictor of dispersal.*** Multiple backwards stepwise regression analyses including all predictors of AD eliminated both wind speed and aphids as significant predictors of variation in monthly ladybird AD, identifying temperature as the most important predictor of ladybird flight (Table 2.1).



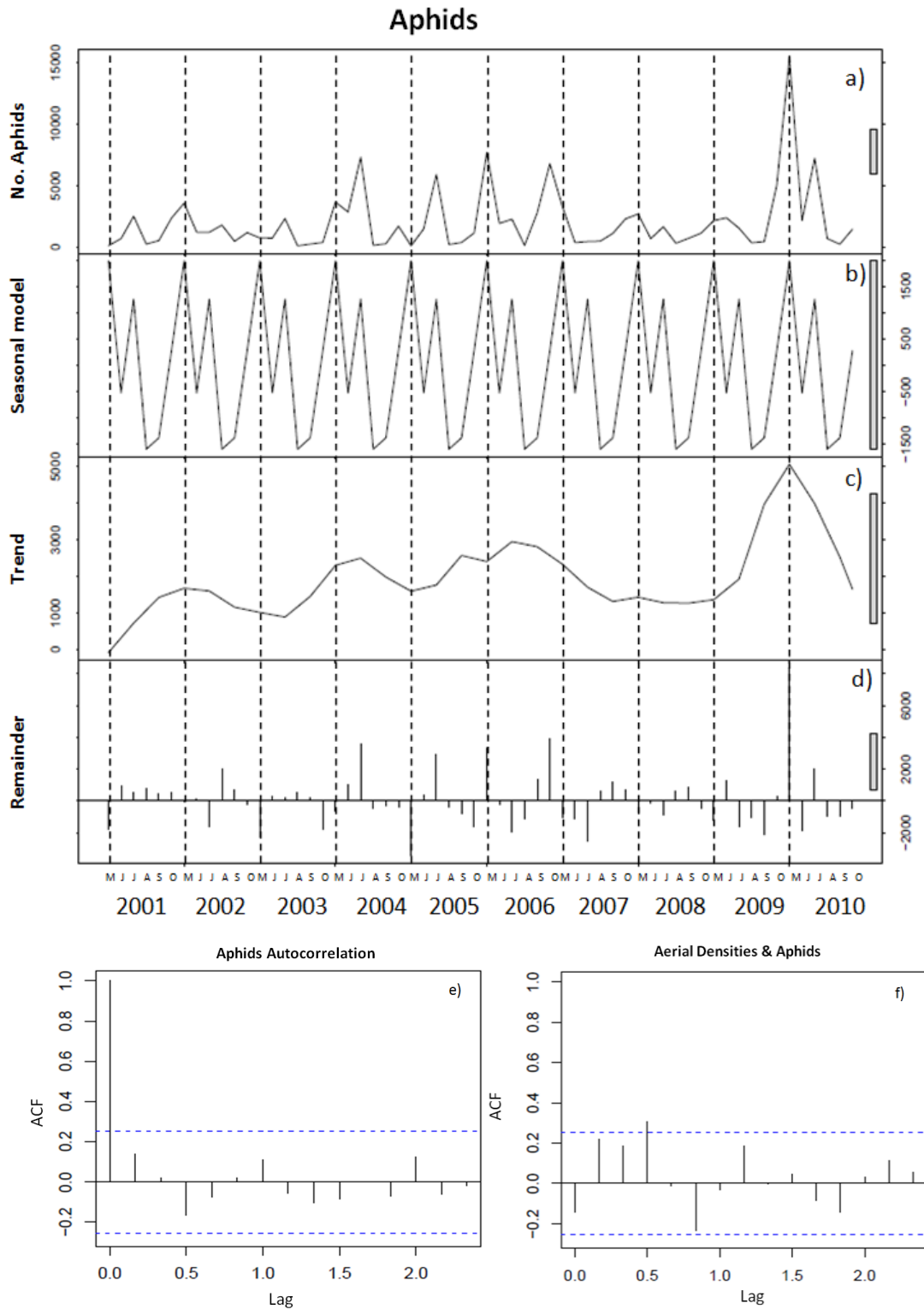
**Figure 2.4** a) Comparison of time series for a) summed monthly AD, b) average monthly temperature and c) average monthly ground level wind speed for months May – Oct. 2001 – 2008. d) cross-correlation plot for summed monthly AD and average monthly temperature time series. e) Cross correlation plot for summed monthly AD and average monthly ground level wind speed. Y axis describes correlation coefficient (-1 - +1), Y axis describes lag period; a lag unit of 1.0 equates to 1 year. 6 months (May-Oct) were used for each year therefore the 6 peaks within each lag unit represent a lag of one month each. Peaks crossing the horizontal dashed blue lines represent significant autocorrelation at the 0.05 level.



**Figure 2.5** Summed aerial densities of 700 insect detections sub-sampled from the Rothamsted VLR data at temperatures between 0°C - 29°C.



**Figure 2.6** Scatter plot relationship between daily aerial density totals and average ground level wind speeds for 74 days where the temperature was between 16 – 21°C. Data taken from Rothamsted for May - Oct between 2003-2008. The regression line (with equation  $y = -20.93x + 148.59$ ) is shown in black ( $R^2 = 0.22$ ,  $n = 74$ ,  $p < 0.001$ )



**Figure 2.7 a)** Time series decomposition for Aphid suction trap catches at Rothamsted research centre for 2001-2008 inc. X axis vales 1-8 relate to years 2001-2008. **b)** Seasonal decomposition model for time series. **c)** Overall annual trend after accounting for seasonal cycles. **d)** Remaining variation not accounted for by seasonal model or overall trend. Vertical grey bar represents relative scale of Y axes. **e)** Autocorrelation plot for Aphids. **f)** Cross correlation for Aphids and AD. Y axis denotes correlation coefficient (-1 - +1), Y axis denotes lag period; a lag unit of 1.0 equates to 1 year. 6 months (May-Oct) were used for each year therefore the 6 peaks within each lag unit represent a lag of one month each. Peaks crossing dashed blue lines represent significant autocorrelation at the 0.05 level.

**Table 2.1** Multiple backwards linear regression between total monthly AD for May-October (2000-2010) and all predictors. Temperature and wind speed values were ground level averages for each month. Aphid + 1 represents the aphid abundance for the previous month.

Model	Explanatory Variables	R <sup>2</sup>	F	Sig.
1	Temperature, Wind speed, Aphids+1	0.38	2.81	0.078
2	Temperature, Aphids+1	0.37	4.47	0.030
3	Temperature	0.30	6.78	0.019

Note: for Model 2\*,  $\Delta R^2 = 0.01$ , for Model 3\*  $\Delta R^2 = 0.07$

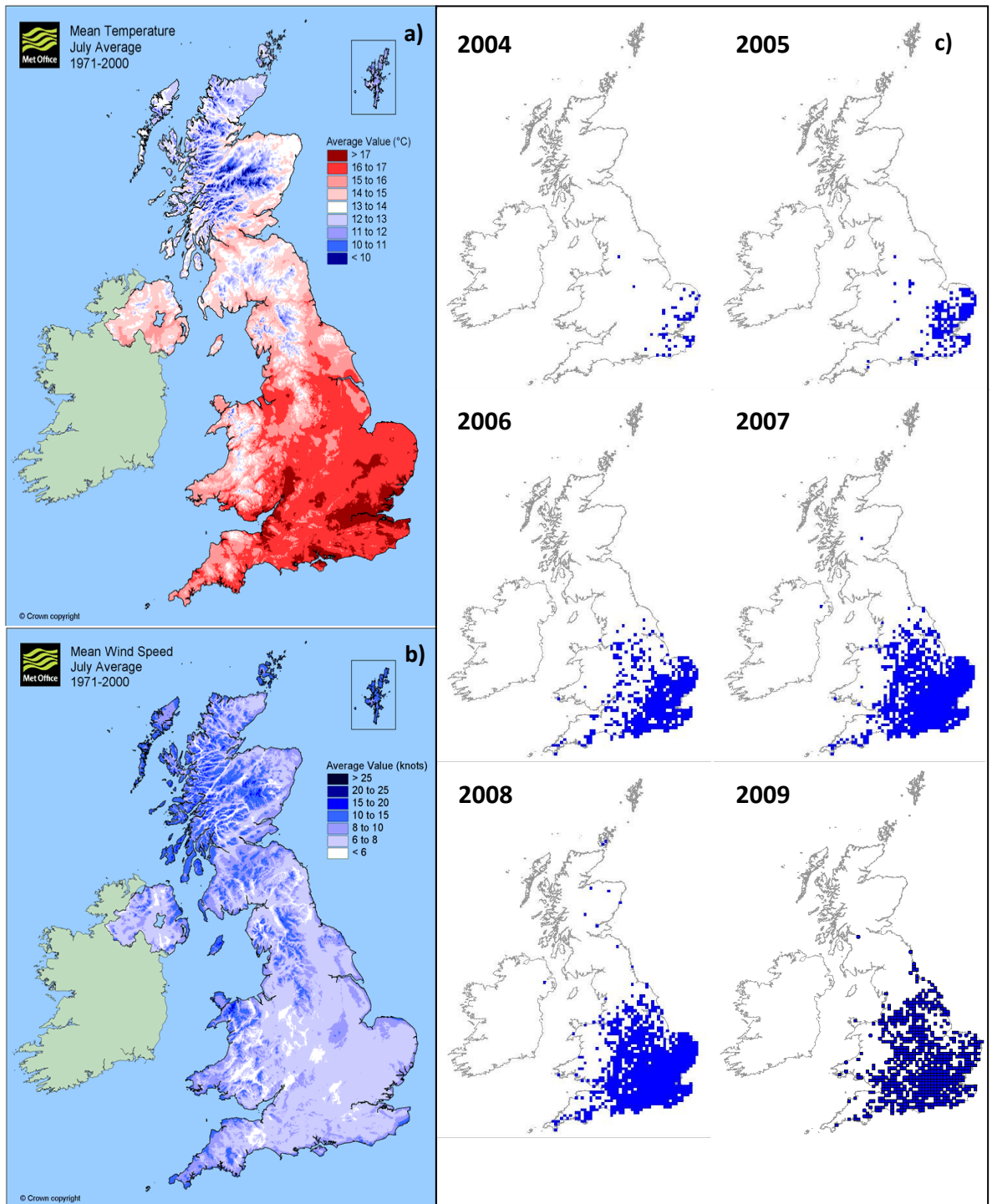
### 2.3.3 Implications for invasion

The north-westerly spread of *H. axyridis* across the U.K. did not occur at a constant rate (Fig. 2.8c) (Brown et al., 2008); there was a noticeably larger range expansion distance between 2005 and 2006 than in other years. This corresponds to a temperature and AD peak for 2006 as shown in the time series (Fig. 2.3a). The range boundary expanded a much shorter distance in 2007; numbers of 10km squares occupied did increase, however almost all additional occupied areas were within the range of the previous year. This trend was similar in 2008 and 2009 with little range boundary expansion. The range boundary for *H. axyridis* in 2006 associates strongly with areas of high average temperature and low average wind speed in the U.K. (Fig. 2.8a, b). There was little expansion past these boundaries in the two following years, which also saw low temperatures and AD. A number of additional occupied areas were detected in Scotland, however, as there are few records between the lower range and these outlying populations these may not represent the range boundary of the invasive population.

Tethered flight tests produced a mean uninterrupted flight duration of 36 minutes with maximum flight durations of 2 hours at which time flights were stopped (Jeffries unpublished



data, Chapter 3). The average insect displacement speed as shown by the VLR was  $9.9 \text{ ms}^{-1}$ , which equates to approximately  $35.6 \text{ kmh}^{-1}$ . Therefore, although displacement distances will depend greatly on changes in flight altitude, wind speed and wind direction, ladybirds migrating at altitudes above 150m AGL are likely able to travel an average of approximately 21km and possibly up to around 70 km in a 2 hour flight.



**Figure 2.8** The distribution and spread of *H. axyridis* in relation to the geographic distribution of temperature across the UK. Average **a)** temperature, **b)** wind speed distribution across the U.K. for Jul (1971 – 2000) (data from the U.K. Met Office ([www.metoffice.gov.uk](http://www.metoffice.gov.uk))). **c)** Number of 10 km squares occupied by *H. axyridis* in 2004-2009. Data from the HLS (accessed at [www.harlequin-survey.org](http://www.harlequin-survey.org)).

## 2.4 Discussion

The overall aim of this study was to identify the most important predictor of ladybird dispersal and to relate this to the spread of the highly invasive *H. axyridis* in the U.K. The modern VLR and Met office tools used here points to significant relationships between dispersal and temperature and wind speed whereas no significant relationship with local abundance of aphids was found. Of these environmental conditions, temperature was identified as the main predictor of dispersal. There is evidence to suggest that temperature and wind speed may have reduced the rate of spread of *H. axyridis* in the U.K. in areas where abiotic conditions are unfavourable for flight. Below, these results are discussed in light of the current knowledge surrounding coccinellid flight and their implications for the spread of the U.K. invasion of *H. axyridis*. The limitations of this study are also highlighted and future avenues of research are proposed.

### 2.4.1 Characteristics of ladybird dispersal

The altitudinal distribution of insect detections shows no layering effect that could be attributed to certain flight types as in Chapman et al. (2004). Ladybird flight (excluding migration) may therefore exist on a spectrum from short distance, low altitude appetitive flight, to long distance, high altitude dispersal (Fig. 2.9) This contrasts with the more discrete classifications of short and long distance flights which likely arose as a result of the difficulties in observing high altitude flight (Elliott et al., 2000; Osawa, 2000; Hodek & Michaud, 1993).

As the average FBL for ladybirds is lower than 150m AGL it seems safe to assume here that the flight observed is long distance. Insects flying above their FBL will have little directional control over their flight (Taylor, 1974), heights above the FBL would therefore seem inappropriate for appetitive trivial flight. Although thermal currents can on occasion carry insects involuntarily to higher altitudes (J. Chapman, *pers. comm.*), an insect must expend energy to maintain high altitude when flying (Chapman et al., 2006). Therefore, for the purposes of short trivial flights

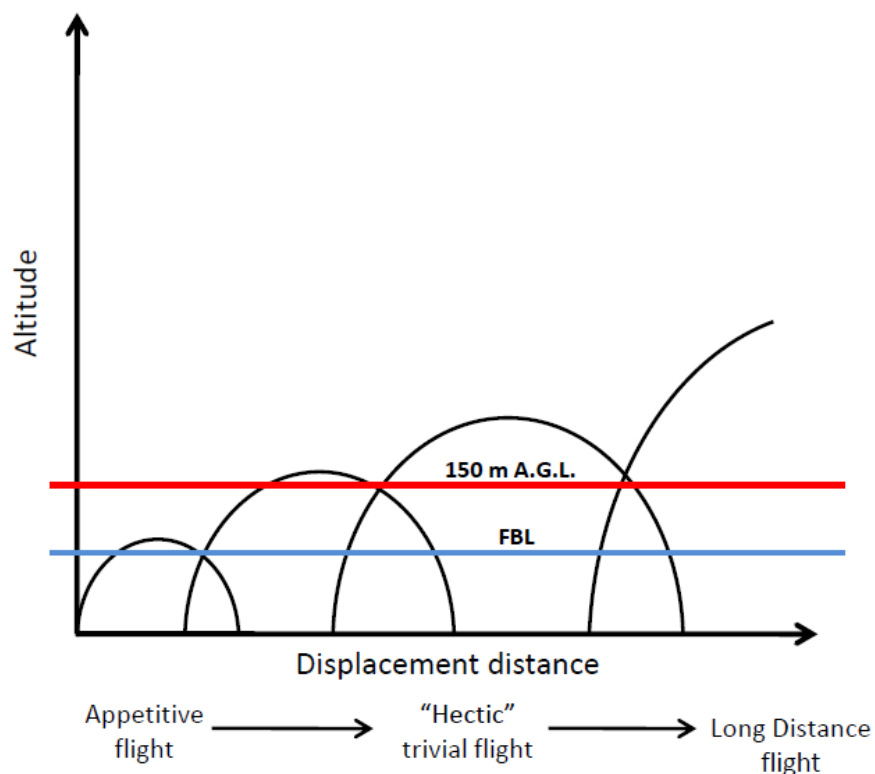
often less than 10m (Elliot et al., 2000; Osawa, 2000), intentionally flying above 150m would be a waste of valuable energy.

Due to the minimum detection altitude of the VLR it is not possible to observe flight close to the ground and so no conclusions can be drawn for trivial flight from the results of this study. A number of authors have looked at the low altitude flight of coccinellids using field observations, however the difficulty in flight classification is noted (Elliot et al., 2000; Osawa, 2000). A solution may be the use of harmonic radar, which uses diode transponders to track insects in a local area (Riley & Smith, 2002). This tool has previously been used to provide evidence of the waggle dance phenomenon in the honey bee *Apis mellifera* (L.) (Hymenoptera: Apidae) (Capaldi et al., 2000) and could be extremely useful in monitoring ladybird trivial flight close to ground level.

The temporal trends in ladybird dispersal seen here correspond, in most years, with the seasonal trends in temperature; a good example of this is the corresponding peaks in temperature and AD in the summer of 2006. The peak ladybird flight was generally July and dispersal was lowest in May and October. *Coccinella septempunctata* and *H. axyridis* undergo migration to overwintering sites in autumn in the U.K. (Hodek & Honěk, 1996). As migratory flight to dormancy sites has been observed to be long distance in *H. axyridis* and a proportion of the *C. septempunctata* population (Hodek & Honěk, 1996) a peak in ladybird dispersal in autumn might therefore be expected in the VLR detections. However none is seen in the results here. This suggests that migratory flights take place at altitudes below 150m AGL. Chapman et al. (2006) also noted an absence of this peak when looking for evidence of migratory flights in lacewings. A potential explanation put forward was that this is a result of a behavioural adaptation in the insects that predisposes them to fly at altitudes below 150m in order to increase the probability that they will come into contact with their favoured overwintering sites (e.g. trees). In their invasive range, *H. axyridis* are renowned for their inconvenient presence in man-made structures (Riddick et al., 2000) which, in urban and sub-

urban areas (like Rothamsted) are often not far from foraging sites. Also, a proportion of *C. septempunctata* populations are observed to overwinter in nearby forest edges or even on field boundaries in their summer foraging and breeding sites (Hodek et al., 1993). It may be that in the U.K. long distance migratory flights are not necessary and thus, short distance flights below 150m are performed that cannot be detected by the VLR.

As no migratory flight peak is observed here and would only occur in Britain in autumn just prior to overwintering, the flights observed in the present study best fit the description of hectic trivial flight. Hodek et al, (1993) imply that hectic trivial flight is uncommon; however the numerous detections of ladybirds above 150m by the VLR suggest that it is a more common occurrence.



**Figure 2.9** Suggested model for classification of coccinellid flight behaviour. Short flights of lower altitude are associated with appetitive trivial flight, longer flights have higher maximal altitude and are associated with migratory flight. Red line indicates the altitude at which the VLR can detect insects. (Diagram not drawn to scale)

### ***2.4.2 Predictors of ladybird dispersal***

Temperature was identified as the main predictor of dispersal in *C. septempunctata* and *H. axyridis*; however the low predictive ability of the minimum adequate multiple regression model when all predictors were included (Table 2.1) suggests that temperature is certainly not the only predictor of ladybird flight. Taylor (1974) and Elliot et al. (2000) noted that above the flight-inhibiting minimum threshold, temperature no longer has a significant effect on the flight behaviour of most insects. Therefore above a certain threshold other environmental factors may be predicting the variation within ladybird aerial densities. For example, the annual trends in AD are well predicted by annual temperatures however, in 2007 and 2008 ADs crash when temperatures remain relatively warm. These low AD correspond to high rainfall which would produce poor flying conditions (Rainey, 1976) in these years (See Appendix 7).

Matching detections with the temperature at that time and altitude in the sub-sampled data set showed a favoured flight temperature range for ladybird flight 17-20°C, however the associated curve (Fig. 2.5) is somewhat different to that found in previous studies of insect flight in relation to temperature. Hagen (1962) found that the convergent ladybird *Hippodamia convergens* had a minimal flight threshold temperature of approximately 17°C, above which dispersal remains high. Other coccinellid studies have identified a lower and upper threshold at which a drop in flight efficiency occurs; with a plateau in the amount of dispersal in between the two thresholds (Elliot et al., 2000; Taylor 1963). The analyses of thresholds by Taylor (1963) show relatively narrow ranges within temperature thresholds for example, approx 14-20°C for the lower threshold in the common red soldier beetle *Rhagonycha fulva*. However the lower temperature threshold for *H. axyridis* and *C. septempunctata* spans 14°C between the point at which flight is possible (6°C) and the peak flight temperature (19°C). A lot of high altitude flight therefore occurs below the favoured temperature for flight (17-20°C). A possible explanation for this may be that as the ambient

temperatures drop below the minimum threshold dispersal does not cease instantaneously. Ectothermic insects are able to maintain body temperatures above ambient levels using the metabolic heat generated by their wing muscles as observed by Marden (1995) in the winter moth, *Operophtera bruceata*. If ambient temperatures drop below minimum thresholds there will likely be a number of already airborne insects capable of maintaining flight due to higher body temperatures.

A second explanation for the shallow rise in aerial densities at lower temperatures could be behavioural adaptations. In the morning, as the temperature is rising, insects will not initiate flight before the take-off threshold is reached (Taylor, 1963). However, 2-spot ladybirds, *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) can increase their body temperature by up to 7-10°C through basking (Brakefield & Willmer, 1985). This would allow them to reach body temperatures adequate for flight at lower ambient temperatures and is a behaviour also exhibited in *H. axyridis* (personal observations). In relation to this, solar radiation intensity has been found to influence numbers of long distance flights in *Hippodamia tridecimpunctata* (L.) and *Hippodamia convergens* (Elliot et al., 2000).

The temperature range at which aerial densities peak falls between  $17^{\circ}\text{C} \leq t \leq 20^{\circ}\text{C}$ , but instead of plateauing after a minimum flight threshold is reached, AD decreases as temperature continues to increase. This seems a relatively narrow and low range of flight temperatures. An explanation may be the production of metabolic heat during flight, which at high ambient temperatures might result in body temperatures that are too high. However it may in fact be a result of too few occasions within the data at which temperature reached these heights. Therefore the upper threshold seen in this graph may be an underestimation of the true value.

Although the VLR data here provides a rough estimate of the minimum and maximum flight thresholds within which *H. axyridis* fly, these thresholds may not be consistent over populations outside or even within the UK. Prasad et al. (1999) showed that the temperature at which an insect is reared can have an effect on the lower flight initiation threshold. In the

parasitoid *Trichogramma sibericum* it was found that insects reared at lower temperatures initiated flight at lower temperatures and those reared at higher temperatures initiated flight at higher temperatures. If this phenomenon is also present in *H. axyridis* then it would be likely that the flight initiation and the optimal and maximum thresholds may differ between populations, depending on the climate in which they live. Flight tests of *H. axyridis* individuals reared in culture at different temperatures could also provide an effective way of testing this phenomenon. Phenotypic plasticity such as this has been highlighted as one of the important determinants for invasion success (Michie et al, 2010; Peacor et al., 2006) and this ability to fly at broad temperature ranges may be one factor that has contributed to the success of *H. axyridis* in its climatically diverse invasive range.

The lack of a significant relationship between monthly ADs and ground level wind speeds suggests that wind speed is not a strong predictor of ladybird flight. However, linear regressions of the sub-sampled data identified a negative relationship between densities of ladybirds flying and the ground level wind speed at the time of detection. As wind speeds increase, the variation in the number of flying ladybirds decreases. This may represent an inhibitory effect of high wind speeds on ladybird flight (Fig. 2.6). Linear regression showed that between temperatures of 17-21°C there is no significant effect of temperature on AD. This suggests that there is no effect of minimum temperature thresholds as found by Taylor (1974) on AD in these detections, therefore the variation in AD found here can be confidently attributed to wind speed. Chapman et al (2006) report similar results in their study of lacewing migration in which dispersal was not observed at wind speeds above 3ms<sup>-1</sup>.

In light of the discrepancy between the results of cross correlations and the linear regressions here; future work might consider controlling for minimum thresholds in all but one variable at a time in order to examine the predictive effects of environmental factors.

The temporal peaks in aphid abundance as shown by the seasonal decomposition of the aphid time series likely represent the migratory phases of aphid population cycles. Spring migrations



of aphids often occur when aphid populations move from overwintering hosts to their first spring/summer hosts (Osawa, 2000).

Time series and regression show no relationship between *C. septempunctata* and *H. axyridis* dispersal and aphid suction trap catches of the same month. However, in order to relate this to *C. septempunctata* and *H. axyridis* dispersal behaviour it is necessary to understand the link between suction trap catches and aphid population densities.

Aphids exist in polyphenic populations that are made up of varying proportions of alate (winged) and apterous (non-winged) individuals so as to maximise the efficient use of resources for dispersal or fecundity. The proportions of these two polymorphisms within a population are subject to change influenced mainly by two factors; the quality of the host plant as a food source and the population density, i.e. overcrowding. In response to a reduction in host quality or to high population densities an aphid population will produce larger numbers of alate individuals that then disperse in search of alternative foraging sites (Müller et al., 2001). It is these alates that are caught in the RIS suction trap network. Fabre et al. (2010) show through hierarchical Bayesian modelling that suction trap data similar to that used in this report acts as a reliable proxy for estimates of aphid population size for the immediate area around Rothamsted research centre. Although an area of 100km<sup>2</sup> is often quoted as the range for which suction trap aphid abundances are indicative, within this area population densities can be affected by environmental factors such as host plant condition, rain and aphid crowding (Dixon, 1985). High RIS aphid suction trap catches therefore likely point to high numbers of aphids in the nearby surrounding area at that time.

If the assumption that RIS aphid trap catches are a reliable proxy for local aphid abundances were to hold true for the whole summer, then it is possible to infer relationships between aphid population characteristics and the dispersal behaviour of *C. septempunctata* and *H. axyridis*. In early and mid summer, high aphid catch number would infer overcrowding of aphids on host plants. At these times, aphid colonies would be large; therefore the long

distance dispersal of *C. septempunctata* and *H. axyridis* from this area would be suppressed. However, this would logically predict a negative correlation between AD and aphid catches for the same month, which is not seen in the results of this report.

It is important here to discuss the movement of ladybirds *into* an area. Although emigration is known to be suppressed by the presence and abundance of aphids (Evans, 2003; Osawa, 2000; Hodek et al., 1993; Ives et al., 1993) there is still debate over the effects of aphid abundance on the rates of immigrations to an area. It is thought by some that coccinellids are not able to actively fly towards areas of high aphid abundance (Hodek & Honěk, 1996 and references therein) and that their movement into an area is therefore, at least in relation to prey, random. However in contrast to this, Osawa (2000) found evidence that *H. axyridis* are efficient prey trackers and that this may play a part in the synchronisation of *H. axyridis* life cycles with aphid population cycles. Additionally, it has been shown that honeydew from aphids can be a cue for the aggregation of coccinellid larvae (Carter & Dixon 1984) but not for short distance movement (Osawa, 2000). During long distance dispersal, appetitive cues are most likely to be in the form of volatiles from plants infested by aphids or from aphids themselves (Osawa, 2000; Obata, 1986) however there is no knowledge of the effects that these volatiles may have on the dispersal behaviour of *H. axyridis* during long distance dispersal. We suggest however that even if volatiles do act as cues during ladybird flight, ladybirds flying at the altitudes observed here (i.e. above 150m AGL) would not be able to track them to their source (upwind) as wind speeds at these altitudes will, for the most part, exceed the maximum self powered flight speed of ladybirds and thus carry them downwind (see FBL above). Therefore, it is likely that immigration rates for the ladybirds observed in the present study are independent of aphid abundances in the field; they may instead be predicted by the aphid abundance (or lack of) at the site from which they emigrate.

This may partly explain the lack of a significant relationship observed here between number of dispersing ladybirds and aphid abundances at this site. Perhaps a future study in this area

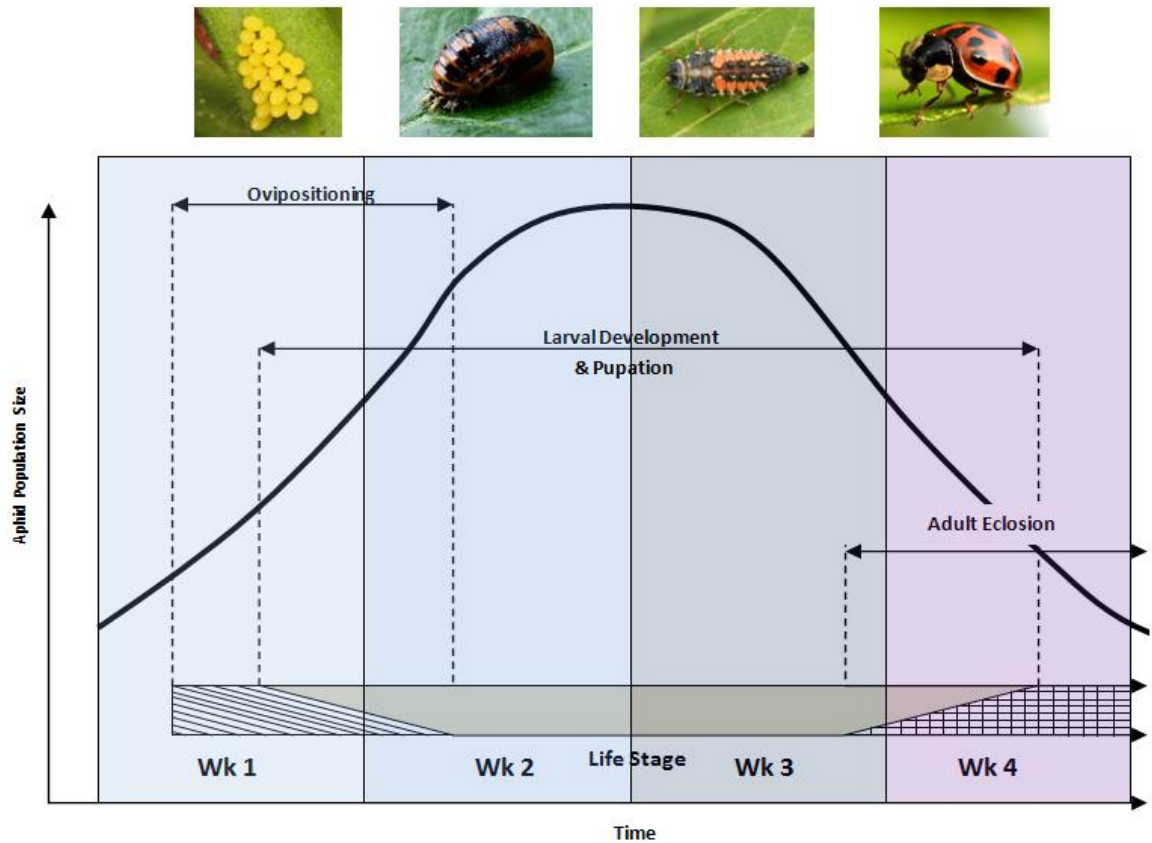
could look for a relationship between coccinellid aerial densities and aphid abundances for sites upwind of the VLR.

During preliminary analyses excluding 2009 and 2010 (containing an anomalously high peak in aphid abundance), a significant relationship between ladybird AD and the aphid abundance for the previous month was found (Appendix 8). Chapman et al. (2006) found a one month lag, between high altitude flight in lacewings, *Chrysoperla carnea* and the aphid catches from the previous years. The postulated explanation for this was the development time of lacewings; resulting in the migration from breeding sites of adults that emerged into an area of low prey abundance. As suggested above, the ladybird flight observed in this study, being above 150m AGL may not provide adequate information to make conclusions about the importance of aphids in long distance ladybird dispersal however evidence presented in other studies would predict a relationship between them. Osawa et al. (2000) and Hemptinne et al. (1992) have found that ovipositing occurred at or just before peaks in aphid population density. The development time of *C. septempunctata* and *H. axyridis* in the U.K. is roughly 3-4 weeks (*personal observations*) depending on temperature and diet (Koch, 2003). During development the highly voracious larval stages could significantly deplete the numbers within an aphid colony (Elliot et al., 2000). On the basis of this information, 3 - 4 weeks after the start of ovipositioning (just before peak aphid abundance) newly eclosed adult ladybirds would start to emerge into environments with low prey density which may then result in a high propensity for long distance flight in search of new foraging and ovipositioning sites (Fig. 2.10). (Dixon & Agarwala, 1999) also point out the similarity between the duration of larval development of ladybirds and the period that an infested area remains suitable for the larvae. Similar hypotheses have been formed by Hodek et al. (1993); Osawa (2000) and Elliot et al. (2001).

The above discussion is however only a preliminary look at what is likely to be a very complex system. In order to draw more concrete conclusions many factors require additional study and the data must be validated thoroughly. For example, the suction trap data used here is made

up of data from 23 aphid species, some of which may not be predated upon by *H. axyridis* and *C. septempunctata*. The list of prey species compiled by Hodek & Honěk (1996) may be incomplete or may not apply to *H. axyridis* in its invasive range. Being polyphagous, *H. axyridis* also have a number of alternative food sources that may be available (Koch, 2003); although not the preferred food, this could further prolong the time it takes for appetitive dispersal to occur and so would need to be factored into any investigation. Furthermore, if an agricultural patch is ideal for aphids, i.e. the host plants are highly nutritious; the area may be heavily infested whilst at the same time having low proportion of alates resulting in low numbers of suction trap catches. Conversely, mass aphid migrations may result from host senescence or crop harvests and so be seasonal (Losely & Eubanks, 2000; R. Harrington, *pers. comm.*).

This complex area of study is made even more so by the interactions between the environmental factors affecting aphid population dynamics. For instance, on poor hosts aphids become restless and more mobile; in both instances the chances of tactile contact with each other increases, which is the proposed mechanism by which overcrowding is judged by aphids (Müller et al., 2001). Therefore it seems likely that overcrowding will be more probable on host plants that are highly nutritious and therefore confer a greater fecundity. Unfortunately this study does not allow for a detailed look at these complex issues; to further investigate the expected importance of aphids in the flight of *C. septempunctata* and *H. axyridis* analyses of ladybird flight at ground level would be required in conjunction with data of aphid abundances which include counts of apterous aphids in the field.



**Figure 2.10** Suggested model for the relationship between aphid population size and *H. axyridis* phenology over 4 weeks. Thick black line indicates aphid population size. ▨ = Ovipositioning phase of *H. axyridis* life-cycle. ■ = Larval phase. ▩ = Newly Eclosed Adult phase. Ovipositioning occurs just before the peak aphid abundance, a few days later larvae hatch and begin foraging. The later the instar stage, the higher their aphid consumption. After approximately 3 – 4 weeks adults eclose as aphid populations are low. Flight propensity is therefore high which results in high observed aerial densities.

#### 2.4.4 Implications for invasion

An often quoted route of entry into the U.K. is the natural flight of *H. axyridis* across the English Channel from continental Europe (Brown et al., 2008) however no studies exist that have tested the ability of *H. axyridis* to make flights of this length. The rough calculations using flight speed and time performed here suggest that, with a maximum displacement distance of approximately 70km or more per flight, natural long distance flight is indeed a plausible explanation for the arrival of *H. axyridis* in the U.K. Studies using Met office modelling in conjunction with VLR data have in the past allowed for the back-tracking of the dispersal trajectories of high altitude insect flights (Chapman et al., 2010). Similar techniques could

provide support for this conclusion, which would not only explain their spread to the UK but also shed more light on their global invasion routes.

This ability to displace large distances in a short amount of time could also account for their rapid spread through the U.K. However, as the prevailing winds in England are South-Westerly, the larger component of the flight involved in the spread of invasion is likely to be directional trivial flight below the FBL as opposed to wind-borne flight above the FBL.

Looking at the spread of the *H. axyridis* invasion in the U.K. (Fig. 2.8) it can be seen that the range boundary reached as far as the Cambrian Mountains and the Pennines in 2006, and further spread in 2006-2010 was minimal. This could be a direct result of the topography of this range boundary and the resulting meteorological conditions. Height above sea level in these areas increases and therefore so does wind speed (Durran, 1990; Mahrt et al., 1979), whereas temperature decreases (Harding, 1978). Both of these conditions are suggested by the results of this Chapter to be deleterious for *C. septempunctata* and *H. axyridis* flight. Brown et al. (2008) propose another reason for the reduced rate of spread in Northern England; the authors suggest that there will be recruitment of *H. axyridis* from mainland Europe which may have aided in the spread of invasion in the South East of England, but that the benefits of this would not be felt in the North.

One limitation of this study has been the inability to say with certainty which species of coccinellid are being observed. Hodek (2008) states that coccinellids differ in their prey and habitat specificity and dispersal behaviour, therefore to group these two species is by no means ideal. Although the characteristics used to identify the species in the VLR data are assumed to be adequately diagnostic for *C. septempunctata* and *H. axyridis* there is no way of knowing for certain what insect species are in the data and in what proportions. Additionally, *C. septempunctata* is native to the U.K. whereas *H. axyridis* is invasive, thus conclusions about variation in dispersal behaviour in relation to invasion should be treated with caution. Reassuringly however the large range expansion between 2005 and 2006 as documented by

the Harlequin Ladybird Survey (Brown et al., 2008) corresponds to the highest peak in AD seen in the VLR data for 2000-2010, supporting the assumption that a large proportion of the insects observed here are *H. axyridis*.

#### ***2.4.5 Conclusions***

The results of this study show that the numbers of *H. axyridis* and *C. septempunctata* performing long distance flight is affected by both temperature and wind speed and that there are likely to be a range of favourable abiotic conditions for flight. No relationship between coccinellid aerial densities and aphid abundances was found, it therefore seems that although aphids may provide motivation for flight, it is governed predominantly by temperature. The estimated flight capabilities of *H. axyridis* support the suggestion that natural dispersal was at least one mechanism of their arrival in the U.K. Meteorological conditions differ with the topography of the U.K. and evidence suggests that this may have hindered the spread of invasion in areas where conditions are unfavourable for flight.

## **Chapter 3.**

# ***An in vitro* method for analysing coccinellid flight.**



## Abstract

The study of insect flight is important yet problematic due to difficulties associated with observations in the field. As a result, *In vitro* flight tests have been previously used to examine insect dispersal in relation to environmental and ontological factors. A new flight test method is described here and used to test the hypothesis that, at range expansion boundaries (U.K.), there will be selection for increased dispersal ability in *Harmonia axyridis*. This was done by comparing 20 samples from the native range (Japan) with 20 from the invasive range (U.K.). The method was easily repeatable between tests. Flight times in 40 test subjects were highly variable however no significant difference between the dispersal ability of invasive and native populations were found. This could be explained by the trade off between dispersal ability and fecundity at the range expansion boundary.

**Keywords:** Flight test, dispersal, tethered, ladybird, invasive species, range expansion boundary.

### 3.1 Introduction

Dispersal is fundamental in the establishment and persistence of populations (Roff, 1974) and in the case of species invasions, it will have a large influence on the success of a species in an exotic range. Travis et al. (2009) explain how dispersal strategies may change in response to a change in selection pressures at range expansion boundaries. Hughes et al. (2003; 2007) found evidence that supports this in the selection for increased dispersal traits at the expanding range boundaries of the speckled wood butterfly *Pararge aegeria*. In this way dispersal can drive range expansions and is therefore essential to understanding the mechanisms of species invasions (Travis et al., 2009) and in predicting future spread (Kokko & López-Sepulcre, 2007).

The harlequin ladybird, *Harmonia axyridis* is a highly invasive species; native to Eastern Russia, Eastern Kazakhstan, Siberia, China, Korea and Japan it is now present on almost every continent, having been introduced in its invasive ranges through a mixture of intentional introductions for biological control and unintentional introductions (Koch, 2003). Natural dispersal by flight has also been suggested as a mechanism of the spread of *H. axyridis*, however no direct evidence of this could be found in the literature. Although it is a plausible claim for much of the spread across terrestrial habitats, the ability of *H. axyridis* to move naturally across dispersal barriers such as water bodies and mountains is not as certain.

The flight ability and behaviour of an insect is dependent on a diverse number of environmental factors. Of these, temperature is considered the most important (Heinrich, 1995, also see Chapter 2 of this thesis). Wind currents are also important in insect flight and are used by a number of species in long distance migrations in order to maximise displacement distances (Johnson, 1969; Chapman et al., 2006; 2008). Aphids are arguably the main behavioural motivation behind the dispersal of female *H. axyridis*, and males follow as motivated by their desire to reproduce (Hodek & Honěk 1996). Osawa (2000) and Elliott et al. (2000) found that the density of *H. axyridis* in a local area was strongly predicted by the abundance of aphid prey to their tendency to reduce coccinellid emigration from an area. The

above environmental factors are known to be important predictors of insect dispersal however; information on how *H. axyridis* dispersal changes in relation to these variables is limited at best.

*In vitro* flight experiments have proven a valuable method of analysing physiological flight parameters and flight behaviours in insects; overcoming the difficulties in observing them in the field. *In vitro* methods allow for the testing of environmental conditions on the flight ability and behaviour of insects (Rankin et al., 1994; Dingle, 1966; Miller et al., 2008). Previous studies have used a number *in vitro* methods, for example, flight mills are used to observe insect flight against an air current of known speed. This method has been previously used to examine the duration, distance (Schumacher et al., 1997; Sarvary et al., 2008) and speed of flight (Rowley et al., 1968), the effects of environmental conditions (Ishiguri & Shirai, 2004) and ontological characters such as age, body size and reproductive status (Ishiguri & Shirai, 2004; Sarvary et al., 2008; Stewart & Gaylor, 1994).

Tethered flight without the use of a flight mill, although less sophisticated, has the advantage of not requiring expensive computer monitoring equipment and software. Dingle (1965, 1996) used such a method in which he tethered milkweed bugs to a short stick using paraffin wax to look at the effects of age on flight duration. Rankin et al. (1994) tested for the “oogenesis flight syndrome” in the boll weevil *Anthonomus grandis* using a similar method and also tested the reliability of this method by comparing the tethered flight ability between insects caught at 2 sites differing in distance from breeding sites. A significant difference was found between the insects caught at these sites supporting the assumption that tethered flight tests were indicative of flights in the field. More sophisticated tethered flight tests have used force-displacement transducers in order to quantify the force produced by insects during flight (Coelho, 1991).

Flight tests have previously been used by Tourniaire et al. (2000) to examine the flight of *H. axyridis* individuals that had been selectively bred for a mutation affecting the wing muscle

that resulted in flightlessness. This was done with the aim of producing biological control stocks that were unable to disperse from an area intended for biological control; an innate behaviour of coccinellids at times of low prey abundance. This method used a “take-off stick” placed at the centre of a 5m<sup>2</sup> grid located outdoors. Flightless strains climbed and jumped off the take-off stick but were unable to fly.

The latest advance in monitoring insect flight is the use of radar technology. High altitude insect dispersal (above 150m) is now observable using vertical looking radar (VLR) (see Chapman et al., 2003 for a review; and Chapter 2 of this thesis). Low-level insect flight can be observed with the use of harmonic radar, which uses diode transponders that re-emit a signal emitted from a ground based scanning station, allowing the tagged insect to be identified amongst environmental interference (O’Neal et al, 2004). Harmonic radar has a range of approximately 1km and produces accurate flight path measurements that can be superimposed onto landscape maps. This technique allows for the study of low altitude insect flight which is not possible with field observations or VLR. Capaldi et al. (2000) and Riley et al. (2005) have used harmonic radar to study foraging in bees; an extremely important part of insect ecology which is essential to their conservation and their role as pollinators. Harmonic radar has also provided a demonstration of the Nobel-prize-winning hypothesis behind the honey bee “waggle dance” (Riley et al., 2005).

The large and heterogeneous established range of *H. axyridis* will incorporate large variations in the above factors. Understanding how these factors can influence the flight of *H. axyridis* is therefore essential to predicting its future range expansion in both space and time. The main aim of this Chapter is to test a newly conceived tethered flight method with which flight ability the effects of environmental factors on flight of *H. axyridis* can be examined *in vitro*. The method differs from those described above in that insects are tied to a tether of fine fishing line and suspended within a “flight cube”. This method will then be used to look for evidence of increased dispersal capacity in *H. axyridis* samples from the invasive population in the U.K.

compared to samples from the native range, Japan. The hypothesis tested being; dispersal ability would be increased at the invasive range expansion boundary due to the importance of flight in the spread of populations as found by Hughes et al. (2003) in the speckled wood butterfly *Pararge aegeria*.

## **3.2 Methods**

### ***3.2.1 Method development***

Flight tests were carried out in a purpose built steel framed “flight cube” exactly 1m in length, width and height, covered with clear Perspex panels (Fig. 3.1). The flight cube was situated in a controlled temperature room kept at 18°C at all times which is within the optimum flight temperature range for *H. axyridis* (see Chapter 2 of this thesis). It has been observed that air currents can provoke and direct insect flight (Gewecke & Philippen, 2008) therefore it was important that air movement within the flight cube was minimal. All sides were closed off and the door was magnetically sealed while flights were underway.

A video camera (Panasonic SDR-S26, capable 25 frames per second (fps)) was suspended approximately 2 feet above the upper surface of the cube pointing down with a “bird’s-eye view” in order to record ladybird flights.

Initial attempts used super glue to attach the tether to the ladybirds, however due to the waxy nature of the *H. axyridis* cuticle the superglue and attached line was shed usually within only 2-3 minutes. It was therefore necessary to find an alternative means of tethering.



**Figure 3.1** Flight Cube used for tethered flight tests of *H. axyridis*.

A solution was to tie fine fishing line (0.1mm diameter) over the head, pronotum and forelegs. Ladybirds were placed in a petri dish in an ice-filled container to cool for approximately 3 minutes. Once docile, ladybirds were then removed and placed on a flat surface. A slip knot tied in the end of the line was placed over the head and ladybirds and behind the front pair of legs, the knot was pulled tight behind the pronotum and line was kept under tension. Samples were then left to warm up for 1 minute to ensure that body temperature was sufficient to meet the minimum temperature threshold for flight. There were no signs that cooling had any lasting negative effects on ladybirds, individuals were active quickly after warming.

The tether was threaded through a piece of clear Perspex tubing approx. 15cm in length, which was fastened to the upper cube surface along with the tether in order to move the point at which the line could pivot away from the cube ceiling. This prevented the ladybirds from landing on the inside ceiling surface. Tethered insects were suspended directly below the camera. The loss of tarsal contact usually resulted in immediate flight; subjects were therefore placed on a small piece of card whilst the line was fastened to ensure no flight time was unrecorded. As the card was removed from under the test subject, the camera was set to record for 2 hours. Videos were recorded onto a micro SD card in the camera and later transferred to a portable hard drive for storage. Each video was 120 minutes in length and was shot in black and white (60i, 25 fps) to conserve storage space whilst maintaining enough picture quality to track flying ladybirds.

Flight videos of tested harlequins were preliminarily analysed in ImageJ (Abràmoff et al., 2004) with the hope of developing the tool for examining flight velocity and distance travelled. ImageJ is a video analysis suite which makes use of various plug-ins designed to analyse particular types of video/scenario. In this project the plug-in “particle tracker” (Sbalzarini & Koumoutsakos, 2005) was tested which allows the user to tag and automatically track a particle frame by frame throughout a video. In this way the distance travelled per frame can be measured (in pixels) which can be translated into actual distance and used with time to calculate velocity. In order to convert the video into one that could be used by ImageJ, Mpeg video files were converted to jpeg “stacks” consisting of individual images for each frame. Utilities were used to enhance these images; firstly images were thresholded to convert to black and white. These colours were then inverted to give a white particle on a black background, which is more efficiently detected in particle tracker. Areas outside of the flight area were cropped from the zone of detection so as to reduce the possibility of additional “false” particles being recognised. These were often shadows or discolorations of the lower surface of the flight cube. Contrast was also maximised between background and particle. These parameters were then applied to all images within the stack.

### ***3.2.2 Flight comparison between native & invasive populations***

Samples used in this comparison comprised of 20 *H. axyridis* individuals from Hull in the U.K. invasive range and 20 from Fuchu, Japan; the native range. U.K. samples were collected from Hull, U.K. approximately 2 weeks before flight tests were performed to mimic the time between capture of the Japanese samples and their arrival in the U.K. All samples were therefore in captivity for the same period of time. As both sets of samples were collected as adults it was not possible to determine and so control for age. All ladybirds were kept in a controlled temperature room at 18°C at all times and fed on an artificial food containing yeast, liver, vitamins, sugar agar and water (Majerus & Kearns, 1989).

In order to compare the flight performance of test subjects from native and invasive *H. axyridis* populations a simple approach was taken; flights were scored in terms of period of time spent flying/not flying. Flight was categorised in 3 ways, flying, stopped and intermittent. Intermittent describes periods where flight started and stopped in quick succession (less than 2 minutes in each state, flying and stopping).

Analysis of variance (ANOVA) was performed to test for differences in flight ability between native *H. axyridis* (JAP) and individuals from the invasive population (U.K.). In order to fulfil the requirements for the ANOVA data was first log transformed to overcome heterogeneity of variances as identified by the Levene test ( $P = 0.04$ ) (Fig. 3.2b). The function  $\ln(X + 1)$  was used, where  $X$  = Flight time and 1 was added to each data point to account for flight times of 0. Analyses used here were performed in SPSS (IBM SPSS for windows release 19.0.0).



## 3.3 Results

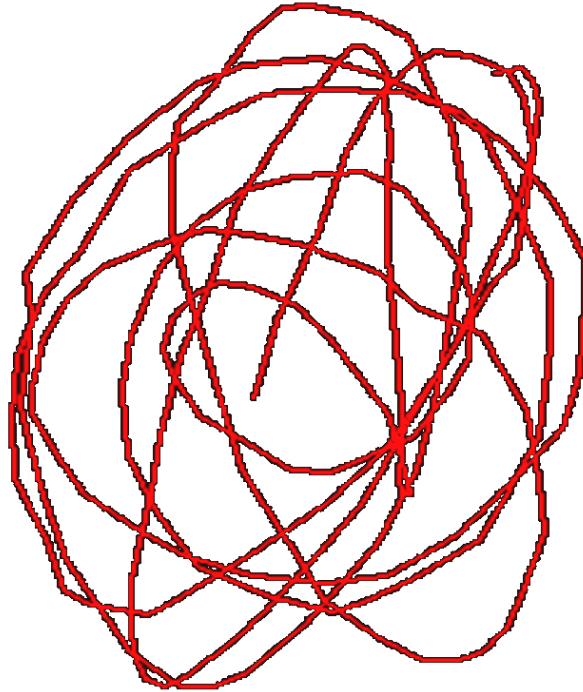
### *3.3.1 Method development*

The tethering procedure itself was quick, easily repeatable and left little room for error. The position of the tether behind the pronotum and forelegs allowed for an appropriate suspended body position and was not observed to prevent or hamper the opening of the elytra.

Although the test subjects were cooled in order to make them docile for tethering; they were active enough to initiate flight after 1 minute of being held in the hand. The loss of tarsal contact with a surface was, in most cases, enough to stimulate immediate flight. In the majority of the remaining subjects; movement through the air during fixing of the tether to the inside of the flight cube provoked flight.

After flight testing, individual subjects were easily removed from the tether with no observed ill effects besides fatigue. Although it was not done here, this would allow for repeated tests of the same individuals if required. The simple apparatus could be re-used for multiple samples with no change in test conditions.

ImageJ analyses proved impractical in this study due to the limitations of the camera used, particularly the shutter speed in relation to the speed at which the ladybirds flew. The shutter speed of the camera available in this study was too slow, resulting in blurred particles in each video image. It was therefore not possible to automatically detect ladybirds in particle tracker. A facility is available to manually track particles in each frame (Fig. 3.2) and this was used for small video samples however, it was not feasible to manually track each 2 hour flight.



**Figure 3.2** Flight trajectory of 8 seconds of *H. axyridis* flight recorded manually tracked by using the Image particle tracker plug-in.

### ***3.3.2 Flight comparison between native & invasive populations***

Mean flight times for U.K. and JAP populations were  $36.5 \pm 35.5$ min and  $54.5 \pm 45.67$ min

respectively with a positive skew for both populations (Fig. 3.3). Maximum flight time was the full 120 minutes and was accomplished by only one individual from the U.K. population.

However flight times of over 100 minutes occurred several times in the Japanese population.

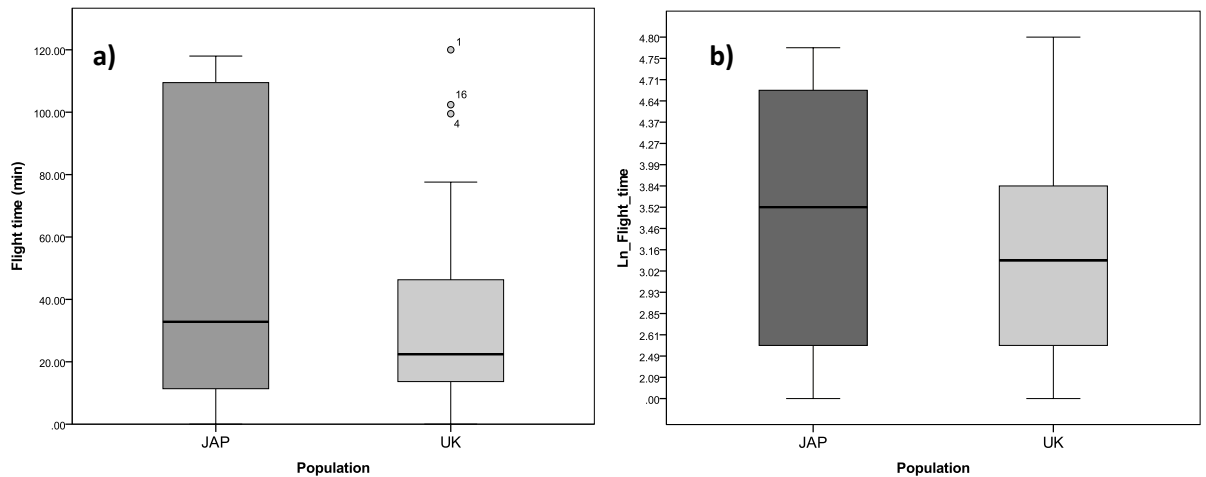
In contrast, a number of test subjects, mostly from the Japanese samples exhibited no flight; thus the Japanese population displayed the greatest variation in flight behaviour (Fig. 3.3a,b.)

Levene's statistic confirmed that the variances for the log transformed data were not

significantly different ( $P = 0.176$ ). Analysis of variances showed that there was no significant

difference between the population means ( $F_{1,40} = 0.127$ ,  $P = 0.723$ ). Power analyses for ANOVA

analyses on the log transformed data showed that the sample sizes given were adequate to have identified a significant relationship at the 0.05 confidence level if one existed (Power (1- $\beta$  err prob) = 0.083, required sample size, 18/18).



**Figure 3.3 a)** Distribution of total flight times for JAP and U.K. populations from flight tests of 20 individuals per population. **b)** Log transformed total flight times using the function  $(\ln X + 1)$  where  $X =$  flight time.

### 3.4 Discussion

The method examined here was successful in producing total flight time data with a large variation between individuals. Due to the simplicity of the method and the ease in which conditions can be replicated, this variation in flight times can confidently be attributed to differences between individuals as opposed to methodological inconsistencies. Therefore this method represents a potentially informative way of analysing the flight behaviour of *H. axyridis* and possibly other species. Additionally this method would allow to control for and test many environmental factors such as temperature, wind currents, diet, age and sex. A point of contention amongst scientists studying coccinellid flight has in the past been the cues for dispersal to overwintering sites. Some evidence suggests that migratory ladybird flight is driven hypostatically, i.e. ladybirds fly towards visual cues, whereas other authors postulate that it is driven by volatile cues (Hodek & Honěk, 1996). The blank sides of the flight cube used

here may provide an opportunity to test such hypotheses as could the introduction of air currents containing volatiles.

Contrary to the prediction of increased dispersal capacity in the invasive (U.K.) population; the comparison of flight between the invasive and native *H. axyridis* populations in the present study showed no significant difference between them. Hughes et al. (2003) explain the increased dispersal ability found in *P. aegeria* at range boundaries as driven by natural selection. However, the authors also point out that the trade-offs between dispersal and fecundity complicate matters. If it is more important to reproduce quickly at range boundaries as opposed to being highly dispersive, then there may more selection for high fecundity than high dispersal ability. The trade-off between dispersal ability and fecundity may therefore result in similar, if not reduced, dispersal ability at range expansion boundaries relative to populations from the range core. To test this alternative hypothesis, the fecundity of populations at range boundaries could be examined in parallel to dispersal ability.

The results of flight times from this Chapter were used in conjunction with displacement speeds (calculated in Chapter 2) to estimate the displacement distance per flight (average and maximum) for *H. axyridis*. This information could prove important in predicting the future spread of invasion in this species. However, the conclusions pertaining to *in vivo* flight drawn from this study should be treated with caution, as there were many conditions that were not controlled for due to impracticality. Firstly, the age of test subjects of both populations was not known as they were collected as adults in the field. Insect flight ability has been found to decrease as age increases due to degradation of flight muscles (Miller et al., 2008) therefore age should be controlled in any formal examination of insect flight ability. Additionally, the potential ill effects of the long travel time of live Japanese samples to the U.K. could mean that the results presented here are an underestimation of the flight ability for this population. The use of F1 generation test subjects raised *in vitro* would allow for the effect of age on flight to

be examined or to be controlled for if other conditions are of interest. Further a larger sample size would incorporate more natural variation.

In order to use the method described here to make inferences about *in vivo* flight, additional factors should be controlled for such as diet and photoperiod, both of which have been shown to be important in dispersal (see Hodek & Honěk 1996; Palmer, 2008 respectively).

As well as the ability to control for the above flight predictors, the method examined here provides a means of studying the effects they have on *H. axyridis* flight in a way that would be impossible in the field. This could potentially yield information on the dispersal of *H. axyridis* to help predict future range expansions and the mechanisms behind them.

## **Chapter 4.**

### **Factors affecting the dispersal of the invasive ladybirds *Coccinella septempunctata* and *Harmonia axyridis* :**

#### **General Discussion**

## General Discussion

This thesis has addressed a fundamental question of invasion biology; what is the importance of dispersal in invasions? Firstly the effects of environmental factors, namely temperature, wind speed and local aphid abundance, on the dispersal of *C. septempunctata* and *H. axyridis* were examined. These factors are highly variable in both space and time and therefore knowledge of how these changing conditions effect the dispersal of *C. septempunctata* and *H. axyridis* is integral to understanding the dispersal of these species in their invasive range. Secondly, a novel *in vitro* method for analysing ladybird flight was designed to allow for the controlled testing of many aspects of dispersal. This was then used to test the hypothesis that high dispersal would be selected for at range expansion boundaries as a result of the fitness increase that it confers.

### 4.1 Abiotic and biotic predictors of ladybird dispersal

The results presented in Chapter 2 of this thesis point to temperature as the most important predictor of flight in *C. septempunctata* and *H. axyridis*. There was a favoured range of between 17-21°C, however these species were observed flying at a broad range of temperatures from 6-29°C. A look at the current invasive range of *C. septempunctata* and *H. axyridis* also suggests a tolerance for a broad range of temperatures. *Harmonia axyridis* is invasive in some of the warmest countries in the world, for example Brazil (Brown et al., *in press*) where the average summer (January- March) temperature for is 27.6°C in Vitoria, central Brazil ([www.worldclimate.com/](http://www.worldclimate.com/)). However, this ladybird is also invasive in Canada (Brown et al., *in press*) where the average summer temperature in June-August at Ennadai Lake, Central Canada is 10.4°C ([www.worldclimate.com/](http://www.worldclimate.com/)). Similarly *C. septempunctata* is also invasive in a diverse range of climates which differ from the South to the North of the Nearctic ecozone (Hodek & Michaud, 2008) (Average temperatures for Jul-Sept is 17.7°C in Mexico City, Mexico ([www.worldclimate.com/](http://www.worldclimate.com/)) and see above for Central Canada). As discussed in Chapter 1 of this thesis, dispersal is of high importance in species invasions; both in range expansions (Travis et al., 2009, Hughes et al., 2008) and in the persistence of established populations

(Bowler & Benton, 2005). The ability of *H. axyridis* and *C. septempunctata* to disperse at a broad temperature range as indicated above suggests phenotypic plasticity associated with their dispersal, which has been suggested to result in an increase in fitness in unfavourable conditions encountered during invasions (Peacor et al., 2006). As a result, the potential for phenotypic plasticity in a source population has been suggested as one way in which a species may be predisposed to invasion success (Lee et al., 2007). *Coccinella septempunctata* and *H. axyridis* are native to heterogeneous landscapes containing both plains and mountains. Within such landscapes meteorological conditions are known to change with the topography; for instance temperature decreases as height increases above sea level (Harding, 1978) and wind speeds, which were found in Chapter 2 to have a negative impact on the number of flying ladybirds, increases as a result of “mountain waves” created by air rising over hills and mountains (Durrant, 1990). Thus within their native ranges, it is likely that *C. septempunctata* and *H. axyridis* have encountered a broad range of temperatures and wind speeds. The fact that both species have evolved in such landscapes may have equipped them with the ability to adapt to the wide range of environmental conditions described above, which may in turn have provided the phenotypic plasticity on which selection during invasions could act.

Despite this suggested ability to adapt to unfavourable environmental conditions, however the comparison of historical *H. axyridis* distribution data ([www.harlequin-survey.org](http://www.harlequin-survey.org)) for 2000-2009 suggests that the spread of this species across the U.K. has been slowed by topographical barriers. According to this distribution data, the Cambrian Mountains and the Pennines, both of which are mountainous areas, have represented the range boundary of the *H. axyridis* invasion from 2006-2009; since 2006, little further range boundary expansion has taken place. Therefore, in light of the negative influence of low temperatures and high wind speeds on dispersal; one explanation for this lack of spread may be the unfavourable meteorological conditions for flight at these locations. It is likely however that these topographical barriers will only slow, not stop the spread of *H. axyridis* in the U.K. Anthropogenic means of transport may allow them to bypass these areas of unfavourable dispersal conditions; the first record of



*H. axyridis* in Scotland for example, was the result of inadvertent transport of in a ladybird in a suitcase (Brown et al., *in press*). A number of outlying records in Scotland and Ireland in 2008 which are a long way from the range boundary (Brown et al., 2008) suggest that similar instances of anthropogenic transport have taken place and thus, are likely to again.

One previously suggested route of *H. axyridis* introduction into the U.K. has been via natural dispersal (Brown et al., 2008). However, direct evidence of the ability of *H. axyridis* to perform such long distance flights has been lacking. Combining the displacement speed recorded by the VLR in Chapter 2 with the *H. axyridis* flight times observed in Chapter 3 an average displacement distance of 21km was calculated, and in a 2 hour flight this distance was found to be as much as 70km. It may even be that Chapter 2 underestimates flight time, thus the maximum possible displacement distances could be larger. Therefore given the right wind direction and temperatures; the suggested (Brown et al., 2008) natural dispersal of *H. axyridis* into the U.K. from continental Europe does indeed seem plausible. This question however would benefit from the genetic analyses of populations from both regions. As performed by Rollins et al. (2011) in invasive starlings, analyses of mitochondrial DNA could help to track the origins of the invasive U.K. *H. axyridis* population. Additionally, mitochondrial DNA analyses can also be used to calculate the minimum number of introductions of an invasive species (Ficetola et al., 2008; Hänfling et al., 2005), from which it might be possible to infer the frequency at which these natural entries occurs.

As well as examining the effects of abiotic factors, Chapter 2 also compared the number of ladybirds flying with the local abundance of aphids, however no significant relationship between them was found. This is surprising as many authors have described the negative relationship of short distance coccinellid flight with aphid abundance (Elliott et al, 2000; Osawa; 2000; Hodek & Honěk, 1996). The observations of Chapter 2 however are of high altitude flight (above 150m AGL) and so the lack of a significant relationship between ladybird AD and aphid abundance suggests that this high altitude flight may be predicted by other

factors. Despite the fact that the flight observed here is likely motivated by low aphid abundance, it could be that the less favourable conditions at higher altitudes i.e. low temperature and high wind speeds (Harding, 1978; Durran, 1990) impose stricter constraints on when and where the ladybirds can fly than they do at ground level. Therefore, any relationship between aphid abundance in the local area and ladybird flight may be masked.

The problem described above highlights one of the major limitations of the VLR method used in Chapter 2; the inability to observe ladybird flights at altitudes below 150m AGL. To address this, field counts and observations of both ladybirds and aphids at ground level could be used to supplement VLR detections. This would link high altitude flights to those at low levels and enable more detailed inferences about the dispersal dynamics of ladybirds in relation to aphids. Ives et al. (1993) highlights the importance of studying such ecological relationships at different scales; describing how the cumulative effect of a number of individuals will produce population-level patterns that would not be observed at the individual scale. The author also mentions that at larger scales, more variables are introduced and could confound or mask patterns in the data, which as previously suggested, may be the case in the present study. Harmonic radar might also be used to track the low level flight of ladybirds, allowing for more direct observations of ladybird flight in relation to aphid abundances. It may also provide a better understanding of the spatial scales associated with the different flight behaviours of with coccinellids, i.e. appetitive, hectic trivial (long distance) and migratory flight.

Another important limitation of Chapter 2 is the inclusion of both *H. axyridis* and *C. septempunctata* in the VLR data, which was unavoidable due to the ways in which VLR identifies insect targets and the physical similarities between *H. axyridis* and *C. septempunctata*. As *C. septempunctata* are not invasive in the U.K., no solid conclusions pertaining specifically to the invasion of the U.K. could be made. Therefore an obvious avenue for future work would include the discrimination between these two species by the VLR, perhaps through a more strict calibration protocol.

## 4.2 An *in vitro* method for examining flight.

The method described in Chapter 3 was successful in providing a repeatable standardised way of examining ladybird flight. The simple method required no expensive monitoring equipment as used in flight mill tests or tethered tests using force-transducers and although not practical here, the use of video analyses software could provide an effective means of quantifying these flights.

Chapter 3 also focused on the comparison between native and invasive populations of *H. axyridis*; a significant increase in dispersal was predicted in the invasive population due to the inherent fitness advantages of being highly dispersive at range expansion boundaries (Travis et al., 2009, Hughes et al., 2007; Travis & Dytham, 2002). The lack of a significant difference in the flight ability between the native and invasive populations studied in Chapter 3 may be a result of the conditions that were not controlled for in this study. However, an alternative explanation may be that a selection for increased fecundity can also take place at a range boundary. Therefore due to trade-offs between dispersal and fecundity, this may result in no change, or even a reduction in dispersal at range expansion boundaries (Hughes et al., 2007). The method used here was assumed to be an adequate comparative test as the conditions for all test subjects were kept consistent. However one step that was not taken in this Chapter was to test whether or not this *in vitro* method was indicative of flight behaviours and capabilities in the field as was done by Rankin & Rankin (1980). It is known that many factors can affect insect activity and in turn insect flight; for example diet and hypostatic cues (Hodek & Honěk, 1996) that could not be reproduced in the laboratory. Therefore the average and maximum flight times that were quoted in Chapter 2 for the calculation of displacement distances per flight must be treated with caution. However, comparisons between individuals with this method would be enough to test many aspects of ladybird flight, from the effects of diet, wind direction and hypostatic cues to the acclimatisation to temperature among others. Provided that other factors such as age and sex are controlled for in an appropriate way.

The underlying question of this project has been what is the importance of dispersal in invasions? However, the results of this study have highlighted a more specific question; what is the importance of the evolution of dispersal at range expansion boundaries during invasions? As Travis et al. (2009) explain; the evolution of density dependent dispersal strategies in which organisms disperse at low population densities, as opposed to above a population density threshold, could result in higher dispersal at range expansion boundaries. However as postulated above, the selection of higher fecundity in exchange for reduced dispersal ability or propensity (Hughes et al., 2008) may result in lower dispersal at range expansion boundaries. Information on the evolutionary processes during invasions could help predict future range expansions, invasion routes and potential invasive species, however in light of life-history trait trade-offs; these predictions must be informed by detailed knowledge of selective pressures at range expansion boundaries and species ecology. The results of this thesis add to the knowledge surrounding the dispersive ability and behaviour of *H. axyridis* and *C. septempunctata* and may therefore go some way in helping understand the traits that can determine invasion success and in predicting future invasions.

### **4.3 Conclusions**

Dispersal is important in invasions as it can be affected by highly variable meteorological factors which change over time and landscape topography. The limitations imposed on dispersal by these environmental factors are likely to influence the spread and route of invasions across a landscape. However, it is a process with a high degree of plasticity in these species and the potential for this may be one way in which *C. septempunctata* and *H. axyridis* are predisposed to invasion success in large and heterogeneous invasive ranges. At range expansion boundaries the dispersal traits of these species are likely to be selected upon by changing pressures at range expansion boundaries. This could result in the evolution of dispersal strategies that are different from those in the native populations. Due to dispersal-fecundity trade-offs however higher dispersal may not always be selected for at range expansion boundaries. In order to examine these multi-scale analyses of dispersal should be

applied to account for differences in the effects of dispersal strategies at the individual and population levels. And *in vitro* tests may provide a valuable controlled means with which to address the effects of both environmental and ontological factors that affect dispersal.

## References

- Abràmoff, M. D., Hospitals, I., Magalhães, P. J. (2004). Image Processing with ImageJ. *Biophotonics International*, July.
- Berkvens, N., Baverstock, J., De Clercq, P., Majerus, M. E. N., Pell, J. K., Roy, H. E. (2009). Good and bad traits of *Harmonia axyridis* - from biological control to invasion. Proceedings of the Third International Symposium on Biological Control of Arthropods, Christchurch, New Zealand.
- Blackburn, T.E., Cassey, P., Duncan, R.P., Evans, K.L., Gaston, K.J. (2004). Avian extinction and mammalian introductions on oceanic islands. *Science*, 305(5692), 1955-1958.
- Blossey, B., & Notzold, R. (1995). Evolution of Increased Competitive Ability in Invasive Nonindigenous Plants: A Hypothesis. *The Journal of Ecology*, 83(5), 887.
- Bowler, D.E. & Benton, T.G. 2005. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biological Reviews*, 80, 205 – 225.
- Brakefield, P. M., Willmer, P.M. (1985). The basis of thermal melanism in the ladybird *Adalia bipunctata*: differences in reflectance and thermal properties between the morphs. *Heredity*, 54, 9–14.
- Briers, R., Cariss, H. M., Gee, J. H. R. (2003). Flight activity of adult stoneflies in relation to weather. *Ecological Entomology*, 28(1), 31-40.
- Brown, P. M. J., Frost, R., Doberski, J., Sparks, T., Harrington, R., Roy, H.E. (2011). Decline in native ladybirds in response to the arrival of *Harmonia axyridis*: early evidence from England. *Ecological Entomology*, 36(2), 231-240.
- Brown, P. M. J., Roy, H.E., Rothery, P., Roy, D. B., Ware, R. L., Majerus, M.E.N. (2008). *Harmonia axyridis* in Great Britain: analysis of the spread and distribution of a non-native coccinellid. *BioControl*, 53(1), 55-67.
- Capaldi, E. A., Smith, A. D., Osborne, J. L., Fahrbach, S. E., Farris, S. M., Reynolds, D. R. (2000). Ontogeny of orientation flight in the honeybee revealed by harmonic radar. *Nature*, 403(6769), 537-540.
- Carroll, S. (1996). The biology of post-invasion events. *Biological Conservation*, 78(1-2), 207-214.
- Carter, M. C., Dixon, A.F.G. (1984). Foraging behaviour of coccinellid larvae: duration of intensive search. *Entomologia Experimentalis et Applicata*, 36(2), 133-136.

- Chapman, J. W., Drake, V.A. (2010). Insect Migration. In: *Encyclopedia of Animal Behavior* 2010, 2, 161-166
- Chapman, J. W., Nesbit, R. L., Burgin, L. E., Reynolds, D R, Smith, A D, Middleton, D. R., et al. (2010). Flight Orientation Behaviors Promote Optimal Migration Trajectories in High-Flying Insects. *Science*, 327(5966), 682-685.
- Chapman, J. W., Reynolds, D. R., & Smith, A. D. (2003). Vertical-looking radar: A new tool for monitoring high-altitude insect migration. *Bioscience*, 53(5), 503-511.
- Chapman, J. W., Reynolds, D. R., Smith, A. D., Riley, J. R., Pedgley, D. E., & Woiwod, I. P. (2002). High-altitude migration of the diamondback moth *Plutella xylostella* to the UK: a study using radar, aerial netting and ground trapping. *Ecological Entomology*, 27, 641-650.
- Chapman, J.W., Reynolds, D.R., Mouritsen, H., Hill, J.K, Riley, J.R., Sivell, D. (2008). Wind selection and drift compensation optimize migratory pathways in a high-flying moth. *Current Biology*, 18(7), 514-8.
- Chapman, J.W., Reynolds, D.R., Brooks, S. J., Smith, A.D., & Woiwod, I. P. (2006). Seasonal variation in the migration strategies of the green lacewing *Chrysoperla carnea* species complex. *Ecological Entomology*, 31(4), 378-388.
- Chapman, J.W., Reynolds, D., Smith, A. (2004). Migratory and foraging movements in beneficial insects: a review of radar monitoring and tracking methods. *International Journal of Pest Management*, 50(3), 225-232.
- Clavero, M., & García-Berthou, E. (2005). Invasive species are a leading cause of animal extinctions. *Trends in Ecology & Evolution*, 20(3), 110.
- Coelho, J.R. (1991). The effect of thorax temperature on force production during tethered flight in honebee (*Apis mellifera*) drones, workers and queens. *Physiological Zoology*, 64(3) 823-835.
- Cohen, A. N., & Carlton, J. T. (1998). Accelerating invasion rate in a highly invaded estuary. *Science*, 279(5350), 555-558.
- Colautti, R. I., MacIsaac, H. J. (2004). A neutral terminology to define “invasive” species. *Diversity and Distributions*, 10(2), 135-141.
- Colautti, R. I., Ricciardi, A., Grigorovich, I. a, & MacIsaac, H. J. (2004). Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, 7(8), 721-733.
- Crawley, M.J. (2007). *The R Book*. John Wiley & Sons Ltd., London.

- Didham, R. K., Ewers, R. M., & Gemmell, N. J. (2005). Comment on "Avian extinction and mammalian introductions on oceanic islands". *Science*, *307*(5714), 1412-1412.
- Dingle, B. Y. H. (1965). The relation between age and flight activity in the milkweed bug, *Oncopeltus*. *Journal of Experimental Biology*, *42*, 269-283.
- Dingle, B. Y. H. (1966). Some factors affecting flight activity in individual milkweed bugs (*Oncopeltus*). *Journal of Experimental Biology*, *44*(2), 335-43.
- Dixon, A.F.G. (1985). Aphid ecology. Chapman and Hall, London.
- Dixon, a. F. G., & Agarwala, B. K. (1999). Ladybird-induced life-history changes in aphids. *Proceedings of the Royal Society B: Biological Sciences*, *266*(1428), 1549-1553.
- Dixon, A.F.G., Horth, S., Kindlmann, P. (1993). Migration in Insects: Cost and Strategies. *The Journal of Animal Ecology*, *62*(1).
- Durrant, D. R. (1990). Mountain waves and downslope winds. *Atmospheric processes over complex terrain*, *23*(45), 59-62.
- Elliott, N. (2000). Adult coccinellid activity and predation on aphids in spring cereals. *Biological Control*, *17*(3), 218-226.
- Elliott, N., Kieckhefer, R., Kauffman, W. (1996). Effects of an invading coccinellid on native coccinellids in an agricultural landscape. *Oecologia*, *105*(4), 537-544.
- Evans, E.W. (2000). Habitat displacement of North American ladybirds by an introduced species. *Ecology*, *85*, 637-647.
- Evans, E. W. (2003). Searching and reproductive behaviour of female aphidophagous ladybirds (Coleoptera : Coccinellidae): a review. *European Journal of Entomology*, *100*(1), 1-10.
- Fabre, F., Dedryver, C.-A., Plantegenest, M., Hullé, M., & Rivot, E. (2010). Hierarchical bayesian modelling of plant colonisation by winged aphids: Inferring dispersal processes by linking aerial and field count data. *Ecological Modelling*, *221*(15), 1770-1778.
- Ferran, A., Niknam, H., Kabiri, F., Picart, J.-L., De Herce, C., Brun, J. (1996). The use of *Harmonia axyridis* larvae (Coleoptera: Coccinellidae) against *Macrosiphum rosae* (Hemiptera: Sternorrhyncha: Aphididae) on rose bushes. *European Journal of Entomology*, *93*, 59-67.



- Ficetola, G. F., Bonin, A., & Miaud, C. (2008). Population genetics reveals origin and number of founders in a biological invasion. *Molecular ecology*, 17(3), 773-82.
- Fritts, T. H., & Rodda, G. H. (1998). The role of introduced species in the degradation of island ecosystems: A Case History of Guam. *Annual Review of Ecology and Systematics*, 29(1), 113-140.
- Gewecke, M., Philippen, J. (2008) Control of the horizontal flight-course by air-current sense organs in *Locusta migratoria*. *Physiological Entomology*, 3(1), 43-52.
- Goldwasser, L., Cook, J., & Silverman, E. D. (1994). The effects of variability on metapopulation dynamics and rates of invasion. *Ecology*, 75(1), 40-47.
- Gordon, R. D. (1985). The coccinellidae (Coleoptera) of America north of Mexico. *Journal of the New York Entomological Society*, 93(1), 1-912.
- Gurevitch, J., & Padilla, D. K. (2004). Are invasive species a major cause of extinctions? *Trends in Ecology & Evolution*, 19(9), 470-4.
- Hagen, K.S. (1962). Biology and ecology of predaceous Coccinellidae. *Annual reviews in Entomology*, 7, 289-326.
- Hanfing, B., Bolton, P., Harley, M., & Carvalho, G. R. (2005). A molecular approach to detect hybridisation between crucian carp (*Carassius carassius*) and non-indigenous carp species (*Carassius* spp. and *Cyprinus carpio*). *Freshwater Biology*, 50(3), 403-417.
- Harding, R. J. (1978). The variation of the altitudinal gradient of temperature within the British Isles. *Geografiska Annaler, Series A, Physical Geography*, 60(1), 43-49.
- Hawley, D. M., Hanley, D., Dhondt, A. a, & Lovette, I. J. (2006). Molecular evidence for a founder effect in invasive house finch (*Carpodacus mexicanus*) populations experiencing an emergent disease epidemic. *Molecular Ecology*, 15(1), 263-75.
- Heinrich, B. (1995). Insect thermoregulation. *Endeavour*, 19(1), 28-33.
- Hemptinne, A. J., Dixon, A. F. G., & Coffin, J. (2010). International association for ecology attack Strategy of ladybird beetles (Coccinellidae): factors shaping their numerical response. *Ecology*, 90(2), 238-245.
- Heulsman, M.F., Kovach, J., Jasinski, J., Young, C., Easley, B. (2002). Multicoloured Asian Lady Beetle (*Harmonia axyridis*) as a nuisance pest in households in Ohio. In Jones, C., Zhai, J., Robinson, Wm. H. (Eds.) *Proceedings of the 4<sup>th</sup> international conference on urban pests*, 243-250.

- Hodek & Honěk (Eds.) (1996). Ecology of Coccinellidae Series Entomologica, 54. Dordrecht: Kluwer Academic Publishers.
- Hodek, I., Ipert, G., & Hodkova, M. (1993). Long-distance flights in coccinellidae (Coleoptera). *European Journal of Entomology*, 90, 403-414.
- Hodek, I., & Michaud, J. P. (2008). Why is *Coccinella septempunctata* so successful? (A point-of-view). *European Journal of Entomology*, 105, 1-12.
- Hughes, C. L., Hill, Jane K, & Dytham, C. (2003). Evolutionary trade-offs between reproduction and dispersal in populations at expanding range boundaries. *Proceedings. Biological sciences / The Royal Society*, 270 (Suppl).
- Hughes, C. L., Dytham, C., & Hill, Jane K. (2007). Modelling and analysing evolution of dispersal in populations at expanding range boundaries. *Ecological Entomology*, 32(5), 437-445.
- Huxel, G. (1999). Rapid displacement of native species by invasive species: effects of hybridization. *Biological Conservation*, 89(2), 143-152.
- Ishiguri, Y., & Shirai, Y. (2004). Flight activity of the peach fruit moth, *Carposina sasakii* (Lepidoptera: Carposinidae), measured by a flight mill. *Applied Entomology and Zoology*, 39(1), 127-131.
- Ives, A. R., Kareiva, P., & Perry, R. (1993). Response of a Predator to Variation in Prey Density at Three Hierarchical Scales Lady Beetles Feeding on Aphids. *Ecology*, 74(7), 1929-1938.
- Johnson, C.G., Taylor, L.R. (1955). The development of large suction traps for airborne insects. *Annals of Applied Biology*, 43(1), 51-62.
- Johnson, C. G. (1969). Migration and dispersal of insects by flight. Methuen, London.
- Kliber, A., & Eckert, C. G. (2005). Interaction between founder effect and selection during biological invasion in an aquatic plant. *Evolution; International Journal of Organic Evolution*, 59(9), 1900-1913.
- Koch, R L. (2003). The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts. *Journal of Insect Science*, 3(1971).
- Koch, Robert L, Venette, R. C., & Hutchison, W. D. (2006). Invasions by *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in the western hemisphere: implications for South America. *Neotropical entomology*, 35(4), 421-34.

- Koch, Robert L., & Galvan, T. L. (2008). Bad side of a good beetle: the North American experience with *Harmonia axyridis*. *BioControl*, 53(1), 23-35.
- Kokko, H., & López-Sepulcre, A. (2007). The ecogenetic link between demography and evolution: can we bridge the gap between theory and data? *Ecology Letters*, 10(9), 773-82.
- Kot, M., Lewis, M.A., van den Driessche, P. 1996. Dispersal data and the spread of invading organisms. *Ecology*, 77, 2027–2042.
- Krivan, K. (2008). Dispersal dynamics: distribution of lady beetles (Coleoptera: Coccinellidae). *European Journal of Entomology*, 105, 405-409.
- Lambrinos, J. G. (2004). How interactions between ecology and evolution influence Contemporary Invasion Dynamics. *Ecology*, 85(8), 2061-2070.
- Lee, C. E., Remfert, J. L., & Chang, Y.-M. (2007). Response to selection and evolvability of invasive populations. *Genetica*, 129(2), 179-92.
- Losey, J.E., Eubanks, M.D. (2000). Implications of pea aphid host-plant specialization for the potential colonization of vegetables following post-harvest emigration from forage crops. *Environmental Entomology* 29, 1283-1288.
- Lucas, É., Gagne, I., & Coderre, D. (2002). Impact of the arrival of *Harmonia axyridis* on adults of *Coccinella septempunctata* and *Coleomegilla maculata* (Coleoptera: Coccinellidae). *European Journal of Entomology*, 99, 457-463.
- Macaulay, E. D. M., Tatchell, G.M., Taylor, L.R. (1988). The Rothamsted Insect Survey '12 metre' suction trap. *Bulletin of Entomological Research*, 78, 121-9.
- Mahrt, L., Heald, R. C., Lenschow, D. H., Stankov, B. B., & Troen, I. B. (1979). An observational study of the structure of the nocturnal boundary layer. *Boundary Layer Meteorology*, 17, 247-264.
- Majerus, M.E.N. (1994). Ladybirds, 81, New Naturalist series. HarperCollins, London
- Majerus MEN, Kearns P (1989) Ladybirds. Richmond Publishing, Slough
- Marden, J. (1995). Evolutionary adaptation of contractile performance in muscle of ectothermic winter-flying moths. *The Journal of experimental biology*, 198, 2087-94.
- McPeck, M. A., & Holt, R. D. (1992). The evolution of dispersal in spatially and temporally varying environments. *Environments*, 140(6), 1010-1027.

- Michie, L. J., Mallard, F., Majerus, M. E. N., & Jiggins, F. M. (2010). Melanic through nature or nurture: genetic polymorphism and phenotypic plasticity in *Harmonia axyridis*. *Journal of Evolutionary Biology*, 23.
- Miller, M. S., Lekkas, P., Braddock, J. M., Farman, G. P., Ballif, B. a, Irving, T. C., et al. (2008). Aging enhances indirect flight muscle fiber performance yet decreases flight ability in *Drosophila*. *Biophysical journal*, 95(5), 2391-401.
- Mooney, H. a, & Cleland, E. E. (2001). The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences of the United States of America*, 98(10), 5446-51.
- Müller, C. B., Williams, I. S., & Hardie, J. (2001). The role of nutrition, crowding and interspecific interactions in the development of winged aphids. *Ecological Entomology*, 26(3), 330-340.
- Neubert, G., & Caswel, H. (2002). Demography and dispersal: calculation and sensitivity analysis of invasion speed for structured populations. *Ecology*, 81(6), 1613-1628.
- Obrycki, J.J., Elliott, N.C., Giles, K.L. (2000). Coccinellid introductions: potential for and evaluation of non target effects. In Follet, P.A. & Duan, J.J., (Eds.). Nontarget effects of biological control. Kluwer Academy Publishers, Boston, MA.
- Obata, S. (1986). Mechanisms of prey finding in the aphidophagous ladybird beetle, *Harmonia axyridis* (Coleoptera, Coccinellidae). *Entomophaga*, 31(3), 303-311.
- Oilivieri, I., Michalakis, Y., & Gouyon, P.-H. (1995). Metapopulation genetics and the evolution of dispersal. *The American Naturalist*, 146(2), 202-228.
- O'Neal, M.E., Landis, D.A., Rothwell, E., Kempel, L., Reinhard, D. 2004. Tracking Insects with Harmonic Radar: a Case Study. *American Entomologist*. 50(4), 212-218.
- Osawa, N. (2000). Population field studies on the aphidophagous ladybird beetle *Harmonia axyridis* (Coleoptera : Coccinellidae): resource tracking and population characteristics. *Population Ecology*, 42(2), 115-127.
- Palmer, J.O. (2008). Photoperiodic control of reproduction in the milkweed leaf beetle, *Labidomera clivicollis*. *Physical Entomology*, 8(2), 187-194.
- Peacor, S. D., Allesina, S., Riolo, R. L., & Pascual, M. (2006). Phenotypic plasticity opposes species invasions by altering fitness surface. *PLoS biology*, 4(11).

- Peterson, a T., & Vieglais, D. a. (2001). Predicting species invasions using ecological niche modeling: new approaches from bioinformatics attack a pressing problem. *BioScience*, 51(5), 363.
- Petit, R. J. (2004). Biological invasions at the gene level. *Diversity and Distributions*, 10(3), 159-165.
- Pimentel, D. (2001). Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems & Environment*, 84(1), 1-20.
- Pimentel, D., Zuniga, R., & Morrison, D. (2004). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*, 52, 273 - 288.
- Prasad, R.P., Roitberg, B.D., Henderson, D. (1999). The effect of rearing temperature on flight initiation of *Trichogramma sibericum* Sorkina at ambient temperatures. *Biological control*. 16(3), 291-298.
- R Development Core Team. 2011. R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org. Packages: zoo, lme4, matrix.
- Rainey, R.C. 1976. Insect flight. Wiley-Blackwell Publishing, London.
- Rankin, M. A. N., & Rankin, S. (1980). High reproductive potential and great capacity for rapid exploitation of a newly invaded habitat. *Biological Bulletin*, 158, 356-369.
- Rankin, M. A., Hampton, E. N., & Summy, K. R. (1994). Investigations of the oogenesis-flight syndrome in *Anthonomus grandis* (Coleoptera: Curculionidae) using tethered flight tests. *Journal of Insect Behavior*, 7(6), 795-810.
- Reynolds, D.R., Riley, J.R., 1997. The Flight behaviour and migrations of insect pests: Radar studies in developing countries. Chatham, U.K: Natural resources Institute. NRI Bulletin no. 71.
- Reznick, D.N. & Ghalambor, C.K. (2001). The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica*, 112-13, 183-198. Springer.
- Riddick, E. W., Aldrich, J. R., De Milo, A., & Davis, J. C. (2000). Potential for modifying the behavior of the multicolored asian lady beetle (Coleoptera: Coccinellidae) with plant-derived natural products. *Annals of the Entomological Society of America*, 93(6), 1314-1321.

- Riley, J.R. (1985). Radar cross sections of insects. *Proceedings of the Institute of Electrical and Electronic Engineers*, 73(2), 228-232.
- Riley, J. R., Greggers, U., Smith, A. D., Reynolds, D. R., Menzel R. 2005. The flight paths of honeybees recruited by the waggle dance. *Nature*, 435, 205-207
- Riley, J.R., Smith, A.D. 2002. Design considerations for harmonic radar to investigate the flight of insects at low altitude. *Computers and Electronics in Agriculture*. 35, 151-169.
- Rivas, G.-G., Zapater, M.-F., Abadie, C., & Carlier, J. (2004). Founder effects and stochastic dispersal at the continental scale of the fungal pathogen of bananas *Mycosphaerella fijiensis*. *Molecular Ecology*, 13(2), 471-482.
- Roff, D. a. (1974). The analysis of a population model demonstrating the importance of dispersal in a heterogeneous environment. *Oecologia*, 15(3), 259-275.
- Roff, D. A., & Fairbairn, D. J. (2007). The evolution and genetics of migration in insects. *BioScience*, 57(2), 155.
- Rollins, L. A., Woolnough, A. P., Sinclair, R., Mooney, N. J., & Sherwin, W. B. (2011). Mitochondrial DNA offers unique insights into invasion history of the common starling. *Molecular Ecology*, 2307-2317.
- Rowley, W.A., Graham, C.L. (1968). The effect of temperature and relative humidity on the flight performance of female *Aedes aegypti*. *Journal of Insect Physiology*, 14(9), 1251-1257
- Roy, H.E., Brown, P.M.J., Majerus, M.E.N. (2006) *Harmonia axyridis*: A successful biocontrol agent or an invasive threat? In: *An ecological and societal approach to biological control* Eilenberg, J. & Hokkanen, H. (Eds.). Kluwer Academic Publishers, Netherlands.
- Sbalzarini, I. F. Koumoutsakos, P. (2005) Feature point tracking and trajectory analysis for video imaging in cell biology. *Journal of Structural Biology*, 151(2), 182-195.
- Schaefer, G.W. 1976. Radar observations of insect flight. In: *Royal Entomological Society Symposium 7: Insect Flight*. 1976. Rainey, R.C. (Ed.)
- Sarvary, M.A., Hight, S.D., Carpenter, J.E., Bloem, S., Bloem, K.A., Dorn, S. (2008). Identification of factors influencing flight performance of field-collected and laboratory-reared, overwintered, and nonoverwintered cactus moths fed with field-collected host plants. *Environmental Entomology*, 37(5), 1291-1299.

- Schumacher, P., Weyeneth, A., Weber, D. C., & Dorn, S. (1997). Long flights in *Cydia pomonella* L. (Lepidoptera: Tortricidae) measured by a flight mill: influence of sex, mated status and age. *Physiological Entomology*, 22(2), 149-160.
- Simberloff, D., & Stiling, P. (1996). How risky is Biological control? *Ecology*, 77(7), 1965-1974.
- Solbreck, C. (1974) Maturation of post-hibernation flight behaviour in the coccinellid *Coleomegilla maculata* (DeGeer). *Oecologia*, 17, 265-275.
- Staub, C.S., Snyder, W.E. (2006). Experimental approaches to understanding the relationship between predator biodiversity and biological control. *Progress in Biological Control*. 3, 221-239.
- Stevenson, B. Y. R. D., & Josephson, R. K. (1990). Effects of operating frequency and temperature on mechanical power output from moth flight muscle. *Journal of Experimental Biology*, 149, 61-78.
- Stewart, S.D. & Gaylor, M.J. (1994). Effects of age, sex, and reproductive status on flight by the tarnished plant bug (Heteroptera, Miridae). *Environmental Entomology*, 23,80-84.
- Taylor, L. R. (1963). Analysis of the effect of temperature on insects in flight. *Journal of Animal Ecology*, 29, 45–63.
- Taylor, L. (1974). Insect migration, periodicity and the boundary layer. *Journal of Animal Ecology*, 43(1), 225-238.
- Tedders, W. L., & Schaefer, P. W. (1994). Release and establishment of *Harmonia axyridis* (Coleoptera: Coccinellidae) in the southeastern United States. *Entomol. News*, 105, 228-243.
- Tourniaire, R., Ferran, A, Giuge, L., Piotte, C., & Gambier, J. (2000). A natural flightless mutation in the ladybird, *Harmonia axyridis*. *Entomologia Experimentalis et Applicata*, 96(1), 33-38.
- Travis, J. M. J., & Dytham, C. (2002). Dispersal evolution during invasions. *Evolutionary Ecology*, 1119-1129.
- Travis, J. M. J., Mustin, K., Benton, T. G., & Dytham, C. (2009). Accelerating invasion rates result from the evolution of density-dependent dispersal. *Journal of Theoretical Biology*, 259(1), 151-8.

- Ware, R. L., & Majerus, Michael E. N. (2008). Intraguild predation of immature stages of British and Japanese coccinellids by the invasive ladybird *Harmonia axyridis*. *BioControl*, 53(1), 169-188.
- Wilcove, D. S., Rothstein, D., Dubow, J., Phillips, A., & Losos, E. (1998). Quantifying threats to imperilled species in the United States. *Bioscience*, 48(8), 607-615.
- Williams, C. B. (1940). The analysis of four years captures of insects in a light trap. Part 2. The effect of weather conditions on insect activity; and the estimation and forecasting of changes in the insect population. *Transactions of the Royal Entomological Society of London*, 90, 227-306.
- Williams, C.B. (1948). The Rothamsted light trap. *Proceedings of the Royal Entomological Society of London*, 23(7), 80-85.
- Wood, C. R., Chapman, Jason W, Reynolds, Donald R, Barlow, J. F., Smith, Alan D, & Woiwod, Ian P. (2006). The influence of the atmospheric boundary layer on nocturnal layers of noctuids and other moths migrating over southern Britain. *International Journal of Biometeorology*, 50(4), 193-204.



## Appendices

**Appendix 1.** Peter M.J. Brown, Cathleen E. Thomas, Eric Lombaert, Daniel L. Jeffries, Arnaud Estoup and Lori-Jane Lawson Handley. (*In Press*). The global spread of *Harmonia axyridis* (Coleoptera: Coccinellidae): distribution, dispersal and routes of invasion. *BioControl*.

**Appendix 2. Ladybirds of the U.K.: Identification sheet (Taken from [www.harlequin-survey.org](http://www.harlequin-survey.org)).**

<p><b>Eyed ladybird</b></p>  <p>Length: 7 – 8.5mm Habitat: Conifers</p>	<p><b>Striped ladybird</b></p>  <p>© Frank Botterill Length: 6 – 8mm Habitat: Conifers</p>	<p><b>Harlequin ladybird</b></p>  <p>Length: 6 – 8 mm Habitat: Generalist Note: The two most common forms of this very variable species are shown</p>	<p><b>7-spot ladybird</b></p>  <p>Length: 5 – 8mm Habitat: Generalist</p>
<p><b>Cream-streaked ladybird</b></p>  <p>Length: 5 – 6mm Habitat: Conifers</p>	<p><b>Orange ladybird</b></p>  <p>Length: 4.5 – 6mm Habitat: Trees</p>	<p><b>Cream-spot ladybird</b></p>  <p>© Henry Arnold Length: 4 – 5mm Habitat: Deciduous trees</p>	<p><b>18-spot ladybird</b></p>  <p>Length: 4 – 5mm Habitat: Conifers</p>
<p><b>2-spot ladybird</b></p>  <p>© J. Mentens Length: 4 – 5mm Habitat: Generalist, often urban Note: Two forms of this very variable species are shown</p>	<p><b>Larch ladybird</b></p>  <p>Length: 4 – 5mm Habitat: Conifers</p>	<p><b>Kidney-spot ladybird</b></p>  <p>© Allen Beechey Length: 4 – 5mm Habitat: Deciduous trees</p>	<p><b>11-spot ladybird</b></p>  <p>Length: 4 – 5mm Habitat: Generalist, often coastal</p>
<p><b>Adonis' ladybird</b></p>  <p>Length: 4 – 5mm Habitat: Diverse, usually sandy soils</p>	<p><b>Water ladybird</b></p>  <p>Length: 4mm Habitat: Wetlands</p>	<p><b>14-spot ladybird</b></p>  <p>Length: 3.5 – 4.5mm Habitat: Generalist</p>	<p><b>10-spot ladybird</b></p>  <p>© Mike Peters Length: 3.5 – 4.5mm Habitat: Deciduous trees</p>
<p><b>Pine ladybird</b></p>  <p>Length: 3 – 4.5mm Habitat: Trees</p>	<p><b>22-spot ladybird</b></p>  <p>© J. Mentens Length: 3 – 4mm Habitat: Grass</p>	<p><b>24-spot ladybird</b></p>  <p>Length: 3 – 4mm (hairy) Habitat: Grass</p>	<p><b>16-spot ladybird</b></p>  <p>Length: 3 mm Habitat: Grass</p>

**Appendix 3.** Cross sectional measurements of **a)** 5 *C. septempunctata* **b)** 20 *H. axyridis* samples made using the radar transmission rig at Rothamsted research centre. Mass was measured immediately before the insect was placed into the rig.  $\sigma_{x/x}$  and  $\sigma_{y/y}$  relate to insect axes (length and width respectively), 2 blind backscatter measurements were performed for each and an average taken (not shown in **a)**). A calibration curve made from 6 steel spheres of known size was then used to translate ladybird backscatter measurements into cross sectional measurements. Mass (mg) and Mean cross section Ratio ( $\sigma_{x/x}/\sigma_{y/y}$ ) ranges were then used to produce the species specific selection criteria for *H. axyridis* and *C. septempunctata*.

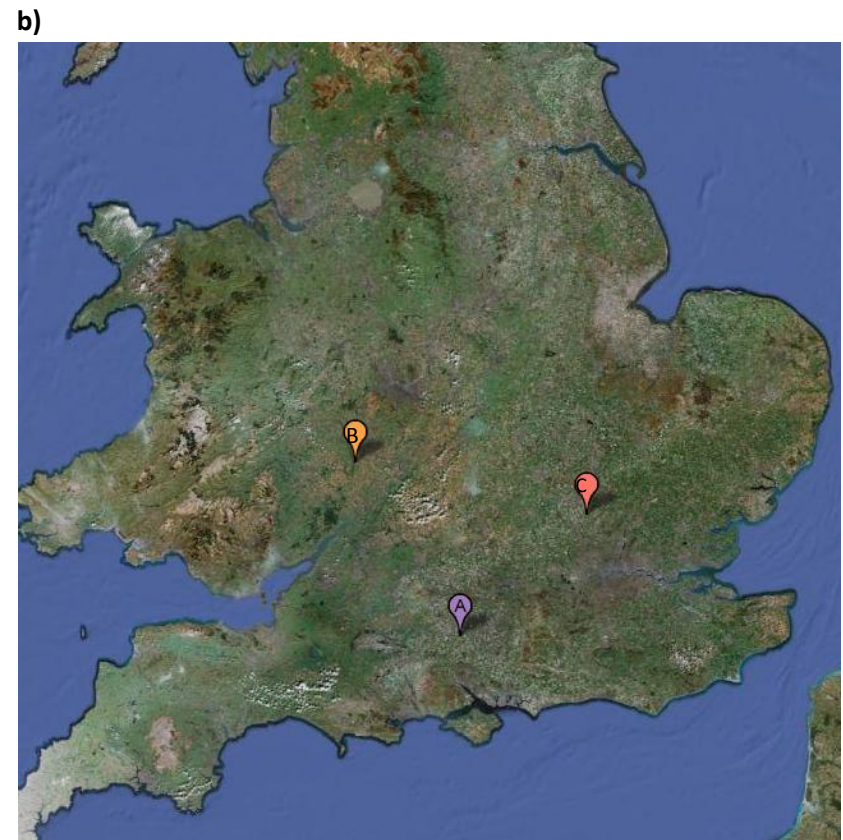
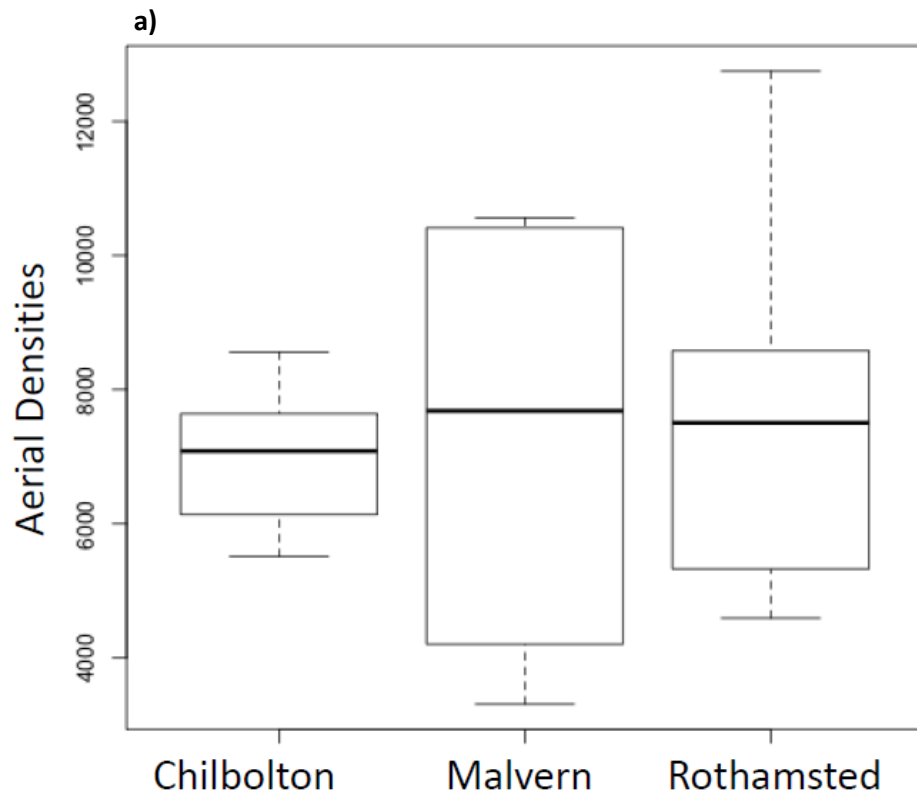
**a)**

Sample #	Mass (mg)	Sigma xx (cm <sup>2</sup> )	Sigma yy (cm <sup>2</sup> )	Ratio
<b>1</b>	29.4	$3.4 \times 10^{-2}$	$2.3 \times 10^{-2}$	1.5
<b>2</b>	26.6	$3.4 \times 10^{-2}$	$1.5 \times 10^{-2}$	2.3
<b>3</b>	24.0	$4.6 \times 10^{-2}$	$1.8 \times 10^{-2}$	2.6
<b>4</b>	26.7	$4.6 \times 10^{-2}$	$2.5 \times 10^{-2}$	1.8
<b>5</b>	32.2	$3.4 \times 10^{-2}$	$2.5 \times 10^{-2}$	1.4
<b>Mean</b>	<b>27.8</b>	<b><math>3.9 \times 10^{-2}</math></b>	<b><math>2.1 \times 10^{-2}</math></b>	<b>1.9</b>
<b>SD</b>	<b>3.122819</b>			<b>0.51672</b>

b)

Sample #	Mass (mg)	$\sigma$ x/x (mV)	Mean $\sigma$ x/x (mV)	$\sigma$ x/x (cm2)	$\sigma$ y/y (mV)	Mean $\sigma$ y/y (mV)	$\sigma$ y/y (cm2)	Ratio ( $\sigma$ x/x)/( $\sigma$ y/y)
1	39	2.1	2.175	0.082816797	1.2	1.22	0.014571709	5.683396299
		2.25			1.24			
2	35	2.2	2.18	0.083448566	1.52	1.43	0.022486589	3.711037146
		2.16			1.54			
3	31	2.04	2.03	0.066014018	1.4	1.43	0.022486589	2.935706175
		2.02			1.46			
4	47	2.76	2.77	0.185940414	1.64	1.66	0.034919241	5.32486985
		2.78			1.68			
5	41	2.46	2.48	0.128319143	1.76	1.75	0.041094004	3.122575813
		2.5			1.74			
6	44	2.64	2.64	0.158277669	1.82	1.82	0.046476114	3.40557017
		2.64			1.82			
7	38	2.2	2.21	0.087315244	1.54	1.53	0.027346428	3.192930461
		2.22			1.52			
8	40	2.14	2.14	0.078494423	1.4	1.4	0.021176464	3.706682221
		2.14			1.4			
9	42	2.48	2.47	0.126591659	1.68	1.69	0.036888134	3.431771846
		2.46			1.7			
10	37	2.4	2.27	0.095447018	1.46	1.46	0.023862996	3.999791857
		2.14			1.46			
11	28	1.6	1.61	0.031827314	1.08	1.09	0.010944779	2.907990621
		1.62			1.1			
12	36	2.02	2.01	0.063918415	1.42	1.41	0.02160596	2.958369563
		2			1.4			
13	35	1.98	1.99	0.061874384	1.48	1.47	0.024336958	2.542404177
		2			1.46			
14	38	2.34	2.34	0.105625487	1.68	1.66	0.034919241	3.024850578
		2.34			1.64			
15	35	2.14	2.13	0.077291277	1.46	1.46	0.023862996	3.238959465
		2.12			1.46			
16	40	2.5	2.52	0.135397066	1.82	1.83	0.047288574	2.863208904
		2.54			1.84			
17	30	1.94	1.96	0.058903405	1.28	1.29	0.01690022	3.485363216
		1.98			1.3			
18	37	2.22	2.21	0.087315244	1.3	1.32	0.017988182	4.854033973
		2.2			1.34			
19	31	1.82	1.87	0.050651237	1.34	1.36	0.019528478	2.593711437
		1.92			1.38			
20	40	2.42	2.41	0.116574339	1.72	1.72	0.038945517	2.993267213
		2.4			1.72			
Mean	37.2							3.498824549
SD	4.7969							0.86374728

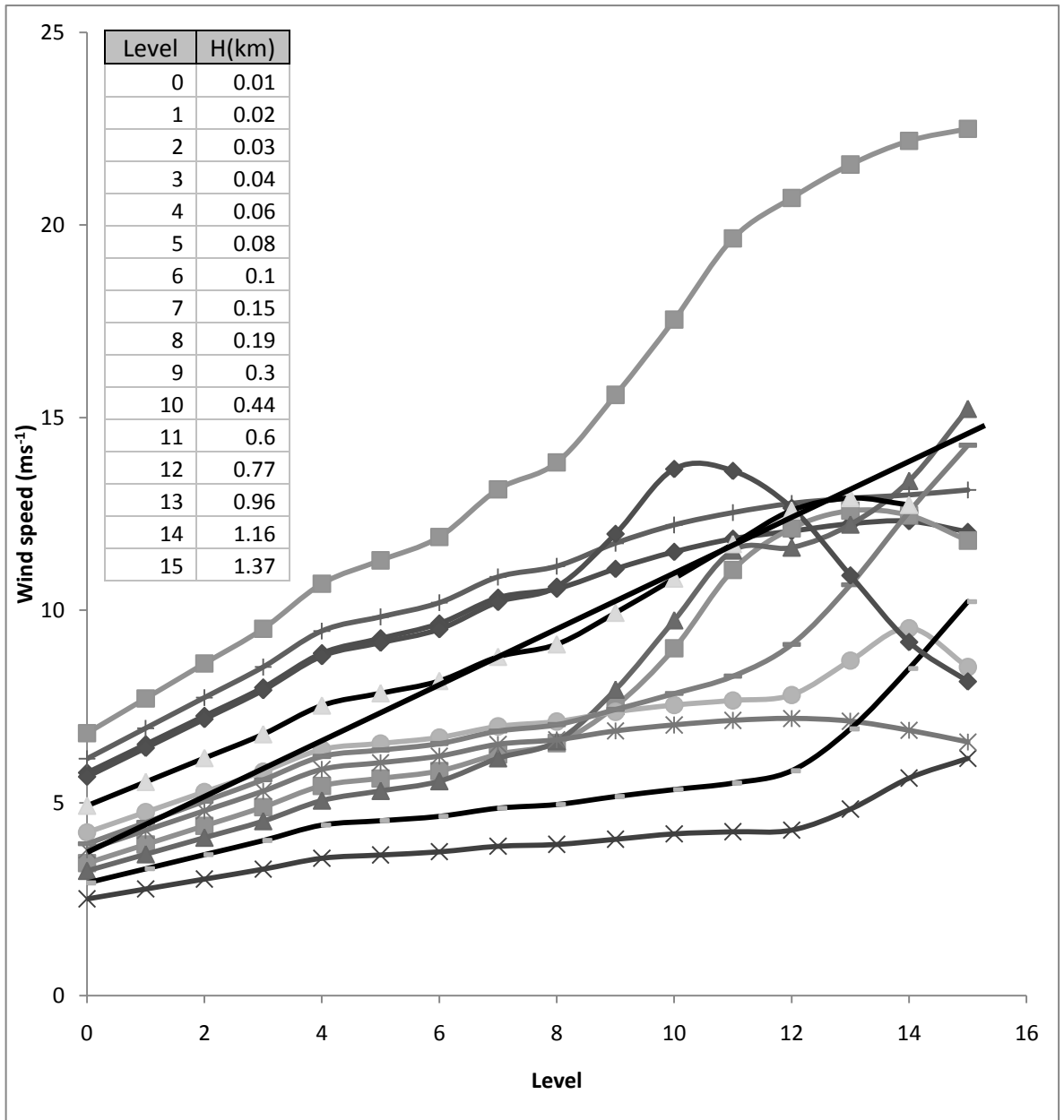
**Appendix 4. a)** Box and whisker plot comparison of VLR detections of *H. axyridis* & *C. septempunctata* from 3 sites; Chilbolton, Hampshire, (A), Malvern, Worcestershire, (B) & Rothamsted research centre, Hertfordshire(C) (locations shown in **b)**([www.google.com/maps](http://www.google.com/maps))).



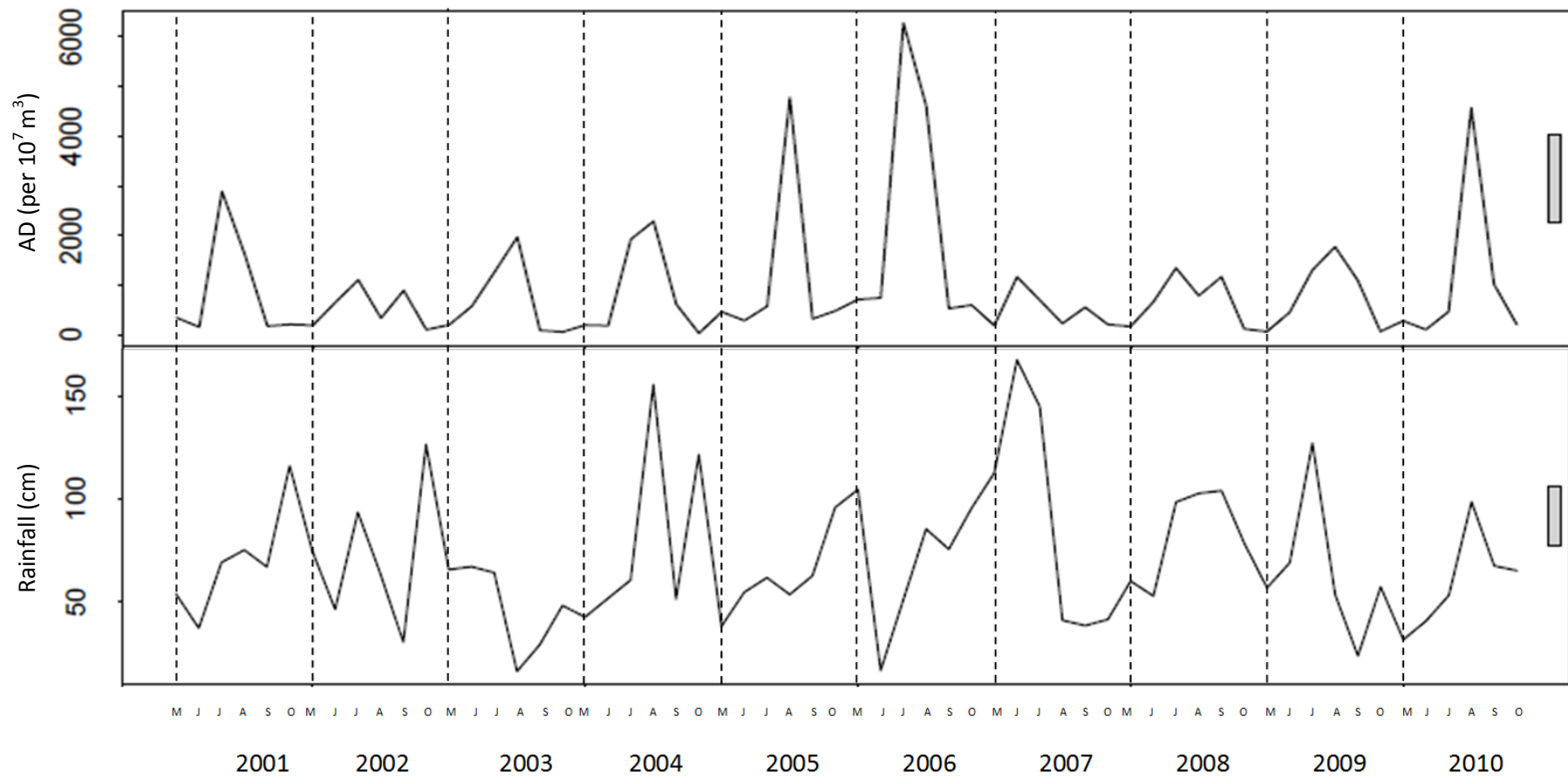
**Appendix 5.** All aphid species recorded by the Rothamsted Insect Survey (RIS) including the percentage of total aphid species caught by suction traps at Rothamsted research centre, Hertfordshire between 2000-2010, and some known prey preferences of *C. septempunctata* and *H. axyridis* in the U.K. (Hodek & Honek, 1996)

Aphid species recoded by RIS	<i>Harmonia axyridis</i> prey	<i>Coccinella septempunctata</i> prey	% abundance per species of total aphids caught 2000-2009
<i>A. pisum</i>	+	+	3.69
<i>A. fabae</i> gp.		+	4.90
<i>A. solani</i>			0.21
<i>B. helichrysi</i>			11.0
<i>B. brassicae</i>	+	+	2.20
<i>C. aegopodii</i>			2.99
<i>D. platanoidis</i>			25.37
<i>E. abietinum</i>			1.19
<i>H. pruni</i>		+	3.30
<i>H. lactucae</i>			0.619
<i>M. euphorbiae</i>			0.349
<i>M. dirhodum</i>			2.49
<i>M. ascalonicus</i>			0.20
<i>M. persicae</i>	+	+	1.71
<i>N. ribisnigri</i>			0.055
<i>P. humuli</i>			2.80
<i>R. insertum</i>			4.50
<i>R. maidis</i>			0.03
<i>R. padi</i>		+	13.97
<i>S. avenae</i>			16.71
<i>S. fragariae</i>			1.78

**Appendix 6.** Relationship between wind speeds at 15 different altitudes (see table) estimated by the Met office UK Unified Model at Rothamsted research centre, Harpenden. Data taken from 12 days (1<sup>st</sup>, 15<sup>th</sup> of each month May – Oct in 2006). Linear regression for average wind speed of all days against altitude (level) showed significant positive relationship ( $R^2$  0.631,  $n = 12$ ,  $p = 0.012$ ).



**Appendix 7.** Comparison of AD for *H. axyridis* and *C. septempunctata* (VLR) and rainfall (www.metoffice.gov.uk) for 2001-2010 (inc.) showing high rainfall peaks in the early summer of 2007 and high rainfall in June-August of 2008 which may be partly responsible for the low AD during these years (see text).





**Appendix 8.** Multiple backwards linear regression between AD and aphids from the same month (Aphids), 1 month previous (Aphids +1) and 2 months previous (Aphids +2) to test for a lagged predictive effect of aphids on AD.

Model	Explanatory Variables	R <sup>2</sup>	F	Sig.
1	Aphids, Aphids +1, Aphids +2	0.517	4.996	0.015
2	Aphids +1, Aphids +2	0.5	7.490	0.006
3	Aphids +1	0.424	11.789	0.003

Note: For Model 1,  $\Delta R^2 = -0.017$  for Model 2,  $\Delta R^2 = -0.075$  for Model 3. \*\*  $p < 0.01$