ANGLIA RUSKIN UNIVERSITY

THE SPREAD OF THE HARLEQUIN LADYBIRD *HARMONIA AXYRIDIS* (COLEOPTERA: COCCINELLIDAE) IN EUROPE AND ITS EFFECTS ON NATIVE LADYBIRDS

PETER MICHAEL JAMES BROWN

A thesis in partial fulfilment of the requirements of Anglia Ruskin University for the degree of Doctor of Philosophy

Submitted: March 2010

Acknowledgements

I dedicate this thesis to Professor Michael Majerus, one of my supervisors, who tragically died in early 2009. Mike's enthusiasm and amazing knowledge of ladybirds was, and is (through his books and papers), an inspiration. He was a wonderful mentor and friend and had a logical answer to any question about ladybirds, insects in general or pretty much any other biological topic.

Very special thanks go to my other supervisors – Dr Helen Roy, Dr Julian Doberski and Dr Alison Thomas. Helen, with Mike, gave me the initial opportunity to work on this project by employing me to co-ordinate the Harlequin Ladybird Survey. Helen has been a constant source of encouragement and inspiration from the moment that she suggested I register for the PhD. This work would not have been completed without her enthusiasm, guidance and friendship.

Julian was my undergraduate supervisor and is responsible for sparking my interest in beetles. His advice and questioning from the point of view of a non-ladybird ecologist was extremely useful. Alison has been a wonderful source of encouragement and guidance, particularly during the final year of this project. She also helped to broaden the scope of the thesis.

I would also like to extend special thanks for support and friendship to Dr Remy Ware (University of Cambridge), who runs the UK Ladybird Survey with Helen and myself, Dr David Roy (NERC CEH) who has provided a lot of help to the UK Ladybird Survey, and Mr Robert Frost, an amazing volunteer ladybird recorder.

There are many others to thank:

Anglia Ruskin University, Natural Environment Research Council Centre for Ecology & Hydrology, National Biodiversity Network Trust, Department for Environment Food and Rural Affairs, University of Cambridge, University of Hull, Joint Nature Conservation Committee and Rothamsted Research, for funding, support and/or access to data.

Cathleen Thomas and Lori Lawson-Handley (University of Hull), especially for the opportunity to study *H. axyridis* in Japan; Philip Pugh, Dawn Hawkins and Mike Cole (Anglia Ruskin University); Mark Hill, Björn Beckmann, Steph Ames, Jim Bacon, Peter Rothery, Chris Preston, Henry Arnold and Richard Comont (NERC CEH); Trevor James, Jim Munford and Mandy Henshall (National Biodiversity Network); Laura-Jane Michie, Emma Rhule, Ian Wright and Francis Rowland (University of Cambridge); Jason Baverstock, Richard Harrington, Sue Parker, Judith Pell and Patricia Wells (Rothamsted Research); Tim Sparks (Poznań University of Life Sciences).

Finally, this work would certainly not have been possible without the patience, support and love from my partner Clare, our children Cameron and Jodie, my mother, father and Marleen. I sincerely thank them all.

Specific acknowledgements of assistance with work included in this thesis

I acknowledge and thank all of the following for their assistance with work included within this thesis:

Collaborators in Europe, for sharing their data and insights on *H. axyridis* from their countries (Chapter 2) - Tim Adriaens, Horst Bathon, Piotr Ceryngier, Jan Cuppen, Arturo Goldarazena, Tord Hägg, Marc Kenis, Bernhard Klausnitzer, Ivo Kovář, Antoon Loomans, Viktor Marko, Olda Nedved, Jan Pedersen, Wolfgang Rabitsch, Arnstein Staverloekk, Vincent Ternois, Ragheb Thalji and Ilia Zakharov.

Gavin Hatt, Ken Dolbear and Remy Ware for permission to use their images (Chapter 2).

The thousands of volunteer recorders and naturalists across Europe who have provided records (Chapters 2 to 4) and especially Paul Mabbott , Roger Hawkins and Bill Phillips.

Remy Ware, Mike Majerus, Emma Rhule, Laura-Jane Michie and Helen Roy for identifying some of the *H. axyridis* samples (Chapters 3 and 4); throughout the project I verified photographs submitted to the Harlequin Ladybird Survey. In the earlier years I also identified most of the specimens received by post, but in later years this was mostly done by the others at Cambridge. In later years Helen verified *H. axyridis* records that were received by the UK Ladybird Survey.

David Roy for assistance with mapping (Chapters 2 and 3) and permission to use CEH Land Cover Map 2000 data (Chapter 4).

Peter Rothery (Chapter 3) and Tim Sparks (Chapter 5) for advice and help with statistical analyses.

Robert Frost for carrying out the fieldwork at sites E to G (Chapter 5).

Richard Harrington and Sue Parker (Rothamsted Research) for permission to use aphid data from the Rothamsted Research Insect Survey (Chapter 5).

Alison Thomas, Styliana Philippou, Heather Kitson and Angela Wheatley for carrying out some of the DNA extractions, all of the PCR reactions, and assisting with some of the feeding trials (Chapter 6).

Remy Ware, Emma Rhule and Laura-Jane Michie for rearing ladybirds for the PCR feeding trials, which I carried out at the University of Cambridge (Chapter 6).

ANGLIA RUSKIN UNIVERSITY

ABSTRACT

FACULTY OF SCIENCE AND TECHNOLOGY

DOCTOR OF PHILOSOPHY

THE SPREAD OF THE HARLEQUIN LADYBIRD *HARMONIA AXYRIDIS* (COLEOPTERA: COCCINELLIDAE) IN EUROPE AND ITS EFFECTS ON NATIVE LADYBIRDS

By PETER MICHAEL JAMES BROWN
March 2010

Native to Asia, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is an invasive non-native ladybird in Europe and North America, where it was widely introduced as a biological control agent of aphids and coccids. There were three main objectives of this study: firstly, to investigate the spread and distribution of *H. axyridis* in Europe from the start of the invasion process; secondly, to study its ecology in Great Britain; and thirdly, to examine its effects on native ladybirds in Britain.

In Europe, collaborations with a network of scientists allowed the collation of *H. axyridis* occurrence data from across the continent. In Great Britain, a web-based public survey (www.harlequin-survey.org) was used, leading to the receipt of over 10000 verified records of *H. axyridis* between 2004 and 2008. National land cover data enabled the habitats used by *H. axyridis* to be identified across Great Britain. These datasets were analysed in order to study the spread and ecology of *H. axyridis* in Europe and Britain. Fieldwork over a three year period (2006 to 2008) was carried out to examine changes in ladybird species assemblages during the invasion phase of *H. axyridis* in eastern England. Laboratory work was conducted to detect intraguild predation by *H. axyridis*, through PCR analyses of gut contents of field-collected ladybirds.

In Europe, *H. axyridis* has spread since 2001 at the rate of approximately 200km yr^{-1}. It has become established in at least 23 European countries. The established range extends from Norway in the north to southern France in the south, and from Ukraine in the east to Great Britain in the west. In the first five years of establishment *H. axyridis* spread north through Britain at the rate of 105km yr^{-1} and west at the rate of 145km yr^{-1}. Evidence of the production of two generations per year was found, giving *H. axyridis* an advantage over most native ladybirds in Britain. Although *H. axyridis* was very common in urban habitats, it increasingly used semi-natural habitats. In addition, whilst the species was most common on deciduous trees, it was increasingly found on herbaceous plants. Aceraceae, Rosaceae and Malvaceae were the dominant plant families used by *H. axyridis*, especially for breeding. In eastern England *H. axyridis* increased from 0.1% to 40% of total ladybirds in three years, whilst native aphidophagous ladybirds declined from 84% to 41% of total ladybirds in the same period. Three species in particular experienced declines: *Adalia 2-punctata*, *Coccinella 7-punctata* and *Propylea 14-punctata*. *Harmonia axyridis* was the most abundant species by the end of the study. Detection of intraguild predation by one coccinellid on another, in the field in Europe, was shown for the first time using PCR techniques: *A. 2-punctata* DNA was detected in the gut of one of 112 field-collected *H. axyridis*.

Harmonia axyridis has spread very quickly since 2001 and has become one of the most widely distributed coccinellids in Europe. Populations of native aphidophagous ladybirds were negatively affected by the arrival of *H. axyridis*, partly through intraguild predation.

Table of Contents

List of Figures

List of Tables

Notation

dNTP	Deoxyribonucleotide triphosphate
DTT	Dithiothreitol
EGP	Extraguild predation
GC-MS	Gas chromatography mass spectrometry
IGP	Intraguild predation
MAb	Monoclonal antibody
PCR	Polymerase Chain Reaction
TBE	Tris Borate EDTA (ethylenediaminetetraacetic acid)

List of Appendices

1. Introduction

1.1 Non-native insects in Great Britain and continental Europe

1.1.1 Detection and recording of non-native species

The negative impacts of non-native species[1] have been cited as one of the biggest risks to global biodiversity (Glowka et al., 1994). While this may be an over-statement in terms of Great Britain (Pearman & Walker, 2009), a small proportion of non-native species become invasive[2] and have major effects (Williamson, 1996). There has been substantial work in Europe in recent years to assess the extent of non-native species presence and spread, and start to assess their impacts. Two major European Community projects were set up to tackle these issues: Delivering Alien Invasive Species Inventories for Europe (DAISIE) (www.europe-aliens.org) and Assessing Large Scale Risks for Biodiversity with Tested Methods (ALARM) (www.alarmproject.net). The DAISIE project identified 1296 alien terrestrial invertebrate species in Europe, of which over 90% were insects (Roques et al., 2009). A further 964 species of European origin were introduced from one region of Europe to another. Other invertebrate phyla, generally being less well studied than insects, were probably under-represented in the DAISIE figures (Roques et al., 2009). Of 100 species (of any taxa) identified by the DAISIE project as being amongst the most harmful non-native species in Europe, 13 were insects and four were beetles (www.europe-aliens.org). After the assessment stage, the next step is to minimise further spread of high risk species, and attempt to manage their effects.

---

[1] Non-native species: refers to a species introduced (i.e. by human action) outside its natural past or present distribution (GB NNSS - www.nonnativespecies.org) and is the equivalent of 'alien species' as used by the Convention on Biological Diversity (www.cbd.int).

[2] Invasive non-native species: refers to any non-native animal or plant that has the ability to spread causing damage to the environment, the economy, our health and the way we live (GB NNSS - www.nonnativespecies.org).

Scientists and policy makers in Great Britain played a significant role in the European initiatives, and Britain has an increasingly strong infrastructure for dealing with non-native issues at a national level. Funded by government sources in England, Scotland and Wales, the GB Non-native Species Secretariat (GB NNSS) (www.nonnativespecies.org) and Great Britain Non-native Species Information Portal (GBNNSIP) are important initiatives. The latter is led by the Natural Environment Research Council Centre for Ecology & Hydrology (NERC CEH) and aims to establish a web portal for the detection and reporting of non-native species, to allow effective decision making (GBNNSIP, 2010). In a recent review in England, a total of 2722 non-native species were identified (Hill et al., 2005); however, an updated version of this report for Great Britain has added a further 1068 species to date (GBNNSIP, 2010), with insects representing 866 (23%) of the total species. Thus in a European context, by comparison with the figures from the DAISIE project (above), Great Britain appears to have a high number of non-native species relative to its land area. In Europe, for their size, islands in general were found to have proportionately more non-native species than continental countries (Roques et al., 2009). In addition, the high volume of biological recording in Great Britain (Chapter 3) may have led to a higher detection rate of non-native species.

1.1.2 Representation of insect taxa

Within the insects, beetles (order Coleoptera) represent the largest group in terms of both native and non-native fauna; the number of non-native beetle species reported was: Europe: 378 (Roques et al., 2009); Great Britain: 261 (GBNNSIP, 2010). Some insect orders (notably Hemiptera) were over-represented in the non-native fauna in Europe, i.e. the proportion of all non-native insect species that were in Hemiptera was greater than the proportion of all native insect species that were in Hemiptera. Some insect orders (e.g. Diptera) were under-represented, whilst Coleoptera was evenly represented (Table 1.1).

Table 1.1. Percentage of native and non-native insect species in Europe by order (selected orders only) (from Roques et al., 2009).

Insect order	% of total **native** insect species in Europe	% of total **non-native** insect species in Europe
Coleoptera	30	29
Hymenoptera	25	15
Diptera	21	7
Lepidoptera	10	10
Hemiptera	8	26
Thysanoptera	0.6	4
Psocoptera	0.3	3
Blattodea	0.2	2

Similarly, 30% of Great Britain's non-native insects were beetles (Figure 1.1). However, this pattern is not entirely consistent throughout Europe: in Austria and Switzerland non-native Coleoptera were over-represented (30% native vs. 41% non-native) (Kenis et al., 2007).

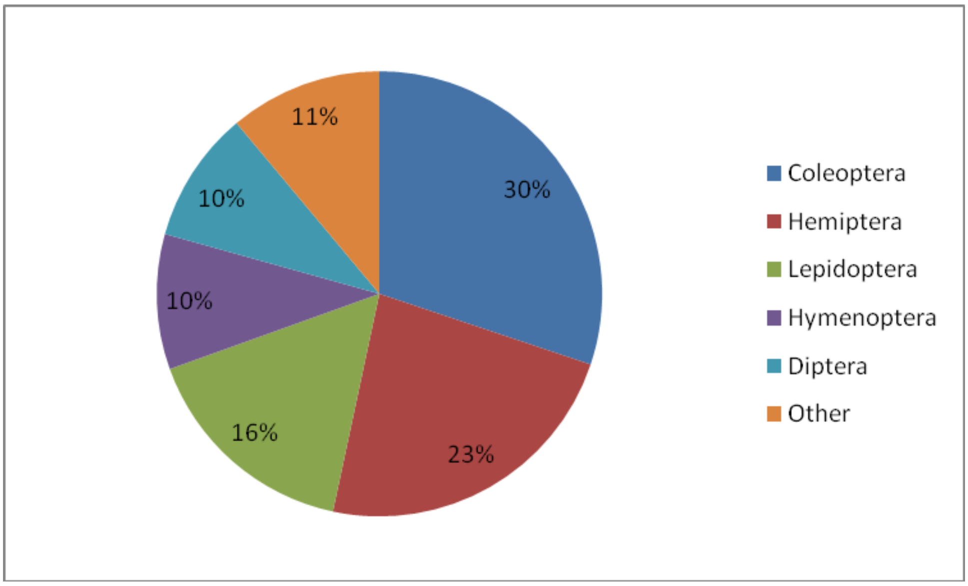


Figure 1.1. Percentages of non-native insect species in Great Britain, by order (n = 866). Source: GBNNSIP, 2010.

1.1.3 Feeding niches

Whilst 48% of non-native insects in Europe were phytophagous, 22% were predators or parasitoids (Roques et al., 2009), which seemed to travel or establish less successfully (Kenis et al., 2007). Sap feeders (mainly Sternorrhyncha) and detritivores were the dominant feeding niches of Austrian and Swiss non-native insects (Kenis et al., 2007). However, these analyses were likely subject to recording biases for two main reasons. Firstly, species causing serious negative impacts on people (e.g. crop pests) were likely to be over-recorded. Secondly, taxonomic expertise and interest was generally not even amongst the different insect groups; a point illustrated for Great Britain by the list of national recording schemes in place (http://www.brc.ac.uk/recording_schemes.asp), which shows wide taxonomic coverage but with many families nevertheless unrepresented. Some non-native phytophagous beetles are serious pests of agriculture or forestry – e.g. western corn rootworm *Diabrotica virgifera* LeConte (Coleoptera: Chrysomelidae) (Miller et al., 2005), Colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) (http://www.eppo.org/QUARANTINE/insects/Leptinotarsa_decemlineata/LPTNDE_ds.pdf) and Asian long-horn *Anoplophora glabripennis* Motschulsky (Coleoptera: Cerambycidae) (Herard et al., 2005).

The effects of non-native predators may also be significant. For example, the Argentine ant *Linepithema humile* (Mayr) (Hymenoptera: Formicidae) displaced native ants wherever it was introduced, indirectly affecting other animals and plant communities; e.g. in South Africa, seed dispersal of myrmecochorous shrubs was affected when native ants were reduced (Suarez et al., 2001). Through an association with humans, *L. humile* has spread widely around the world and become established on six continents (Suarez et al., 2001).

1.1.4 Ecological effects

There is evidence that most non-native insects do not cause negative ecological effects. This particularly applies to those species deliberately introduced, e.g. biological control agents: in continental U.S., under 1% of intentionally introduced biological control insect species had negative population level non-target effects[3], whilst 3-5% may have caused lesser effects (van Lenteren et al., 2006). Further, despite the enormous scale of non-native arthropod releases in biological control programmes, there is no unequivocal evidence that any have directly caused species extinctions (De Clercq, 2002). Thirty-six terrestrial invertebrates were risk assessed in a review of non-native animal species with the potential to have negative effects in England. Two thirds of these were assessed as having low environmental risk, with 19% medium risk and 14% high risk (Parrott et al., 2009). This study was unlikely to be fully representative of non-native terrestrial invertebrates as a whole: it was necessarily targeted towards species likely to be invasive. Even amongst these, most had low predicted environmental impacts.

However, detailed studies of the ecological effects of non-native species have historically been limited, and many of them focused on North America (Kenis et al., 2009). Competitive displacement of native bees (and spread of disease) by non-native bees was identified as a global problem (Goulson, 2003). Beyond Europe, examples of non-native predatory insects having negative impacts include social wasps in New Zealand, the crazy ant *Anoplolepis gracilipes* (Jerdon) (Hymenoptera: Formicidae) on

---

[3] Non-target effect: in biological control, refers to an effect on a species other than the one targeted for control.

Christmas Island and blow flies in America (Kenis et al., 2009). Kenis et al. (2009) identified ten non-native beetle species for which there were studies on their ecological effects; two weevils, four carabids and four coccinellids. In some cases no negative impacts were found, e.g. the European carabid *Pterostichus melanarius* Illiger (Coleoptera: Carabidae), in North America (Niemela et al., 1997). However, negative effects caused by coccinellids were more common (see below). Non-native beetles may have ecological impacts as disease vectors; for example the European bark beetle *Scolytus multistriatus* (Marsham) (Coleoptera: Curculionidae) as a vector of Dutch elm disease (Kenis et al., 2007). Whilst there is limited evidence of deliberate insect introductions causing serious ecological problems (van Lenteren et al., 2003), negative effects may have occurred that remain undetected due to lack of investigation (De Clercq, 2002), or lack of understanding of the ecosystem; indeed, this is very likely the case.

1.1.5 Mechanisms of spread

Non-native species arrive through three broad mechanisms: natural spread from a neighbouring region (where the species is non-native); importation with a commodity; and entry with transport (Hulme et al., 2008). The latter two mechanisms can be accidental or deliberate. In Europe these mechanisms were studied by the DAISIE project and reveal that about 90% of non-native terrestrial invertebrates arrived through transport (inadvertent or with a commodity), with about 9% intentionally released and only 1% arriving by dispersal (www.europe-aliens.org). Horticulture, ornamental use and stored products were the dominant pathways for the importation of non-native terrestrial invertebrates to Europe (Roques et al., 2009). Stowaways accounted for 15% of insect introductions in Europe (Roques et al., 2009). The association of non-native species with human habitation and disturbed landscapes is common and applies to coccinellids (Roy & Migeon, in press). In Nordic countries, at least 34 non-native beetles became established on compost heaps in the 20th century (Odegaard & Tommeras, 2000). In Europe, 34% of non-native insects were associated with buildings (Roques et al., 2009); whilst in Austria and Switzerland the figure was nearly 40% (Kenis et al., 2007). However, these figures may partly reflect a bias in recording towards urban areas. Importantly, 20% of non-native insect species in Europe were associated with natural environments, mostly in forests (Roques et al., 2009). The dominant areas of origin of non-native arthropods in Europe were Asia (29% of total) and North America (20%) (Roques et al., 2009), although this was likely biased by differing trade and transport between the continents.

1.1.6 Non-native coccinellids

Coccinellids have received substantial attention in the literature because of their beneficial role as feeders of pest insects, particularly aphids and coccids (scale insects). As such, coccinellids have been used as biological control agents around the world (Majerus, 1994a). Indeed, modern biological control was effectively initiated by a very successful control programme using a coccinellid: the well documented case of the Australian vedalia ladybird *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae), introduced in 1887 to control cottony cushion scale in California (Obrycki & Kring, 1998). This success led to over 40 other coccinellid species being introduced to North America, with many releases also in Europe (Roy & Migeon, in press). Europe has 11 established non-native coccinellids, all of which were introduced for biological control, mostly from 1975 to 1999 (Roy & Migeon, in press), thus the mechanism of arrival of non-native coccinellids is rather atypical (see above). *Rodolia cardinalis* was the first of the non-native coccinellids to be used in Europe, initially released in Portugal and Italy between 1888 and 1901, and later in at least nine other European countries, including Great Britain (Roy & Migeon, in press). However, coccinellids have not usually been successful in fully controlling the target pest species, although more successes have been achieved with coccids than aphids (Dixon, 2000). This lack of effectiveness is partly explained by asynchrony between aphid and coccinellid occurrence (Dixon, 2000).

Rhyzobius lophanthae (Blaisdell) (Coleoptera: Coccinellidae) (native to Australasia) was used throughout the 20th century to control armoured scale insects and was recently reported as established in London (Roy & Migeon, in press). *Rhyzobius forestieri* (Mulsant) (Coleoptera: Coccinellidae), *Nephus reunioni* Fürsch (Coleoptera: Coccinellidae), *Chilocorus nigrita* (Fabricius) (Coleoptera: Coccinellidae) and *Chilocorus kuwanae* Silvestri (Coleoptera: Coccinellidae) were all introduced as biological control agents of coccids in Europe (Roy & Migeon, in press), but none of these are known to be established in Great Britain. *Hippodamia convergens* (Guérin-Méneville) (Coleoptera: Coccinellidae) and *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) are the two main species introduced in Europe for aphid control (Roy & Migeon, in press), but neither was deliberately introduced in Britain. The other species introduced, but not established in Britain, is *Scymnus impexus* Mulsant (Coleoptera: Coccinellidae), a coccinellid native to parts of Europe, introduced to control adelgids (http://archives.eppo.org/EPPOStandards/biocontrol_web/classical/coleop_class.htm). This small species probably did not establish because of its specialist niche and the fact that it was apparently released only once (http://archives.eppo.org/EPPOStandards/biocontrol_web/classical/coleop_class.htm). Similarly, *S. impexus* did not persist for long after introduction in Canada (Majka & McCorquodale, 2006).

Great Britain has four established non-native coccinellids: *H. axyridis* (common and widespread); *R. lophanthae* (rare); *Henosepilachna argus* (Geoffroy) (Coleoptera: Coccinellidae) (localised in south-east England) (see Chapter 7); and *Scymnus suturalis* Thunberg (Coleoptera: Coccinellidae) (common and widespread) (UK Ladybird Survey data). The latter two are native species elsewhere in Europe. *Henosepilachna argus* is a herbivore that feeds on, and seems restricted to, white bryony *Bryonia dioica* Jacq. (Cucurbitaceae). This large coccinellid was first recorded in Surrey (south-east England) in 1997 (Menzies & Spooner, 2000) and whilst locally common there (P. Brown, personal observation) and in London, there is very little evidence of further spread. *Scymnus suturalis* is a very widespread species in Great Britain that can be common on pine trees (Majerus, 1994a). It is a very small species of conifers and has had no recorded impacts on native biodiversity. It has been part of the British fauna for at least 100 years (UK Ladybird Survey data).

Occasional records of other non-native coccinellids have been received by the UK Ladybird Survey, often associated with imported produce. The most commonly recorded was *Cheilomenes lunata* (Fabricius) (Coleoptera: Coccinellidae), generally transported with grapes from Africa (Mabbott, 2005). *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) (an Australian species) has also been recorded, and in 2009 the steelblue ladybird *Halmus chalybeus* Boisduval (Coleoptera: Coccinellidae) was reported from London (Brown et al., 2010). This Australian species has been used in biological control programmes in New Zealand and Hawaii (Sheppard et al., 2004). There is no evidence of establishment of any of these species in Great Britain.

The best documented examples of non-native coccinellids affecting native species are from North America. *Coccinella 7-punctata* L. (Coleoptera: Coccinellidae) was widely introduced in the USA between 1957 and 1990 (Elliott et al., 1996) and has been implicated in the displacement of native species (Evans, 2004). For example, in Manitoba, Canada, four native species - *Coccinella transversoguttata richardsonii* Brown (Coleoptera: Coccinellidae), *Hippodamia parenthesis* (Say) (Coleoptera: Coccinellidae), *Coccinella trifasciata perplexa* Mulsant (Coleoptera: Coccinellidae) and *H. convergens* - declined after invasion by *C. 7-punctata*. In arable crops in South Dakota, USA, *C. transversoguttata* and *A. 2-punctata* declined to under 5% of their original populations after *C. 7-punctata* arrived (Elliott et al., 1996). A 31-year study in potato crops in Maine, USA, found that following the arrival of *C. 7-punctata*, *P. 14-punctata* and *H. axyridis*, significant declines in the native species *C. transversoguttata* and *Hippodamia 13-punctata* (L.) (Coleoptera: Coccinellidae) were observed (Alyokhin & Sewell, 2004). Aphids were also substantially reduced after establishment by *H. axyridis* (Alyokhin & Sewell, 2004). The latter study found an overall increase in coccinellid diversity after the non-native species arrived. However, this was accounted for simply by the addition of the non-native species and not by native species thriving.

The general pattern observed in the studies from North America is that the invading coccinellid(s) reduced the abundance of previously dominant native coccinellids, which after invasion, tended to survive at low population densities and/or were displaced into other habitats. Evans (2000) argued that habitat suppression could be the main effect of invasive coccinellids on natives, i.e. the native species would retreat from arable habitats into ancestral habitats, where they may have an advantage over the new arrivals

(Evans, 2000). The generalist nature and ecological flexibility of *C. 7-punctata* (e.g. in terms of wide variations in the body size of *C. 7-punctata* individuals, implying good adaptation to differing resource availability) helped increase its dominance in agricultural systems in North America (Evans, 2000), at least until *H. axyridis* arrived. The impact of *H. axyridis* on native coccinellids in Europe is one of the main topics of this thesis.

1.2 Introduction to Harmonia axyridis

1.2.1 Geographic range

Variously known as the multicolored Asian lady beetle, Halloween beetle and harlequin ladybird, *Harmonia axyridis* is native to China, Japan, Korea, Mongolia, Kazakhstan and Siberia (Dobzhansky, 1933, Kuznetsov, 1997), although its entire native range, particularly in parts of the former Soviet Union, has not been clearly recorded. Although usually stated in the literature to be semi-arboreal (Hodek, 1973), it occupies many habitats, and in parts of both its native and introduced ranges has been recorded in meadows, heathlands and riparian zones (Adriaens et al., 2008), reed beds (Ware et al., 2005) and crop systems (Colunga-Garcia & Gage, 1998, Jansen & Hautier, 2008). In Japan *H. axyridis* is one of the most common species (Sakurai et al., 1992) and can dominate the coccinellid fauna: for example, in urban habitats in spring *H. axyridis* formed 80% (n = 763) of all adult coccinellids on trees, shrubs and hedges (P. Brown & C. Thomas, unpublished data).

1.2.2 Introduction and subsequent spread

Harmonia axyridis has a long history of use as a classical biological control[4] agent of aphids and coccids in North America, where it was first introduced in 1916 (Gordon, 1985). The species has also been used in augmentative biological control[5] (Trouve et al., 1997). It has been widely used for pest control in crops as diverse as pecans (Tedders & Schaefer, 1994) and red pines (McClure, 1987). As a biological control agent *H. axyridis* has incidentally succeeded in controlling pest aphid species on other crops, including apples (Brown & Miller, 1998) and citrus fruits (Michaud, 2002). *Harmonia axyridis* has also had a pest-controlling role in other crop systems, including

---

[4] Classical biological control: the release of exotic natural enemies (with the aim of establishment) for the long term control of exotic pests (van Lenteren, et al., 2003; Babendreier, 2007).

[5] Augmentative biological control: the release of natural enemies whose affects are inundative (control from periodically released agents only, without necessarily the aim of establishment) and may be inoculative (control also from progeny of released agents) (van Lenteren, et al., 2003; Babendreier, 2007).

soybean, maize, alfalfa, tobacco, winter wheat and cotton (reviewed in Koch, 2003 and Koch & Galvan, 2008). Despite releases in fourteen US states between 1964 and 1982 (Gordon, 1985) *H. axyridis* was not reported as established in the country until 1988 (Chapin & Brou, 1991). There is uncertainty over the source of the established USA population, which may originate from a single intentional release or accidental introduction (Krafsur et al., 1997). It was suggested that the source could have been accidental seaport introductions (Day et al., 1994). After establishment in North America, *H. axyridis* spread at a rate of 442km yr^{-1} (McCorquodale, 1998), and became the dominant coccinellid in many areas (Colunga-Garcia & Gage, 1998, Tedders & Schaefer, 1994, Smith et al., 1996). The spread rate calculated by McCorquodale (1998) may be high partly because of possible multiple introductions (i.e. *H. axyridis* did not necessarily spread from a single starting point) and other confounding variables (Koch et al., 2006). *Harmonia axyridis* became established in Canada by 1994 (Coderre et al., 1995) and quickly spread across much of the southern part of the country (Majka & McCorquodale, 2006). *Harmonia axyridis* was also introduced in Hawaii (Coderre et al., 1995).

In Europe, early introductions of *H. axyridis* occurred in the east, including to Ukraine from 1964 (for control of aphids on fruit trees) (Katsoyannos et al., 1997) and Belarus from 1968 (Sidlyarevich & Voronin, 1973). In western Europe, *H. axyridis* was first used as a biological control agent in 1982 in France and first marketed in 1995 (Coutanceau, 2006). Various companies, including Koppert (www.koppert.com), Biobest (www.biobest.be) and Biotop (www.biotop.fr), made the species commercially available (Adriaens et al., 2003). It established in the late 1990s and expanded its range rapidly, especially from 2002. Full details of the spread and distribution of *H. axyridis* in Europe and Great Britain are provided in Chapters 2 and 3.

Harmonia axyridis has recently been recorded in other regions of the world. In South America, *H. axyridis* was used as a biological control agent in Argentina in the late 1990s (Saini, 2004), and has since been found in Brazil (de Almeida & da Silva, 2002), Paraguay, Chile and Peru (Grez et al., in press). In Central America, the species was introduced in several states in Mexico and subsequently established and spread (Koch et al., 2006). In Africa, *H. axyridis* was introduced in Egypt (Ferran et al., 2000) where it became established around Cairo (S. Elnagdy, personal communication) and, although not deliberately introduced, became widely established in South Africa (Stals, 2008) and was found in Lesotho (Stals, 2009). In South Africa, the species quickly spread into six of the country's nine biomes at a rate of approximately 500km yr^{-1}, and has been recorded at altitudes of up to 2500m (Stals, 2009). *Harmonia axyridis* may well be present, but unreported, in other regions of Africa. Other areas of northern, southern and eastern Africa (including Madagascar) were assessed as climatically suitable for *H. axyridis*, as were large parts of Australia and New Zealand (Poutsma et al., 2008); thus some vulnerable ecosystems beyond the current known distribution are potentially under threat of invasion by *H. axyridis*. The species was inadvertently imported with excavation equipment to Australia (Bunbury) in 2008, but fortunately all specimens were dead (Smith, 2008).

1.2.3 Species traits and impacts

There is no doubt that *H. axyridis* is an effective aphid predator, and in reducing the need for chemical insecticides, its presence may be welcomed by some. It is little affected by new target specific pesticides (Koch, 2003). However, in Europe and elsewhere there is concern about the rapid increase in distribution and abundance of *H. axyridis*, and its impact on non-target species (Adriaens et al., 2003, Majerus et al., 2006b, Roy et al., 2006). Although most biological control companies have stopped selling *H. axyridis* in Europe, a non-flying variety is still available from Biotop (www.biotop.fr). Flightlessness is an advantage not only for effective biological control (where keeping the controlling species in the target area is key), but also for localising any non-target effects.

In a risk assessment of 31 exotic natural enemies of pest species used in biological control in Europe, *H. axyridis* had the second highest environmental risk index. This was based on its wide host range (i.e. multiple prey species), ability to establish and disperse, and direct and indirect effects on non-target species (van Lenteren et al., 2003). Further, it was concluded that there are no easy ways to mitigate or reduce the risk of *H. axyridis* and that it should not have been released in northwest Europe (van Lenteren et al., 2008).

Harmonia axyridis has the potential to contribute to biotic homogenization (McKinney & Lockwood, 1999) and to negatively impact on up to 1000 species in Great Britain (Majerus, 2007). These are primarily insects (including non-target Homoptera, alternative prey, members of aphidophagous and coccidophagous guilds) and parasites, parasitoids, pathogens and symbionts of these species (Majerus, 2007). See Chapter 7 for further discussion on this.

The traits that make *H. axyridis* an effective biological control agent include large size, high fecundity and fertility, habitat and climate adaptability, voraciousness (Babendreier, 2007) and, in some respects, wide feeding niche (Berkvens et al., 2008a). Unfortunately these are the very traits that make *H. axyridis* a very strong intraguild[6] [7] (IG) predator (Pell et al., 2008). Negative effects on other aphidophagous species are the result of asymmetric interactions (which are usually in favour of *H. axyridis*) in terms of both competition for food, and direct predation on IG prey (e.g. Michaud, 2002, Takahashi, 1989, Ware & Majerus, 2008). Aphids tend to be host specific (Dixon, 1998) and therefore most are not pests, but *H. axyridis* will readily eat non-target species of aphid (Majerus et al., 2006a). Other extraguild[8] prey is also sometimes exploited; *H. axyridis* feeds on other invertebrates as alternative foods, including Lepidoptera eggs & larvae (Harlequin Ladybird Survey data, Koch et al., 2003) and possibly chrysomelid beetle larvae (Sebolt & Landis, 2004) and syrphids (Ingels & De Clercq, 2009, Fremlin, 2008). Large size has been proposed as an indicator of invasiveness in coccinellids (Dixon & Hemptinne, 2009), but this is only one of the

---

[6] Guild: a group of organisms that use the same ecological resource in a similar way, e.g. the guild of animals that feed on aphids.

[7] Intraguild: within an ecological guild. Used in the context of intraguild predation (IGP).

[8] Extraguild: outside an ecological guild. Used in the context of extraguild predation (EGP).

traits of *H. axyridis* that provide an advantage over many other aphidophagous insects. For example, *H. axyridis* is well defended both physically (e.g. larvae possessing thick dorsal spines) (Ware & Majerus, 2008), and chemically (Sato & Dixon, 2004). Further, there is evidence that *H. axyridis* is affected less by parasitoids (Koyama & Majerus, 2008) and fungal pathogens (Roy et al., 2008b) than are native coccinellids. The species can also survive and reproduce on non-arthropod diets, including pollen (Berkvens et al., 2008a).

In the USA, *H. axyridis* has been declared a potential pest of fruit production and processing, as the species sometimes feeds on the juices of ripe fruits, such as grapes, apples, peaches and plums, especially in autumn (Kovach, 2004). Feeding on grapes has been a particular focus of attention, as if harvested with *H. axyridis*, about one beetle per kg of fruit is enough to taint the wine (Linder et al., 2009). This has caused concern in North America (Hutchison & Galvan, 2009), Europe (Linder et al., 2009) and South Africa (Stals, 2008).

Harmonia axyridis may become seriously pestilent to native fauna in Europe (Majerus et al., 2006b). This pest status may extend directly to humans, as *H. axyridis* tends to form very large overwintering aggregations of thousands of individuals, often on or in buildings (Adriaens et al., 2003, Kidd et al., 1995). Such aggregations have been observed in England since 2006 (Brown & Roy, 2007). Damage to furnishings caused by the reflex blood of *H. axyridis* has been reported (Harlequin Ladybird Survey data), and rarely but more seriously, the beetles cause an allergic reaction (Yarbrough et al., 1999, Davis et al., 2006).

Controlling *H. axyridis* in Europe in order to prevent further spread and reduce non-target impacts on biodiversity would be extremely difficult (Kenis et al., 2008) and even if practical, very costly. However, investigations in Europe into various possible control mechanisms using natural enemies have taken place; for example a native parasitoid (Koyama & Majerus, 2008), a fungal pathogen (Roy et al., 2008b) and an ectoparasitic mite (Rhule et al., 2009). The sexually-transmitted mite *Coccipolipus hippodamiae* (McDaniel & Moril) (Acaridae: Podopolipidae), that naturally occurs in Europe, currently represents the best hope. Infection by *C. hippodamiae* makes the females of *H. axyridis* sterile (Rhule et al., 2009). However, a very thorough risk assessment would be needed before any deliberate releases of *C. hippodamiae* are considered, as the mite can infect other coccinellids which have over-lapping generations.

1.2.4 Public surveys

The arrival of *H. axyridis* in Great Britain was used as a model system in terms of public participation in an environmental project or "citizen science". This is currently an important issue in science: for example, in Britain the Open Air Laboratories (OPAL) network (www.opalexplorenature.org), a very large-scale (£11.75 million) project was recently launched with the aim "to create and inspire a new generation of nature-lovers by getting people to explore, study, enjoy and protect their local environment" (www.opalexplorenature.org/?q=aboutOPAL). With the Harlequin Ladybird Survey, the use of a website and online recording was a highly effective way of engaging members of the public, and has been replicated in other countries (e.g. Denmark (http://www.dpil.dk/dpil2005/harlekin/harlekinmariehone.htm), Hungary (www.coleoptera.hu/harlekin), Ireland (www.habitas.org.uk/ladybirds) and Poland (www.cbe-pan.pl)). *Harmonia axyridis* was a very suitable species for public recording because of its high visibility (particularly when aggregating in buildings), relative ease of identification, and, compared to most other insects, the prominence and appeal of ladybirds.

Data quality issues are discussed later (Chapter 3), but were overcome by the submission of photographs or specimens. A high level of media interest in Great Britain and elsewhere (e.g. Serbia, Denmark and Poland) was the key to encouraging public participation. *Harmonia axyridis* has been used as a flagship invasive species and has encouraged interest in other, less prominent species. For example, in Britain the Harlequin Ladybird Survey model was recently used to launch a public outreach project targeting six invasive non-native animal and plant species (www.nonnativespecies.org/recording).

2. *Harmonia axyridis* in Europe: spread and distribution of a non-native coccinellid

2.1 Introduction and Method

The aim of this chapter is to present a thorough examination of the history, spread and distribution of *H. axyridis* in Europe. The data presented are an amalgamation of records of the occurrence of *H. axyridis* in Europe, mapped at 50km square resolution. Records were obtained from a network of scientists working in Europe and Russia, and were either verified by them, derived from published papers, and/or made by other experienced naturalists. There was much variation between countries in the way that records were collected, and some specific information on this is provided in sections 2.2 and 2.3. Some countries, e.g. Belgium, had a strong network of volunteers recording ladybirds in general. Others, e.g. France, had a well organised public survey for *H. axyridis*. Various countries, e.g. Denmark and Poland, set up online surveys for *H. axyridis*, some modelled on the British Harlequin Ladybird Survey. Many countries had no such infrastructure for collecting *H. axyridis* records, and in such cases the data was generally collected by one or a few scientists. I obtained limited additional data, e.g. for Germany and Slovakia, by searching wildlife recording websites for verifiable photographic records of *H. axyridis*.

A working group ('Benefits and risks of exotic biological control agents') was set up under the umbrella of the International Organization for Biological Control of Noxious Animals and Plants (IOBC) (http://www.iobc-wprs.org/expert_groups/index.html), with a major benefit being the resultant collaborative work and sharing of data on *H. axyridis* across Europe. Specifically, the working group aims to assess the characteristics of exotic natural enemies which: (i) are considered to be successful biological control agents; (ii) subsequently become invasive alien species. A further aim is to develop guidelines on assessing environmental benefits and risks of releasing exotic biological control agents.

An earlier version of this chapter was published as: Brown, P.M.J., Adriaens, T., Bathon, H., Cuppen, J., Goldarazena, A., Hägg, T., Kenis, M., Klausnitzer, B.E.M., Kovar, I., Loomans, A.J.M., Majerus, M.E.N., Nedved, O., Pedersen, J., Rabitsch, W., Roy, H.E., Ternois, V., Zakharov, I.A. and Roy, D.B., 2008. *Harmonia axyridis* in Europe: spread and distribution of a non-native coccinellid. *Biocontrol* 53: 5-21.

The above paper contained data to the end of 2006. Here this was updated and mapped to the end of 2008, with some further unmapped updates from 2009. Many of the sections on specific countries have been updated, and sections for eight new countries (Ukraine, Bulgaria, Hungary, Ireland, Poland, Romania, Serbia and Slovakia) added. All figures and the table have been updated. Figure 2.4 is substantially different to the previously published version, now containing data from several additional European countries plus Japan.

2.2 Countries in which **H. axyridis** *was introduced as a biological control agent*

2.2.1 Belgium

Harmonia axyridis was used as a biological control agent in Belgium from 1997 (Adriaens et al., 2003). A large-scale ladybird field survey (Coccinula – Belgian ladybird working group, www.inbo.be/content/page.asp?pid=EN_FAU_INS_LAD_start) was launched in 1999 in the Walloon region, and 2001 in the Flemish region (Adriaens et al., 2003). By 2007 Coccinula had in excess of 500 volunteer surveyors (Adriaens et al., 2008), hence coccinellids are very well recorded in Belgium. *Harmonia axyridis* was first found in the wild in Belgium in autumn 2001, in both Ghent and Brussels (Adriaens et al., 2008) and the invasion probably originated from populations in the north of the country. By 2003, *H. axyridis* was found in large overwintering aggregations comprising 500+ individuals (Adriaens et al., 2008) and was invading semi-natural ecosystems (Adriaens et al., 2003). *Harmonia axyridis* was recorded most commonly in gardens and parks, road verges, forests and woodland fringes, but it also occurred in other habitats such as heathlands, meadows and wetlands (Adriaens et al., 2008). By 2006, *H. axyridis* was recorded in all regions of Belgium (Figure 2.1). The Coccinula database (data from 1999 to 2007) shows that *H. axyridis* was the most abundant coccinellid in Flanders, in terms of the total number of individuals recorded (excluding overwintering groups, which would have made *H. axyridis* abundance even higher). *Harmonia axyridis* was recorded in 76% (426 of 560) of sampled 5km squares in Flanders, making it the second highest occurring coccinellid after *Coccinella 7-punctata*. In Belgium as a whole, *H. axyridis* was recorded in 49% (557 of 1139) of sampled 5km squares, making its occurrence fifth highest after *C. 7-punctata, Propylea 14-punctata* (L.) (Coleoptera: Coccinellidae), *Adalia 2-punctata* (L.) (Coleoptera: Coccinellidae) and *Thea 22-punctata* L. (Coleoptera: Coccinellidae).

2.2.2 Czech Republic

The flightless strain of *H. axyridis* was introduced into hop gardens in north-western Czech Republic in 2003, but the species did not apparently establish at that time. The easterly spread of *H. axyridis* from Germany is assumed to be the main cause of the species arriving in the Czech Republic, where it was first recorded in the wild in 2006 in Prague and nearby rural areas (O. Nedved and I. Kovář, unpublished data). In 2007, intentional monitoring was started, resulting in records from all parts of Bohemia and northern Moravia (Figure 2.1). Records of ovipositing females and mature larvae confirmed that the species had established. Most *H. axyridis* were found on aphid infested woody plants: cherry *Prunus avium* L. (Rosaceae), mock-orange *Philadelphus coronarius* L. (Hydrangeaceae), steeple-bush *Spiraea douglasii* Hook (Rosaceae), willow *Salix alba* L. (Salicaceae), crab apple *Malus sylvestris* (L.) Mill. (Rosaceae) and pear *Pyrus communis* L. (Rosaceae). Based on the findings of mature larvae and ovipositing females, five prey species; *Aphis philadelphi* Börner (Hemiptera: Aphididae), *Aphis spiraecola* Patch (Hemiptera: Aphididae), *Dysaphis plantaginea* (Passerini) (Hemiptera: Aphididae), *Tuberolachnus salignus* (Gmelin) (Hemiptera: Aphididae) and *Cacopsylla pyri* (L.) (Hemiptera: Psyllidae); may be considered as suitable essential, *sensu* Hodek (1973), prey for *H. axyridis*. By early 2008 *H. axyridis* was widespread in the Czech Republic, especially in cities, and had been reported from 57 of 650 grid squares (Nedved et al., 2008).

2.2.3 Denmark

Harmonia axyridis was marketed in Denmark for biological control use until 2005 (Steenberg & Harding, 2009b). The first known record of *H. axyridis* in the wild in Denmark was in Copenhagen in July 2006 (Steenberg & Harding, 2009b). Then and on five subsequent occasions in August 2006, October 2006 and June 2007, a total of 14 adults of *H. axyridis* were found in a light trap. Similarly, a single adult was found in a light trap at Mandemarke, in each of August 2006 and July 2007 (Steenberg & Harding, 2009b) (Figure 2.1). Larvae and pupae were first recorded in summer 2007 (Steenberg & Harding, 2009b). A public survey (http://www.dpil.dk/dpil2005/harlekin/harlekinmariehone.htm) was launched in 2008, and by April 2009 *H. axyridis* was widespread in the southern half of Denmark, with larvae found in Copenhagen and several other localities (Steenberg & Harding, 2009b). Most records were from urban locations dominated by deciduous trees (Steenberg & Harding, 2009b). By December 2009 the species had also been found in northern Denmark (http://www.dpil.dk/dpil2005/harlekin/udbredelseskort.htm). Whilst the deliberate releases of *H. axyridis* in Denmark may account for its establishment there, an alternative hypothesis, favoured by Steenberg and Harding (2009), is that the species spread into Denmark from Germany and/or Poland.

2.2.4 France

Harmonia axyridis was first introduced to France from China by the Institut National de la Recherche Agronomique (INRA) (www.inra.fr) in 1982 (Iperti & Bertand, 2001), but was in quarantine until 1990 (Kabiri, 2006). Between 1990 and 1997 field experiments and releases were carried out, especially in south-eastern France, but also in the north of the country and near Paris (Coutanceau, 2006). Mass production of *H. axyridis* in France began in 1992 and the first open-air commercial field releases were in 1995 (Kabiri, 2006). The species is regarded to have become acclimatized in France by 1991 (Coutanceau, 2006). The earliest known record in the wild is of two individuals, both of the colour form f. *spectabilis* (Figure 2.2), found at Saint-Aubin (Lot-et-Garonne) in south-western France in November 1991 (Coutanceau, 2006). *Harmonia axyridis* was recorded at five overwintering sites in southern France between 1992 and 1993 (Coutanceau, 2006) and at Pas-de-Calais in northern France, where it was released in hops in 1994 (Trouvé, 1995). There are very few known records of *H. axyridis* in France from 1995 to 2003, but from 2004 it was widely established (Coutanceau, 2006) and by 2007 was found across much of northern France (Figure 2.1). It is unknown whether the sparseness of records prior to 2004 is a result of lack of recording effort, or genuine scarcity of *H. axyridis* in France at that time. Certainly, since 2004 the recording effort in France has increased, principally due to the launch of a dedicated survey (Observatoire *H. axyridis* - http://perso.orange.fr/vinc.ternois/cote_nature/Harmonia_axyridis). This is now a well organised survey with over 1000 contributors in 17 French regions, each having a regional co-ordinator. *Harmonia axyridis* has spread into most regions of France, although records from the south-west of the country are sparse (Figure 2.1).

2.2.5 Germany

Although officially never sold in Germany, *H. axyridis* was released in Frankfurt between 1997 and 1998 to control aphids on roses (H. Bathon, unpublished data). It was first recorded in the wild in 1999 in Hamburg (Tolasch, 2002) and Frankfurt-Niederrad (H. Bathon, unpublished data). By 2000, *H. axyridis* was common in the Rhein-Main region and there was major expansion of the species in Germany by 2002 (Klausnitzer, 2002). By 2006, *H. axyridis* occurred in all regions of western Germany and was common in many cities (F. Köhler, personal communication). The species is well recorded in central eastern Germany from 2006, but there are few records from the north-east or south-east of the country (Figure 2.1). This is probably due to lower recording effort rather than genuine absence of the species.

2.2.6 Greece

French stock of *H. axyridis* was introduced into four citrus-growing areas of Greece in 1994 (Katsoyannos et al., 1997). From 1995 to 1999 over 100000 adult *H. axyridis* were released in central and southern Greece and on several islands, to control aphids on various crops (including citrus fruit, vegetables, beans and maize) (Kontodimas et al., 2008b). Further releases took place from 1997 to 2002, mainly in urban areas (Attica and Peloponessos regions, Corfu, Rhodes and Crete) (Kontodimas et al., 2008b). Despite these major releases and four overlapping generations of *H. axyridis* being reported in Greece (Katsoyannos et al., 1997), there is little evidence of establishment of the species, although small numbers (<50) of overwintered adults were found in Attica in spring 1998 and 1999 (Kontodimas et al., 2008b) (Figure 2.1).

2.2.7 Italy

Harmonia axyridis was used as a biological control agent in greenhouses in northern Italy in the late 1990s (Burgio et al., 2008). The first known sighting of *H. axyridis* naturalised in Italy, was in Turin in October 2006, when approximately 30 specimens, including pupae, were found on trees of an *Acer* sp. (Aceraceae) (I. Zakharov, unpublished data) (Figure 2.1). It is unknown whether these ladybirds originated from escaped specimens within Italy, or are immigrants from southern France or Switzerland. In July 2007, a few adult *H. axyridis* were found at two sites in the Piedmont region (M.

Kenis, unpublished data), presenting further evidence of establishment in north-eastern Italy. *Harmonia axyridis* was first found near Verucchio (Emilia-Romagna region) in summer 2008, and subsequent intensive sampling revealed over 1000 specimens at various localities that year. Habitats included orchards, arable crops, hedgerows, isolated trees, field margins, buildings and gardens (Burgio et al., 2008). The species is regarded as established in much of northern Italy (Burgio et al., 2008).

2.2.8 Netherlands

Harmonia axyridis was marketed as a biological control insect in the Netherlands from approximately 1996 (Cuppen et al., 2004) until 2003. The first record of *H. axyridis* naturalised in the Netherlands was in October 2002, when a pupa was found on an English ivy *Hedera helix* L. (Araliaceae) leaf in Nijmegen. A larva of *H. axyridis* was confirmed in Rotterdam the following month. Initial distribution was reported primarily in the southern half of the country. Numbers of *H. axyridis* rose dramatically from 2004 (Loomans, 2004) and survey effort was increased from that year, with records requested via three Dutch websites (www.stippen.nl, www.knnv.nl and www.nev.nl). The species appears to have spread from south to north, and by 2006 all mainland 50km squares in the Netherlands had records of *H. axyridis* (Figure 2.1). Over 2000 records of the species were received between 2002 and 2007.

2.2.9 Portugal

Between 1984 and 1985, *H. axyridis* was used to control aphids on citrus crops in the Algarve province of Portugal, and on the Portuguese administered islands of the Azores (Katsoyannos et al., 1997, Soares et al., 2008). There is no evidence of subsequent establishment. However, it was concluded that if re-introduced to the Azores, *H. axyridis* would present a risk to the native species *Coccinella 11-punctata* L. (Coleoptera: Coccinellidae) (Soares & Serpa, 2007).

2.2.10 Spain

In 1995 *H. axyridis* was introduced to control aphids in greenhouses in Almeria, south-eastern Spain (Servicio de Información Fitosanitaria de Almería, 2004). It established in greenhouses (Jacas et al., 2006), but there is no evidence of subsequent establishment in the wild. In 2003 and 2004 two specimens of *H. axyridis* were found in a garden on Tenerife, Canary Islands and it was found again on the island in 2006 (S. Eizaguirre, personal communication). There are no official reports of introductions of *H. axyridis* for agricultural purposes from the Canary government, and the species is not believed to be present in large numbers (Machado, 2005). In 2007, two adult *H. axyridis* were collected from flowers of a *Tilia platyphyllos* Scop. (Malvaceae) tree in a park in Loiu, Bilbao, Basque country of northern Spain (Goldarazena & Calvo, 2007) (Figure 2.1). This was the first record of naturalised *H. axyridis* in the Iberian peninsula. There is no evidence that *H. axyridis* was released for biological control purposes in the Basque country (R. Amenabar, personal communication). The specimens are likely to have either originated from France, or from imported goods entering the nearby Bilbao airport. A second *H. axyridis* record from closeby, at Irun, was made in 2008, but with no further known records (A. Goldarazena, personal communication).

2.2.11 Switzerland

Harmonia axyridis was available commercially in Switzerland for a short period in the late 1990s, and was used in a small field trial on roses in Grossdietwil in 1996 (Andermatt, 1996). However, *H. axyridis* did not apparently establish as a result, and applications for the commercial release of the species were refused by the Swiss Pesticide Regulatory Authority in 1997 (van Lenteren et al., 2008). No adults were found in the wild in Switzerland until 2004, when a single specimen was found at an exhibition of Asian plants in Basle (Klausnitzer, 2004). *Harmonia axyridis* was not found during surveys in 2005, but in 2006 it was recorded in ten cantons in northern Switzerland (Eschen et al., 2007), and larvae of the species were found for the first time in Switzerland. The high abundance of the beetle near the northern border suggests that it invaded the country from southern Germany and/or eastern France. In a large survey for ladybirds in north-western Switzerland, *H. axyridis* was found to be the seventh most abundant species on trees and shrubs, and the first cases of aggregations on buildings were notified in autumn 2006 in Basle (Eschen et al., 2007). The monitoring continued in 2007 and a dedicated website was launched (www.cabi-e.ch/harmonia). By 2008, *H. axyridis* was found in all Swiss cantons (Figure 2.1) and the species is now one of the most common and widespread coccinellids in the country (M. Kenis, personal communication). Tests on Swiss wines deliberately tainted by *H. axyridis* were conducted, concluding that the beetle, at fairly low levels, would have a serious negative impact if harvested with grapes (Linder et al., 2009).

2.2.12 Ukraine

Ukraine, (at the time part of USSR), was the first European country to introduce *H. axyridis*, in 1964 (Chernovitskaya, south-west Ukraine) and 1969 (Crimea, south-east Ukraine) (Poutsma et al., 2008). Little information is known about any subsequent establishment, but the first documented records for the country were in June 2009: many larvae and pupae were found on lime *Tilia* sp. (Malvaceae) trees in Berehove, and the species was found on elder *Sambucus nigra* L. (Adoxaceae) in Csop (Marko & Pozsgai, 2009, V. Marko, personal communication). From the pattern of European spread it seems most probable that presence in the country is a result of easterly spread from Europe (Poland, Slovakia and/or Hungary), rather than from biological control stocks.

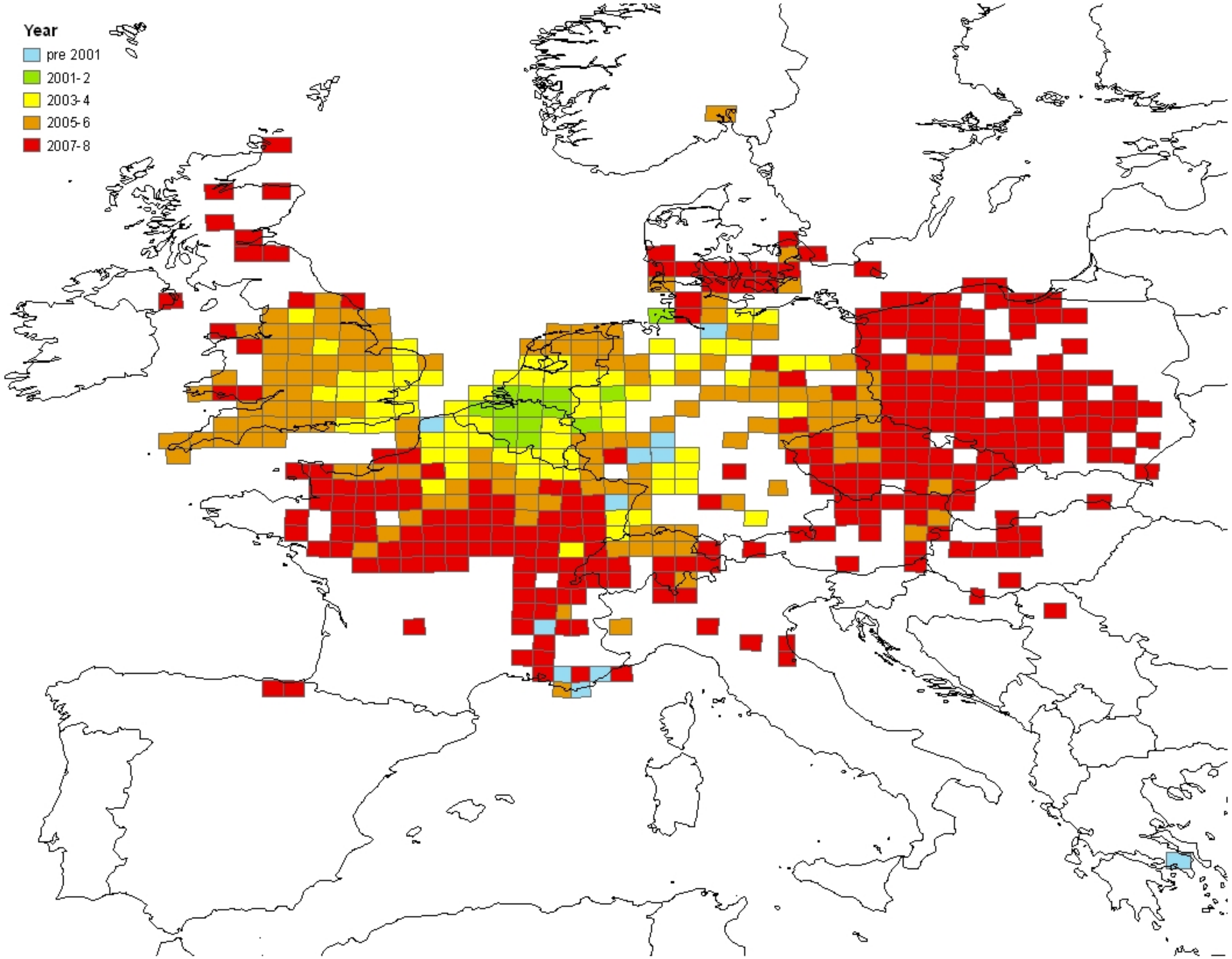


Figure 2.1. *Harmonia axyridis* occurrence in 50km squares in Europe to 2008. Where a square has been recorded in more than one year class, occurrence in the earliest year class is shown.

2.3 Countries in which **H. axyridis** *has been found in the wild without evidence of deliberate introduction*

2.3.1 Austria

In Europe and North America, the attraction of *H. axyridis* to buildings for use as overwintering sites (Koch, 2003) has increased the number of sightings of the species. The first published record in Austria (October 2006; Wiener Neustadt, eastern Austria) was one such sighting, where several adult specimens of *H. axyridis* were found on a house wall searching for an overwintering site (Rabitsch & Schuh, 2006). An earlier record subsequently came to light, in which *H. axyridis* larvae were found on plants on a balcony in nearby Vienna in July 2006, and there are several later records of adults in Vienna in 2006 and 2007 (W. Rabitsch, personal communication). *Harmonia axyridis* was clearly established in Austria by 2007. Independent observations in the west (Götzis, October 2006; Rankweil, April 2007; Vorarlberg, August 2007), centre (Salzburg, May 2007), and south (Herberstein, July 2007) of the country (W. Rabitsch, personal communication), support the hypothesis of spread from Switzerland and/or Germany (Figure 2.1). There is no known evidence that *H. axyridis* was used for biological control purposes in Austria, making it less likely that the spread resulted from (illegally) imported specimens escaped from greenhouses or gardens. Whilst the species is common and widespread in lowland areas throughout Austria, distribution in the Alps is less well-known; however, several *H. axyridis* were found at an alpine hut above 2000m altitude (W. Rabitsch, personal communication).

2.3.2 Bulgaria

First recorded in July 2009, *H. axyridis* was found in eight of 45 localities during structured surveys carried out that summer. These were mostly in the west of the country, but also along the southern border with Greece (Tomov & Kenis, 2009). However, the species was not recorded in surveys in the eastern half of the country. With one exception (Kresnia) all localities with *H. axyridis* were urban, and the largest number of specimens (180 adults, pupae and larvae) was in Sofia, associated with *Eucallipterus tiliae* (L.) (Hemiptera: Aphididae) on *Tilia cordata* Mill. (Malvaceae) (Tomov & Kenis, 2009).

2.3.3 Great Britain and the Channel Islands

The first record of *H. axyridis* in Great Britain, in September 2004, initiated considerable media and public interest, and led to the launch of the web-based Harlequin Ladybird Survey (www.harlequin-survey.org) (Roy et al., 2006, Majerus et al., 2006a). Very recently, an earlier record was discovered, from October 2003 (R. Comont, personal communication). Thanks to thousands of contributors across Britain, the species has been recorded and mapped at high resolution (Roy et al., 2005, Chapter 3) and its rapid spread north and west from the south-east is clear (Figure 2.1). By 2009, *H. axyridis* was recorded in all English and most Welsh counties, with sparse records from Scotland. It was also found on the following islands: Jersey, Guernsey and Sark (Channel Islands); Isle of Wight (southern England); Isle of Man (near southern Scotland) (Harlequin Ladybird Survey data); Orkney (north-eastern Scotland) (Ribbands et al., 2009). Three colour forms have been recorded in Britain; f. *succinea*, f. *spectabilis* and f. *conspicua* (Figure 2.2). A detailed account and analysis of the spread of *H. axyridis* in Great Britain is provided in the next chapter.

2.3.4 Hungary

Harmonia axyridis has frequently been found to overwinter with *A. 2-punctata* in Europe, often in buildings (Majerus, 2008). A single *H. axyridis* overwintering with a group of *A. 2-punctata* under loose bark of a poplar tree, was the first reported sighting of *H. axyridis* in Hungary, near Budapest in February 2008 (Merkl, 2008). A second record was reported in July 2008, and increased recording effort and rapid expansion of the species led to records from 103 10km squares across most of the country by July 2009 (Marko & Pozsgai, 2009, www.coleoptera.hu/harlekin). The species was never released for biological control purposes in Hungary, and it is assumed to have arrived from the west (Austria) (Merkl, 2008), and possibly from the north (Marko & Pozsgai, 2009). In less than two years from first detection, *H. axyridis* was considered to be one of the most common ladybird species in Hungary (Marko & Pozsgai, 2009).

2.3.5 Ireland

Harmonia axyridis was first reported from Northern Ireland in November 2007 (Murchie et al., 2008) and an online survey is in place (www.habitas.org.uk/ladybirds).

The species was inadvertently imported from England: a live adult beetle arrived with celery grown in eastern England that was delivered to a supermarket in Lisburn, Co. Antrim (Murchie et al., 2008). This highlights an additional mechanism by which the species may spread; indeed, there are further examples of the spread of *H. axyridis* within Britain on transported produce (Ribbands et al., 2009, W. Grange, personal communication). The species has subsequently been reported twice more from Northern Ireland, but with no evidence of establishment (Brown et al., 2010) and there are no known records from the Republic of Ireland. However, establishment in both Irish countries in the near future seems inevitable.

2.3.6 Liechtenstein

Harmonia axyridis was found for the first time in the tiny principality of Liechtenstein in August 2007 (A. Loomans, unpublished data). The whole principality is contained within a single 50km square in which there were earlier records for both Austria and Switzerland.

2.3.7 Luxembourg

Considering that the border areas of France, Belgium and Germany that surround Luxembourg were all known to have populations of *H. axyridis* (Figure 2.1), it was inevitable that the species would arrive in this small country. It was present by September 2004, when first recorded on *Acer pseudoplatanus* L. (Aceraceae) in the south of Luxembourg (Schneider & Loomans, 2006). Further sightings of *H. axyridis* were made in October, November and December 2004 in urban locations (Schneider & Loomans, 2006) and large reproducing populations were recorded in August 2005 in the north (Clervaux) and south (Luxembourg City) of the country (M. Majerus, unpublished data).

2.3.8 Norway

After risk assessment, *H. axyridis* was turned down for use as a biological control agent in greenhouses in Norway. However, it was first recorded there in April 2006, in Oslo, where a gravid female was found on horticultural plants imported from the Netherlands (Staverloekk et al., 2007) (Figure 2.1). Also in Oslo, an autumn aggregation of >20 adult *H. axyridis* was found in November 2007, adults survived that winter and larvae were found in summer 2008 (A. Staverloekk, personal communication). The distance between Denmark and Norway was thought to be too great to allow a northward invasion from Denmark (Staverloekk et al., 2007), the closest country with established *H. axyridis* populations. Thus accidental importation with commodities was assessed as the likeliest entry method (Staverloekk et al., 2007). Indeed, further *H. axyridis* arrived on plants from the Netherlands, and over 1000 adult *H. axyridis* (many alive) arrived with timber from Pennsylvania, USA in 2008 (A. Staverloekk, personal communication). Survival of *H. axyridis* in the Norwegian climate, at least in the south of the country, concurred with CLIMEX modelling for the species (Poutsma et al., 2008). Recording of *H. axyridis* is being encouraged via an online survey (http://www.bioforsk.no/ikbViewer/page/prosjekt/forside?p_menu_id=19738&p_sub_id=19729&p_dimension_id=19728&p_dim2=19729).

2.3.9 Poland

Three adult *H. axyridis* in Wielkopolska-Kujawy Lowland (west-central Poland) in late 2006 were the first documented sightings in the country: the first in a university garden in Poznań-Ogrody in September, followed in October by two beetles on a house wall in Mokrz ad Wronki (Przewozny et al., 2007). After much media interest a survey was set up (www.cbe-pan.pl) and records were received from 12 new 10km squares (mostly in the west) in 2007, and at least 200 further 10km squares in 2008, extending across all but the north-east of Poland (Figure 2.1) (P. Ceryngier, personal communication). Thus *H. axyridis* appears to have spread extremely rapidly in Poland.

2.3.10 Romania

Harmonia axyridis was first recorded in Romania in April 2009, with one ladybird found at Oradea (western Romania near the Hungarian border) and then >20 individuals found further south, at Gurani, in August 2009 (Marko & Pozsgai, 2009). Subsequent records submitted to an online survey (www.harlequin.ro) show distribution in the north-west and centre of the country. *Harmonia axyridis* was common in Transylvania (northern Romania) in autumn 2009, with larvae and thousands of adults recorded (V. Marko, personal communication).

2.3.11 Serbia

The earliest record of *H. axyridis* in Serbia was of three adults caught in a light trap in the province of Vojvodina (National Park Fruška Gora) in August 2008 (Thalji & Stojanovic, 2008). Despite extensive media coverage after this, no formal survey has so far been set up for collating sightings of *H. axyridis* in Serbia. However, personnel from several National Parks have provided records (Thalji, personal communication). Although *H. axyridis* larvae have not been noticed in Serbia, there was a large expansion of the species in 2009, and it is now estimated to be present in 70% of the country, extending to the Romanian border in the east, the Bulgarian border in the south-east (Thalji, personal communication) and Subotica, near the Hungarian border, in the north (Marko & Pozsgai, 2009). The beetle seems to have spread eastwards then southwards. It was most abundant in arboreal habitats, including apple orchards. It has been found feeding on ripe fruits (e.g. peaches and figs grown in gardens) in the city of Novi Sad and other areas close by (Thalji, personal communication).

2.3.12 Slovakia

Harmonia axyridis was found in Pezinok (Bratislava region, close to the Austrian border) in August 2008 and establishment in Slovakia was confirmed with a larva of the species in Kostolná (Trenčín region, close to the border with the Czech Republic) in September 2008 (O. Nedved, personal communication). *Harmonia axyridis* was also found in Banská Bystrica region (close to the Hungarian border) in 2009 (V. Marko, personal communication). The species was clearly widespread in Slovakia by the end of 2009, with records (including juveniles) as far east as Kosiče region (www.foto-net.sk), which borders Ukraine.

2.3.13 Sweden

Harmonia axyridis was first recorded in Sweden in Malmö in April 2007 (T. Hägg, personal communication), when a single adult was found in a house (Figure 2.1). Subsequently, a dead adult was found at the same location, and it is thought that the species had been overwintering in the house, suggesting that it arrived in 2006, possibly from Germany or Denmark. Near the south coast of Sweden, four adult *H. axyridis* were caught, attracted to light at night in August 2008. Establishment in Sweden was only confirmed in September 2009, when a large number of larvae were found in a garden in central Malmö. In early December there were still pupae and adults in the same garden (T. Hägg, personal communication). There is no formal survey for *H. axyridis* in Sweden, and the species, though locally distributed, is probably more common than the sparse records reflect.

Figure 2.2. *Harmonia axyridis* colour forms: (a) f. *succinea*; (b) f. *conspicua*; (c) f. *spectabilis*; (d) f. *axyridis*. Photographs courtesy of: (a) and (b) Gavin Hatt, John Innes Centre Entomology; (c) Ken Dolbear; (d) Remy Ware.

Table 2.1. Summary of *H. axyridis* occurrence in Europe.

Country	Year of first record in the wild	Deliberately introduced? (Earliest year of introduction)	Evidence of establishment?
France	1991	Yes (1982)	Yes
Greece	1998	Yes (1994)	Limited
Germany	1999	Yes (1997)	Yes
Belgium	2001	Yes (1997)	Yes
Netherlands	2002	Yes (1996)	Yes
England & Channel Islands	2003	No	Yes
Switzerland	2004	Yes (1996)	Yes
Luxembourg	2004	No	Yes
Italy	2006	Yes (1990s)	Yes
Czech Republic	2006	Yes (2003)	Yes
Denmark	2006	Yes (2000s)	Yes
Austria	2006	No	Yes
Norway	2006	No	Yes
Poland	2006	No	Yes
Wales	2006	No	Yes
Spain	2007	Yes (1995)	No
Liechtenstein	2007	No	Yes
N. Ireland	2007	No	No
Scotland	2007	No	Yes
Sweden	2007	No	Yes
Hungary	2008	No	Yes
Serbia	2008	No	Yes
Slovakia	2008	No	Yes
Ukraine	2009	Yes (1964)	Yes
Bulgaria	2009	No	Yes
Romania	2009	No	Yes
Belarus	Unknown	Yes (1968)	Unknown
Portugal	None	Yes (1984)	No

2.4 Establishment and spread

Harmonia axyridis has spread in Europe at a maximum rate of approximately 200km per year (Table 2.1; Figure 2.3). This calculation is based on a south-easterly spread from Belgium, the Netherlands or northern France to Bulgaria (approximately 1600km) in an eight year period. It is not possible to fully explain the mechanisms of spread leading to the current distribution, because of the spatial and temporal aspects of the deliberate releases of *H. axyridis*. This involved multiple introduction sites in at least 13 European countries over a period of approximately 40 years (Table 2.1).

Time lags may occur throughout the invasion process, including the arrival, establishment and impacts of the invading species (Crooks, 2005). These data indicate a variable time lag between initial establishment and major expansion, at least in the countries where *H. axyridis* was deliberately introduced. In France this time lag was approximately 13 years (i.e. 1991 to 2004), in the Netherlands approximately six years (i.e. 1996 to 2002), and in Belgium approximately four years (i.e. 1997 to 2001). In countries where the species has not known to have been introduced, there seems to be very little time lag between the first record of establishment and major expansion (e.g. less than one year in England). The period between establishment and rapid spread in the USA is difficult to assess because of multiple intentional releases over many years (Gordon, 1985), uncertainty over the date of establishment, and ambiguity over whether an intentional release or an accidental introduction was the source of establishment (Koch, 2003, Day et al., 1994).

The reasons for the time lag between establishment of *H. axyridis* and major expansion in Europe are as yet unknown. However, it is possible that it is related to the genetic make-up of releases, and to differences between environmental conditions in the locations where released stocks originated and where they were released. The genetic make-up of released stocks would be a function of selection acting to locally adapt populations in their native range before collection, and random genetic drift and selection acting on laboratory cultures before release. It is likely that some cultures passed through genetic bottlenecks while in culture. Moreover, there are reports of rapid changes in some obvious genetically controlled traits in laboratory cultures. For example, the phenotypic frequencies of melanic forms (f. *conspicua* and f. *spectabilis*) of a laboratory population increased from 0.5 to >0.99 in 50 generations (Berkvens et al., 2008a). As many laboratory cultures are maintained on diets that *H. axyridis* would not encounter in the wild (i.e. *Ephestia* sp. (Lepidoptera: Pyralidae) eggs), it is inevitable that released beetles will have been exposed to abnormal selection pressures in captivity. In consequence, it is highly unlikely that released individuals will be precisely adapted to the conditions that they face in their introduced range. In many cases, releases therefore do not lead to establishment, as recorded for *H. axyridis* in the USA (Gordon, 1985) and Greece (Kontodimas et al., 2008a, 2008b). However, if we assume that at least some individuals do survive and reproduce, the expectation would be that these would take time to start increasing in number significantly, for two reasons. First, because of their as yet imperfect adaptation to local conditions, and second because at low population size they are likely to suffer the effects of inbreeding depression, which is considerable in many aphidophagous coccinellids (Hodek, 1973, Majerus, 1994a, 2003).

There will thus be a period after establishment during which the average fitness of members of the population increase as the population is purged of deleterious recessives (Haldane, 1927), and slowly becomes better locally adapted to the new conditions, through selection acting on the genotypic variation generated by recombination and mutation (Fisher, 1930). It is only once the population has become locally adapted that it will start to increase in number. However, the rate of increase may then be rapid for two reasons. First, the population may have been purged of deleterious recessive alleles, as mentioned above. Second, while in culture the released beetles may have been inadvertently selected for rapid reproduction, high fecundity and broad dietary range; biological control companies reared large numbers as efficiently as possible for commercial reasons. Once numbers in a released population increased sufficiently to approach carrying capacity, increased rate of dispersal would be inevitable as the beetles sought new resources. The rate of this process may vary greatly depending on factors such as the size of the original samples collected from the native range, the number of different populations that such samples were collected from, the length of time and number of generations that cultures were kept in captivity for before release, the number of individuals that founded each culture generation, and the number of beetles finally released. If releases involved stocks with quite different origins, then the speed of local adaptation would be faster than if all released beetles were from a single stock. This is because matings between individuals from different locally adapted populations would produce a greater range of genetic variants among their offspring for selection to act upon.

The initial need for adaptation before increase in number and spread may explain the lack of a time lag between establishment and major expansion in countries where *H. axyridis* was not deliberately introduced; individuals of *H. axyridis* arriving in these countries from parts of the introduced range had already been through the adaptation phase. So after a period of adaptation of one or several populations in France, Netherlands, Belgium and/or Germany, a rapid spread of *H. axyridis* occurred throughout Europe.

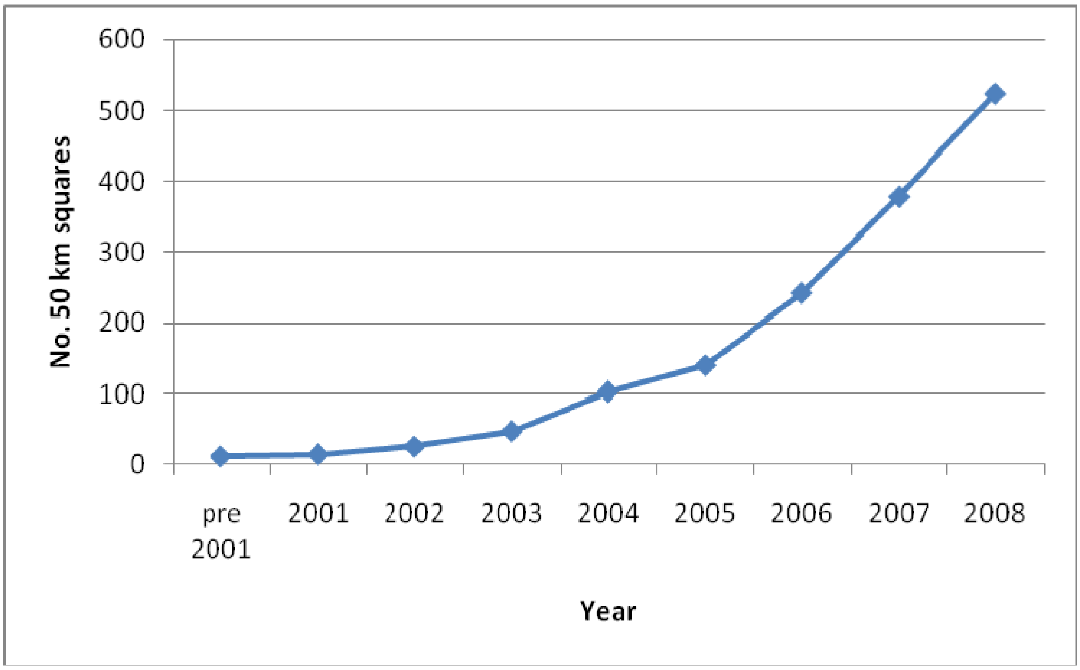


Figure 2.3. Cumulative occurrence of *H. axyridis* in 50km squares in Europe to the end of 2008.

2.5 Genetic variation and the origins of **H. axyridis** *in Europe*

Four main colour forms of adult *H. axyridis* have been found in Europe: f. *succinea*, f. *spectabilis*, f. *conspicua* and f. *axyridis* (Figure 2.2). The *succinea* complex has been divided into many sub-forms (e.g. *siccoma* – 0 spots; *frigida* – 6 spots; *novemdecimsignata* – 19 spots) (Dobzhansky, 1933), but is regarded as a single form here. Thus f. *succinea* has elytra with a ground colour of yellow, orange, or red, and 0-21 black spots, which may or may not be fused. Forma *spectabilis* has black elytra with four yellow, orange, or red spots or other shaped markings, which sometimes contain a central black spot. Forma *conspicua* is as f. *spectabilis*, but with only the anterior pair of spots. Forma *axyridis* has a chequered appearance of yellow, orange or red on black.

The typical colour form, f. *axyridis*, which predominates in central Asia (Dobzhansky, 1933, I. Zakharov & M. Majerus, personal observation), was not recorded in Europe until 2007. However, since then f. *axyridis* has been found in Denmark (Nedved et al., 2008), the Czech Republic (Nedved et al., 2008), Slovakia (www.foto-net.sk), Serbia (R. Thalji, personal communication) and Bulgaria (Tomov & Kenis, 2009). Where frequencies for this colour form are known, they are very low (e.g. less than 0.3% in Denmark and the Czech Republic) (Nedved et al., 2008). It is notable that with the exception of Denmark, all known records of f. *axyridis* occurred in eastern Europe, a point worthy of further investigation. In widespread surveys in Japan in 2009, f. *axyridis* occurred at a frequency of 4% (n = 786) (P. Brown & C. Thomas, unpublished data) (Figure 2.4).

There is strong evidence of a link between climate and colour form in coccinellids (Majerus, 1994a, Majerus, 1998). Whilst the basis of difference in distinct colour form (e.g. f. *succinea* cf. f. *spectabilis*) is genetic, difference in colouration within a form is at least partly environmental. For example, in many species of coccinellid, inverse correlations between temperature and extent of melanic patterning have been reported (e.g. Dobzhansky, 1933, Abbas et al., 1988, Majerus, 1998). Indeed, in Great Britain, specimens of *H. axyridis* f. *succinea* that emerge late in the year often exhibit a high degree of melanism, and are occasionally almost completely black (P. Brown, personal observation). Also in *H. axyridis* f. *succinea*, the amount of melanism was found to increase at high elevations (Nalepa et al., 1996).

European populations of *H. axyridis* generally include a mix of the three main colour forms, but with f. *succinea* dominating (Figure 2.4). Conversely, the frequency of colour forms found in Japan was strikingly different to that in Europe, with f. *conspicua* dominant and f. *succinea* uncommon (Figure 2.4). Interestingly, in England the percentage of melanic specimens declined markedly from the year of establishment of *H. axyridis*, 2004 (45%, n = 344) (Majerus & Roy, 2005) to the following year, 2005 (20%, n = 6180) (M. Majerus, unpublished data). The broad consistency in the colour form frequency data from Europe (Figure 2.4) provides some evidence of the genetic similarity of populations in different European countries. Further, I suggest that this possible genetic similarity is the result of spread of the species from very limited points of origin in Europe. Genetic analyses of *H. axyridis* samples from different countries are needed in order to confirm this; indeed, work on this is underway (Lombaert et al., 2009, Loiseau et al., 2009, Thomas et al., in press). Lombaert, et al. (2009) concluded that Eastern USA populations of *H. axyridis* were the source of the European invasive populations.

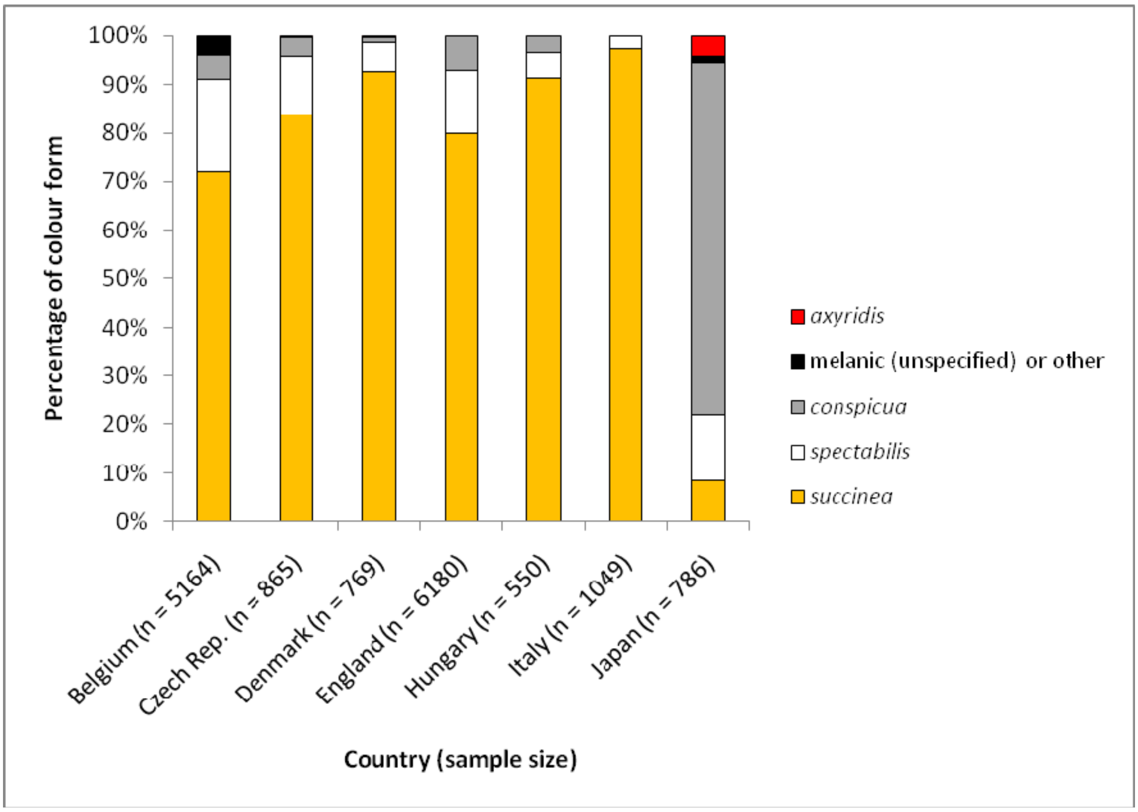


Figure 2.4. Percentages of *H. axyridis* colour forms in various countries. Belgium data from 2004-06 (Adriaens et al., 2008); Czech Republic data from 2006-08 (Nedved et al., 2008); Denmark data from 2006-08 (Nedved et al., 2008); England data from 2005 (M. Majerus, unpublished data); Hungary data from 2008-09 (Marko & Pozsgai, 2009); Italy data from 2008 (Burgio et al., 2008); Japan data from 2009 (P. Brown & C. Thomas, unpublished data).

2.6 Traits that give **H. axyridis** *a competitive advantage over native coccinellids*

In Europe, *H. axyridis* has been shown to be multi-voltine[9], with evidence of two generations per year in Great Britain (Chapter 3) and Denmark (Steenberg & Harding, 2009b), three per year in Hungary (Marko & Pozsgai, 2009) and four per year in Greece (Katsoyannos et al., 1997). Production of multiple generations helps to explain the rapid spread of *H. axyridis* in Europe. In northern Europe, many native coccinellids are uni-voltine (Majerus, 1994a, Klausnitzer & Klausnitzer, 1997), thus *H. axyridis* has a significant advantage over them in terms of potential population growth. Dispersal of *H. axyridis* in northern Europe appears to occur primarily in autumn, when the species moves to aggregate at overwintering sites. This pattern is similar to that observed in North America (Koch, 2003). Large overwintering aggregations have been observed in many European countries including Belgium, the Netherlands, France, Germany and England.

Recent European research has provided further evidence of the adaptability and resilience of *H. axyridis*. Many coccinellids sometimes feed on pollen and nectar as supplementary food sources (Majerus, 1994a); indeed, LaMana, & Miller (1996) reported *H. axyridis* feeding on pollen and nectar in Oregon. In laboratory experiments, 35-48% of *H. axyridis* larvae fed a pollen-only diet successfully reached adulthood (Berkvens et al., 2008a). Although fitness was reduced, approximately 40% of these adult females produced viable eggs (Berkvens et al., 2008a). One study found that only the highest dose (10^9 spores ml^{-1}) of the fungal pathogen *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) killed *H. axyridis*, whereas two other species included in the study (*A. 2-punctata* and *C. 7-punctata*) experienced high mortality (70% and 80% respectively) at a medium dose (10^7 spores ml^{-1}) of the pathogen (Roy et al., 2008b). Another study found that *H. axyridis* had lower susceptibility than *C. 7-punctata* to the parasitoid wasp *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) (Koyama & Majerus, 2008). Recent work on IGP (Pell et al., 2008, Ware & Majerus, 2008, Ware et al., 2008a) suggests that *H. axyridis* may have a negative

---

[9] Multi-voltine: production of more than one generation per year (cf. uni-voltine and bi-voltine).

impact on native coccinellids in Europe.

2.7 Conclusion

Harmonia axyridis is now one of the most widely distributed coccinellids in Europe. I predict that the species will continue to spread in Europe, particularly eastwards, through Ukraine and Belarus. It is entirely possible, (indeed likely), that the species is already present, but undetected, in countries such as Slovenia, Croatia and Bosnia. Some northward spread within Norway and Sweden is likely, as is spread into the Baltic states and Finland. Given that *H. axyridis* survives as a native species in parts of Siberia, the climate in these countries should not be a barrier to establishment; indeed, winter temperatures as low as approximately -17°C allow survival of European field populations of *H. axyridis* (Berkvens et al., 2010). The Republic of Ireland is the only remaining country left to be invaded from the westward spread, and I predict that *H. axyridis* will reach there, from Great Britain or Northern Ireland, in the near future, and once established will spread across the whole country. The spread southwards is less certain, although as the species is adapted to Mediterranean and sub-tropical climates in parts of its native range (southern Japan and China), it may gradually adapt to the warmer climes of southern Europe, the Iberian peninsular and north Africa, and eventually establish and spread there. The recent fast spread in Bulgaria may be a sign of this. There are already initial reports of establishment in Egypt (S. Elnagdy, personal communication). It remains to be seen what effect this very rapid spread will have on native coccinellids in Europe, but mounting evidence suggests a seriously detrimental impact.

3. *Harmonia axyridis* in Great Britain: analysis of the spread and distribution of a non-native coccinellid

3.1 Introduction

Biological recording in Great Britain has a long tradition; detailed recording of many taxa has been carried out since the nineteenth century (Harding, 1990). Biological records for Great Britain and Ireland are integrated and distributed through the National Biodiversity Network (NBN) Gateway (http://data.nbn.org.uk/), an interactive mapping website holding almost 50 million records for over 8500 species. The biological recording infrastructure in Great Britain provides an effective tool for monitoring species undergoing rapid range expansions. Analysis of long-term datasets showed that various taxa (e.g. dragonflies, butterflies, spiders and fish), are moving northwards in Great Britain in response to climate change (Hickling et al., 2006). Invasive species are regarded as one of the most serious threats to biodiversity (Glowka et al., 1994). The monitoring of biological invasions is vital, in order to understand the population dynamics, habitat tolerance and impact on native taxa, of the invading species. In Great Britain, monitoring projects for invasive insects include the horse chestnut leafminer *Cameraria ohridella* Deschka and Dimic (Lepidoptera: Gracillariidae) (www.forestresearch.gov.uk/leafminer), and the rosemary beetle *Chrysolina americana* L. (Coleoptera: Chrysomelidae) (www.rhs.org.uk/Science/Plant-pests/Rosemary-beetle).

Harmonia axyridis is not known to have been deliberately introduced to Great Britain, but arrived by various means, including flight and on produce from mainland Europe and in packing cases from Canada (Majerus et al., 2006a, 2006b). Uniquely, the early detection of *H. axyridis* in Britain presented the opportunity to study the spread of an invasive animal from the year of its establishment (Majerus et al., 2006a). Through detailed mapping of adult and juvenile stages, the objective of my study was to quantify and analyse the spread of *H. axyridis* in its early stages of invasion in Great Britain. A further objective was to investigate the voltinism of *H. axyridis* in Great Britain, to determine whether there was evidence of multiple generations per year.

An earlier version of this chapter was published as: Brown, P.M.J., Roy, H.E., Rothery, P., Roy, D.B., Ware, R.L. and Majerus, M.E.N., 2008. *Harmonia axyridis* in Great Britain: analysis of the spread and distribution of a non-native coccinellid. *Biocontrol* 53: 55-67.

Figures 3.1 and 3.2 and Table 3.1 have been updated from the versions published above. Other figures are unaltered. The northerly rate of spread calculation has been updated to include data to 2008 (it was previously to 2006).

3.2 Method

3.2.1 Ladybird data collection

The spread of *H. axyridis* was monitored by utilising the extensive biological recording community in Great Britain, coupled with engagement of the general public. Recent advances in technology, and high levels of public access to the internet and digital photography, enabled a web-based biological survey to be set up. The Harlequin Ladybird Survey (www.harlequin-survey.org) was one of the first online surveys of its kind in Britain. It was launched in March 2005 in response to the first report of *H. axyridis* in Britain, in September 2004 (Majerus, 2004) and I have co-ordinated the survey since the start. The Harlequin Ladybird Survey benefited from high levels of media interest, including the front page of The Times newspaper on 15 March 2005. Members of the British public showed great willingness to look for *H. axyridis*, and to register their sightings with the survey.

The dataset presented here comprises 10609 species records of *H. axyridis* in Great Britain between 2004 and 2008. Until very recently, the earliest record of *H. axyridis* in Britain was from 3 July 2004, hence for 2004 only six months of data are available. A single earlier record has subsequently been discovered; one *H. axyridis* adult was found in the insect catch from a suction trap (part of the Rothamsted Research Insect Survey – see Chapter 5) in the extreme south-east of England in October 2003 (R. Comont, personal communication). Each record represents a verified sighting of *H. axyridis* on a given date, and comprises one or more individual ladybirds observed from one or more life stages (larva, pupa and adult; records of eggs were not included). Approximately 75% of these records were received from members of the British public by post, or entered online at the Harlequin Ladybird Survey website or UK Ladybird Survey website (www.ladybird-survey.org); the latter was set up to encourage the recording of native ladybirds. Verification of the records was made by receipt of either a specimen or photograph. The remaining records were received from coleopterists and other naturalists, are regarded as accurate, and have been included in the dataset. Approximately 19000 further online records were received that remain unverified (i.e. no photograph or specimen was sent), or were verified as another species, and so are not included in the analyses. Verified records were regularly uploaded to the NBN Gateway. There they could be viewed via online maps, which helped to encourage further recording.

Each species record includes the following data: recorder name; location of sighting (the grid reference of the Ordinance Survey British national grid reference system); locality/site name (not included for all records); date of sighting; life stage observed (larva, pupa, adult); number of each life stage observed (assumed to equal 1, if not specified); and determiner name. Additional optional attributes include the number of each colour form of any adults observed (included for approximately 80% of records), and any supplementary comments provided, such as habitat, host plant and behavioural information. Three main colour forms of adult *H. axyridis* have been found in Great Britain: f. *succinea*, f. *spectabilis* and f. *conspicua* (see Chapter 2 for descriptions of each).

The spatial resolution of the records is variable. While approximately 20% include a grid reference, enabling resolution to 100 metres, the other approximate 80% of records were derived at 1km resolution from a UK postal code (UK Government Schemas and Standards, www.cabinetoffice.gov.uk/govtalk/schemasstandards/e-gif/datastandards/address/postcode.aspx). The option on the online recording form to enter the location via a UK postal code was provided to make the entry of records easier for members of the public unfamiliar with the grid reference system. Whilst the resolution is thus reduced for these records, the reduction in user error (e.g. the problem of grid reference eastings and northings being transposed), is an advantage (Majerus et al., 1990). The postal code method was applicable for sightings of *H. axyridis* made within 200 metres of a specified postal code, so could not be used for a minority of records where the ladybird was seen in a semi-natural habitat.

Variability in recording effort (both temporally and spatially) is clearly an issue when analysing a dataset of the kind presented here. Other factors being equal, more records will come from areas with a higher density of recorders. Across Britain there were a number of particularly active local groups or individuals, which contributed hotspots of recorder activity, potentially biasing the results for certain areas, e.g. London, because of the high activity of the London and Essex Ladybird Survey. The Harlequin Ladybird Survey benefited from a high profile and enjoyed frequent local and national media attention, thus increasing the volume of records received at certain times. To minimise these effects, the data have mostly been analysed in terms of the presence of *H. axyridis* in 10km or 1km squares by year. Thus, whether the species was recorded just once or many times in any given square in a year, is not reflected in the analyses. To many recorders, juvenile stages (especially pupae and early instar larvae) were less noticeable and more difficult to identify than the adult stage, thus limiting their recording.

The possibility of a reporting bias towards sightings early in the season also existed (i.e. some recorders may have reported their first sighting of *H. axyridis*, but not subsequent sightings). In order to minimise this effect, the importance of recording multiple sightings was stressed to recorders. The peaks in record numbers observed late in each year also suggest that any effect of this potential bias was minor.

3.2.2 Rate of spread

The location of the northern range margin of *H. axyridis* in Great Britain was measured by calculating the mean northing of the ten most northerly 10km squares occupied each year (2004 to 2008) (Hickling et al., 2006). The location of the western range margin was calculated for 2004 to 2006 only, using the same method. By the end of 2006, *H. axyridis* had reached the west coast of Britain, so westerly spread calculations for 2007 and 2008 were redundant.

3.2.3 Seasonal pattern

The seasonal patterns of *H. axyridis* sightings in 2005 and 2006 were examined. The data are weekly counts of the occurrence of *H. axyridis* in grid squares. For adult *H. axyridis* 10km squares were used, but for juveniles (i.e. larvae and pupae), because of a lower number of records, 1km squares were used.

Seasonal patterns were analysed using a Generalized Additive Model (GAM) in which the counts varied randomly about a smooth trend described by a cubic smoothing spline (Green & Silverman, 1994). The degrees of freedom for the fitted GAM were determined using cross-validation (leaving out each data point in turn) and minimising the cross-validation Poisson deviance. A GAM was fitted separately to data from 2005 and 2006 to describe the seasonal pattern of observations in each year. The number of sightings was generally higher throughout 2006 compared to 2005, but the GAM curves allow comparison of the shape of the seasonal pattern each year. The null hypothesis of equality of shape in 2005 and 2006 implies that curves differ by some constant factor, i.e. parallel on a log scale. The alternative hypothesis allows different curves in each year. The test-statistic is $F = \{(D_{null} - D_{alt})/(df_{null} - df_{alt})\}/ D_{alt} /df_{alt}$, where D denotes the residual deviance and df is the corresponding degrees of freedom. On the null hypothesis, the statistic follows an F distribution with $(df_{null} - df_{alt})$ and df_{alt} degrees of freedom. The analysis was performed using the statistical package Genstat 6 (Payne & members of the Genstat 6 committee, 2002).

3.3 Results

3.3.1 Abundance and rate of spread

Harmonia axyridis has spread rapidly in Great Britain (Figure 3.1).

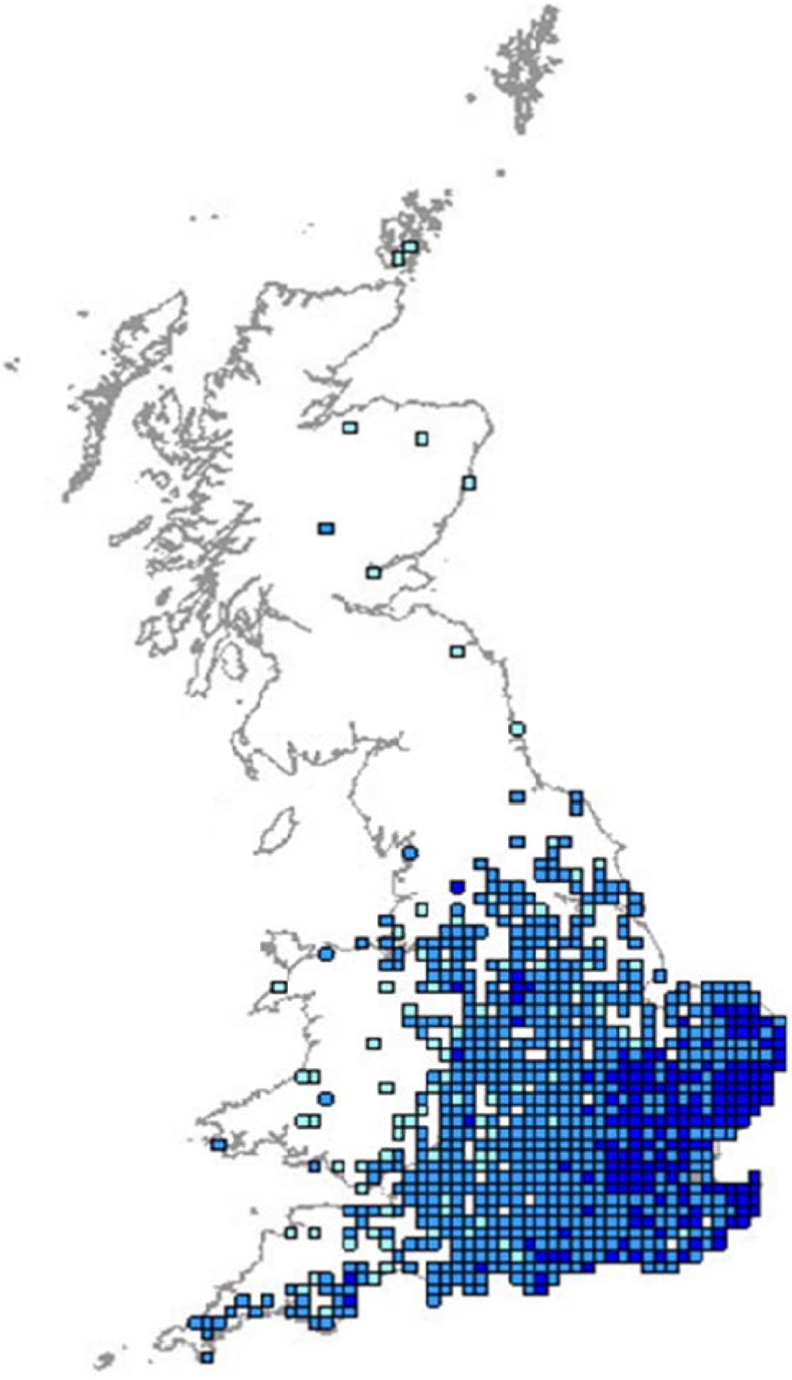


Figure 3.1. *Harmonia axyridis* occurrence in 10km squares in Great Britain from 2004 to 2008. Where a square has been recorded in more than one year class, occurrence in the earliest year class is shown (dark blue = 2004-05; medium blue = 2006-07; pale blue = 2008).

Recorded occurrence of *H. axyridis* in 10km squares increased from 51 (of which 8 had records of juveniles) in 2004 to 710 (231 with juveniles) in 2007 (Figure 3.2). The number of squares decreased to 578 (118 with juveniles) in 2008. In 2004, evidence of *H. axyridis* breeding was restricted to London and East Anglia, with juveniles recorded in a wider range of counties in 2005 (notably Kent and Derbyshire). Further evidence of breeding was recorded across south-east England, and west as far as Devon, and north as far as Yorkshire, in 2006. The most northerly breeding record was from Edinburgh (south-east Scotland) in 2007.

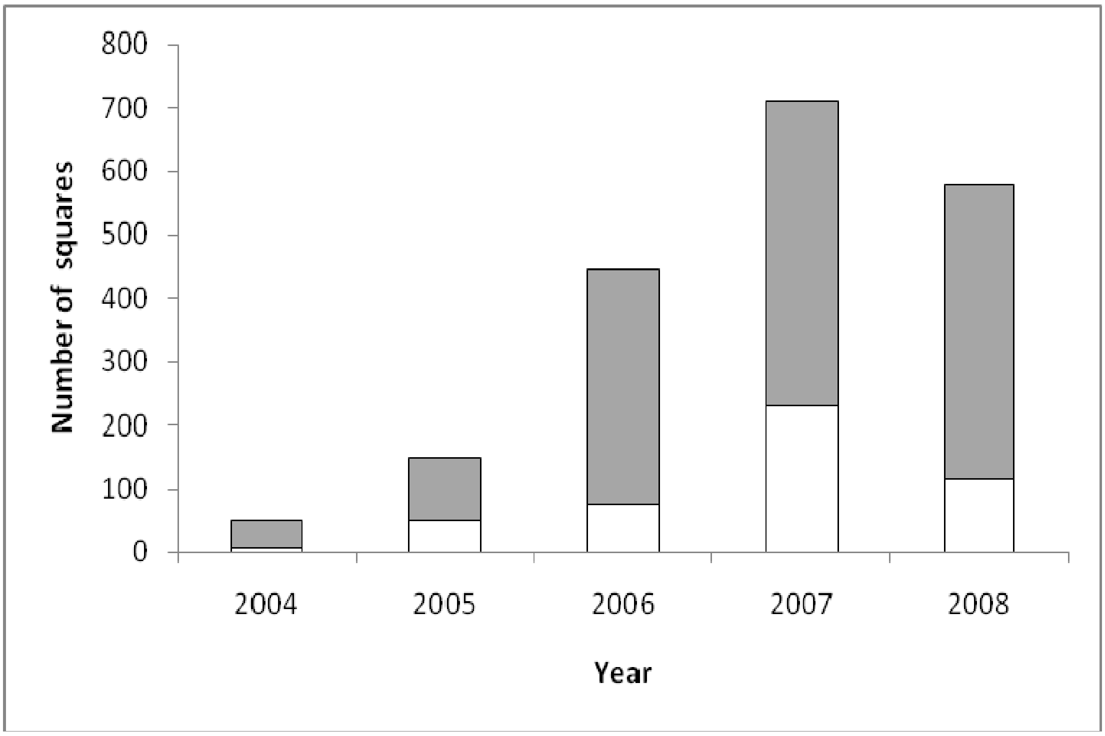


Figure 3.2. The number of 10km squares in Great Britain per year with *H. axyridis* records (squares that included juveniles shown in white).

The mean number of adults per record increased year on year, from 2.9 in 2004, to 17.3 in 2008. The trend was similar for larvae, although there was a slight decrease from 2007 to 2008 (Table 3.1).

Table 3.1. Summary of Harlequin Ladybird Survey data from Great Britain. For some large aggregations, counts of ladybirds were approximate.

Life stage	Year	No. verified records	Total no. *H. axyridis* recorded	Mean no. *H. axyridis* per record	Maximum no. *H. axyridis* recorded
Larva	2004	13	54	4.2	10
	2005	199	1942	9.8	266
	2006	187	1795	9.6	200
	2007	511	7673	15.0	1000
	2008	169	2287	13.5	200
Adult	2004	119	344	2.9	25
	2005	1045	6180	5.9	399
	2006	2825	17641	6.2	689
	2007	4569	78025	17.1	10000
	2008	1652	28561	17.3	2000

Northerly spread distances per year were: 19km between 2004 and 2005; 97km between 2005 and 2006; 98km between 2006 and 2007; 206km between 2007 and 2008; mean – 105km yr^{-1}. Westerly spread distances per year were: 107km between 2004 and 2005; 182km between 2005 and 2006; mean – 144.5km yr^{-1}.

The density of records received was highest in and around London, and was also high in parts of East Anglia and along the south coast of England, particularly in Kent, Sussex, Hampshire and Devon (Figure 3.3). There is also a notable hotspot in Derby, the only location in central-north England with a large number of verified records in the first three years of invasion (Figure 3.3).

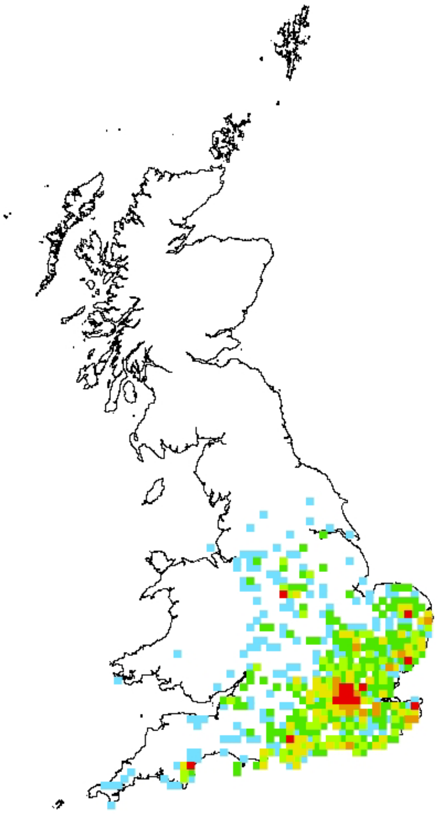


Figure 3.3. Density of *H. axyridis* records in 10km squares in Great Britain from 2004 to 2006 (red = 50+ records per square; orange = 20 to 49; yellow = 10 to 19; pale green = 6 to 9; dark green = 2 to 5; blue = 1)

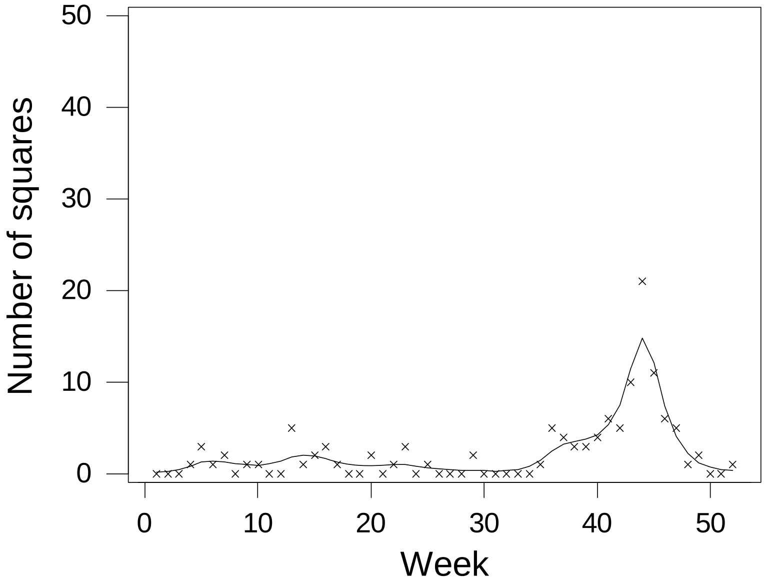
3.3.2 Seasonal pattern

3.3.2.1 Adults

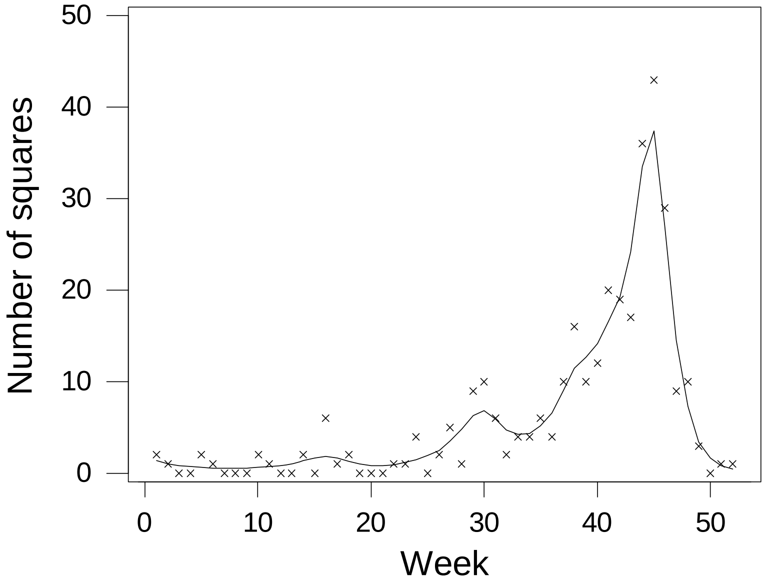
The seasonal patterns of spread of adult *H. axyridis* in 2005 and 2006 were significantly different ($F_{12,78} = 2.52$, $p = 0.007$). The main difference in the smoothed curves was during the period 23-34 weeks, where there was a seasonal peak observed in 2006, which was not observed in 2005 (Figure 3.4).

3.3.2.2 Juveniles

The seasonal patterns of spread of juvenile *H. axyridis* in 2005 and 2006 were significantly different ($F_{6,89} = 3.57$, $p = < 0.001$). The main difference is the extra peak around week 25 in 2006, and the earlier date of the autumn peak in 2006. For the fitted GAMs, the main peaks occur at week 44 (2005) and week 40 (2006) (Figure 3.5).



(a)

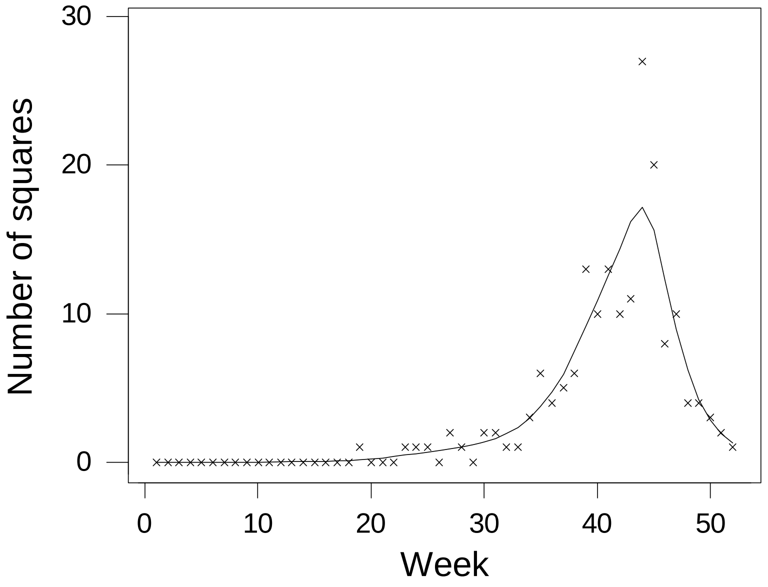


(b)

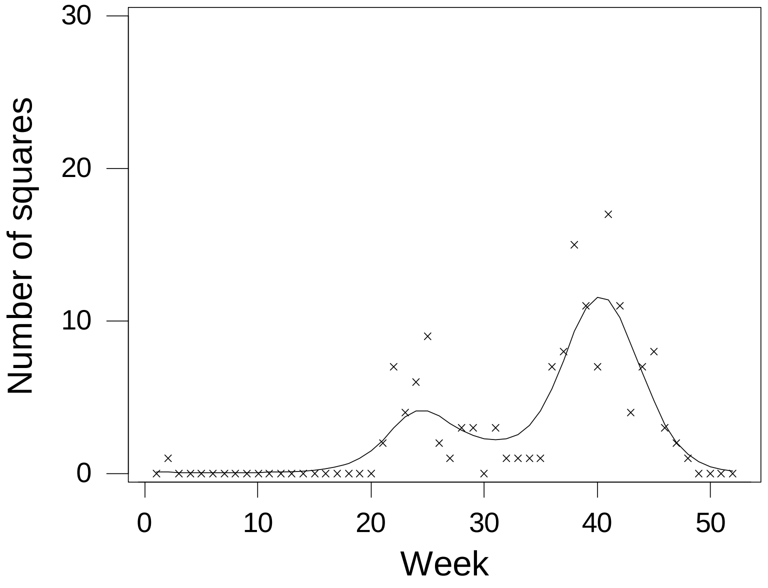
Figure 3.4. Adult *H. axyridis* occurrence in 10km squares in Great Britain with fitted GAMs and degrees of freedom (df)

(a) 2005 (df = 12)

(b) 2006 (df = 13)



(a)



(b)

Figure 3.5. Juvenile *H. axyridis* occurrence in 1km squares in Great Britain with fitted GAMs and degrees of freedom (df)

(a) 2005 (df = 6)

(b) 2006 (df = 6)

3.4 Discussion

3.4.1 Rate of spread

The rate of spread and increase in abundance of *H. axyridis* in Great Britain was dramatic. By the end of 2006 the species had spread to the west coast of England and Wales, and by the end of 2008 it had spread to the far north of Scotland. Westerly spread was faster than northerly spread by a factor of approximately 2.5, and this may partly be explained by the likelihood of repeated recruitment of *H. axyridis* from mainland Europe. Indeed, in 2006, eight of the ten most westerly 10km squares occupied were in the extreme south-west of England and close to the coast, possibly representing new recruits from abroad, rather than spread from the existing British range. This factor is presumed not to apply to the most northerly squares occupied, because of their greater distance from mainland Europe and, in most cases, their lack of proximity to the British coast. Ecological factors such as climate and habitat type are also likely to have influenced the faster westerly spread. In late October and early November 2006 there was an influx of reports of large aggregations of *H. axyridis* adults, mainly at locations close to the south coast of England. Aggregations of hundreds and in a few cases, thousands, of *H. axyridis* were observed (Brown & Roy, 2007). Whether these large aggregations represented new recruits from mainland Europe is unknown. Although there were scattered records from Scotland, there is limited evidence of establishment there, with only one record of *H. axyridis* larvae.

The most closely related species to *H. axyridis* in Great Britain is *Harmonia 4-punctata* (Pontoppidan) (Coleoptera: Coccinellidae). This species is also a relatively recent arrival to Britain, but is now regarded as a native species, having expanded its native range northwards within Europe, and is assumed to have reached Britain by natural means (Majerus, 1994a, Majerus & Kearns, 1989). It is not considered invasive. Although *H. 4-punctata* is far more habitat-specific than *H. axyridis*, and the mechanisms of spread may not be the same, a comparison of the rate of spread of the two species is enlightening. Majerus and Kearns (1989) outlined the spread of *H. 4-punctata*. It was first recorded in Great Britain in West Suffolk (East Anglia) in 1937, and based on earliest records for each vice county, took fifty years to spread west as far as Devon, in south-west England. My data show that *H. axyridis* took just two years to spread to Devon from a similar starting point in East Anglia.

In Great Britain, the northerly range expansion of some insect groups was calculated, giving figures of up to 4.2km yr^{-1} (over a 25 year period) for the most dispersive taxa (dragonflies); but these range expansions were for native species responding to climate change, rather than for invasive species (Hickling et al., 2006). There is limited quantitative data on the spread of invasive insects in Europe. The spread of the horse chestnut leafminer *C. ohridella* in Germany in the late 1990s was modelled, and the rate of spread calculated to vary between 54km yr^{-1} and 330km yr^{-1} (Gilbert et al., 2004), figures of the same order of magnitude as my calculations for *H. axyridis* in Britain. In eastern North America the rate of range expansion of *H. axyridis* was estimated at 442km yr^{-1} (McCorquodale, 1998). As it was probably confounded by many intentional releases at various locations (McCorquodale, 1998), this very fast expansion is not a fair comparison with that observed in Great Britain. Expansion was thought to be slower in other parts of North America (Koch et al., 2006).

3.4.2 Abundance and mechanism of spread

Abundance of *H. axyridis* in Great Britain increased rapidly from 2004 to 2007. The observed reduction in the number of juvenile records from 2005 to 2006 is not believed to reflect a true reduction in breeding activity. Rather, this is likely to be the effect of reduced recorder effort. In particular, recorders with the London and Essex Ladybird Survey worked diligently in 2005 to gather juvenile records, and provided 46% of all such records; this effort was reduced to 9% of the total in 2006. A 47% increase in the number of 10km squares with juvenile records from 2005 to 2006 suggests that there was in fact an increase in breeding activity.

Although there was a decline in *H. axyridis* in 2008, this was probably largely due to poor weather conditions and a knock-on effect on aphid numbers (see Chapter 5 for further discussion on this). 2008 was a year when abundance of most ladybird species in Britain was low (Majerus et al., 2009).

There is very strong demographic evidence that the spread of *H. axyridis* in Great Britain did not originate from a single immigration event, and that the species arrived at different locations at various times, and by various means. Apart from the spread (by flight) from continental Europe, some specimens of *H. axyridis* are known to have arrived in England on imported flowers from the Netherlands, and in packing cases from Canada (Majerus et al., 2006a). By 2004, *H. axyridis* had sufficient time to adapt to conditions in mainland Europe and was building-up in number and spreading fast. Crossing the English Channel to reach Great Britain was not a major step. It is a coincidence that specimens from Canada were found in Great Britain for the first time in the same year, and it is possible that it had arrived by similar mechanisms in earlier years, but failed to be noticed and failed to establish.

A separate population of *H. axyridis* was evident in Derby (central England) in 2004, which is thought to have originated from specimens arriving with produce to a supermarket in the city (Ribbands et al., 2009). Derby was not the most northerly verified location of *H. axyridis* in 2004 (a single specimen was recorded in Lancashire, but with no evidence of subsequent establishment), but is the only place outside of the south-east with multiple verified records of *H. axyridis* in each of 2004, 2005 and 2006. Large numbers of *H. axyridis* (three records each of 50+ individuals) were recorded in Derby in 2005. The colour form profile of the Derby population almost totally lacked melanics[10] in 2004 and 2005 (99.6% f. *succinea*; n = 495), in contrast to other parts of the country, where melanic specimens formed a significant proportion of the population (a mean of 20% melanic specimens were recorded in 2005). The clear difference in colour form profile strongly suggests a genetically distinct population, and further work is in progress to examine this.

Examples of invasive species in Great Britain exhibiting a ‘boom-and-bust’ population cycle are unusual, although this scenario did apply to the rhododendron lacebug *Stephanitis rhododendri* Horvath (Hemiptera: Tingidae) (Williamson, 1996). The evidence from over 20 years as an established introduced species in North America suggests that this will not apply to *H. axyridis* on either side of the Atlantic.

---

[10] Melanic: refers to a predominantly black colour form of a species. In the context of this thesis, *H. axyridis* f. *spectabilis* and f. *conspicua* are melanic.

3.4.3 Evidence of bi-voltinism

Some of the common and widespread coccinellid species in Britain, e.g. *Coccinella 7-punctata*, are limited by the requirement of a winter dormancy period before they can reproduce (Majerus, 1994a). *Harmonia axyridis* does not have this limit to population growth and may have several generations per year, five having been recorded in Asia (Wang, 1986) and four in southern Europe (Katsoyannos et al., 1997). In the cool and damp maritime climate of Great Britain, the data reveal that *H. axyridis* achieves two generations per year; there is evidence of a second generation in 2006 in the data presented here, with a summer peak (week 24) and an autumn peak (week 40), in juvenile records. Production of two generations would help to explain the observed rapid spread of *H. axyridis*.

3.5 Conclusion

Within five years of its establishment, *H. axyridis* had colonised virtually all of England, much of Wales and had a sparse but widespread distribution in Scotland, extending to the far north. I predict that the spread of *H. axyridis* in Great Britain will continue, and that the species will increase in range and abundance, especially in uncolonised areas of southern and central Scotland. To determine the impact of *H. axyridis* on native species, monitoring of ladybird populations is required in semi-natural habitats and urban habitats, both of which can support high abundance and species richness. Quantitative research at appropriate spatial and temporal scales is essential if we are to objectively assess the ecological impact of *H. axyridis* (see Chapter 5). Field studies need to focus on habitat use by this species and its interactions within the large guild of associated aphidophagous species. *Harmonia axyridis* has many traits that have ensured its status as a successful invasive alien species. The continued population expansion within and beyond its invaded range seems inevitable.

4. The ecology of *Harmonia axyridis* in Great Britain

4.1 Introduction

In both its native and introduced ranges *H. axyridis* is a habitat generalist. The species has the potential to flourish in many parts of Great Britain (Majerus et al., 2006b). Whilst *H. axyridis* has a tendency to occur in arboreal habitats (Hodek, 1973), in Europe it has also commonly been found in meadows, heathlands and riparian zones (Adriaens et al., 2008), crop systems (Colunga-Garcia & Gage, 1998, Jansen & Hautier, 2008) and reed beds (Ware et al., 2005). In part of its native range (Japan), wherever there were coccinellids present in urban trees or herbage, it was rare not to find *H. axyridis*, and usually as the most abundant species (P. Brown & C. Thomas, unpublished data).

Harmonia axyridis is a generalist aphid feeder and a large and voracious species, eating up to 65 aphids per day (Koch, 2003). In Maine, USA, aphids were substantially reduced after the establishment of *H. axyridis* (Alyokhin & Sewell, 2004). The generalist traits of *H. axyridis* fuel concern over its rapid increase in distribution and abundance (Chapters 2 and 3) and its impact on non-target species (Adriaens et al., 2003, Majerus et al., 2006b, Roy et al., 2006). Indeed, a lepidopteran diet (*Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs) increased fitness of the species compared to an aphid diet (Berkvens et al., 2008b).

Harmonia axyridis appears to be bi-voltine in its northern European invaded range, e.g. Belgium (Adriaens et al., 2008), Denmark (Steenberg & Harding, 2009b) and Great Britain (Chapter 3). In warmer European climates *H. axyridis* may produce even more generations, with evidence of three generations per year in Hungary (Marko & Pozsgai, 2009) and four per year in Greece (Katsoyannos et al., 1997). Here I re-examine the British data up to 2008 for evidence of continuing bi-voltinism.

The first European country to undertake substantial habitat surveys for *H. axyridis* was Belgium. Whilst it was found in many different habitats in that country, *H. axyridis* appeared to favour urban habitats over semi-natural habitats such as forest (although sampling biases may partly account for this) (Adriaens et al., 2008). In Belgium, just over half of the *H. axyridis* observations recorded the species on trees, with about a third on herbs and the remainder on shrubs (Adriaens et al., 2008). The Belgian data reveals that *H. axyridis* was found on 139 plant genera, most commonly on *Urtica* spp. followed by five genera mainly comprising deciduous trees (*Acer*, *Salix*, *Tilia*, *Quercus* and *Betula*) (Adriaens et al., 2008). Over 40% of *H. axyridis* were reported from the six genera specified (Adriaens et al., 2008).

The Harlequin Ladybird Survey, which I have co-ordinated from its launch in early 2005 to date, has been very successful in engaging the British public, and has generated a high volume of quality-controlled data (see Chapter 3). The opportunity existed to analyse these data from across Britain, to investigate the ecology of *H. axyridis* in part of its invaded European range. Here I present data on *H. axyridis* habitat use and seasonality in the first five years of its establishment in Great Britain, measured in different ways and at various spatial scales. Specifically, four inter-related areas of *H. axyridis* ecology are investigated. Firstly, the abundance, seasonality and activity period of *H. axyridis* in Britain is analysed, partly through data on the overwintering period of the species in buildings. Next, a landscape scale assessment of *H. axyridis* is provided by associating national land cover data with Harlequin Ladybird Survey data. Habitat use is then assessed, based on information provided with individual records. Finally, plant use by *H. axyridis* is explored.

4.2 Method

4.2.1 Ladybird data collection

Harlequin Ladybird Survey data was used for the analyses in this chapter. For full details, please refer to Section 3.2.1.

4.2.2 Weather data collection

Weather data (UK mean annual temperatures) were obtained from the UK Met Office, (www.metoffice.gov.uk/climate/uk/datasets).

4.2.3 Land use assessment

Land cover data were obtained from NERC Centre for Ecology & Hydrology, from the Land Cover Map 2000 dataset (LCM2000) (www.ceh.ac.uk/sci_programmes/BioGeoChem/LandCoverMap2000.html). LCM2000 is derived from a computer classification of satellite scenes, obtained mainly from Landsat satellites. The dominant land cover category (i.e. the land cover category with the highest percentage) from each 1km square with verified *H. axyridis* occurrence was derived from LCM2000 (Table 4.1). For each year (2004 to 2008), a list of 1km squares with *H. axyridis* occurrence was produced, and matched by square with the associated dominant land cover category. For some analyses, categories have been grouped. Thus 'Woodland' means a combination of two categories: Broad-leaved, mixed and yew woodland; and Coniferous woodland. 'Grassland' means a combination of four categories: Improved grassland; Neutral grassland; Calcareous grassland; and Acid grassland (Table 4.1).

Table 4.1. Land cover categories with definitions from Land Cover Map 2000 (LCM2000), as applied in the land use assessment of ladybirds. Categories not featuring in the ladybird analyses are shown in grey. Littoral habitats not shown. Table adapted from Land Cover Map 2000: Widespread Broad Habitats and LCM2000 Classes (http://www.ceh.ac.uk/sections/seo/documents/leaflet3.pdf).

Land cover category	LCM2000 definition
1. Broad-leaved, mixed and yew woodland	Broad-leaved woodland in stands > 5m high with tree-cover > 20%; or scrub < 5m with cover > 30%. Mixed woodland is included if broadleaved trees in conifers cover > 20%.
2. Coniferous woodland	Coniferous woodland, semi-natural and plantations, with cover > 20%, and recently felled forestry. Once felled areas are colonised by rough grass, heath or scrub, they take that category.
3. Boundaries and linear features	Larger linear features such as shelter belts or motorways.
4. Arable and horticulture	Annual crops, recent leys, freshly ploughed land, rotational setaside, and perennial crops such as berries and orchards. Once setaside is substantially vegetated with weeds or rough grass, it is included in the Improved grassland category.
5. Improved grassland	Improved grasslands in swards dominated by agriculturally 'preferred' species, generally 'improved' by reseeding and/or fertiliser treatment. May be used for agriculture or amenity. Fertile pastures with *Juncus effusus* are included. Setaside grass is included but, where possible, distinguished at the subclass level; abandoned or little-managed Improved grasslands may be confused with semi-natural swards.
6. Neutral grassland 7. Calcareous grassland 8. Acid grassland	Acid, neutral and calcareous semi-natural swards are generally not reseeded or fertiliser-treated; they are dominated by lower productivity grasses, perhaps with many herbs. Grassland management may obscure distinctions from Improved grassland. Neutral, calcareous and acid components are distinguished at subclass level using a soil 'acid sensitivity' map. Pastures with *Juncus effusus* and with semi-natural spectral-characteristics are included with acid swards.
9. Bracken	The Bracken category is, at the height of the growing season, dominated by *Pteridium aquilinum.*
10. Dwarf shrub heath	Ericaceous species and gorse forming > 25% of plant cover.
11. Fen, marsh and swamp	Vegetation which is permanently, seasonally or periodically waterlogged.
12. Bog	Bogs include ericaceous, herbaceous and mossy vegetation in areas with peat > 0.5 m deep.
13. Standing open water and canals	
14. Rivers and streams	Water bodies ≥ 0.5ha are mapped, but only the wider canals and rivers (>50m) are shown.
15. Montane	Prostrate dwarf heath, sedge and rush, moss heaths and snow bed communities.
16. Inland rock	Natural and man-made bare ground, including waste tips and quarries.
17. Built-up areas and gardens	Urban land, rural development, roads, railways, waste and derelict ground, including vegetated wasteland, gardens and urban trees. All larger areas of vegetation (≥ 0.5ha) are identified as the appropriate cover category.

4.2.4 Habitat use assessment

The term ‘habitat use’, is applied to mean *H. axyridis* occurrence in a given habitat. Whether or not any particular *H. axyridis* was actively using the habitat (e.g. for feeding, breeding or overwintering) was not necessarily known. Thus a proportion of the ladybirds may have occurred transiently in a habitat without ‘using’ it. However, the high number of records in the dataset should mask any minor distorting effects caused by this issue.

Assessments were made relating to broad habitats (e.g. buildings, gardens) where *H. axyridis* was recorded, based on comments included with records, and calculations are expressed as a percentage of total records (i.e. 10609). Approximately 65% of records included a habitat use comment of some kind. Thus the percentages are minimum figures, with the remaining 35% of records occurring in an unknown habitat. Few records from 2004 contain information on habitat, so may not be representative.

High prevalence of *H. axyridis* from buildings was used as a surrogate measure for indicating overwintering. The end of overwintering in each of 2005 to 2008 was assessed, based on the principal dip in March / April of the number of records reported from buildings. Similarly, the start of overwintering was assessed, based on the principal peak in October / November of the number of records reported from buildings. The duration of the annual activity period was calculated from the difference each year between the start and end of overwintering.

4.2.5 Plant use assessment

The term ‘plant use’, is applied to *H. axyridis* occurrence on a given plant. See comment above (Section 4.2.4) re ‘habitat use’, which also applies to ‘plant use’.

A proportion of the total 10609 records included details of one or more plants that *H. axyridis* was found on. Plant use was analysed at three levels. Firstly, plants were split into four broad categories: deciduous trees and shrubs, evergreen trees and shrubs, grass/rush/sedge/fern, and other herbaceous plants. Secondly, plants were split into families. Thirdly, they were split into genera. Plants identifiable at least to family level were reported from 1713 records.

4.3 Results

4.3.1 Ladybird abundance

Harmonia axyridis records as a percentage of all coccinellid records increased from 2.5% in 2004 to 76.9% in 2008 (Table 4.2). Overall, the percentage of records from each *H. axyridis* abundance category was: 1 (i.e. a single *H. axyridis* reported): 57.3%; 2 to 9: 23.9%; 10 to 99: 15.8%; 100+: 3.0%. The percentage of records reporting 100+ *H. axyridis* individuals reached a high of 4.7% in 2008 (Figure 4.1).

Table 4.2. 2004 to 2008 annual summary of *H. axyridis* and total coccinellid records received. Only *verified* records received by the Harlequin Ladybird Survey / UK Ladybird Survey are shown, and this also applies to all other tables and figures.

Year	Total no. records of all coccinellid species	No. *H. axyridis* records (% of total coccinellid records)	No. *H. axyridis* records from buildings (% of total *H. axyridis* records)	No. *H. axyridis* records from gardens (% of total *H. axyridis* records)	Mean no. *H. axyridis* individuals per record
2004	4963	124 (2.5)	0 (0.0)	1 (0.8)	3.4
2005	7927	1096 (13.8)	236 (21.5)	121 (10.8)	8.5
2006	8094	2897 (35.8)	1090 (37.6)	248 (8.5)	6.8
2007	7684	4747 (61.8)	1808 (38.1)	429 (8.9)	18.3
2008	2269	1745 (76.9)	727 (41.7)	190 (10.7)	17.7
Total	30937	10609 (34.3)	3861 (36.4)	988 (9.3)	

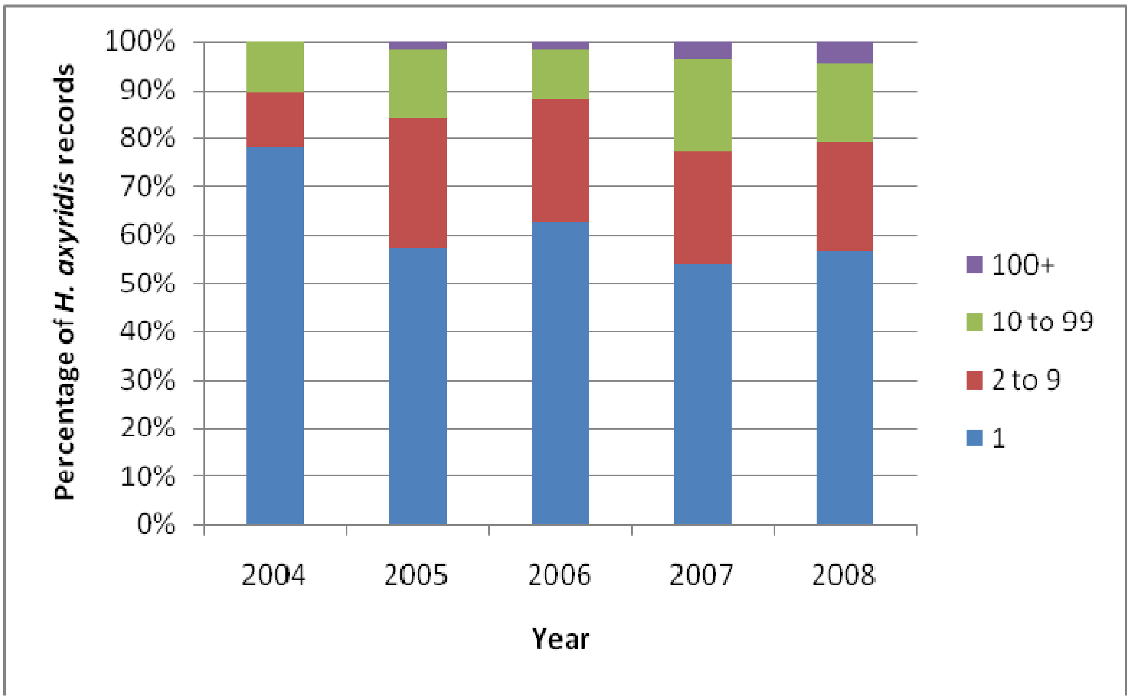


Figure 4.1. Percentage of *H. axyridis* records each year from 2004 to 2008 with: 1 (blue); 2 to 9 (red); 10 to 99 (green); 100+ (purple); *H. axyridis* individuals per record (n = 10609 records).

4.3.2 Ladybird seasonality

The annual peak month for *H. axyridis* records (all life stages) was October in each year except 2006, when the peak was in November (Figure 4.2a). The annual peak month for *H. axyridis* larval records was October in each year except 2008, when the peak was in June (Figure 4.2b).

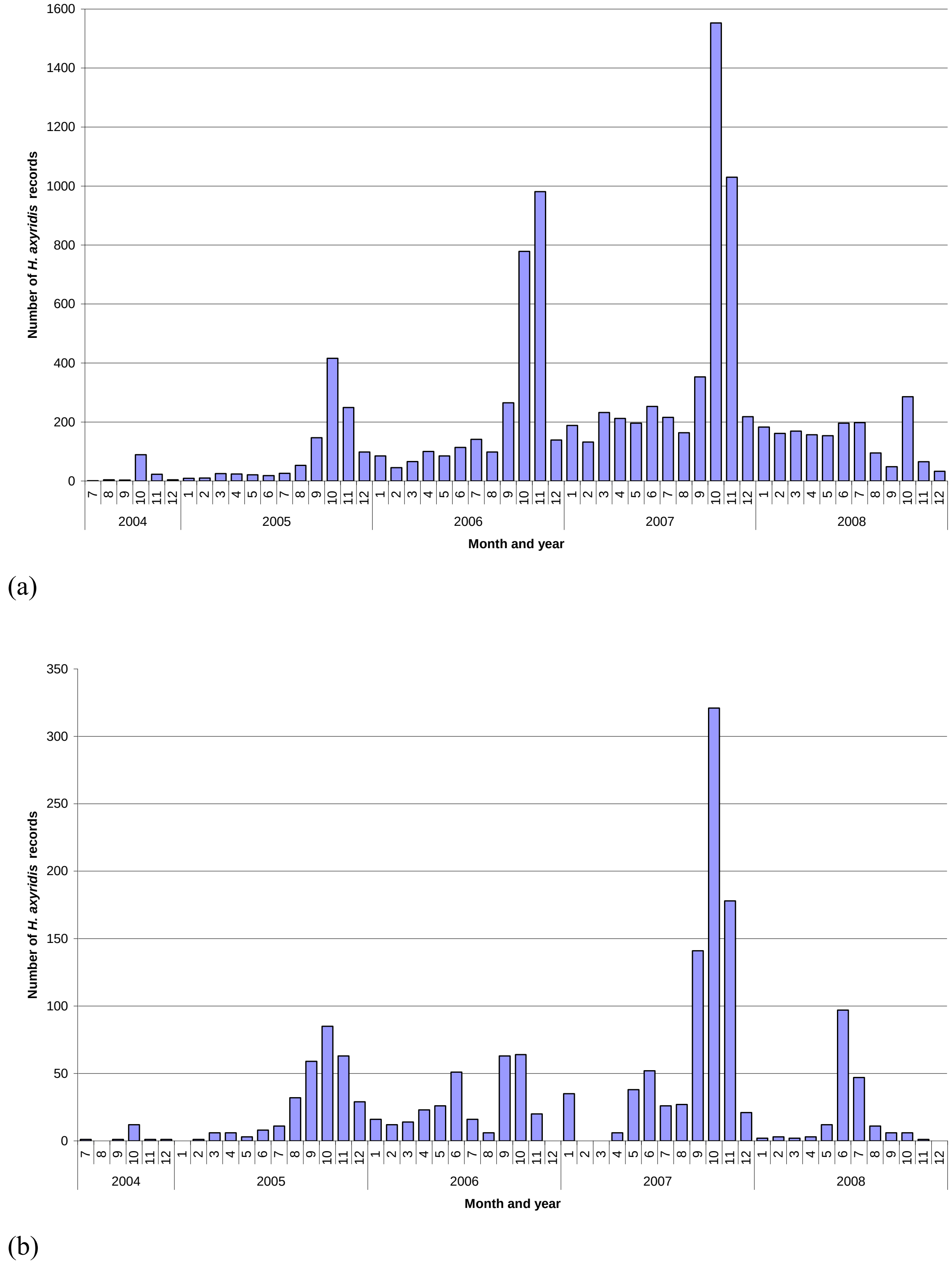
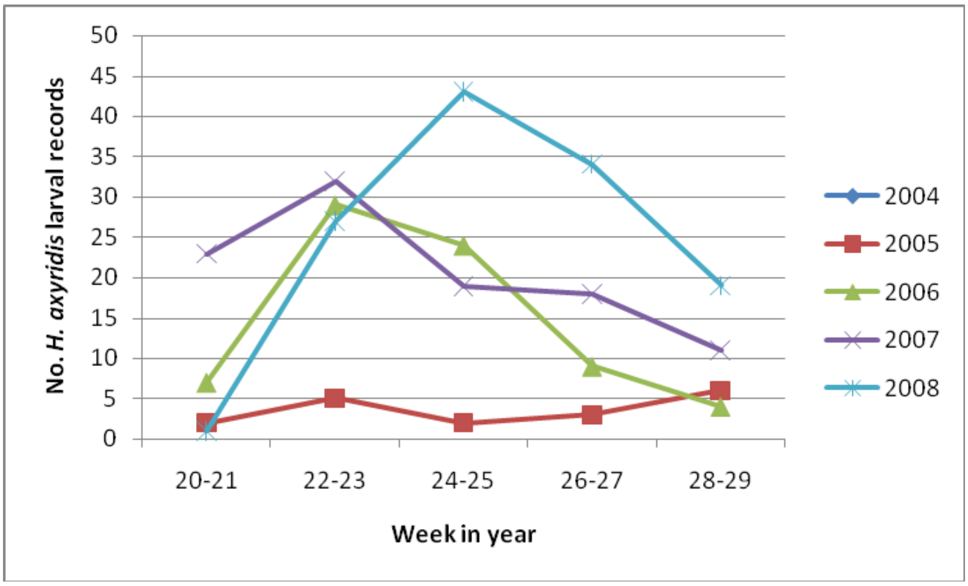


Figure 4.2. Total number of *H. axyridis* records received, by month of sighting in 2004 to 2008: (a) all life stages; (b) larvae only. Note difference in Y axis scales.

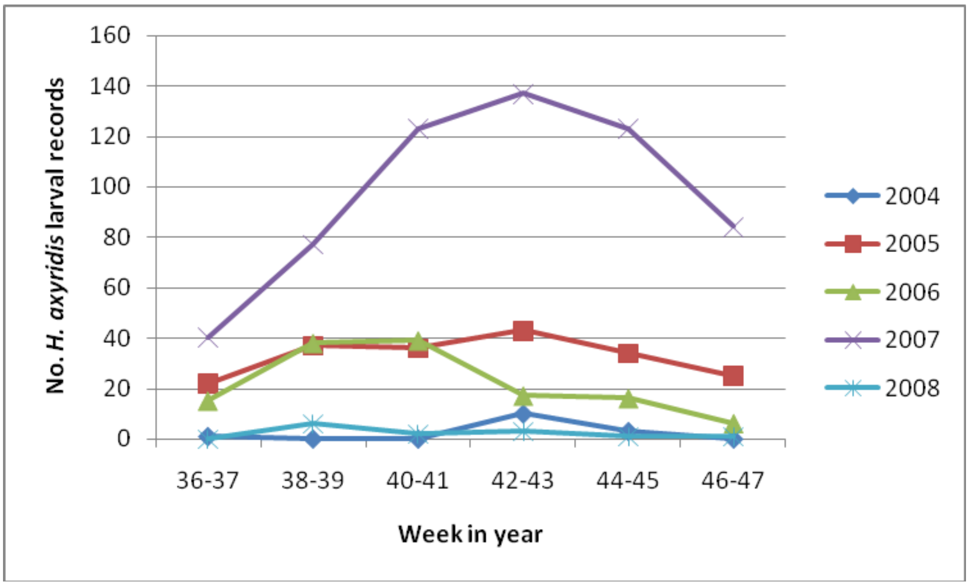
As outlined in Chapter 3, there is evidence that *H. axyridis* is bi-voltine in the UK. Examination of the two peak larval periods per year (in late spring and autumn) revealed that the spring peak in larval records came in weeks 22-23 (i.e. late May – early June) in 2006 and 2007 and in weeks 24-25 in the cooler year of 2008 (Figure

4.3a). There were few spring records in 2005 and no clear pattern is evident. 2008 was the year with highest abundance of larval records in spring.

In three of five years the autumn peak in larval records came in weeks 42-43 (i.e. mid-late October) (Figure 4.3b). The exceptions were 2006, when the peak was in weeks 40-41, and 2008 when the peak was in weeks 38-39 (although the number of autumn larvae in 2008 was very low). 2007 was the year with highest abundance of larval records in autumn.



(a)



(b)

Figure 4.3. Number of *H. axyridis* larval records in: (a) weeks 20 to 29 (late spring / early summer); (b) weeks 36 to 47 (autumn); of each year from 2004 to 2008. Note difference in Y axis scales.

4.3.3 Land use assessment

‘Built-up areas and gardens’ was the dominant land cover category with occurrence of *H. axyridis* (Figure 4.4), followed by ‘Arable and horticulture’, ‘Grassland’ and ‘Woodland’. *Harmonia axyridis* occurrence in squares dominated by ‘Arable and horticulture’ was quite consistent between years. However, from 2005 to 2008, occurrence in ‘Built-up areas and gardens’ decreased each year, from 70.2% in 2005 to 53.4% in 2008. Conversely, occurrence in ‘Grassland’ increased year on year, from 6.0% in 2005 to 20.4% in 2008. Occurrence in ‘Woodland’ was greater in later years (2006-8 mean = 5.6%) than in earlier years (2004-5 mean = 2.5%).

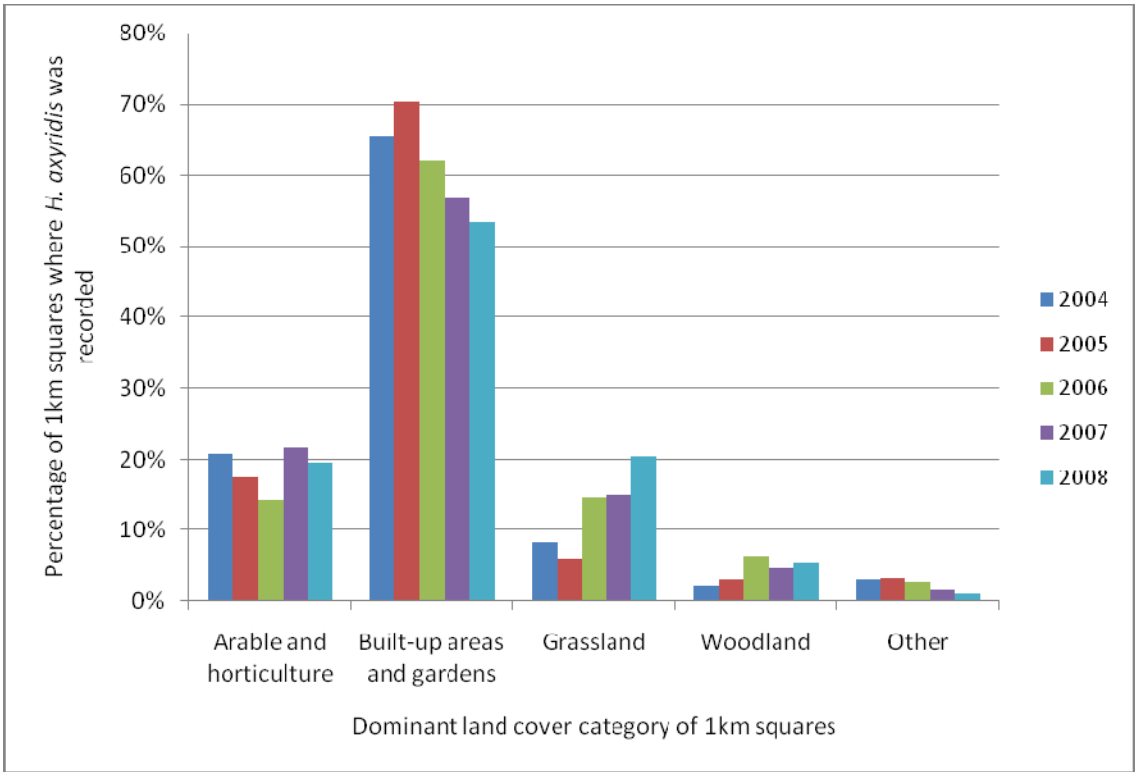


Figure 4.4. Annual percentage over time (2004 to 2008) of 1km squares where *H. axyridis* was recorded, by dominant land cover category. ‘Woodland’ means a combination of two categories: Broad-leaved, mixed and yew woodland; and Coniferous woodland. ‘Grassland’ means a combination of four categories: Improved grassland; Neutral grassland; Calcareous grassland; and Acid grassland. Land cover data from NERC Centre for Ecology & Hydrology, Land Cover Map 2000 dataset.

Aggregating the data from all years, most of the occupied squares dominated by grassland were in the ‘Improved grassland’ land cover category, representing 11.7% of all squares, with fewer in ‘Calcareous grassland’ (2.1%), ‘Neutral grassland’ (1.3%) and ‘Acid grassland’ (0.2%) (Figure 4.5). Most of the occupied squares dominated by woodland were in the 'Broadleaved, mixed & yew' category (4.8%), as opposed to 'Coniferous woodland' (0.3%) (Figure 4.5).

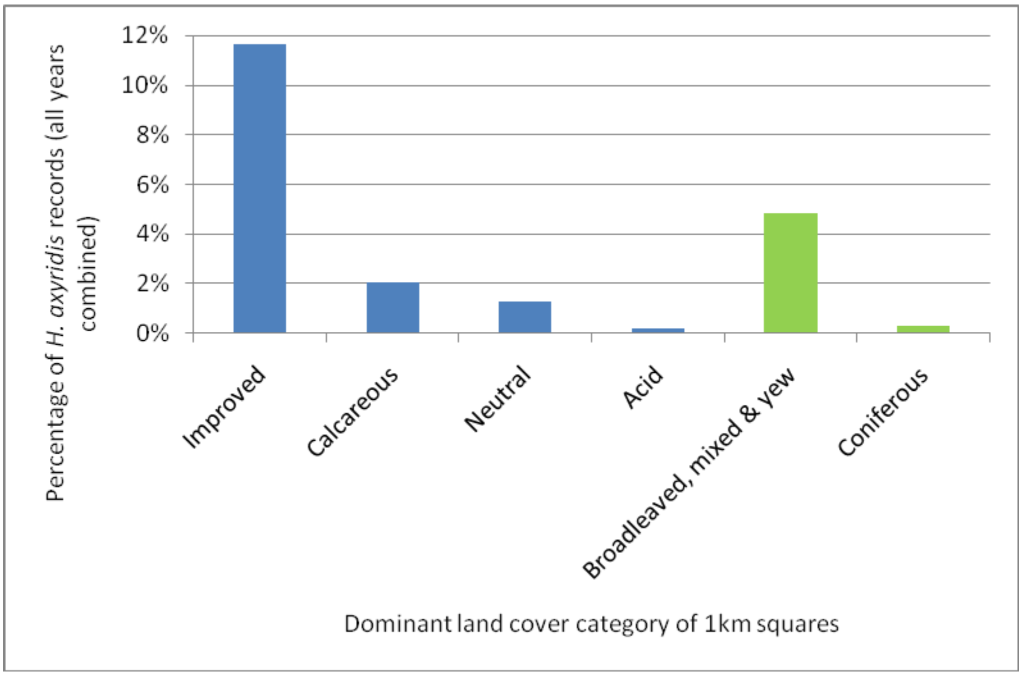


Figure 4.5. Percentage of *H. axyridis* records (with all years combined from 2004 to 2008) in grassland (blue bars) and woodland (green bars) dominant land cover categories. Land cover data from NERC Centre for Ecology & Hydrology, Land Cover Map 2000 dataset.

4.3.4 Habitat use assessment

4.3.4.1 Records from buildings and gardens

36.4% of records reported *H. axyridis* from buildings (Table 4.2). No *H. axyridis* were reported from buildings in 2004 (but see note in Section 4.2.4) and low numbers in 2005. However, from 2006 to 2008 a fairly consistent percentage (mean = 39.1%; minimum = 37.6%; maximum = 41.7%) was recorded from buildings. The mean percentage of records from gardens was 9.3% (Table 4.2).

The association of *H. axyridis* with buildings showed a clear seasonal pattern, with 89.4% of such records occurring in the six month period from October to March (Figure 4.6). Conversely, gardens were mainly used from March to November (Figure 4.6).

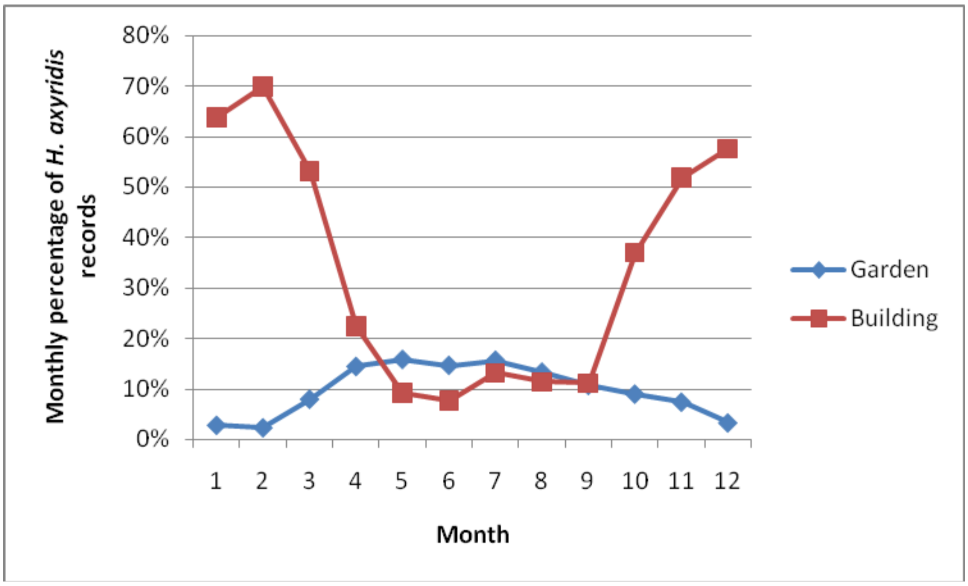


Figure 4.6. Monthly percentage of *H. axyridis* records reported from buildings and gardens (with all years combined from 2004 to 2008).

4.3.4.2 Overwintering and annual activity based on presence in buildings

Based on the difference between the start and end of overwintering, *H. axyridis* had a mean activity period of 30.75 (minimum = 26; maximum = 34) weeks per year (Table 4.3). There was a very strong positive relationship ($R^2 = 0.977$) between the length of activity period per year and the UK mean temperature per year. Differences in activity period based on latitude were explored, and whilst no clear pattern was observed, this was possibly because of an insufficient amount of data from northern Britain.

Table 4.3. Annual dates for the end and start of *H. axyridis* overwintering, with associated annual activity durations and UK mean temperatures. Each annual activity period was calculated as the number of weeks between the end of overwintering in spring and the start of overwintering the following autumn. Annual overwintering start dates were based on the main autumn peak in records reported from buildings. Annual overwintering end dates were based on the main spring trough in records reported from buildings.

Year	End of overwintering date	Start of overwintering date	Duration of activity period (no. weeks)	UK mean annual temperature (°C)
2005	29 March	24 October	30	9.45
2006	13 March	06 November	34	9.73
2007	12 March	29 October	33	9.59
2008	07 April	07 October	26	9.05

4.3.4.3 *Harmonia axyridis* attraction to light sources

Early on in the survey it was apparent that *H. axyridis* was attracted to moth traps and other light sources. 173 records (1.6% of total) were received from moth traps from 2004-8, with a further 237 (2.2% of total) records reporting *H. axyridis* on or close to artificial lights or lamps, suggesting that the species had a tendency to be attracted to artificial light.

4.3.5 Plant use assessment

4.3.5.1 Plant types

Recorded occurrence of *H. axyridis* on evergreen trees and shrubs was fairly low, ranging from 7% to 17% per year (Figure 4.7). Occurrence on herbaceous plants was more common (15% to 36%) and with signs of an increasing trend. *Harmonia axyridis* most commonly occurred on deciduous trees and shrubs (42% to 77%) (Figure 4.7). The mean percentage occurrence per year from 2005 to 2008 was: deciduous trees and shrubs – 55.9%; herbaceous plants – 29.1%; evergreen trees and shrubs – 11.2%; grass/rush/sedge/fern – 3.7%.

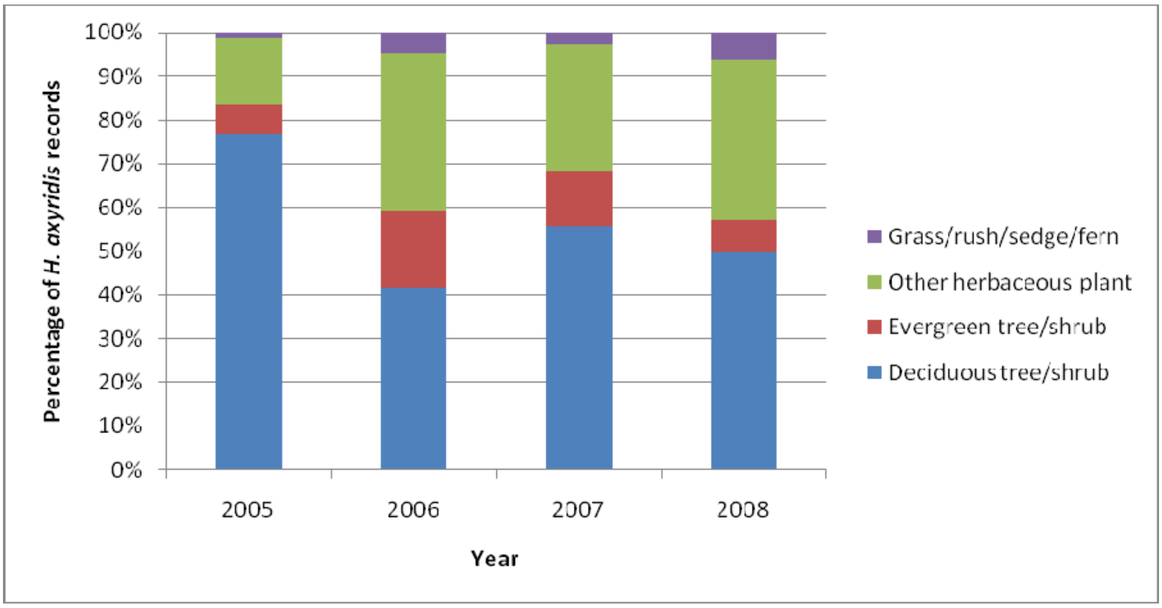
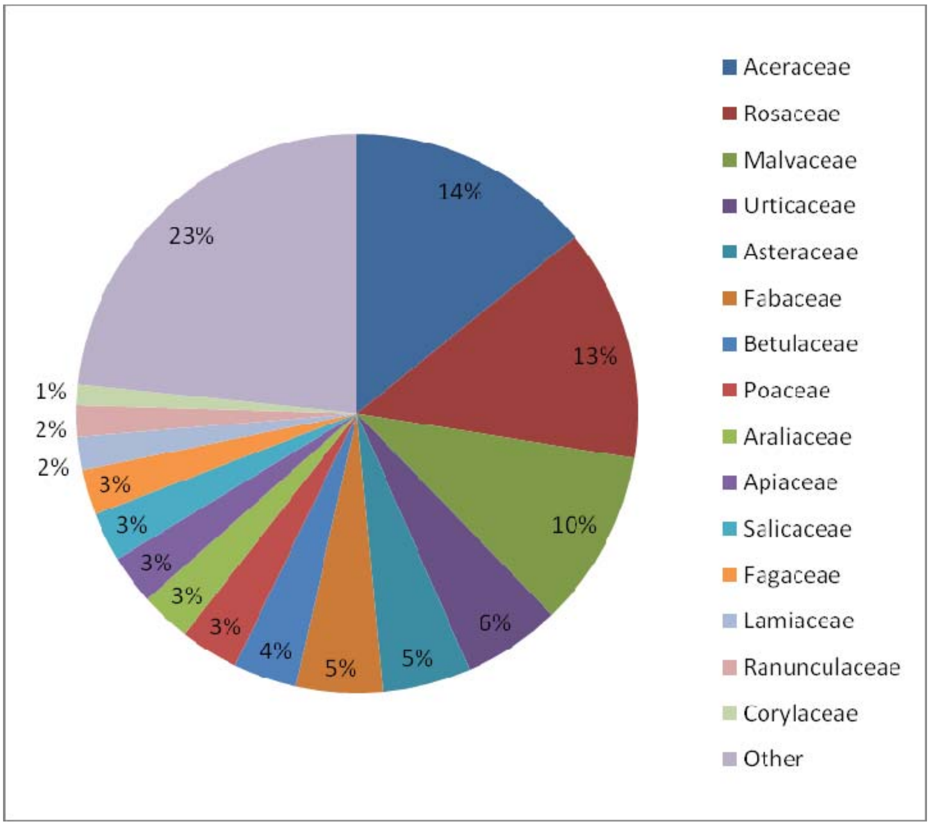


Figure 4.7. Percentage of *H. axyridis* records in four plant type categories from 2005 to 2008. 2005: n = 226; 2006: n = 373; 2007: n = 735; 2008: n = 379.

4.3.5.2 Plant families

Harmonia axyridis was recorded from a total of 75 families of flowering plant, three families of coniferous plant and at least one family of fern (ferns were generally not fully identified). 53.5% of all such records were reported from the six most represented plant families (Aceraceae, Rosaceae, Malvaceae, Urticaceae, Asteraceae and Fabaceae) (Figure 4.8a). *Harmonia axyridis* larvae were recorded on a total of 50 plant families, with 50.2% of all such records reported from the three most represented plant families (Aceraceae, Malvaceae and Rosaceae) (Figure 4.8b).

(a)



(b)

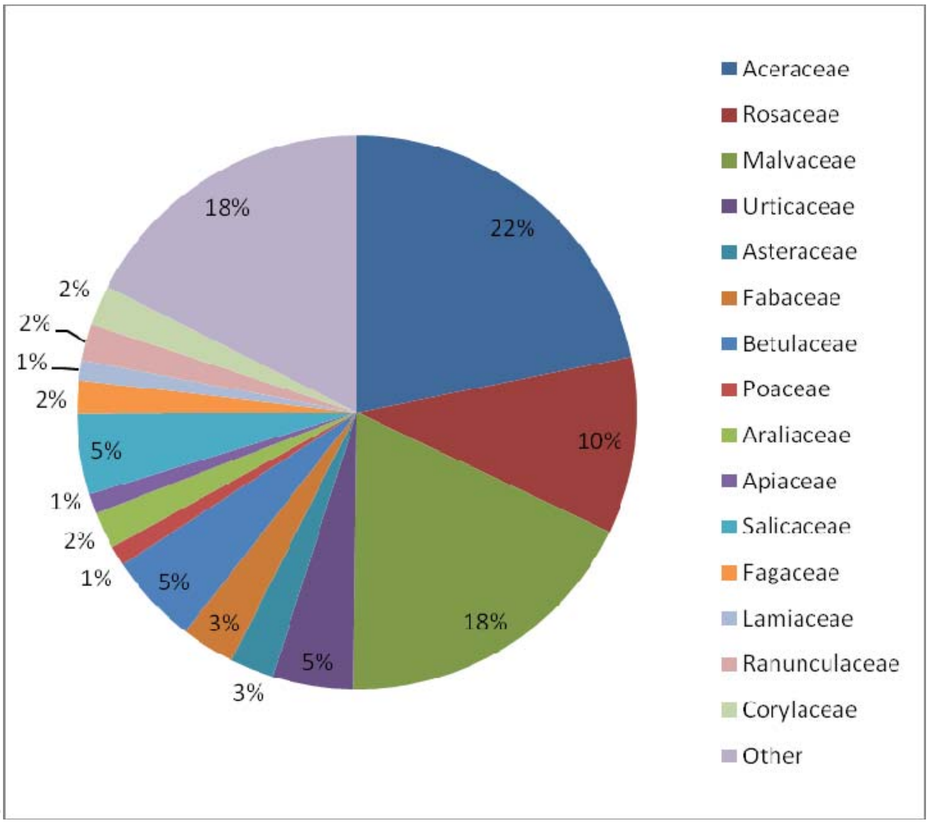


Figure 4.8. Proportion of records of *H. axyridis* associated with plant families (with all years combined from 2004 to 2008): (a) all life stages (n = 1713); (b) larvae only (n = 514).

4.3.5.3 Plant genera

The dominant plant genera in terms of *H. axyridis* use were *Acer* and *Tilia* (Table 4.4). These genera show a clear seasonal pattern of use, with an autumn peak for both (September for *Tilia* and October for *Acer*) and low usage from April to July (Figure 4.9a). No clear trend was evident for the other two main genera principally comprising deciduous trees, i.e. *Betula* (Betulaceae) and *Salix* (Salicaceae) (Figure 4.9b). The Rosaceae genera (*Prunus*, *Rosa* and *Rubus*) were primarily used from April to August (Figure 4.9c). Conversely, *Hedera* (Araliaceae) (the sole plant recorded for this genus being English ivy, *Hedera helix*) was primarily used from September to March (Figure 4.9d). *Urtica* (the sole plant recorded for this genus being stinging nettle, *Urtica dioica* L. (Urticaceae)) was primarily used in spring and autumn (Figure 4.9d).

Table 4.4. The ten most recorded plant genera on which *H. axyridis* was found (with all years combined from 2004 to 2008).

Rank	Genus	Total records (2004-8)	Family
1	*Acer*	243	Aceraceae
2	*Tilia*	166	Malvaceae
3	*Urtica*	97	Urticaceae
4	*Rosa*	64	Rosaceae
5	*Prunus*	58	Rosaceae
6	*Betula*	55	Betulaceae
7	*Phaseolus*	51	Fabaceae
8	*Hedera*	49	Araliaceae
9	*Rubus*	45	Rosaceae
10	*Salix*	44	Salicaceae

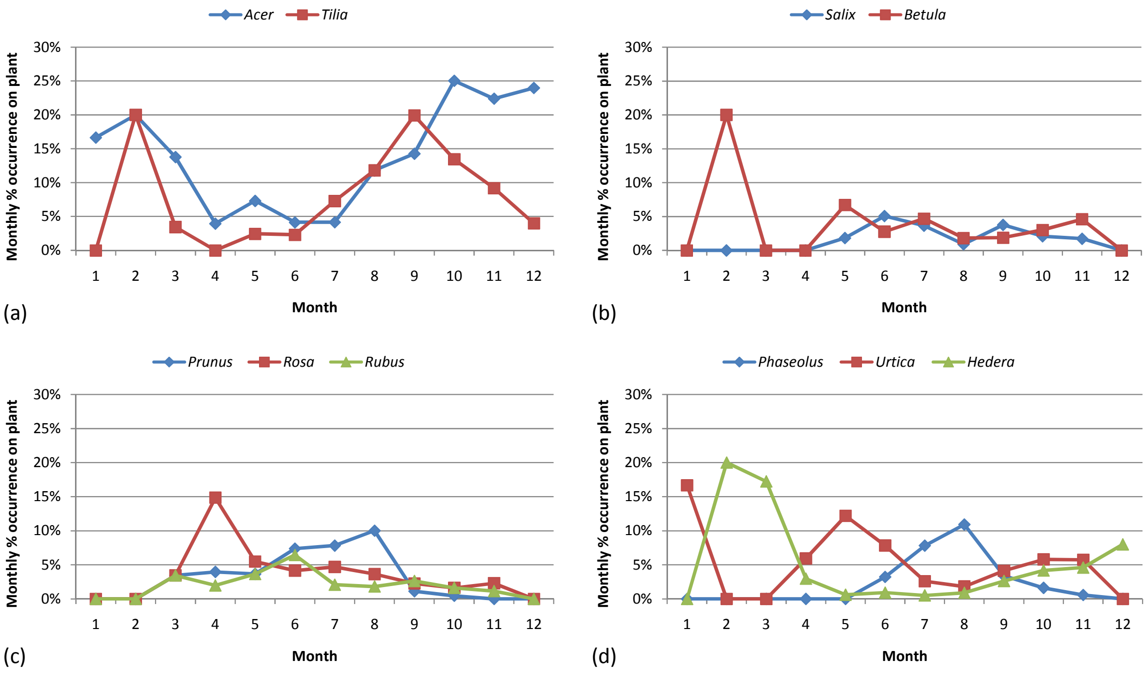


Figure 4.9. Seasonal pattern of records of *H. axyridis* found on certain plant genera, assessed as a percentage per month of all records with plant data (with all years combined from 2004 to 2008): (a) *Acer* and *Tilia*; (b) *Salix* and *Betula*; (c) *Prunus*, *Rosa* and *Rubus*; (d) *Phaseolus*, *Urtica* and *Hedera*.

4.4 Discussion

These data show the increasing abundance and dominance within the coccinellid assemblage of *H. axyridis* during the first five years of the invasion process in Great Britain, and provide insights into the ecology and habitat use of the species. The volume and spatial scale of the coccinellid data provided by the UK Ladybird Survey is very large, with data quality strictly controlled. In addition, access to national land cover data at 1km resolution enabled dominant habitat types used by *H. axyridis* to be identified across Great Britain.

Harmonia axyridis was observed in progressively larger aggregations over time (mean of approximately 18 beetles per record in 2007 and 2008; cf. less than nine beetles per record in 2005 and 2006). The proportion of the records reporting large numbers (>100) of *H. axyridis* together, also increased over time. These data clearly show increasing abundance of *H. axyridis* during the first five years of invasion. Although the number of *H. axyridis* records decreased in 2008, the species' proportion of total coccinellid records increased throughout the five year period (records of all species were low in 2008, in what was the coolest year in Great Britain during the study period).

Each year, the annual peak of *H. axyridis* total records occurred in autumn. The same applied to *H. axyridis* larval records in all years except 2008. In that year, there was a large June peak in larval records but the usual autumn peak was absent. Peaks in *H. axyridis* larvae were observed in May/June and October in Oregon, USA (LaMana & Miller, 1996), and April/May and October in southern France (Ongagna et al., 1993). My results for 2008 may be explained by poor weather and the resultant low aphid supply negatively impacting on the second *H. axyridis* generation of the year. In Japan *H. axyridis* is generally bi-voltine (Osawa, 2000), with larval peaks occurring in May (Osawa, 1992, Sakurai et al., 1993) and October (Sakurai et al., 1993). However, sometimes three generations occur; e.g. one or rarely two generations in spring and one in autumn, in central Japan (Sakurai et al., 1993). In Britain some adults from the second generation would have had sufficient time to feed and build up fat reserves prior to overwintering. However, the abundance of larvae very late in the year in most years in Britain may be taken as a sign of incomplete adaptation; in the British climate (which is colder than many parts of the native range, e.g. much of Japan), many of these larvae would have been unlikely to complete their development before winter, and hence would not have survived to reproduce. Similarly, in Denmark, small larvae were present in November, and live pupae in November did not survive the winter (Steenberg & Harding, 2009a).

The observed proportional decrease of *H. axyridis* in built-up areas between 2005 and 2008, and the corresponding proportional increase of occurrence in grassland (and to some extent woodland), suggest that the species increasingly used semi-natural, rather than urban, habitats. Although much of the grassland use was associated with 'Improved grassland' this category represents the great majority of all grassland found in rural and urban parts of the UK (UK Biodiversity Action Plan - www.ukbap.org.uk/ukplans.aspx?id=50). A fairly constant proportion (around 20%) of *H. axyridis* records came from arable land (a land type that represents a similar percentage of the total land area of Great Britain (Defra - https://statistics.defra.gov.uk/esg/quick/agri.asp). Thus, arable land was an important habitat for *H. axyridis*; when the density of human population in arable, as opposed to urban, squares (see below) is taken into account, 20% is very likely an under estimation of the use of arable land by *H. axyridis*.

Few woodland records came from coniferous woodland, compared to broadleaved and mixed woodland. In contrast, in Belgium, *H. axyridis* was commonly recorded on pine trees (Adriaens et al., 2008). Whilst *H. axyridis* appeared to fare less well on conifers in Great Britain (Chapter 5), the low reported occurrence is partly because there is relatively little coniferous woodland in south-eastern Britain, where *H. axyridis* first invaded. In addition, where large areas of coniferous woodland do occur in south-eastern Britain (e.g. the Breckland area of East Anglia), there is generally low human population density. The land use analyses should not be taken to represent absolute occurrence of *H. axyridis* in the different land types; clearly, because of the way the data were collected, there was a bias towards urban areas (i.e. because of human population density, an urban 1km square was more likely to generate a record of *H.*

axyridis than a rural square, other variables being equal). However, the changes over time are meaningful, as the data were collected consistently throughout.

Over a third of all records were from buildings, most of these occurring between October and March (overwintering period). The annual activity period in Great Britain, based on lack of occurrence in buildings, was very strongly related to mean annual temperature, and varied from 26 weeks in the coolest year to 34 weeks in the warmest. The overwintering period of *H. axyridis* in Britain appears to be broadly similar to that in its native range, e.g. Japan (Obata, 1986, Watanabe, 2002), although may start earlier than in some native areas; *H. axyridis* was reported flying to overwintering sites in late November in Gifu, central Japan (Sakurai et al., 1993).

The data show that *H. axyridis* was increasingly found on herbaceous plants. Together with the observed increasing occurrence in squares dominated by grassland, a trend emerged towards low herbaceous vegetation and away from trees. This could be taken as a sign of adaptation to local conditions and use of additional aphid species, or may have been caused by population pressure in the trees (i.e. the carrying capacity of the trees was reached). Just over half of all *H. axyridis* records with the associated plant family recorded came from six plant families (Aceraceae, Rosaceae, Malvaceae, Urticaceae, Asteraceae and Fabaceae). Further, about half of the larval records were from just the first three of these families, indicating a strong preference that was presumably aphid-induced.

The data on the genera of plants used by *H. axyridis* have strong similarities to that from other European countries, particularly Belgium. For example, the top four genera in Belgium (*Urtica*, *Acer*, *Salix* and *Tilia*) represented 31.7% of all *H. axyridis* records (Adriaens et al., 2008), whilst the same four genera represented 32.2% of the British records. Six of the ten most used plant genera in Belgium are in the British top ten. In Belgium, *U. dioica* was an important plant for *H. axyridis* (Alhmedi et al., 2006) and its use was higher than in Britain, whereas that of *Acer* spp. was lower (Adriaens et al., 2008). This may be due to sampling effects, or may be a sign that herbaceous vegetation (*Urtica*) was increasingly used in Belgium, where the invasion by *H.*

axyridis was several years ahead of Britain. Although there is much less data available, early results on plant use by *H. axyridis* in Denmark are similar to those from Britain (Steenberg & Harding, 2009b) and there is some similarity between my results and preliminary data from Italy (Burgio et al., 2008) and Hungary (Marko & Pozsgai, 2009).

Some seasonal differences in the use of plants by *H. axyridis* in Britain are evident. Whilst there is no corresponding aphid data here, it is reasonable to assume that some of the observed differences were influenced by the seasonality of aphid species on particular plants; indeed, this was observed for native coccinellids on *U. dioica* in Britain (Zhou et al., 1994). However, some plants may have been favoured for other reasons; for example, use of *H. heli*x corresponded to the overwintering period and was possibly used as an overwintering site and/or late season nectar source; *H. helix* flowering occurs between September and November in Britain, and it is an important autumn nectar source for many insects (Jacobs et al., 2010). Similarly, the use of *Acer* spp. and *Tilia* spp. may be connected not only with a late-season aphid supply, but also with overwintering, as observed in Denmark (Steenberg & Harding, 2009b).

To summarise, the survey data presented provide useful evidence on the seasonal dynamics of *H. axyridis* habitat use over a large spatial and temporal scale. From this it is possible to present a hypothesis of *H. axyridis* seasonal dynamics at a landscape scale across Great Britain, accepting that there will be much spatial variation. On emergence from over-wintering, *H. axyridis* was not only using arboreal habitats, but also low vegetation, because of the presence of early season aphids on some herbaceous plants (e.g. *U. dioica*) and/or alternative food supplies such as nectar and pollen. These plants could be in field margins, woodland edges or urban areas (e.g. gardens) close to overwintering sites. Some *H. axyridis* then used arable field crops or herbaceous garden plants in the summer. The first generation could be produced in any of the habitats mentioned. In late summer the species tended to move to deciduous trees, as in Italy (Burgio et al., 2004), and produce the second generation, which either stayed in trees for overwintering, or more often, moved to nearby buildings, forming large overwintering aggregations. Osawa (2000) found that *H. axyridis* could make effective use of patchy habitats, and the habitat mosaic in Great Britain means that the hypothesised seasonal movements above could all occur in a relatively small area (e.g. 1km square). However, depending on food availability, some *H. axyridis* and their offspring probably stayed in a single habitat patch of even smaller area, at least until dispersal to overwintering sites. This would perhaps principally have occurred in arboreal habitats, especially those with higher diversity, which would likely increase the seasonal availability of food. In a British study, native ladybirds were relatively common on trees in May, but not in June and July, when they tended to move to grassland and woodland edge habitats (Leather et al., 1999), but as with *H. axyridis* on potato crops in Belgium (Jansen & Hautier, 2008), not in synchrony with aphid supplies. In the USA *H. axyridis* exhibited high mobility and dispersed in and out of forest patches throughout the growing season; and landscapes with more forest supported more *H. axyridis* in the soybean system (Gardiner et al., 2009). My data reinforce that *H. axyridis* is a highly adaptable species that is able to utilise a wide range of habitats in its introduced range.

4.5 Conclusion

These data show the increasing abundance and dominance of *H. axyridis* during the first five years of the invasion process in Great Britain. Evidence of bi-voltinism in *H. axyridis* (Chapter 3) is reinforced, and each year, the annual peak in records of the species occurred in autumn. The data indicate that although *H. axyridis* was very common in urban habitats, it increasingly used semi-natural habitats. In addition, whilst the species was most common on deciduous trees, it was increasingly found on herbaceous plants. Aceraceae, Rosaceae and Malvaceae were the dominant plant genera used by *H. axyridis*, especially for breeding.

Collection of data as presented in this chapter is ongoing. Additional detailed work would be useful at a local scale, to investigate habitat use and the seasonal dynamics of *H. axyridis*. Mark-recapture studies can provide useful data on the details of habitat use at a small spatial scale (Osawa, 2000). The use of radio-tracking to detect insect movements is not new (Hayashi & Nakane, 1989) and this or other hi-tech detection techniques could be used to track *H. axyridis* movements and habitat use. Vertical-looking radar systems have recently been used to assess insect (including bee, butterfly, moth and carabid) movements at increasing resolution (Riley et al., 2007) and in the future may be used in research on coccinellid dispersal and habitat use. Work has started on modelling *H. axyridis* in relation to aphids, other coccinellids and climate (Comont et al., in press) and will provide insights applicable to other invasive taxa.

5. Decline in native ladybirds in response to the arrival of the harlequin ladybird *Harmonia axyridis*: early evidence from eastern England

5.1 Introduction

Harmonia axyridis may cause negative impacts on native ladybirds through various mechanisms, including competition for prey, IGP and disease transmission. The breadth of prey range of *H. axyridis* (Koch, 2003) means that it may affect the food supply of a wide range of both generalist and specialist ladybirds in Great Britain. In Maine, USA, aphids were substantially reduced after establishment by *H. axyridis* in potato crops (Alyokhin & Sewell, 2004). The current study investigates changes to species assemblages in arboreal habitats, including deciduous and coniferous trees. Coniferous trees provide important habitats for some of the specialist ladybirds found in Britain (Majerus, 1994a), e.g. *Anatis ocellata* (L.) (Coleoptera: Coccinellidae), *Myrrha 18-guttata* (L.) (Coleoptera: Coccinellidae), *Aphidecta obliterata* (L.) (Coleoptera: Coccinellidae), *Myzia oblongoguttata* (L.) (Coleoptera: Coccinellidae) and *Harmonia 4-punctata*. The aphidophagous species occurring on deciduous trees tend, to a greater or lesser extent, to be more generalist in terms of habitat specificity and diet (Majerus, 1994a). For example, *Adalia 2-punctata*, *Propylea 14-punctata* and *Coccinella 7-punctata* occur on a wide range of deciduous trees, plus herbage including nettles, and *Adalia 10-punctata* (L.) (Coleoptera: Coccinellidae) commonly occurs on hedgerows as well as on deciduous trees (P. Brown, personal observation). Effects of the arrival of *H. axyridis* on these and other species are explored. Majerus (2008) presented the first evidence of decline in a population of *A. 2-punctata* close to the study area, based on long-term overwintering records.

IGP can be an important factor shaping changes in species assemblage structure (Polis & Holt, 1992). This will depend on the degree of niche overlap (i.e. temporal and spatial co-occurrence) of the species at different life stages, and the relative strengths (i.e. defence and attack capabilities) of the species that encounter each other. So, the species most at risk of suffering negative impacts are those which are 'weak' and have a high degree of niche overlap with a 'strong' invading species such as *H. axyridis*. Many studies have investigated IGP involving *H. axyridis* and other ladybirds and have found asymmetric advantage in favour of *H. axyridis* (Ware & Majerus, 2008, Soares & Serpa, 2007, Burgio et al., 2005).

Harmonia axyridis is more resistant to one of the important fungal pathogens that affect coccinellids, *Beauveria bassiana* (Roy et al., 2008b, Cottrell & Shapiro-Ilan, 2003), and it is possible that, by acting as a vector, *H. axyridis* could enhance disease transmission of this and other pathogens.

Studies in North America have shown declines in native ladybirds in response to the arrival of *H. axyridis* (Colunga-Garcia & Gage, 1998, Michaud, 2002), *C. 7-punctata* (Elliott et al., 1996, Evans, 2004, Turnock et al., 2003, Turnock et al., 2003) or both (Alyokhin & Sewell, 2004, Brown & Miller, 1998). This chapter aims to take the ecological study of *H. axyridis* in Great Britain (Chapter 4) a step further, by looking at detailed changes to the ladybird community structure at a local scale, before and after the arrival of *H. axyridis*. The specific aims of the study were to: (i) assess changes over a three year period in ladybird species assemblages at sites representing a range of habitat types suitable for *H. axyridis* in eastern England; (ii) assess the impact of *H. axyridis* and other factors (weather and prey availability) on native ladybirds.

The study was initiated at the start of the invasion process of *H. axyridis* in the study area. *Harmonia axyridis* was first recorded in eastern England in 2004 and there were three records in Cambridgeshire in that year (Brown et al., 2006). Whilst the species was present in the study area in 2006 (year 1 of this study), it was not abundant and would have had little if any impact within the coccinellid assemblage to that point.

5.2 Method

5.2.1 Ladybird surveys

Surveys were carried out from 2006 (year 1) to 2008 (year 3) at eleven sites representing six different habitat types in Cambridgeshire and Suffolk, eastern England (Table 5.1). The target time of day for surveys was between 1000 and 1600 hours, and target weather dry and preferably sunny, with air temperature of at least 14°C (achieved for 87% of surveys - the remainder carried out when air temperature was 10.5°C to 13.5°C, most such surveys being early or late in the year). The surveys were conducted nine times at each site per year between April and October (one per month, except two in both May and June). At sites A to D and H to K each survey was of 20 minutes duration. Sites E to G were larger and more diverse and the surveys were of longer duration (approximately one hour), thus the data from them have been time-adjusted to allow direct comparison with the other sites. On occasions when high numbers of ladybirds were found, additional time for identification was added, so that a standard sampling effort was maintained. Thus when over ten larvae were recorded, an extra minute per each additional five larvae was added; when over 20 adults were recorded, an extra minute per each additional ten adults was added.

The surveyed vegetation is listed in Table 5.1. Different sampling techniques had to be used for surveying the different forms of vegetation. However, a standard sampling effort was maintained by the strict time allocation for each survey. For sampling trees, branches were beaten with a stick above a 110cm x 86cm white canvas beating tray. For sampling herbage, a 46cm diameter sweep net was used. For sampling reeds, stems were beaten above a 46cm diameter sweep net. The supplier of sweep nets and beating trays was Watkins and Doncaster (www.watdon.co.uk). Before the start of recording in year 3, the trees at site B (Fordham) were pollarded[11], thus severely affecting the ladybird catch. Whilst the site continued to be surveyed, a substitute site (Worlington) was added and it is the data from there that has been used for year 3. Fordham and Worlington are approximately 7km apart and are similar churchyard sites dominated by

---

[11] Pollarding: in woodland management, the method of encouraging the growth of lateral branches by cutting off a tree stem 2-3m above ground level.

mature lime trees *Tilia* x *europaea* (Malvaceae).

In year 3, only seven surveys were carried out at sites E and F, so estimated data for these have been calculated using least square methods. This involved forming a site by survey data matrix for each species and year and using a two-way ANOVA to estimate the missing surveys, based on the seasonal patterns at the remaining sites and the records made at the incomplete sites in the remainder of the year.

Table 5.1. The sites surveyed for ladybirds from 2006 to 2008 in eastern England, including grid reference, site description, vegetation type and species surveyed. Sites H to K shown in grey as they were excluded from the analyses.

Site code	Site name	Grid reference	Site description	Vegetation type surveyed	Vegetation species (where mixed, shown in order of importance)
A	St Ives churchyard	TL309716	Churchyard	Lime trees	*Tilia* x *europaea*
B	Fordham / Worlington churchyard	TL633707 / TL691738	Churchyard	Lime trees	*T.* x *europaea*
C	Chippenham Fen 1	TL646698	Border of fenland National Nature Reserve and meadow	Plantation Scots pine trees	*Pinus sylvestris* L. (Pinaceae)
D	Brampton Wood	TL177698	Mixed woodland	Plantation Scots pine trees	*P. sylvestris*
E	Houghton Grange	TL296723	Mixed parkland	Mixed	*P. sylvestris*, *T.* x *europaea* and herbage (mainly grasses, *Urtica dioica*, *Cirsium* Mill. spp. (Asteraceae))
F	Huntingdon	TL253725	Mixed parkland strip adjacent to suburban road	Mixed	*Acer pseudoplatanus*, *Sorbus intermedia* (Ehrh.) Pers. (Rosaceae), *P. sylvestris*, *Fraxinus excelsior* L. (Oleaceae), *Carpinus betulus* L. (Betulaceae), *Hedera helix*
G	St Ives garden	TL306727	Suburban garden	Mixed	*A. pseudoplatanus*, *Prunus spinosa*, *Rhamnus cathartica* L. x *Frangula alnus* Mill. (Rhamnaceae), *Ulmus glabra* Huds. (Ulmaceae), *H. helix*
H	Chettisham Meadow	TL542833	Track verge adjacent to hedgerow, meadow and arable field	Nettle bed	*U. dioica*
I	Upwood	TL253826	Track verge adjacent to hedgerow, meadow and arable field	Nettle bed	*U. dioica*
J	Chippenham Fen 2	TL650692	Fenland National Nature Reserve dominated by *Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae)	Reed bed	*P. australis*
K	Woodwalton Fen	TL230852	Fenland National Nature Reserve dominated by *P. australis*	Reed bed	*P. australis*

Conspicuous coccinellid (i.e. sub-families Epilachninae, Coccinellinae and Chilocorinae - ladybirds *sensu* Majerus, 1994) adults and larvae were recorded to species. Exceptions were first and second instar larvae (which could not be identified to species and have been excluded), *Adalia* larvae (*A. 2-punctata* and *A. 10-punctata* could not reliably be separated so were recorded as *Adalia* spp.), and third instar *Harmonia* larvae (*H. axyridis* and *H. 4-punctata* could not be separated until fourth instar, so third instar larvae were recorded as *Harmonia* spp.). Inconspicuous coccinellid species such as *Rhyzobius litura* (Fabricius) (Coleoptera: Coccinellidae) and *Scymnus suturalis* were found only in small numbers and have been excluded from further analyses. Ladybirds were re-released on-site.

For some analyses the ladybirds were subdivided into three groups: *H. axyridis* only, aphidophagous natives (i.e. *A. 10-punctata*, *A. 2-punctata*, *Anatis ocellata*, *Coccinella 7-punctata*, *Calvia 14-guttata* (L.) (Coleoptera: Coccinellidae), *Harmonia 4-punctata*, *Myrrha 18-guttata* and *Propylea 14-punctata*) and non-aphidophagous natives (i.e. *Aphidecta obliterata*, *Chilocorus renipustulatus* (Scriba) (Coleoptera: Coccinellidae), *Exochomus 4-pustulatus* (L.) (Coleoptera: Coccinellidae), *Halyzia 16-guttata* (L.) (Coleoptera: Coccinellidae), *Subcoccinella 24-punctata* (L.) (Coleoptera: Coccinellidae), *Tytthaspis 16-punctata* (L.) (Coleoptera: Coccinellidae) and *Thea 22-punctata*).

5.2.2 Aphid data

Aphid capture data from the Rothamsted Research Insect Survey (www.rothamsted.ac.uk/insect-survey/) was used as a measure of aphid availability. Insects were captured by 12.2m tall suction traps, sampling 0.75m^3 air per second and running continuously. The trap at Rothamsted dates back to 1964. Currently the UK suction trap network comprises 16 traps, whilst 73 traps based on the Rothamsted design are operated in 20 European countries (www.rothamsted.ac.uk/insect-survey/). Weekly total aphid catch (all species) collected from 2006 to 2008 in the two traps closest to the ladybird survey sites (Rothamsted (TL133134) and Brooms Barn (TL754656)) was used. The ladybird survey sites are situated between Rothamsted and Brooms Barn, which are approximately 80km apart. The mean of the two traps over four weeks of aphid catch (the ladybird survey week, plus the three preceding weeks) was used as the aphid abundance measure to compare with ladybird numbers.

5.2.3 Weather data

Shade air temperature at the time of each survey was recorded on-site using a mercury in glass thermometer. Broader weather data (monthly mean maximum temperature, and monthly total sunshine hours and rainfall) for NIAB Cambridge (TL432603) for 2006-2008 were obtained from the UK Met Office (www.metoffice.gov.uk/climate/uk/stationdata/cambridgedata.txt). The NIAB Cambridge weather station is a maximum of 32km from the ladybird survey sites. For statistical analyses, weather variables were used for both the calendar month of the survey and a mean for a three calendar month period ending in the month of the survey.

5.2.4 Statistical analyses

Differences between years in annual abundances of ladybird species at sites A to G were compared (Friedman test using SPSS 16.0 software). Using combined data for sites A to G, the most abundant ladybird species were ranked each year in order of abundance (i.e. species rank 1 was the most abundant). The change in rank for each species between years 1 and 3 was then calculated. Only the eleven most abundant species were used for this analysis; data for the remaining species were too limited. The annual totals per site of each native ladybird species (i.e. excluding *H. axyridis*) were used to calculate the native species diversity in each of years 1 to 3 (Shannon-Weaver method, log base e, calculated using MVSP software). Pearson correlations were calculated for ladybird abundance (ladybirds grouped as outlined in Section 5.2.1) with aphid abundance, survey air temperature, monthly and three-monthly mean maximum temperature, and monthly and three-monthly total sunshine hours and rainfall. Pearson correlations were also calculated for ladybird abundance (species assessed individually) with aphid abundance. Pearson correlations were calculated using SPSS 16.0 software.

5.3 Results

5.3.1 Ladybirds

Summaries of species numbers are shown in Appendix 1. Ladybird abundance at the nettle and reed sites (H to K) was low and these have been excluded from the main analyses; thus the analyses are of primarily arboreal habitats (lime trees, pine trees or mixed trees and vegetation).

5.3.1.1 Validity of replacement site B

The ladybird species assemblages at Fordham and Worlington (site B) in year 3 were very similar, with a very high proportion (84% Fordham, 92% Worlington) of all ladybirds comprising *A. 10-punctata*, *A. 2-punctata* and *H. axyridis*, indicating that Worlington is a suitable substitute site for Fordham. Overall ladybird abundance at Fordham and Worlington was broadly similar, and in line with observed annual trends at other sites: total ladybirds in year 1: 310 (Fordham); year 2: 571 (Fordham); year 3: 339 (Worlington) (Appendix 1a).

5.3.1.2 Lime tree sites (A and B)

In year 1 a single specimen of *H. axyridis* was recorded (a larva at site B). By year 2 *H. axyridis* was the most abundant ladybird species at sites A and B. It continued to dominate in year 3 and was the only species to increase over the three years (Figure 5.1a). *Adalia 2-punctata*, *C. 7-punctata* and *P. 14-punctata* declined between years 1 and 3.

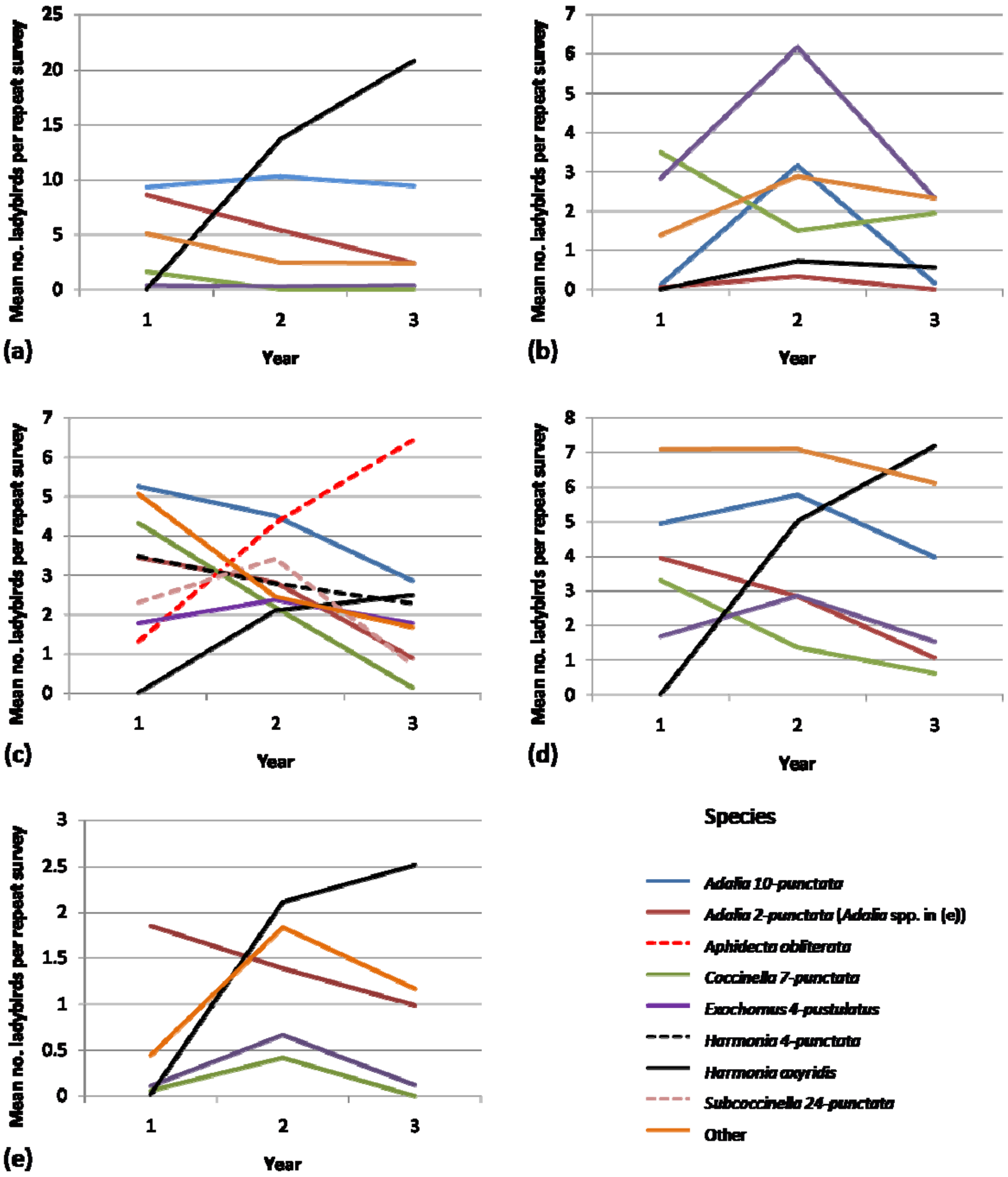


Figure 5.1. Mean number of ladybirds per repeat survey, for each year (nine repeat surveys per site per year), grouped by habitat: (a) Lime tree sites (A and B) adults; (b) pine tree sites (C and D) adults; (c) mixed sites (E, F and G) adults; (d) sites A to G adults; (e) sites A to G larvae. For standard errors see Appendix 1d. Note difference in Y axis scales.

5.3.1.3 Pine tree sites (C and D)

Years 1 and 3 appeared to be generally poor for ladybirds, with higher abundance of most species in year 2: this was the case for *A. 10-punctata*, *A. 2-punctata*, *E. 4-pustulatus* and *H. axyridis*, but not for *C. 7-punctata* (Figure 5.1b). Adults and larvae of

H. axyridis were recorded at site C in year 2. However, in contrast to the lime trees, *H. axyridis* did not dominate on the pines and was less abundant than the conifer specialists *M. 18-guttata* and *H. 4-punctata* and a third species commonly found on pines, *E. 4-pustulatus*. *Harmonia axyridis* larvae were not recorded at either of the sites in year 3, and the species was not recorded at all at site D (although ladybirds in general were less abundant at this site).

5.3.1.4 Mixed sites (E, F and G)

Abundance of various species (including *A. 10-punctata*, *A. 2-punctata*, *C. 7-punctata* and *H. 4-punctata*) declined year-on-year, as *H. axyridis* increased in abundance, ending up as the third most abundant species in year 3 (Figure 5.1c). The only other species to increase in abundance over the three year period was the adelgid and coccid feeder *A. obliterata*, which became particularly abundant on the *P. sylvestris* trees at site F.

5.3.1.5 Arboreal sites (A to G)

Across sites A to G, from very low numbers in year 1, *H. axyridis* became the most abundant ladybird by year 3 (Figure 5.1d). *Adalia 2-punctata* and *C. 7-punctata* were abundant in year 1 and decreased in each of years 2 and 3, whereas the abundance of *A. 10- punctata* and *E. 4-pustulatus* peaked in year 2, before declining in year 3.

Three species declined over the three year period (Figure 5.1d) and showed a significant difference in abundance between years: *A. 2-punctata* ($\chi^2_2 = 9.333$, n = 7 sites, p = 0.009); *C. 7-punctata* ($\chi^2_2 = 8.615$, n = 7 sites, p = 0.013); and *P. 14-punctata* ($\chi^2_2 = 7.000$, n = 7 sites, p = 0.030). One species increased over the three year period (Figure 5.1d) and showed a significant difference in abundance between years: *H. axyridis* ($\chi^2_2 = 9.000$, n = 7 sites, p = 0.011).

The only species which increased in larval abundance in both years 2 and 3 was *H. axyridis* (Figure 5.1e). Some of the other species (notably *C. 7-punctata* and *E. 4-pustulatus*) experienced an increase in larval abundance in year 2, followed by a major decline in year 3. *Adalia* spp. larvae decreased in abundance in each of years 2 and 3, although not significantly so.

The abundance of *H. axyridis* in relation to all native ladybirds (aphidophagous and non-aphidophagous species grouped separately) is shown in Figure 5.2. In year 1, *H. axyridis* was present but not abundant in the survey area and was recorded in very small numbers (0.1% of total ladybirds recorded; n = 1480). In year 3, *H. axyridis* was dominant (accounting for 40% of total ladybirds recorded; n = 1586). Conversely, native aphidophagous species declined from 84% of total ladybirds in year 1, to 41% in year 3.

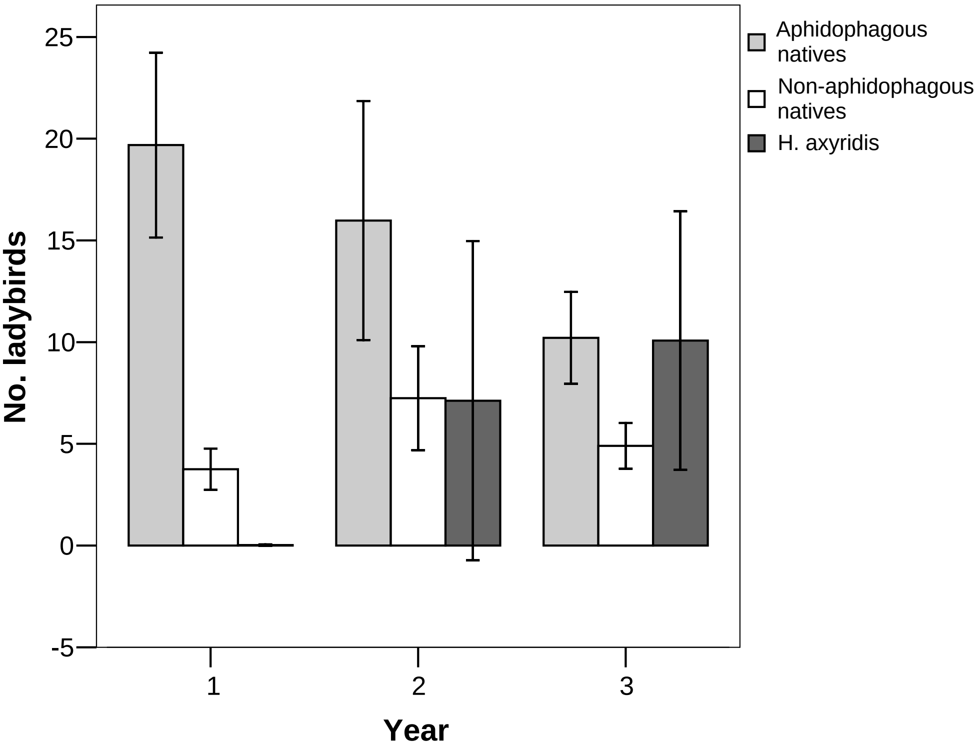


Figure 5.2. Mean number of ladybirds (adults and larvae) per repeat survey, for each year (nine repeat surveys per site per year), at arboreal sites (A to G). Error bars indicate 95% confidence intervals. Ladybirds grouped as follows: ***H. axyridis*** only; **Aphidophagous natives**: *A. 10-punctata*, *A. 2-punctata*, *A. ocellata*, *C. 7-punctata*, *C. 14-guttata*, *H. 4-punctata*, *M. 18-guttata* and *P. 14-punctata*; **Non-aphidophagous natives**: *A. obliterata*, *C. renipustulatus*, *E. 4-pustulatus*, *H. 16-guttata*, *S. 24-punctata*, *T. 16-punctata* and *T. 22-punctata*.

Only two species experienced an increase in abundance rank; *H. axyridis* (changing from rank 14 in year 1 to rank 1 in year 3) and *A. obliterata*. Four species had no change in rank and five experienced a decrease in rank (Figure 5.3).

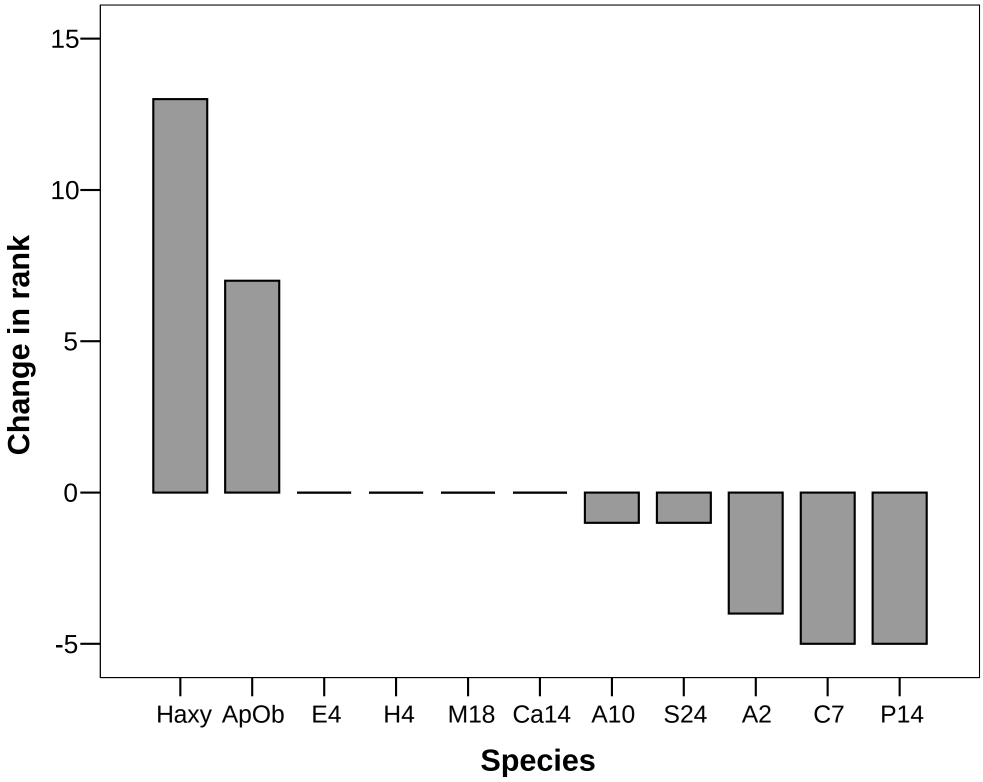


Figure 5.3. Change in abundance ranking from year 1 to 3 of the eleven most numerous ladybird species at arboreal sites (A to G). Haxy: *H. axyridis*; ApOb: *A. obliterata*; E4: *E. 4-pustulatus*; H4: *H. 4-punctata*; M18: *M. 18-guttata*; Ca14: *C. 14-guttata*; A10: *A. 10-punctata*; S24: *S. 24-punctata*; A2: *A. 2-punctata*; C7: *C. 7-punctata*; P14: *P. 14-punctata*.

Whilst there was no significant change in native species richness, between years 1 and 3 a mean loss per site of 1.0 native species was observed (Table 5.2).

Table 5.2. Native ladybird (i.e. *H. axyridis* excluded) species richness and diversity (Shannon-Weaver method, log base e) recorded per year at arboreal sites (A to G).

Site code (Habitat)	Year 1		Year 2		Year 3	
	Richness	Diversity	Richness	Diversity	Richness	Diversity
A (Lime)	8	1.35	5	0.99	6	1.27
B (Lime)	7	1.50	8	1.27	7	1.02
C (Pine)	7	1.39	10	1.55	6	1.41
D (Pine)	5	0.91	5	0.97	5	1.13
E (Mixed)	11	1.77	11	1.65	12	1.65
F (Mixed)	12	2.10	13	1.83	10	1.43
G (Mixed)	9	1.75	10	1.47	6	0.91
Mean	8.43	1.54	8.86	1.39	7.43	1.26

A mean reduction in native species diversity of 18% was observed from year 1 to year 3 (Table 5.2), although this was not significant.

5.3.1.6 Co-occurrence of *Adalia* spp. larvae and *H. axyridis* larvae

At the lime tree sites (A and B), out of 21 surveys in years 2 and 3 when *Adalia* spp. larvae and/or *H. axyridis* larvae were recorded, 52% of the surveys had both species recorded, 24% had *Adalia* spp. only (all such occasions between May and July) and 24% had *H. axyridis* only (four of the five such occasions in September or October). Thus there was a reasonably high degree of overlap of larval stages at the sites. *Adalia* spp. tended to breed earlier with a single peak in abundance; most larvae being recorded in June and July in years 1 and 3, but occurrence was more prolonged (late May to August) in year 2. In year 2 the larval peak for *Adalia* spp. and *H. axyridis* was in August. *Harmonia axyridis* exhibited two peaks in larval abundance, suggesting bi-voltinism (Chapter 3), but the timing of the peaks changed: from August and October in year 2 to late June and September in year 3.

Table 5.3. Significance of Pearson correlations for ladybird abundance with aphid abundance and weather data in years 1 to 3 at arboreal sites (A to G). Correlations based on mean data for 7 sites for each survey period (i.e. 9 survey periods in each of 3 years). Degrees of freedom = 25. Significant values shown in red (*: P < 0.05; **: P < 0.01). Negative correlations indicated. For details of species in Aphidophagous native and Non-aphidophagous native groups, see Figure 5.2.

	Survey temperature	Monthly mean maximum temperature	3-monthly mean maximum temperature	Monthly total sunshine	3-monthly total sunshine	Monthly total rainfall	3-monthly total rainfall	Aphids	*H. axyridis* adults	*H. axyridis* larvae	Aphidophagous native adults	Aphidophagous native larvae	Non-aphidophagous native adults
H. axyridis adults	-.364 .062	-.257 .196	.242 .223	-.448 .019*	-.194 .333	-.048 .812	.125 .534	-.144 .472					
H. axyridis larvae	-.035 .864	.048 .814	.290 .142	-.161 .421	.061 .761	-.077 .703	.194 .332	-.216 .279	.351 .073				
Aphidophagous native adults	.348 .076	.248 .213	-.191 .339	.257 .196	.048 .812	.120 .552	-.156 .438	.310 .116	-.580 .002**	-.460 .016*			
Aphidophagous native larvae	.370 .057	.407 .035*	.078 .698	.500 .008**	.304 .123	-.195 .329	-.019 .926	.231 .246	-.315 .109	.030 .883	.220 .269		
Non-aphidophagous native adults	-.054 .788	.102 .613	.397 .040*	-.098 .627	.219 .273	-.147 .464	.327 .096	-.275 .165	.406 .036*	.581 .001**	-.310 .116	-.079 .697	
Non-aphidophagous native larvae	.218 .274	.271 .171	.043 .830	.172 .390	.179 .373	.232 .245	.272 .170	.147 .465	-.286 .148	-.109 .587	.308 .119	.578 .002**	-.089 .659

The seasonal patterns of the three ladybird subgroups and aphids were plotted (Figure 5.4). This emphasises the decline in aphidophagous native species in year 3 and the corresponding rise in *H. axyridis*.

5.3.2 Relationships

5.3.2.1 Relationships between ladybird groups

Significant negative correlations were found for aphidophagous native adults with both *H. axyridis* adults and larvae. Significant positive correlations were found for non-aphidophagous native adults with both *H. axyridis* adults and larvae. A further significant positive correlation was found between aphidophagous native larvae and non-aphidophagous native larvae (Table 5.3).

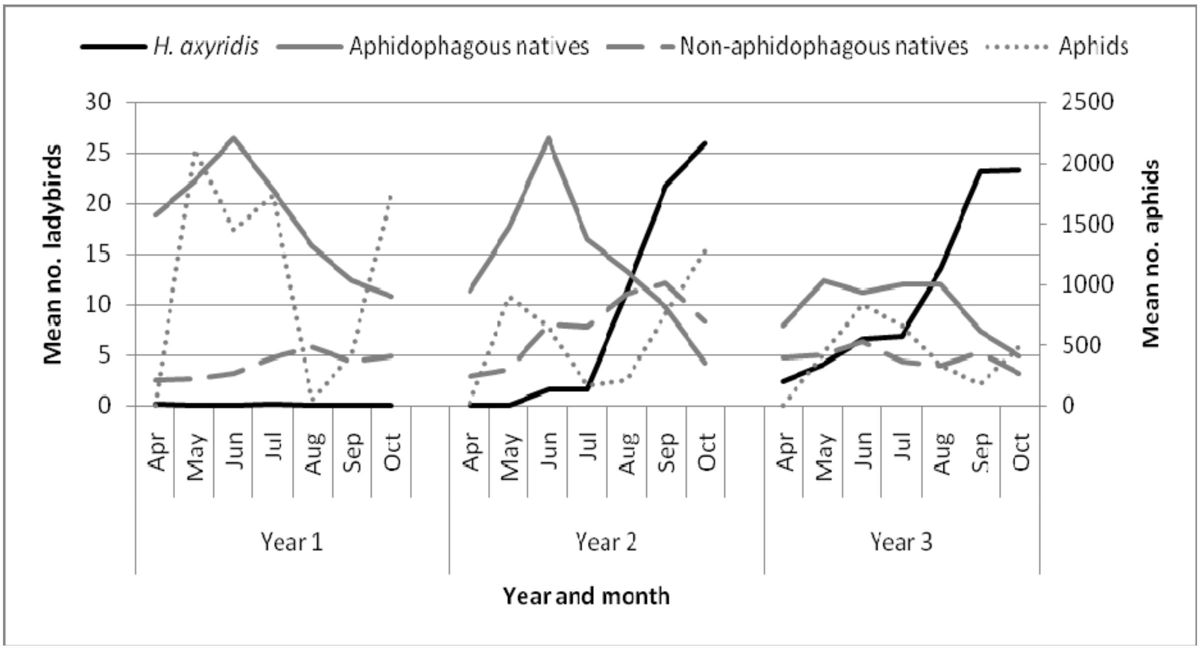


Figure 5.4. Mean number of ladybirds (adults and larvae) per repeat survey, for each month and year (nine repeat surveys per site per year), at arboreal sites (A to G), with corresponding mean number of aphids from Rothamsted Research Insect Survey (Rothamsted and Brooms Barn suction traps). For details of species in Aphidophagous native and Non-aphidophagous native groups, see Figure 5.2.

5.3.3.2 Relationships between ladybirds and aphids

The weekly aphid abundance totals for the two suction traps (Rothamsted and Brooms Barn) were strongly related ($R^2 = 0.673$) over the three year survey period, suggesting a broad consistency in aphid abundance across the region. There were no significant correlations between aphid abundance and abundance of the three ladybird subgroups (Table 5.3). Significant correlations between aphid abundance and only two ladybird species were found: *A. 2-punctata* adults ($r_{25} = 0.431$, P = 0.025) and *P. 14-punctata* adults ($r_{25} = 0.576$, P = 0.002) (Pearson correlation test).

5.3.2.3 Relationships between ladybirds and weather

Neither the monthly or the three-monthly rainfall total was significantly correlated to any of the ladybird measures, whilst monthly total sunshine was significantly positively correlated to aphidophagous native larvae and significantly negatively correlated to *H. axyridis* adults (Table 5.3). Monthly mean maximum temperature was significantly positively correlated to the abundance of aphidophagous native larvae, and three-monthly mean maximum temperature was significantly positively correlated to non-aphidophagous native adults (Table 5.3).

5.4 Discussion

Aphid abundance is strongly associated with weather conditions (Dixon, 1998, Dixon & Kindlmann, 1999, Masterman et al., 1996, Zhou et al., 1997), although the mechanism (e.g. winter temperature (Westgarth-Smith et al., 2007), or winter precipitation (Estay et al., 2009)) may vary depending on the aphid species and ecosystem. In East Anglia, winter temperature correlated with the abundance of cereal aphids in early summer (Dixon, 1998). The significant positive correlation between *A. 2-punctata* adults and aphid abundance that was not the case for *H. axyridis*, suggests that *A. 2- punctata* was more reliant on aphids than *H. axyridis*. *Adalia 2- punctata* strongly prefers habitats with high aphid density (Honek, 1985), whilst *H. axyridis* is a highly effective aphid predator and successfully tracks prey seasonally within a heterogeneous habitat (Osawa, 2000). Monthly mean maximum temperature had a stronger positive correlation to the abundance of aphidophagous native ladybirds (significantly so for larvae) than to *H. axyridis*. This is partly explained by the high abundance of *H. axyridis* late in the year, when temperatures tended to be lower. In year 3, *H. axyridis* increased in abundance despite fewer aphids than in years 1 and 2, in contrast to native aphidophagous species.

Harmonia axyridis abundance peaked late in the year, and although aphids were recorded in October of each year, they were in decline by then. Therefore alternative foods are assumed to have been utilised, particularly at the lime tree sites, with high densities of *H. axyridis* causing high pressure on limited aphids. This presumably included a significant level of cannibalism (which was observed at the lime tree sites, but not quantified). Adult *H. axyridis* are also known sometimes to feed on fruit juices late in the year (Koch & Galvan, 2008), providing late-emerging adults with an additional food resource to build up fat reserves and enhance their chances of overwintering survival. Whilst it is common for predatory ladybirds to use secondary foods, frugivory in this group is rare (Hodek & Honek, 1996). The high abundance of *H. axyridis* larvae very late in the year (larvae were sometimes present in November and December (P. Brown, personal observation)), with little chance of survival to adulthood, suggests that the species is not yet fully adapted to conditions in Britain. Similarly, larvae and pupae were observed in November in Denmark, and thousands of pupae that were alive in November did not survive the winter (Steenberg & Harding, 2009b).

Overwintering mortality in ladybirds is often very high (Majerus, 1994a) but also very variable. For example, for *H. axyridis*, mortality was about 10% in Japan (Watanabe, 2002) but for *C. 7-punctata*, *A. 2-punctata* and *P. 14-punctata* about 85% mortality was estimated in Great Britain (Zhou et al., 1994). These studies may not be representative of the general situation and it is likely that there are great differences between years. In the current study, increasing abundance of *H. axyridis* in years 2 and 3 suggests that overwintering survival by *H. axyridis* adults was relatively high. In Italy, *H. axyridis* (31.9% mortality) survived overwintering significantly better than *A. 2-punctata* (61.3% mortality) and had a higher post-overwintering rate of increase (a measure which provides an estimate of capacity to colonise the environment) (Bazzocchi et al., 2004). *Harmonia axyridis* multi-voltinism was a further advantage to population growth in the current study; in Great Britain the species seems to have two generations per year (Chapter 3), whereas many native species (including *C. 7-punctata* and *A. 2-punctata*) are uni-voltine in most years (Majerus, 1994a).

Year 1 represents a baseline year, with native ladybirds at the study sites presumably affected little, if at all, by *H. axyridis*. The data provide evidence that *H. axyridis* subsequently started to displace some native aphidophagous ladybirds in eastern England. *Harmonia axyridis* increased (with a significant difference between years), whilst *A. 2-punctata*, *C. 7-punctata* and *P. 14-punctata* decreased (also with a significant difference between years) over the three year period. The latter three species exhibit high niche-overlap with *H. axyridis* in England (P. Brown, unpublished data) and elsewhere in Europe (Adriaens et al., 2008). These three species suffered 100% asymmetric IGP by *H. axyridis* in confrontations of fourth instar larvae (Ware & Majerus, 2008). A similar situation was observed in the Florida citrus ecosystem, where the native *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) was displaced by *H. axyridis*, with evidence of asymmetric IGP (Michaud, 2002). The significant negative correlations between aphidophagous native adult ladybirds and *H. axyridis* (adults and larvae) provide further evidence that the latter was at least partially the cause of the observed decline in the former. Because of the particularly high niche overlap with *H. axyridis*, *A. 2- punctata* faces the highest risk of further decline as a result of *H. axyridis*. There is already some evidence from Britain that *A. 2- punctata* has declined as a consequence of the arrival of *H. axyridis* (Majerus, 2008) and strong evidence that *A. 2- punctata* seriously declined in response to the arrival of adventive species *C. 7-*

punctata, and particularly *H. axyridis*, in North America (Colunga-Garcia & Gage, 1998, Harmon et al., 2007). *Harmonia axyridis* is more voracious (Lucas et al., 2002) and a stronger IG predator (Takahashi, 1989, Yasuda et al., 2004) than *C. 7-punctata* and represents a greater threat to native ladybirds in North America (Yasuda et al., 2004).

Adult female ladybirds are deterred from ovipositing by the presence of conspecific larval tracks (Doumbia et al., 1998) to restrict cannibalism, but not by heterospecific tracks (Doumbia et al., 1998, Yasuda et al., 2000) to restrict IGP. Reasons to explain this apparent lack of adaptation include lack of habitat overlap by the species concerned (Doumbia et al., 1998) or unpalatabilty in the case of two species that do coexist (Yasuda et al., 2000). The latter scenario is suggested for *H. axyridis* in Japan, which is unpalatable to *C. 7-punctata* (Yasuda et al., 2000). *Harmonia axyridis* may enjoy a double benefit in England. Firstly, its own eggs have chemical defences and are unpalatable to species such as *A. 2- punctata* and *C. 7-punctata* (Sato & Dixon, 2004), whereas *A. 2- punctata* and *C. 7-punctata* eggs are more palatable (Sato & Dixon, 2004, Ware et al., 2008a). Secondly, the evidence of Doumbia, et al. (1998) and Yasuda, et al. (2000) suggests that *A. 2- punctata* will not be deterred from ovipositing by the presence of *H. axyridis* larvae, thereby increasing the vulnerability of its eggs to IGP. Moreover, *A. 2- punctata* tends to oviposit where there is high aphid density, leading to higher levels of egg predation (Schellhorn & Andow, 1999). In North America, Cottrell (2004) showed that there is asymmetric IGP of eggs in favour of *H. axyridis* compared to both native species tested, and concluded that *H. axyridis* is likely to have a negative impact on native species, especially when aphids are in short supply (Cottrell, 2004). Egg predation by *H. axyridis* will vary by prey species; for example *C. 14-guttata* eggs contain surface chemicals which are unpalatable to *H. axyridis* larvae (Sato & Dixon, 2004, Ware et al., 2008a). *Calvia 14-guttata* did not decline in the current study. Intuitively, high aphid availability should lead to lower IGP, and vice versa, and this was observed for *H. axyridis* larvae acting as IG predators of American native ladybird larvae (Yasuda et al., 2004); in the current study this may partly explain why, in contrast to the native aphidophagous species, *H. axyridis* continued to increase in abundance in year 3, despite lower aphid abundance. *Harmonia axyridis* is a highly efficient IG predator (Sato et al., 2008) and indications of a high level of IGP of native species, including *A. 2- punctata* and *C. 7-punctata*, has recently been shown in wild caught *H. axyridis* larvae in Europe (Hautier et al., 2008).

The significant positive correlations between non-aphidophagous native adults and *H. axyridis* adults and larvae are likely caused by the most abundant non-aphidophagous species, *A. obliterata*, increasing over the three years and / or low interaction of non-aphidophagous native species with *H. axyridis*. It is not surprising that four of the five native species with an unaltered or improved ranking over the three year period were either conifer/pine specialists (*H. 4-punctata*, *M. 18-guttata* and *A. obliterata*) or commonest on pines (*E. 4-pustulatus*); *H. axyridis* did not thrive on the pine trees and, based on the low number of larvae recorded, breeding there was limited. *Harmonia axyridis* can dominate on pines in its introduced (Sloggett et al., 2009) and native (McClure, 1986) ranges. In field surveys in various Japanese cities in May 2009, 84% (n = 110) of all ladybirds recorded on pine trees were *H. axyridis* (P. Brown & C. Thomas, unpublished data). Whilst this may not be representative of non-urban habitats or of the year as a whole, *H. axyridis* did not dominate on pines at any time at the current study sites. Pine trees are a secondary habitat of *H. axyridis* in Japan, where it coexists with the sympatric species *Harmonia yedoensis* Takizawa (Coleoptera: Coccinellidae) (Osawa & Ohashi, 2008) and may feed on coccids as well as aphids. Whilst *H. axyridis* may adapt better to the pine trees in Britain in the long term, it will similarly face competition from a sympatric species in *H. 4-punctata*, as well as from the largest and best-defended native ladybird, *Anatis ocellata*, which may be an IG predator of *H. axyridis* (Ware & Majerus, 2008). Unlike *H. axyridis*, *H. 4-punctata* is dorsally-ventrally flattened, allowing easier movement amongst pine needles, and also exhibits a degree of crypsis in this habitat (Majerus, 1994a).

5.5 Conclusion

Over a three year period encompassing the invasion phase of *H. axyridis* in eastern England, *H. axyridis* increased from 0.1% to 40% of total ladybirds, whilst native aphidophagous species declined from 84% to 41% of total ladybirds. The actual number of native aphidophagous ladybirds per repeat survey decreased from a mean of 19.7 ladybirds in year 1, to 10.2 ladybirds in year 3. *Harmonia axyridis* was clearly the most abundant species in year 3. The mechanisms by which *H. axyridis* impacted on aphidophagous native species are not apparent from the data presented here. However, they likely include asymmetric competition for aphid prey and asymmetric IGP of eggs, larvae and pupae, in favour of *H. axyridis*. Furthermore, physiological and behavioural traits of *H. axyridis*, including multi-voltinism and polyphagy, confer additional advantages over native species. Cannibalism is also likely to play a major role in the survival of *H. axyridis*, especially late in the year. However, whilst early changes are evident, three years is insufficient time for the full pattern of changing species assemblage dynamics to emerge; thus surveys are ongoing at some sites. In an attempt to quantify levels of IGP at the lime tree sites, genetic studies to detect *A. 2-punctata* in the guts of *H. axyridis* are described in the next chapter.

6. Detection of *Adalia 2-punctata* in *Harmonia axyridis* gut contents using PCR analyses

6.1 Introduction

IGP is the killing and eating of a species that uses similar, often limiting, resources (thus the prey is also a competitor) (Polis et al., 1989). So, IGP differs from classical predation in that the predation event reduces potential exploitation competition (Polis et al., 1989). IGP is widespread in many trophic systems (Polis & Holt, 1992), including the guild of aphidophagous species that includes many coccinellids (Dixon, 2000). Since direct observation of predation events (including IGP) in the field is a labour intensive process and may disturb the study system (Sunderland, 1988), alternative methods of detecting predation have been developed. For example, predators or their faeces may be field collected and analysed later in the laboratory. Indirect detection of predation may thus be made, particularly by studying the gut contents of the predator. Whilst gut contents may be analysed by microscopic observation of prey fragments, this method is very time-consuming (Greenstone et al., 2007). In recent years molecular techniques have been developed to make the process of prey identification more efficient. These techniques include monoclonal antibody (MAb) based assays, gas chromatography mass spectrometry (GC-MS), and increasingly, detection of prey DNA using the Polymerase Chain Reaction (PCR). In general, MAb has been displaced by PCR, partly as it is not cost-effective (Chen et al., 2000), although there is one recent study involving coccinellids using MAb (Fournier et al., 2006).

In coccinellids, GC-MS was used to detect alkaloids of intraguild prey (Hautier et al., 2008, Sloggett et al., 2009), but this technique requires complex facilities that are not available in most laboratories. GC-MS also has features that made it unsuitable for the current study. Firstly, the endogenously produced alkaloids detected in prey are not necessarily species specific. Whilst this may not matter for some studies, it did for mine; adaline is the principal alkaloid of *Adalia* species (Sloggett et al., 2009), and since both *Adalia 2-punctata* and *Adalia 10-punctata* frequently co-occur temporally and spatially (on the same trees) in eastern England, GC-MS would not enable identification of a particular *Adalia* species. Secondly, the very long detection times of coccinellid alkaloids in *H. axyridis* (Hautier et al., 2008) may be a disadvantage in trying to assess the extent of IGP in the field; quantifying IGP is more difficult when the time span is longer (Fournier et al., 2008).

PCR is a method for making many copies of a specific DNA sequence. It allows detection and identification of prey by analysis of prey DNA present in the predator gut. PCR has increasingly been used to detect predation in a range of invertebrate taxa, including spiders (Agusti et al., 2003) and beetles (Pons, 2006, Zaidi et al., 1999). Tree-beating, one of the standard ladybird sampling techniques outlined in the previous chapter, is a suitable method for collecting predators for PCR analyses, because there is no opportunity for secondary predation or scavenging to occur after trapping, as with other methods such as pitfall trapping and vacuum sampling (King et al., 2008). The current study is the first to attempt to use PCR to detect IGP of one coccinellid by another in a wild population in Europe. Using PCR, *H. axyridis* has been studied both in its role as predator (Fournier et al., 2008, Hoogendoorn & Heimpel, 2002, Chacon et al., 2008, Zhang et al., 2007a) and potential prey (Harwood et al., 2007, Harwood et al., 2009). Only one study (Gagnon et al., 2005), in North America, investigated the role of *H. axyridis* as an intraguild predator of other coccinellids.

The aim of this study was to detect and quantify the level of predation by *H. axyridis* on *A. 2-punctata* in the wild, on churchyard lime trees in eastern England. Three of the sites from the Chapter 5 were used, along with one additional site.

6.2 Method

6.2.1 Sensitivity of PCR detection

Before field-collected ladybirds were tested, the level of detection of prey consumption was tested through laboratory feeding trials. The variables tested were the amount of prey, and digestion period; i.e. how much *A. 2-punctata* prey a *H. axyridis* larva had to eat in order for it to be detectable by the PCR method, and for how long after feeding it remained detectable. Trials with *A. 2-punctata* eggs and first and fourth instar larvae fed to *H. axyridis* fourth instar larvae were carried out (Table 6.1). Unfed *H. axyridis* controls were also set up. All ladybird stock was of British origin; laboratory-reared at the Department of Genetics, University of Cambridge and fed on pea aphids, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), prior to experimentation. In the 24 hours immediately prior to experimentation, the larvae were starved, except in the case of the experiments carried out on 28/05/2009 (Table 6.1), when they were fed limited pea aphids. The latter case was applied so that a *H. axyridis* larva would have sufficient energy to engage in a potentially hazardous encounter with a fourth instar *A. 2-punctata* larva.

Fourth instar *H. axyridis* larvae (ecdysed within the previous 36 hours) were placed individually in clean, dry 2.5cm or 3.5cm diameter petri dishes in a controlled laboratory environment at 21°C with constant light. Each petri dish also contained the live *A. 2-punctata* prey item(s) (either one, five or ten eggs, or one first or fourth instar larva). *Harmonia axyridis* larvae were left to feed under observation, and the feeding start-time (i.e. when the larva actually started consuming the prey item(s)) and finish-time noted. After feeding, each *H. axyridis* larva was transferred individually to a clean petri dish. After the allocated digestion period (i.e. 0, 2, 4, 8 or 24 hours), the larva was transferred from its petri dish to a small plastic tube and frozen, initially at -20°C, before transfer on ice to longer-term storage at -80°C, ready for DNA extraction for the PCR experiments at a later date.

6.2.2 Field collection

Third and fourth instar *H. axyridis* larvae were field collected by tree-beating lime trees, *Tilia* x *europaea*, at four churchyard sites in Cambridgeshire (St Ives, Wilburton and Fordham) and Suffolk (Worlington), eastern England, in 2008 and 2009. Grid references for three of the sites are given in Chapter 5 (Table 5.1); Wilburton is located at TL477749. All sites were known in advance to have populations of *A. 2-punctata* and *H. axyridis*, thus the opportunity for IGP existed. In order to maximise the chances of finding ladybirds that had recently fed, all surveys were carried out between 1000 and 1600 hours, in dry weather, with air temperature at site of at least 17°C (in summer) or 12°C (in autumn). 1000 to 1600 hours was the main feeding window observed for *H. axyridis* in Japan (Miura & Nishimura, 1980). Further, ladybirds such as *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae) have high activity at 1000 hours in the US (Greenstone et al., 2007) and there is evidence that aphidophagous coccinellids are not nocturnal feeders (Nakamuta, 1987).

Surveys were carried out three times – in late June or early July 2008 and 2009, and in early October 2008 only. Since no native ladybird larvae were found in October 2008, presumably making the opportunity for IGP minimal, the planned collection in October 2009 was cancelled. However, the October 2008 samples were tested. The target number of *H. axyridis* was 20 per site per survey, although this was not always achieved (Table 6.2). In particular, on three occasions (Fordham, summer and autumn 2008; Worlington, autumn 2008) insufficient numbers of *H. axyridis* were found to make collection worthwhile. The ladybird species assemblage and approximate abundance of each life stage were noted for each survey of approximately 20 minute duration. Species ranks were assigned based on the abundance of adults and larvae combined (rank 1 indicates the most abundant species; rank 2 indicates the second most abundant, etc). The DAFOR scale was also used to assess the abundance of each species: D = Dominant (i.e. >50 ladybirds in survey (adults and larvae combined)); A = Abundant (11-50); F = Frequent (6-10); O = Occasional (2-5); R = Rare (1).

Collected ladybirds were placed in individual small dry plastic vials at the field site and immediately put on ice in an insulated box (Agusti et al., 2003). The ladybirds were thus alive but inactive, with very slow metabolism and digestion. Within 90 minutes the collected samples were frozen at -20°C, before being transferred to a -80°C freezer for long-term storage.

6.2.3 DNA extraction

Ladybirds were removed from -80°C storage and snap-frozen in liquid nitrogen. 208µl of the extraction buffer (containing 0.01g of CHELEX-100, 7µl of 1M dithiothreitol (DTT), 1µl of 25mg/ml Proteinase K and 200µl sterile double distilled water) was added to each ladybird in a separate 1.5ml Eppendorf tube. Each ladybird was crushed with a sterile plastic pestle, the tube vortexed for ten seconds and then incubated at 56^{o}C for 90 minutes. After vortexing for ten seconds each sample was incubated at 96^{o}C for ten minutes and then centrifuged at 13000rpm for five minutes. The supernatant (containing the extracted DNA) was drawn off into a 1.5ml Eppendorf tube and the samples refrigerated at 4^{o}C.

6.2.4 PCR amplification

Each PCR reaction (25µl) contained sterile double distilled water (10.7µl), 10x amplification buffer (2µl) (supplied by the manufacturer with the enzyme), 25mM of $MgCl_2$ (2.5µl), Q solution (4µl), 100µM of each dNTP (0.2µl), 10µM of each primer, Ab35F (GAAATTGTTAGGTTAGGATCGGAAG) & R (ATTCACTGTGCGGAGTATTACGTTC) (2µl), 0.1U Taq DNA Polymerase (5u/µl) and 2µl of extracted DNA. Amplification was performed in a TECHNE TC 412 thermocycler with the following cycling conditions: initial denaturation at 94°C for three minutes, 35 cycles of denaturation at 94°C for one minute, annealing at 60°C for one minute and DNA extension at 72°C for one minute. There was a final extension step at 72°C for ten minutes and then the products were stored at 4°C. The PCR products were separated with agarose gel electrophoresis; they were mixed with 3µl gel loading dye and 6µl loaded on a 2.5% agarose gel which contained 1µl Ethidium Bromide (10mg/ml). The gels were run at 70% volts for 2.5 hours in 1% TBE buffer. The PCR products were then photographed on a UV transilluminator for PCR analysis.

6.3 Results

6.3.1 Sensitivity of PCR detection

When the prey was a first instar larva or five or ten eggs, *A. 2-punctata* DNA was detected in the gut of 93% of the *H. axyridis* larvae at 0h digestion period (i.e. when the fed *H. axyridis* larvae were frozen immediately they finished feeding) (Table 6.1). Electrophoretic bands were particularly strong with the larval prey (Figure 6.1). After 2h digestion of the larval prey, four of five replicates yielded weaker bands. A single *A. 2-punctata* egg was not detected at 0h or any other digestion period. Five eggs were not detected after 2h, and ten eggs were only detected in one of four replicates (in fact this particular *H. axyridis* larva had eaten only eight of the ten eggs offered). Detection of fourth instar larva prey was achieved after 4h in three of six replicates. 4h was close to the limit of detection, and the electrophoretic bands for these replicates were weak. There was no detection of any prey after 8h or 24h and no detection of *A. 2-punctata* DNA in any of the unfed controls.

Table 6.1. Summary of detection of *A. 2-punctata* DNA in the gut of *H. axyridis* fourth instar larvae with differing prey items (*A. 2-punctata* egg x1, x5 or x10, or first or fourth instar larva x1), feeding trial dates and digestion periods (0, 2, 4, 8 or 24 hours). For each permutation, the no. positive detections / no. replicates is shown (positive results shown in bold). *The replicates variously consumed 7-10 of the 10 eggs offered.

		Digestion period					
A. 2-punctata prey item	Feeding trial date	0h	2h	4h	8h	24h	Control
Egg x1	22/01/2009	0/5	0/5	0/5	0/6	0/5	0/5
Egg x5	28/05/2009	**4/5**	0/5	0/5	0/6	N/A	0/5
Egg x10*	25/06/2009	**4/4**	**1/4**	N/A	N/A	N/A	N/A
First instar larva x1	22/01/2009	**5/5**	**4/5**	0/5	0/6	0/5	N/A
Fourth instar larva x1	28/05/2009	N/A	N/A	**3/6**	0/5	0/3	N/A

6.3.2 Field collection

In six of nine surveys, *H. axyridis* was the most abundant species and was most commonly assessed as 'Abundant' (Table 6.3). Conversely, *A. 2-punctata* was the third most abundant species in six of the nine surveys and was generally assessed as 'Occasional'. *Adalia 10-punctata* was generally intermediate – i.e. less abundant than *H. axyridis* but more abundant than *A. 2-punctata*, although in three of nine surveys it was the most abundant species (Table 6.3).

PCR amplification of *A. 2-punctata* was detected for one of 112 *H. axyridis* larvae, i.e. a detection rate of 0.89%. This *H. axyridis* larva was collected from St Ives churchyard in summer 2008.

Table 6.2. Numbers of *H. axyridis* fourth instar larvae collected from four field sites in three collection periods.

Field site name	Summer 2008	Autumn 2008	Summer 2009	Total
Fordham	0	0	22	22
St Ives	19	20	11	50
Wilburton	5	0	20	25
Worlington	10	0	5	15
Total	34	20	58	112

Table 6.3. Species ranks and DAFOR scale assessment of abundance (in brackets) for ladybird species at four field sites in three collection periods. Species ranks based on abundance of adults and larvae combined (rank 1 indicates the most abundant species; rank 2 indicates the second most abundant, etc). DAFOR scale: D = Dominant (i.e. >50 ladybirds in survey (adults and larvae combined)); A = Abundant (11-50); F = Frequent (6-10); O = Occasional (2-5); R = Rare (1).

		Rank and DAFOR scale assessment (in brackets) of ladybird species		
Collection period	Field site name	*H. axyridis*	*A. 2-punctata*	*A.10-punctata*
Summer 2008	Fordham	N/A	N/A	N/A
	St Ives	1 (A)	3 (O)	2 (A)
	Wilburton	1 (A)	3 (O)	2 (F)
	Worlington	2 (A)	3 (O)	1 (A)
Autumn 2008	Fordham	N/A	N/A	N/A
	St Ives	1 (D)	none	2 (O)
	Wilburton	1 (A)	4 (O)	none
	Worlington	N/A	N/A	N/A
Summer 2009	Fordham	1 (A)	3 (O)	2 (O)
	St Ives	1 (A)	2 (A)	3 (F)
	Wilburton	2 (A)	3 (O)	1 (A)
	Worlington	2 (F)	3 (R)	1 (A)

6.3.3 Specificity of PCR primers

When the *A. 2-punctata* Ab35 primers were tested with DNA extracted from whole *A. 2-punctata* and *H. axyridis*, the result was a polymorphic electrophoretic band of approximately 218-260bp (Haddrill et al., 2002). Successful amplification was only achieved with *A. 2-punctata* DNA (Figure 6.1). No amplification was detected when the Ab35 primers were tested with DNA extracted from whole *A. 10-punctata*, showing that PCR primers Ab35 are specific to *A. 2-punctata*.

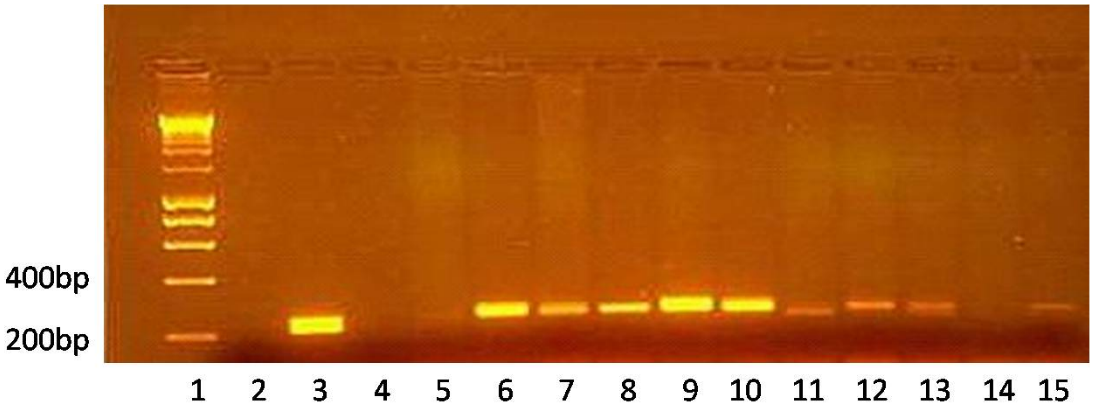


Figure 6.1. PCR amplification of DNA from first instar *A. 2-punctata* larvae fed to fourth instar *H. axyridis* larvae. Lane 1: molecular marker; lane 2: negative PCR control (only *H. axyridis* DNA included in the PCR reaction); lane 3: positive PCR control (amplification of known *A. 2-punctata* DNA); lane 4: reaction blank (i.e. no DNA included in the PCR reaction); lane 5: negative PCR control; lanes 6-10: 0h digestion period; lanes 11-15: 2h digestion period.

6.4 Discussion

The results presented are the first to show detection of IGP by one coccinellid on another, in the field in Europe, using PCR techniques. As outlined in the introduction, alternatives such as MAb and GC-MS were inappropriate. However, there are many variables that affect the detection success of PCR. Haddrill, et al. (2002) isolated and characterised 39 microsatellite loci in *A. 2-punctata*, the first genetic markers developed for any coccinellid species. Of these loci, primers Ab35 had previously been successfully used at Anglia Ruskin University. There were two possible disadvantages of using these primers. Firstly the sequence length (218-260bp) was reasonably long, although some studies (Zhang et al., 2007a, Hoogendoorn & Heimpel, 2001) used similar length, and in some instances longer, fragments. Since prey DNA degrades during predator digestion processes, shorter DNA fragments, on average, will be detectable for longer (Hoogendoorn & Heimpel, 2001, Agusti et al., 1999). For example, with 100-140bp DNA fragments, detection of coccinellid DNA in *H. axyridis* was made 16h after eating five eggs (Gagnon et al., 2005). Secondly, mitochondrial DNA has hundreds or thousands of copies within a cell (King et al., 2008), whilst nuclear DNA such as used here, has just two copies per cell. Thus using mitochondrial DNA may yield a greater number of positive detections (de Leon et al., 2006). Protein encoding genes, and particularly the cytochrome oxidase I and II genes (COI and COII), are good candidates for the design of species-specific primers because they are less conserved than some other genes (King et al., 2008). These genes have been popular for use in several of the studies involving coccinellids (Chen et al., 2000, Hosseini et al., 2008).

Even amongst coccinellids it is evident that differing species and fine details of the method make large differences to prey detection times. For example, a half-life of 17.5h for detection of hemipteran prey in *H. axyridis* was calculated (using a 197bp fragment size of the COI gene) (Fournier et al., 2008), whereas a half-life of just 59 minutes was found for detection of the Colorado potato beetle *Leptinotarsa decemlineata* in *C. maculata* (using a 214bp fragment size of the COI gene) (Weber & Lundgren, 2009). Factors such as the method of sample storage (e.g. ethanol and/or freezing, and at what temperature), temperature whilst feeding, and meal size, may affect DNA detection (King et al., 2008, Hoogendoorn & Heimpel, 2001).

In one sense, none of the variables outlined above matter within an experimental system, as long as the prey is detectable at known levels of these variables. Especially when analysing field-collected samples, it may be advantageous to have a long detection window, in that the chances of detecting prey are maximised (Agusti et al., 2003, Harper et al., 2005). However, a shorter detection window may provide greater insights into the complex trophic interactions within a system (Fournier et al., 2008). For example, knowing that a predator has fed on a certain prey species within a 2h period (as in the present study) is a rather stronger piece of information than knowing that the same predator fed on the prey sometime in the last few days. However, the associated drawback, of obtaining few positive detections, is serious, unless the sample size is very large (which of course makes the study highly labour intensive). A balance is therefore needed, and this study would likely have benefited from a somewhat longer detection window, achieved through detection of a shorter DNA fragment and/or targeting mitochondrial DNA.

Partly because of the short detection window, the apparently low positive IGP rate detected, of 0.89%, may represent a moderate IGP level. Ecological factors need to be taken into account; the relative abundance of the predator and prey species, and hence the probability of encounter, will influence the likelihood of IGP occurring (Hoogendoorn & Heimpel, 2002). In this study, it is assumed that the chance of a positive detection was higher in 2008 than in 2009, as *A. 2-punctata* was generally more abundant in 2008 (Table 6.3). The exception was the site where the one positive detection occurred; at St Ives, *A. 2-punctata* was assessed as ‘Occasional’ in summer 2008 (the survey of positive detection), but ‘Abundant’ in summer 2009. Other factors being equal, the chances of an IGP event occurring between *H. axyridis* and *A. 10-punctata* were higher than between *H. axyridis* and *A. 2-punctata*, since *A. 10-punctata* was generally a more abundant species than *A. 2-punctata*.

An interpretation of my field result is that 0.89% of *H. axyridis* fed on *A. 2-punctata* during a two hour window. Using an estimated feeding window in summer of 12h per day, within a day the estimated IGP level would therefore be 6 x 0.89% = 5.34%. By extrapolating this result, within 18.7 days (i.e. 1 / 0.0534), on average, each *H. axyridis* larva would have fed on *A. 2-punctata*. In the UK, the total duration of the larval stage of predatory ladybirds is typically three to four weeks (Majerus, 1994a). This is relevant in that the *H. axyridis* larval stage represents the highest risk to *A. 2-punctata*, and lasts rather longer than the 18.7 day estimate for each IGP event. Depending on the developmental stage being preyed upon (e.g. a pupa, having achieved much of its development, is a more costly loss to the population than an egg), this arguably represents a level of IGP that could impact upon the local population of *A. 2-punctata*. However, acknowledging that there are several assumptions made in this argument, the extrapolation is debatable when only one of 112 *H. axyridis* tested positive. Only one of 28 field-collected *H. axyridis* tested positive for adaline using GC-MS (Hautier et al., 2008), suggesting a 3.6% IGP rate of *H. axyridis* on *A. 2-punctata* in Belgian potato fields. However, a very long detection window (96h) was calculated for that study (Hautier et al., 2008).

Previous studies have determined that PCR cannot distinguish primary from secondary predation (Sheppard et al., 2005), or scavenging from predation (Foltan et al., 2005, Juen & Traugott, 2005). Detection of secondary predation could occur if a predator ate the target prey for PCR (e.g. *A. 2-punctata*), then that predator was itself eaten by the target predator (e.g. *H. axyridis*), leading to inconclusive results. In my system the likelihood of this occurring was small; firstly, because *A. 2-punctata* was unlikely to be eaten by any species that was itself prey for *H. axyridis*, although possible exceptions were predatory hemipterans; secondly, because of the short detection window and apparently high amount of prey DNA needed for detection with the methods used (King et al., 2008). There is a possibility that *H. axyridis* scavenged *A. 2-punctata*, rather than predated it, as coccinellids are known to scavenge (Majerus, 1994a).

6.5 Conclusion

Detection of IGP by one coccinellid on another, in the field in Europe, was shown using PCR techniques. An apparently low level (0.89%) of IGP by *H. axyridis* on *A. 2-punctata* was observed, but because of the relatively insensitive detection techniques, this may represent a moderate level of predation. This is an important first step, but methods need to be refined, and the study broadened. A technique to identify *A. 10-punctata* is being developed, and the use of real-time PCR, which may be more sensitive than conventional PCR (Schmidt et al., 2009, Zhang et al., 2007b) is being tested. Also, two-way interactions need to be tested, i.e. to determine whether *Adalia* species are preying on *H. axyridis*, although this is an interaction that is less-likely (Ware et al., 2008b).

7. General discussion: assessment of predictions made on the spread and impacts of *Harmonia axyridis* in Great Britain

7.1 Introduction

Professor Michael Majerus of the University of Cambridge was one of the most eminent coccinellid researchers, and had observed the early stages of *H. axyridis* spread in Europe. Thus it was no surprise to him when the first *H. axyridis* reported in Great Britain was brought to him for identification in September 2004. He quickly issued a press release (Majerus, 2004) to alert the public to the arrival of this species and his prediction that it would establish and spread. He realised the potential of encouraging public participation in recording the distribution of *H. axyridis*. The press release drew the attention of the national media, and the coverage led to approximately 100 verified records of the species in 2004. Based on the distribution of these records, and receipt of seven earlier records of *H. axyridis* all from July-September 2004, Majerus believed that the species was new to Great Britain in 2004 (Majerus & Roy, 2005). Funding was obtained (from Defra, through the NBN Trust) to develop a web-based outreach project (www.harlequin-survey.org) which was launched in March 2005 to further media attention, including the front page of The Times newspaper (15 March 2005).

Majerus made two primary predictions regarding *H. axyridis* in Great Britain. Firstly, that the species would quickly establish, and then spread across the entire British mainland by 2008 (Majerus et al., 2006b). Secondly, that it had the potential to negatively affect around 1000 species native to Great Britain (Majerus, 2007). Majerus made these predictions based on extensive knowledge, having worked on coccinellids for over 20 years. Indeed, ten years before *H. axyridis* arrived in Great Britain, Majerus featured it in his regular Cambridge Ladybird Survey newsletter, illustrating its polymorphism with a figure showing various colour patterns (Majerus, 1994b). However, whilst the British media continued to focus on *H. axyridis*, there was scepticism about Majerus' ideas from some entomologists and ecologists, who felt that the predictions were over-stated. Despite the publicity, relatively few confirmed records of *H. axyridis* were received in the first eight months of 2005 (Chapter 4), leading me to sympathise with this view. However, by the end of 2007, *H. axyridis* had dramatically increased in distribution and abundance, spreading north at approximately 100km per year (Chapter 3). Westerly spread was even faster; within two years the species had spread to the far west of England and Wales, and was very widely established. By the end of 2008 there were several records from Scotland, including two in the far north, and some evidence of establishment in that country (Chapter 3). Thus, making an assessment of Majerus' first prediction is straightforward – it has been confirmed.

Resolving the second prediction, that *H. axyridis* would have a negative impact on around 1000 British species, is much more complex and requires critical assessment over many years of study at the landscape scale. However, here I will attempt to interpret the prediction and make a preliminary assessment of it, based on current knowledge.

7.2 Mechanisms of negative impacts

Harmonia axyridis may negatively impact on native species, broadly through the mechanisms outlined below.

7.2.1 Competition for food

Harmonia axyridis is highly polyphagous and is known to feed on many species of aphid and coccid (Koch, 2003). It is also a habitat generalist (Chapters 3 and 4). Thus there is considerable temporal and spatial resource overlap with other aphidophagous and, (to a lesser extent), coccidophagous arthropod species. These may be out-competed for prey by *H. axyridis*, aided by its strong physical and chemical defences. This could result in starvation or displacement of these species. Further, displaced species moving to new territories would have a knock-on effect on existing species in those territories.

7.2.2 Predation

Harmonia axyridis may prey on other arthropods, particularly insects, as alternative foods (Koch et al., 2003, Ingels & De Clercq, 2009, Hautier et al., 2008, Phoofolo & Obrycki, 1998). The insects affected would include many aphidophagous and coccidophagous species (i.e. IGP) and others utilising the same habitats (i.e. EGP). Depending on the prey, predation by *H. axyridis* could be directed to any life stage, but studies have demonstrated that immature stages are particularly vulnerable (Pell et al., 2008). Predation may be bi-directional, especially in the case of EGP. However, evidence shows that IGP is usually uni-directional in favour of *H. axyridis* (e.g. Ware & Majerus, 2008, Soares & Serpa, 2007, Burgio et al., 2005), although this is not always the case (De Clercq et al., 2003).

7.2.3 Disruption of the aphid natural enemy guild

Harmonia axyridis has effects on natural enemies of aphids, such as parasitoid wasps. For example, indirect predation of parasitoids (i.e. *H. axyridis* feeding on parasitized aphids), and/or negative effects on the oviposition of parasitoids, could occur, thus potentially affecting parasitoid populations (Pell et al., 2008).

7.2.4 Disease transmission

Harmonia axyridis may act as a vector for the transmission of bacterial, viral or fungal pathogens. Its rapid spread into new territories and habitats could introduce pathogens that would not otherwise be present in these systems. An example could be the fungal pathogen *Beauveria bassiana*, which *H. axyridis* is more resistant to than native coccinellids (Roy et al., 2008b, Cottrell & Shapiro-Ilan, 2003). *Harmonia axyridis* was found to enhance transmission of the aphid-specific pathogenic fungus *Pandora neoaphidis* (Remaudière and Hennebert) Humber (Entomophthoromycotina: Entomophthorales) (Roy et al., 2008a).

7.3 Contrasting case studies

To help explore the effects of invading insects I will summarise two contrasting case studies, and comment on the differing impacts of these invaders in relation to *H. axyridis* in Great Britain. The two invasions are very dissimilar in terms of the geography of the invaded ranges and the taxonomy and niches of the invaders.

7.3.1 Case study 1 – Argentine ant *Linepithema humile* in Hawaii

The Argentine ant *Linepithema humile* is a notorious invasive insect on several continents (Suarez et al., 2001), but here I will focus only on the Pacific islands of Hawaii. *Linepithema humile* is first known from Hawaii in 1916 (Wetterer et al., 2009). In the 1930s it was known to have arrived on shipped goods from California (where it was also an invasive species) (Medeiros et al., 1986). In Hawaii the ant was over 10000km outside of its native South American range. Its spread in Hawaii was relatively slow (occurring over several decades) partly because, unlike many ant species, *L. humile* queens are flightless and hence walk to form new colonies (Cole et al., 1992). Experimental work carried out in 1985-6, about 20 years post-arrival in the study area, determined that *L. humile* had a very serious negative impact on native arthropods (Medeiros et al., 1986, Cole et al., 1992). Amongst the affected groups were bees, noctuid moths, carabid beetles, earwigs, flies, small hemipterans and hymenopterans and small spiders (Medeiros et al., 1986, Cole et al., 1992). The impact, by nest destruction, on the ground-dwelling bees, was particularly notable and was predicted to have wider implications in the loss of very important pollination services (Medeiros et al., 1986, Cole et al., 1992).

The serious effects of this invasion may be explained in terms of both the strengths of the invading species, and the vulnerability of the invaded ecosystem. *Linepithema humile* is a highly adaptable habitat generalist, tolerant of disturbed environments, and successful at altitudes up to 3000m (Williamson, 1996). It is a polyphagous predator; the chances of an invaded species reaching stable coexistence with an invading species decrease with increasing polyphagy of the invader (Lawton & Brown, 1986). *Linepithema humile* is intolerant of other ant species (Erickson, 1971) and outcompetes them, partly due to the large size of its colonies. In Hawaii, an invasive ant that arrived earlier, *Pheidole megacephala*, was partially displaced by *L. humile* (Elton, 1958), although the former still dominated in some areas (Fluker & Beardsley, 1970). The Hawaiian islands are extremely well-known as hotspots for endemism, caused partly by their isolation from continental land masses and their diverse climates and habitats (Cox & Moore, 2000). Hawaii was particularly vulnerable to ant invasions as it has no native ant species (Williamson, 1996), thus native arthropods have not evolved defence mechanisms against ants (Cole et al., 1992). Indeed, there are many flightless endemic insects, increasing their vulnerability (Cole et al., 1992). *Linepithema humile* was so far from its native range that it had presumably escaped most or all of its natural enemies.

7.3.2 Case study 2 – Bryony ladybird *Henosepilachna argus* in Great Britain

The bryony ladybird *Henosepilachna argus* is a continental European species that was not recorded in Great Britain until 1997, when it was found in Surrey, south-east England (Menzies & Spooner, 2000). It only spread locally at a slow pace (approximately 30km in 12 years). *Henosepilachna argus* has had no reported effects on native species. It is herbivorous and limited to few food plants, notably white bryony *Bryonia dioica*, and these traits alone mean that it could not have the serious widespread impacts of the polyphagous and predatory *L. humile* in Hawaii. It is necessarily a habitat specialist because of the specificity of its food, and can only spread to areas that support the food plant. Other factors probably also limit its spread. Firstly, *H. argus* is close to its native range and is unlikely to have escaped its natural enemies, perhaps especially pathogens. Secondly, *H. argus* may be on the edge of its climatic range; cool temperatures may be a barrier to much further northward spread in Great Britain. The focus of this thesis is the effect of an invasive species on biodiversity, rather than on the economy. Conversely, *H. argus* is more likely to have an economic, rather than a biodiversity, impact; it could conceivably become a pest of cucurbit crops. Indeed, some other members of the Epilachninae, notably the Mexican bean beetle *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae), are serious agricultural pests (http://entnemdept.ufl.edu/creatures/veg/bean/MEXICAN_BEAN_BEETLE.HTM). *Henosepilachna argus* also appears to be spreading in other parts of Europe, e.g. Germany (Hawkins, 2000) and Croatia (Jelovčan et al., 2007).

The invaded ecosystem in Great Britain is much more robust than those in Hawaii. Britain has depauperate ecosystems for many reasons, notably the impact of the last Ice Age, the separation from mainland Europe and the human disturbance of most of the landscape over thousands of years, in an industrial country with high population density. As such there are fewer species in Great Britain for an invader to have a negative impact on, and those that are there are, on average, less vulnerable than endemic species in Hawaii.

7.3.3 Links between case studies and *H. axyridis* in Great Britain

The case studies above have some relevance to the invasion of *H. axyridis* in Great Britain. The generalist, polyphagous and predatory traits of *L. humile* have parallels with *H. axyridis*, but the ecosystems in question (i.e. in Hawaii and Great Britain) are clearly very different. Conversely, *H. argus* has similarities in that it has invaded part of Britain, but the specialist traits of *H. argus* make its invasion very different to that of *H. axyridis*. Thus, the extent of negative effects of *H. axyridis* in Britain is likely to be somewhere between that observed in the two case studies. It is entirely possible that if *H. axyridis* established in Hawaii, it would have negative effects of the same magnitude as those caused by *L. humile*; indeed, there are indications that a different generalist coccinellid predator, the steelblue ladybird *Halmus chalybeus*, may be having serious negative effects on native arthropods in that country (Sheppard et al., 2004, Sheppard et al., 2005). In Great Britain, the effects of *H. axyridis* are anticipated to be serious, but because of the factors outlined above, not on the scale of those occurring due to *L. humile* in Hawaii.

In addition to affects on native biodiversity, *H. axyridis* may affect ecosystem function. Whilst it can be argued that there will be little change in ecosystem function if *H. axyridis* displaces native species, but eats the aphids that those species would otherwise have eaten, this may not be the case. The many aphidophagous species in the ecosystem all have subtly different roles which would not be filled by a single replacement species. Further, even if there was no net change in the aphids consumed, replacing many species with one would severely weaken the ecosystem; if some catastrophic event (e.g. disease) were to eliminate the one remaining species, no aphid control function would remain until such time as a new aphidophagous species arrived. This would clearly have severe knock-on effects on plants.

7.4 Majerus' second prediction: which 1000 species could be affected?

The species that could form the basis of Majerus' second prediction are shown (Table 7.1), with a very coarse assessment ('Most', 'Some' or 'A few') made on the proportion of each group that may be negatively affected.

Table 7.1. An estimate of the number of species and proportion of these that could be negatively affected by the arrival of *H. axyridis* in Great Britain. Approximate no. GB species from Chinery, 1993. Proportions allocated based on the following: 'Most': insect groups in which >75% of species have the potential for a resource overlap with *H. axyridis*; 'Some': about half the species have the potential for a resource overlap; 'A few': a minority of the species have the potential for a resource overlap.

Order	Super-family, family or sub-family	Approximate no. GB species	Proportion that may be affected
Hemiptera	Aphidoidea	550	Most
	Coccoidea	170	Some
	Psylloidea	60	Some
	Anthocoridae	27	Some
Coleoptera	Coccinellidae	45	Most
	Chrysomelidae	250	A few
	Carabidae	350	A few
Lepidoptera		2400	A few
Hymenoptera	Braconidae	1000	A few
Neuroptera	Chrysopidae	14	Most
Diptera	Syrphinae	130	Some

The insect species may be broken into four main categories. Firstly, and most obviously, there are the species that *H. axyridis* is most likely to consume. Since *H. axyridis* is such an abundant, widespread (Chapter 3) and eurytopic (Chapter 4) species, it is possible that it could encounter the majority of the Sternorrhyncha species occurring in Great Britain, and feed upon many of them (Koch, 2003). Secondly, there are the other aphidophagous or coccidophagous species, e.g. coccinellids, chrysopids, syrphids, and (to some extent) carabids, that are likely to suffer direct or indirect effects of *H. axyridis* by the mechanisms outlined in Section 7.2. Thirdly, there are insects that may become secondary prey for *H. axyridis* if primary food supplies run low. Fourthly, there are species, e.g. parasitoid wasps, which may suffer indirectly through some overlap in resource use with *H. axyridis* (Pell et al., 2008).

A total of 650 insect species native to Britain is reached by adding up the following: 75% of the aphids; 50% of the coccids and psyllids; 50% of the anthocorids; 75% of the coccinellids and chrysopids; 50% of the aphidophagous syrphids. In addition, a few braconid species may be affected. I acknowledge that these figures are very speculative.

So, a further 350 species would be needed in order to reach the 1000 species total from Majerus' prediction. Based on evidence of impacts in previous studies, the most likely groups that these would come from are the Lepidoptera, e.g. noctuid moths (W. Phillips, personal communication) and butterflies (e.g. brimstone *Gonepteryx rhamni* (L.) (Lepidoptera: Pieridae) (Stewart, 2008); small tortoiseshell *Aglais urticae* (L.) (Lepidoptera: Nymphalidae) (Wells et al., in press); monarch *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae) (Koch et al., 2003)) and the Coleoptera, e.g. chrysomelids (Sebolt & Landis, 2004) and carabids (Harper et al., 2005). 350 species would represent approximately 12% of the British Lepidoptera, chrysomelid and carabid fauna.

There are also other taxa that may be directly or indirectly affected. For example, depending on their ability to deter *H. axyridis* (and vice versa), some ant species could be affected by aphid predation, reducing an important food supply (honeydew). Little is known about interactions between *H. axyridis* and ants (Pell et al., 2008), although Kabiri (2006) reported ants attacking *H. axyridis* eggs and larvae in France. It is possible that some arachnids could be prey to *H. axyridis*. However, the reverse interaction is more likely (various personal communications through the Harlequin Ladybird Survey, e.g. http://www.pbase.com/louloubelle/image/87155242), and positive impacts on some species are possible. Spiders and other species (e.g. some heteropterans) that adapt to use *H. axyridis* as prey, could benefit. Positive effects could result for parasitic species of *H. axyridis* (e.g. a few phorid species and the braconid *Dinocampus coccinellae*), although those that are shared with other species could possibly incur a fitness cost, if *H. axyridis* is not such a suitable host.

7.5 Extent of negative effects, and assessment of the second prediction

From the species analyses above, I believe that it is quite possible that in the long-term, 1000 species could be negatively affected by *H. axyridis*. However, the extent of effect will be extremely variable between taxa. *Harmonia axyridis* feeding on any of the species mentioned, or their food supplies, may or may not cause them significant negative effects at the population level. For some, there may be long-term, widespread population effects. But I believe that for a far greater number, the scale (either spatial, temporal or both) will be much more limited (Table 7.2). Species experiencing short-term pressures are likely to be able to recover, especially if the effects are localised, when recruitment from a neighbouring area may be possible. Species with high reproductive capacities, i.e. most arthropods (and especially aphids), are likely to suffer fewer long-term negative effects. However, other negative influences on species from factors such as climate change, habitat loss and pollution, are likely to make them more vulnerable to the additional effects of an invader such as *H. axyridis*.

Further factors that will inevitably affect the risk of negative population effects are the geographical spread and habitat specificity of the target species. One which has a very restricted distribution and/or a specialised habitat will face a greater risk of moderate or serious population effects (Table 7.2). An example is the 5-spot ladybird *Coccinella 5-punctata* L. (Coleoptera: Coccinellidae). In Great Britain, this species has a very restricted and patchy geographic range (UK Ladybird Survey data) and only occurs close to the river shingles of fast-flowing streams and rivers (Majerus, 1994a). Studies have shown that the species is vulnerable to IGP by *H. axyridis* (Ware & Majerus, 2008). Further, *H. axyridis* has already become abundant in places close to some of the most important populations of *C. 5-punctata* (Chapter 3). Overall, in the medium term (10-20 years) I believe that *C. 5-punctata* is at risk of extinction in Great Britain due to the negative effects of *H. axyridis*.

Table 7.2. Qualitative model of the predicted negative effects (shown in coloured boxes), at population level, on species with resource overlap with *H. axyridis* at different spatial (local and widespread) and temporal (short- and long-term) scales.

Temporal scale Spatial scale	Short-term	Long-term
Local	**Minimal**	**Moderate**
Widespread (or local for habitat specialists)	**Moderate**	**Serious**

Providing evidence of the predicted extensive impacts of *H. axyridis* on native species is likely to be extremely difficult. This is because the multiple interactions between the species, the spatial scale of the impacts, and the lack of knowledge of trophic interactions between many of the species, all combine to form a very complex scenario.

7.6 Perceptions of the '1000 species' prediction

There is one important criticism regarding Majerus' '1000 species' prediction: that the perception of it differed from the reality. By this, I mean that the way that the prediction was inevitably portrayed by the media was not fully consistent with the detail of the prediction, i.e. it was exaggerated. Journalists tasked with writing an interesting story were bound to focus on the dramatic. Thus 'possible negative effects on up to 1000 species' became in the media coverage: 'A giant ladybird is threatening to wipe out more than 1000 native insect and plant species...' (Gray, 2009). Majerus was predicting negative large-scale impacts on populations of many species, but not a large number of species extinctions. The perception passed from the media to the public was rather different.

However, the result of all the publicity, whether balanced or not, was that a high level of interest was generated in *H. axyridis* and other ladybirds. This led to increased recording activity by entomologists, naturalists and the general public (adults and children). In Great Britain in recent years there has been much concern expressed about declining interest in entomology and a decreasing level of taxonomic expertise amongst entomology students (Key, 2007). Encouraging interest in insects in children and adults, and raising awareness of associated conservation issues (including invasive species) is very important. Majerus' work undoubtedly inspired many (Brown et al., in press), and led to a wealth of new discoveries relating to coccinellids (Majerus, 1994a).

References

Abbas, I., Nakamura, K., Katakura, H. & Sasaji, H., 1988. Geographical variation of elytral spot patterns in the phytophagous ladybird, *Epilachna vigintioctopunctata* (Coleoptera: Coccinellidae) in the province of Sumatra Barat, Indonesia. *Researches on Population Ecology.* 30, pp.43-56.

Adriaens, T., Branquart, E. & Maes, D., 2003. The multicoloured Asian ladybird *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), a threat for native aphid predators in Belgium? *Belgian Journal of Zoology.* 133(2), pp.195-196.

Adriaens, T., San Martin y Gomez, G. & Maes, D., 2008. Invasion history, habitat preferences and phenology of the invasive ladybird *Harmonia axyridis* in Belgium. *Biocontrol.* 53(1), pp.69-88.

Agusti, N., De Vicente, M.C. & Gabarra, R., 1999. Development of sequence amplified characterized region (SCAR) markers of *Helicoverpa armigera*: a new polymerase chain reaction-based technique for predator gut analysis. *Molecular ecology.* 8(9), pp.1467-1474.

Agusti, N., Unruh, T.R. & Welter, S.C., 2003. Detecting *Cacopsylla pyricola* (Hemiptera : Psyllidae) in predator guts using COI mitochondrial markers. *Bulletin of entomological research.* 93(3), pp.179-185.

Alhmedi, A., Haubruge, E., Bodson, B. & Francis, F., 2006. Inter- and intra-guild interactions related to aphids in nettle (*Urtica dioica* L.) strips closed to field crops. *Commun Agric Appl Biol Sci.* 71(2 Pt B), pp.413-423.

Alyokhin, A. & Sewell, G., 2004. Changes in a lady beetle community following the establishment of three alien species. *Biological Invasions.* 6(4), pp.463-471.

Andermatt, M., 1996. Marienkäferlarven als Blattlaus-Bekämpfungsmittel? *Andematt BioControl Journal.* 3, pp.10-11.

Babendreier, D., 2007. Pros and cons of biological control. In: W. Nentwig ed. *Biological invasions.* Dordrecht: Springer, pp.403-418.

Bazzocchi, G.G., Lanzoni, A., Accinelli, G. & Burgio, G., 2004. Overwintering, phenology and fecundity of *Harmonia axyridis* in comparison with native coccinellid species in Italy. *Biocontrol.* 49(3), pp.245-260.

Berkvens, N., Bale, J.S., Berkvens, D., Tirry, L. & De Clercq, P., 2010. Cold tolerance of the harlequin ladybird *Harmonia axyridis* in Europe. *Journal of insect physiology.* 56(4), pp.438-444.

Berkvens, N., Bonte, J., Berkvens, D., Deforce, K., Tirry, L. & De Clercq, P., 2008a. Pollen as an alternative food for *Harmonia axyridis*. *Biocontrol.* 53(1), pp.201-210.

Berkvens, N., Bonte, J., Berkvens, D., Tirry, L. & De Clercq, P., 2008b. Influence of diet and photoperiod on development and reproduction of European populations of *Harmonia axyridis* (Pallas) (Coleoptera : Coccinellidae). *Biocontrol.* 53(1), pp.211-221.

Brown, M.W. & Miller, S.S., 1998. Coccinellidae (Coleoptera) in apple orchards of eastern West Virginia and the impact of invasion by *Harmonia axyridis*. *Entomological news.* 109(2), pp.143-151.

Brown, P.M.J. & Roy, H.E., 2007. The spread of the harlequin ladybird *Harmonia axyridis* in late 2006. *Atropos.* 31, pp.32-33.

Brown, P.M.J., Roy, H.E. & Majerus, M.E.N., 2006. Ladybird recording in Cambridgeshire and Huntingdonshire. *Nature in Cambridgeshire.* 48, pp.12-16.

Brown, P.M.J., Roy, H.E. & Ware, R.L., 2010. Wildlife reports: Ladybirds. *British Wildlife.* 21(3), pp.208-209.

Brown, P.M.J., Ware, R.L. & Roy, H.E., in press. Looking beyond the spots: inspiring the public to record ladybirds. *IOBC Bulletin.*

Burgio, G., Ferrari, R., Pozzati, M. & Boriani, L., 2004. The role of ecological compensation areas on predator populations: an analysis on biodiversity and phenology of Coccinellidae (Coleoptera) on non-crop plants within hedgerows in Northern Italy. *Bulletin of Insectology.* 57(1), pp.1-10.

Burgio, G., Santi, F. & Maini, S., 2005. Intra-guild predation and cannibalism between *Harmonia axyridis* and *Adalia bipunctata* adults and larvae: laboratory experiments. *Bulletin of Insectology.* 58(2), pp.135-140.

Burgio, G., Santi, F., Lanzoni, A., Masetti, A., De Luigi, V., Melandri, M., Reggiani, A., Ricci, C., Loomans, A.J.M. & Maini, S., 2008. *Harmonia axyridis* recordings in northern Italy. *Bulletin of Insectology.* 61(2), pp.361-364.

Chacon, J.M., Landis, D.A. & Heimpel, G.E., 2008. Potential for biotic interference of a classical biological control agent of the soybean aphid. *Biological Control.* 46(2), pp.216-225.

Chapin, J.B. & Brou, V.A., 1991. *Harmonia axyridis* (Pallas), the 3rd species of the genus to be found in the United States (Coleoptera, Coccinellidae). *Proceedings of the Entomological Society of Washington.* 93(3), pp.630-635.

Chen, Y., Giles, K.L., Payton, M.E. & Greenstone, M.H., 2000. Identifying key cereal aphid predators by molecular gut analysis. *Molecular ecology.* 9(11), pp.1887-1898.

Chinery, M., 1993. *Insects of Britain and Northern Europe.* 3rd ed. London: HarperCollins.

Coderre, D., Lucas, E. & Gagne, I., 1995. The occurrence of *Harmonia axyridis* (Pallas) (Coleoptera, Coccinellidae) in Canada. *Canadian Entomologist.* 127(4), pp.609-611.

Cole, F.R., Medeiros, A.C., Loope, L.L. & Zuehlke, W.W., 1992. Effects of the Argentine ant on arthropod fauna of Hawaiian high-elevation shrubland. *Ecology.* 73(4), pp.1313-1322.

Colunga-Garcia, M. & Gage, S.H., 1998. Arrival, establishment, and habitat use of the multicolored Asian lady beetle (Coleoptera: Coccinellidae) in a Michigan landscape. *Environmental Entomology.* 27(6), pp.1574-1580.

Comont, R.F., Harrington, R., Lewis, O.T., Purse, B.V. & Roy, H.E., in press. Modelling *Harmonia axyridis* interactions within the aphidophagous guild. *IOBC Bulletin.*

Cottrell, T.E., 2004. Suitability of exotic and native lady beetle eggs (Coleoptera : Coccinellidae) for development of lady beetle larvae. *Biological Control.* 31(3), pp.362-371.

Cottrell, T.E. & Shapiro-Ilan, D.I., 2003. Susceptibility of a native and an exotic lady beetle (Coleoptera : Coccinellidae) to *Beauveria bassiana*. *Journal of invertebrate pathology.* 84(2), pp.137-144.

Coutanceau, J., 2006. *Harmonia axyridis* (Pallas, 1773): une coccinelle asiatique introduite, acclimatée et en extension en France. *Bulletin de la Société entomologique de France.* 111(3), pp.395-401.

Cox, C.B. & Moore, P.D., 2000. *Biogeography: an ecological and evolutionary approach.* 6th ed. Oxford: Blackwell.

Crooks, J.A., 2005. Lag times and exotic species: The ecology and management of biological invasions in slow-motion. *Ecoscience.* 12(3), pp.316-329.

Cuppen, J., Heijerman, T., van Wielink, P. & Loomans, A., 2004. Het lieveheersbeestje *Harmonia axyridis* in Nederland: een aanwinst voor onze fauna of een ongewenste indringer (Coleoptera: Coccinellidae)? *Nederlandse Faunistische Mededelingen.* 20, pp.1-12.

Davis, R.S., Vandewalker, M.L., Hutcheson, P.S. & Slavin, R.G., 2006. Facial angioedema in children due to ladybug (*Harmonia axyridis*) contact: 2 case reports. *Annals of Allergy Asthma & Immunology.* 97(4), pp.440-442.

Day, W.H., Prokrym, D.R., Ellis, D.R. & Chianese, R.J., 1994. The known distribution of the predator *Propylea quattuordecimpunctata* (Coleoptera, Coccinellidae) in the United States, and thoughts on the origin of this species and 5 other exotic lady beetles in eastern North America. *Entomological news.* 105(4), pp.244-256.

de Almeida, L.M. & da Silva, V.B., 2002. First record of *Harmonia axyridis* (Pallas) (Coleoptera, Coccinellidae): a lady beetle native to the Palaearctic region. *Revista Brasileira de Zoologia.* 19, pp.941-944.

De Clercq, P., 2002. Dark clouds and their silver linings: exotic generalist predators in augmentative biological control. *Neotropical Entomology.* 31(2), pp.169-176.

De Clercq, P., Peeters, I., Vergauwe, G. & Thas, O., 2003. Interaction between *Podisus maculiventris* and *Harmonia axyridis*, two predators used in augmentative biological control in greenhouse crops. *Biocontrol.* 48(1), pp.39-55.

de Leon, J.H., Fournier, V., Hagler, J.R. & Daane, K.M., 2006. Development of molecular diagnostic markers for sharpshooters *Homalodisca coagulata* and *Homalodisca liturata* for use in predator gut content examinations. *Entomologia Experimentalis et Applicata.* 119(2), pp.109-119.

Dixon, A.F.G., 2000. *Insect predator-prey dynamics: ladybird beetles and biological control.* Cambridge: Cambridge University Press.

Dixon, A.F.G., 1998. *Aphid ecology: an optimization approach.* 2nd ed. London: Chapman & Hall.

Dixon, A.F.G. & Hemptinne, J.L. 2009. Ladybird beetle invasions: traits of invasive species. *Harmonia axyridis and other invasive ladybirds (IOBC/WPRS Study Group).* Engelberg, Switzerland, 6-9 September 2009.

Dixon, A.F.G. & Kindlmann, P. 1999. Population dynamics of aphids. In: J.P. Dempster & I.F.G. McLean eds. *Insect populations in theory and in practice.* Dordrecht: Springer, pp.207-230.

Dobzhansky, T., 1933. Geographical variation in ladybeetles. *The American Naturalist.* 67, pp.97–126.

Doumbia, M., Hemptinne, J.L. & Dixon, A.F.G., 1998. Assessment of patch quality by ladybirds: role of larval tracks. *Oecologia.* 113(2), pp.197-202.

Elliott, N., Kieckhefer, R. & Kauffman, W., 1996. Effects of an invading coccinellid on native coccinellids in an agricultural landscape. *Oecologia.* 105(4), pp.537-544.

Elton, C.S., 1958. *The ecology of invasions by animals and plants.* London: Methuen.

Erickson, J.M., 1971. The displacement of native ant species by the introduced Argentine ant, *Iridomyrmex humilis* Mayr. *Psyche.* 78, pp.257-266.

Eschen, R., Babendreier, D., Nauer, S., Bigler, F. & Kenis, M., 2007. Surveys for ladybirds (Coleoptera: Coccinellidae) in Switzerland and confirmation of the presence of the invasive alien ladybird species *Harmonia axyridis* (Pallas). *Mitteilungen der Schweizerischen Entomologischen Gesellschaft.* 80, pp.7-14.

Estay, S.A., Lima, M. & Harrington, R., 2009. Climate mediated exogenous forcing and synchrony in populations of the oak aphid in the UK. *Oikos.* 118(2), pp.175-182.

Evans, E.W., 2004. Habitat displacement of North American ladybirds by an introduced species. *Ecology.* 85(3), pp.637-647.

Evans, E.W., 2000. Morphology of invasion: body size patterns associated with establishment of *Coccinella septempunctata* (Coleoptera : Coccinellidae) in western North America. *European Journal of Entomology.* 97(4), pp.469-474.

Ferran, A., El-Arnaouty, S.A., Beyssat-Arnaouty, V. & Galal, H., 2000. Introduction and release of the coccinellid *Harmonia axyridis* Pallas for controlling *Aphis craccivora* Koch on faba beans in Egypt. *Egyptian Journal of Biological Pest Control.* 10(1), pp.129-136.

Fisher, R.A., 1930. *The genetical theory of natural selection.* Oxford: Oxford University Press.

Fluker, S.S. & Beardsley, J.W., 1970. Sympatric associations of 3 ants - *Iridomyrmex humilis*, *Pheidole megacephala*, and *Anoplolepis longipes* Hymenoptera-Formicidae in Hawaii. *Annals of the Entomological Society of America.* 63(5), pp.1290-1296.

Foltan, P., Sheppard, S., Konvicka, M. & Symondson, W.O.C., 2005. The significance of facultative scavenging in generalist predator nutrition: detecting decayed prey in the guts of predators using PCR. *Molecular ecology.* 14(13), pp.4147-4158.

Fournier, V., Hagler, J., Daane, K., de Leon, J. & Groves, R., 2008. Identifying the predator complex of *Homalodisca vitripennis* (Hemiptera : Cicadellidae): a comparative study of the efficacy of an ELISA and PCR gut content assay. *Oecologia.* 157(4), pp.629-640.

Fournier, V., Hagler, J.R., Daane, K.M., de Leon, J.H., Groves, R.L., Costa, H.S. & Henneberry, T.J., 2006. Development and application of a glassy-winged and smoke-tree sharpshooter egg-specific predator gut content ELISA. *Biological Control.* 37(1), pp.108-118.

Fremlin, M., 2008. Intra-guild predation of hoverfly larvae by harlequin ladybirds. *Bulletin of the Amateur Entomologists' Society.* 67, pp.148-150.

Gagnon, A.E., Heimpel, G.E. & Brodeur, J. 2005. Detection of intraguild predation between coccinellids using molecular analyses of gutcontents. *International symposium on biological control of aphids and coccids.* Tsuruoka, Japan.

Gardiner, M.M., Landis, D.A., Gratton, C., Schmidt, N., O'Neal, M., Mueller, E., Chacon, J., Heimpel, G.E. & DiFonzo, C.D., 2009. Landscape composition influences patterns of native and exotic lady beetle abundance. *Diversity and Distributions.* 15(4), pp.554-564.

GBNNSIP, 2010. *Species register: unpublished data compiled by the Great Britain Non-Native Species Information Portal team*. Wallingford: NERC CEH.

Gilbert, M., Grégoire, J.C., Freise, J.F. & Heitland, W., 2004. Long-distance dispersal and human population density allow the prediction of invasive patterns in the horse chestnut leafminer *Cameraria ohridella*. *Journal of Animal Ecology.* 73(3), pp.459–468.

Glowka, L., Burhenne-Guilmin, F., Synge, H., McNeely, J. & G'ndling, L., 1994. *A guide to the Convention on Biological Diversity (environmental policy and law paper no. 30).* International Union for the Conservation of Nature, Gland, Switzerland.

Goldarazena, A. & Calvo, D., 2007. First record of *Harmonia axyridis* (Coleoptera: Coccinellidae) from the lberian Peninsula. *Boletin de la Sociedad Entomológica Aragonesa.* 41, pp.437-439.

Gordon, R.D., 1985. The Coccinellidae (Coleoptera) of America North of Mexico. *Journal of the New York Entomological Society.* 93(1), pp.1-912.

Goulson, D., 2003. Effects of introduced bees on native ecosystems. *Annual Review of Ecology Evolution and Systematics.* 34, pp.1-26.

Gray, L., 2009. Giant ladybird could threaten 1,000 British species. *Daily Telegraph,* 30 June 2009.

Green, P. & Silverman, B.W., 1994. *Non-parametric regression and generalized additive models.* London: Chapman and Hall.

Greenstone, M.H., Rowley, D.L., Weber, D.C., Payton, M.E. & Hawthorne, D.J., 2007. Feeding mode and prey detectability half-lives in molecular gut-content analysis: an example with two predators of the Colorado potato beetle. *Bulletin of entomological research.* 97(2), pp.201-209.

Grez, A., Zaviezo, T., Gonzalez, G. & Rothmann, S., in press. *Harmonia axyridis* in Chile: a new threat. *Ciencia e Investigación Agraria (Chile).*

Haddrill, P.R., Majerus, M.E.N. & Mayes, S., 2002. Isolation and characterization of highly polymorphic microsatellite loci in the 2-spot ladybird, *Adalia bipunctata. Molecular Ecology Notes.* 2(3), pp.316-319.

Haldane, J.B.S., 1927. A mathematical theory of natural and artificial selection, part V: selection and mutation. *Proceedings of the Cambridge Philosophical Society.* 28, pp.838-844.

Harding, P.T., 1990. National species distribution surveys. In: F.B. Goldsmith ed. *Monitoring for conservation and ecology.* Chapman and Hall, London, pp.133-154.

Harmon, J.P., Stephens, E. & Losey, J., 2007. The decline of native coccinellids (Coleoptera : Coccinellidae) in the United States and Canada. *Journal of Insect Conservation.* 11(1), pp.85-94.

Harper, G.L., King, R.A., Dodd, C.S., Harwood, J.D., Glen, D.M., Bruford, M.W. & Symondson, W.O.C., 2005. Rapid screening of invertebrate predators for multiple prey DNA targets. *Molecular ecology.* 14(3), pp.819-827.

Harwood, J.D., Desneux, N., Yoo, H.J.S., Rowley, D.L., Greenstone, M.H., Obrycki, J.J. & O'Neil, R.J., 2007. Tracking the role of alternative prey in soybean aphid predation by *Orius insidiosus*: a molecular approach. *Molecular ecology.* 16, pp.4390-4400.

Harwood, J.D., Yoo, H.J.S., Greenstone, M.H., Rowley, D.L. & O'Neil, R.J., 2009. Differential impact of adults and nymphs of a generalist predator on an exotic invasive pest demonstrated by molecular gut-content analysis. *Biological Invasions.* 11(4), pp.895-903.

Hautier, L., Gregoire, J., de Schauwers, J., Martin, G.S., Callier, P., Jansen, J. & de Biseau, J., 2008. Intraguild predation by *Harmonia axyridis* on coccinellids revealed by exogenous alkaloid sequestration. *Chemoecology.* 18(3), pp.191-196.

Hawkins, R.D., 2000. *Ladybirds of Surrey.* Woking: Surrey Wildlife Trust.

Hayashi, F. & Nakane, M., 1989. Radio tracking and activity monitoring of the dobsonfly larva, *Protohermes grandis* (Megaloptera: Corydalidae). *Oecologia.* 78, pp.468-472.

Herard, F., Krehan, H., Benker, U., Boegel, C., Schrage, R., Chauvat, E., Ciampitti, M., Maspero, M. & Bialooki, P. 2005. *Anoplophora* in Europe: infestations and management responses. *16th U.S. Department of Agriculture interagency research forum on gypsy moth and other invasive species.* Annapolis, MD., 18-21 January 2005.

Hickling, R., Roy, D.B., Hill, J.K., Fox, R. & Thomas, C.D., 2006. The distributions of a wide range of taxonomic groups are expanding polewards. *Global Change Biology.* 12(3), pp.450–455.

Hill, M., Baker, R., Broad, G., Chandler, P.J., Copp, G.H. & Ellis, J., 2005. *Audit of non-native species in England.* Peterborough: English Nature.

Hodek, I., 1973. *Biology of Coccinellidae.* The Hague: Dr. W. Junk.

Hodek, I. & Honek, A., 1996. *Ecology of Coccinellidae.* Dordrecht: Springer.

Honek, A., 1985. Habitat preferences of aphidophagous coccinellids [Coleoptera]. *Entomophaga.* 30(3), pp.253-264.

Hoogendoorn, M. & Heimpel, G.E., 2002. Indirect interactions between an introduced and a native ladybird beetle species mediated by a shared parasitoid. *Biological Control.* 25(3), pp.224-230.

Hoogendoorn, M. & Heimpel, G.E., 2001. PCR-based gut content analysis of insect predators: using ribosomal ITS-1 fragments from prey to estimate predation frequency. *Molecular ecology.* 10(8), pp.2059-2067.

Hosseini, R., Schmidt, O. & Keller, M.A., 2008. Factors affecting detectability of prey DNA in the gut contents of invertebrate predators: a polymerase chain reaction-based method. *Entomologia Experimentalis et Applicata.* 126(3), pp.194-202.

Hulme, P.E., Bacher, S., Kenis, M., Klotz, S., Kuhn, I., Minchin, D., Nentwig, W., Olenin, S., Panov, V., Pergl, J., Pysek, P., Roques, A., Sol, D., Solarz, W. & Vila, M., 2008. Grasping at the routes of biological invasions: a framework for integrating pathways into policy. *Journal of Applied Ecology.* 45(2), pp.403-414.

Hutchison, W. & Galvan, T. 2009. Pest status and management of *Harmonia axyridis* in North American vineyards. *Harmonia axyridis and other invasive ladybirds (IOBC/WPRS Study Group).* Engelberg, Switzerland, 6-9 September 2009.

Ingels, B. & De Clercq, P. 2009. Intraguild predation between *Harmonia axyridis* and the hoverfly *Episyrphus balteatus*. *Harmonia axyridis and other invasive ladybirds (IOBC/WPRS Study Group).* Engelberg, Switzerland, 6-9 September 2009.

Iperti, G. & Bertand, E., 2001. Hibernation of *Harmonia axyridis* (Coleoptera: Coccinellidae) in South-Eastern France. *Acta Soc Zool Bohem.* 65, pp.207–210.

Jacas, J., Urbaneja, A. & Viñuela, E., 2006. History and future of introduction of exotic arthropod biological control agents in Spain: a dilemma? *Biocontrol.* 51, pp.1-30.

Jacobs, J.H., Clark, S.J., Denholm, I., Goulson, D., Stoate, C. & Osborne, J.L., 2010. Pollinator effectiveness and fruit set in common ivy, *Hedera helix* (Araliaceae). *Arthropod-Plant Interactions.* 4, pp.19-28.

Jansen, J.P. & Hautier, L., 2008. Ladybird population dynamics in potato: comparison of native species with an invasive species, *Harmonia axyridis*. *Biocontrol.* 53(1), pp.223-233.

Jelovčan, S., Igrc Barčić, J. & Gotlin Čuljak, T., 2007. Newly determined ladybugs species (Coleoptera: Coccinellidae) in Croatia. *Entomol. Croat.* 11(1-2), pp.69-74.

Juen, A. & Traugott, M., 2005. Detecting predation and scavenging by DNA gut-content analysis: a case study using a soil insect predator-prey system. *Oecologia.* 142(3), pp.344-352.

Kabiri, F. 2006. *Harmonia axyridis*: a problem or a distraction? *Regulation of Biological Control Agents (REBECA).* Wageningen, Netherlands, 4-6 April 2006.

Katsoyannos, P., Kontodimas, D.C., Stathas, G.J. & Tsartsalis, C.T., 1997. Establishment of *Harmonia axyridis* on citrus and some data on its phenology in Greece. *Phytoparasitica.* 25(3), pp.183-191.

Kenis, M., Auger-Rozenberg, M.A., Roques, A., Timms, L., Pere, C., Cock, M., Settele, J., Augustin, S. & Lopez-Vaamonde, C., 2009. Ecological effects of invasive alien insects. *Biological Invasions.* 11(1), pp.21-45.

Kenis, M., Rabitsch, W., Auger-Rozenberg, M.A. & Roques, A., 2007. How can alien species inventories and interception data help us prevent insect invasions? *Bulletin of entomological research.* 97(5), pp.489-502.

Kenis, M., Roy, H.E., Zindel, R. & Majerus, M.E.N., 2008. Current and potential management strategies against *Harmonia axyridis*. *Biocontrol.* 53(1), pp.235-252.

Key, R., 2007. Another generation of entomologists? *Antenna.* 31(2), pp.87-93.

Kidd, K.A., Nalepa, C.A., Day, E.R. & Waldvogel, M.G., 1995. Distribution of *Harmonia axyridis* (Pallas) (Coleoptera, Coccinellidae) in North Carolina and Virginia. *Proceedings of the Entomological Society of Washington.* 97(3), pp.729-731.

King, R.A., Read, D.S., Traugott, M. & Symondson, W.O.C., 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. *Molecular ecology.* 17(4), pp.947-963.

Klausnitzer, B., 2004. *Harmonia axyridis* (Pallas, 1773) in Basel-Stadt (Coleoptera, Coccinellidae). *Entomologische Gesellschaft Basel.* 54(3/4), pp.115-122.

Klausnitzer, B., 2002. *Harmonia axyridis* (Pallas, 1773) in Deutschland (Col.,Coccinellidae). *Entomologische Nachrichten und Berichte.* 46, pp.177-183.

Klausnitzer, B. & Klausnitzer, H. 1997. 4. überarbeitete auflage. In: *Marienkäfer (Coccinellidae).* Magdeburg: Die Neue Brehm-Bücherei.

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, pp.1-16.

Koch, R.L., Hutchison, W.D., Venette, R.C. & Heimpel, G.E., 2003. Susceptibility of immature monarch butterfly, *Danaus plexippus* (Lepidoptera : Nymphalidae : Danainae), to predation by *Harmonia axyridis* (Coleoptera : Coccinellidae). *Biological Control.* 28(2), pp.265-270.

Koch, R.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), pp.421-434.

Koch, R.L. & Galvan, T.L., 2008. Bad side of a good beetle: the North American experience with *Harmonia axyridis*. *Biocontrol.* 53(1), pp.23-35.

Kontodimas, D.C., Milonas, P.G., Stathas, G.J., Papanikolaou, N.E., Skourti, A. & Matsinos, Y.G., 2008a. Life table parameters of the aphid predators *Coccinella septempunctata*, *Ceratomegilla undecimnotata* and *Propylea quatuordecimpunctata* (Coleoptera : Coccinellidae). *European Journal of Entomology.* 105(3), pp.427-430.

Kontodimas, D.C., Stathas, G.J. & Martinou, A.F., 2008b. The aphidophagous predator *Harmonia axyridis* (Coleoptera : Coccinellidae) in Greece, 1994-1999. *European Journal of Entomology.* 105(3), pp.541-544.

Kovach, J., 2004. Impact of multicolored Asian ladybeetles as a pest of fruit and people. *American Entomologist.* 50(3), pp.159-161.

Koyama, S. & Majerus, M.E.N., 2008. Interactions between the parasitoid wasp *Dinocampus coccinellae* and two species of coccinellid from Japan and Britain. *Biocontrol.* 53(1), pp.253-264.

Krafsur, E.S., Kring, T.J., Miller, J.C., Nariboli, P., Obrycki, J.J., Ruberson, J.R. & Schaefer, P.W., 1997. Gene flow in the exotic colonizing ladybeetle *Harmonia axyridis* in North America. *Biological Control.* 8(3), pp.207-214.

Kuznetsov, V.N., 1997. *Lady beetles of Russian far east.* Gainesville: The Sandhill Crane Press.

LaMana, M.L. & Miller, J.C., 1996. Field observations on *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in Oregon. *Biological Control.* 6(2), pp.232-237.

Lawton, J.H. & Brown, K.C., 1986. The population and community ecology of invading insects. *Philosophical Transactions of the Royal Society of London Series B - Biological Sciences.* 314(1167), pp.607-617.

Leather, S.R., Cooke, R.C.A., Fellowes, M.D.E. & Rombe, R., 1999. Distribution and abundance of ladybirds (Coleoptera : Coccinellidae) in non-crop habitats. *European Journal of Entomology*. 96(1), pp.23-27.

Linder, C., Lorenzini, F. & Kehrli, P., 2009. Potential impact of processed *Harmonia axyridis* on the taste of 'Chasselas' and 'Pinot noir' wines. *Vitis*. 48(2), pp.101-102.

Loiseau, A., Malausa, T., Lombaert, E., Martin, J.F. & Estoup, A., 2009. Isolation and characterization of microsatellites in the harlequin ladybird, *Harmonia axyridis* (Coleoptera, Coccinellidae), and cross-species amplification within the family Coccinellidae. *Molecular Ecology Resources*. 9(3), pp.934-937.

Lombaert, E., Guillemaud, T., Cornuet, J.M., Malausa, T., Facon, B. & Estoup, A. 2009. Worldwide routes of invasion of the biocontrol ladybird *Harmonia axyridis*: recurrent invasions from a bridgehead invasive population and admixture. *Harmonia axyridis and other invasive ladybirds (IOBC/WPRS Study Group)*. Engelberg, Switzerland, 6-9 September 2009.

Loomans, A., 2004. Veelkleurig Aziatisch lieveheersbeestje *Harmonia axyridis* bezig aan een opmars in Nederland. *Coccinula*. 10, pp.62-63.

Lucas, E., 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(4), pp.457-463.

Mabbott, P., 2005. Further reports of *Cheilomenes lunata* (Fab.) (Coleoptera: Coccinellidae) in Britain, winter 2001-2002. *The Entomologist's Record and Journal of Variation*. 117(4), pp.176-177.

Machado, A., 2005. El sarantontón asiático *Harmonia axyridis* (Pallas, 1773) presente en Canarias (Coleoptera: Coccinellidae). *Vieraea*. 34, pp.71-72.

Majerus, M.E.N., 2008. First evidence of a decline in a native ladybird as a consequence of the arrival of the harlequin ladybird, *Harmonia axyridis*, in Britain. *Bulletin of the Amateur Entomologists' Society*. 67, pp.142-147.

Majerus, M.E.N., 2007. Ladybirds of Epping Forest. *British Naturalists' Association Country-Side* 32(6), pp.9-12.

Majerus, M.E.N., 2004. The ladybird has landed. *University of Cambridge press release*.

Majerus, M.E.N., 2003. *Sex wars: genes, bacteria, and sex ratios*. Princeton, New Jersey: Princeton University Press.

Majerus, M.E.N., 1998. *Melanism: evolution in action*. Oxford: Oxford University Press.

Majerus, M.E.N., 1994a. *Ladybirds*. London: HarperCollins.

Majerus, M.E.N., 1994b. Research progress. *Cambridge Ladybird Survey Newsletter*. 17, pp.7-16.

Majerus, M.E.N., Brown, P.M.J., Roy, H.E. & Ware, R.L., 2009. Wildlife reports: Ladybirds. *British Wildlife.* 20, pp.210-212.

Majerus, M.E.N., Forge, H. & Walker, L., 1990. The geographical distributions of ladybirds in Britain (1984-1989). *British Journal of Entomology and Natural History.* 3, pp.153-166.

Majerus, M.E.N. & Kearns, P., 1989. *Ladybirds.* Slough: Richmond Publishing.

Majerus, M.E.N., Mabbott, P., Rowland, F. & Roy, H.E., 2006a. The harlequin ladybird, *Harmonia axyridis*, arrives in Britain. *Entomologist's Monthly Magazine.* 142, pp.87-92.

Majerus, M.E.N. & Roy, H.E., 2005. Scientific opportunities presented by the arrival of the harlequin ladybird, *Harmonia axyridis*, in Britain. *Antenna.* 29(3), pp.196-208.

Majerus, M.E.N., Strawson, V. & Roy, H.E., 2006b. The potential impacts of the arrival of the harlequin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), in Britain. *Ecological Entomology.* 31(3), pp.207-215.

Majka, C.G. & McCorquodale, D.B., 2006. The Coccinellidae (Coleoptera) of the maritime provinces of Canada: new records, biogeographic notes, and conservation concerns. *Zootaxa.* 1154, pp.49-68.

Marko, V. & Pozsgai, G., 2009. Spread of harlequin ladybird (*Harmonia axyridis* Pallas, 1773) (Coleoptera, Coccinellidae) in Hungary, and the first records from Romania and Ukraine. *Növényvédelem.* 45(9), pp.481-492.

Masterman, A.J., Foster, G.N., Holmes, S.J. & Harrington, R., 1996. The use of the Lamb daily weather types and the indices of progressiveness, southerliness and cyclonicity to investigate the autumn migration of *Rhopalosiphum padi*. *Journal of Applied Ecology.* 33(1), pp.23-30.

McClure, M.S., 1987. Potential of the Asian predator, *Harmonia axyridis* Pallas (Coleoptera, Coccinellidae), to control *Matsucoccus resinosae* Bean and Godwin (Homoptera, Margarodidae) in the United States. *Environmental Entomology.* 16(1), pp.224-230.

McClure, M.S., 1986. Role of predators in regulation of endemic populations of *Matsucoccus matsumurae* (Homoptera: Margarodidae) in Japan. *Environmental Entomology.* 15(4), pp.976-983.

McCorquodale, D.B., 1998. Adventive lady beetles (Coleoptera: Coccinellidae) in eastern Nova Scotia, Canada. *Entomological news.* 109(1), pp.15-20.

McKinney, M.L. & Lockwood, J.L., 1999. Biotic homogenization: a few winners replacing many losers in the next mass extinction. *Trends in Ecology and Evolution.* 14, pp.450-453.

Medeiros, A.C., Loope, L.L. & Cole, F.R. 1986. Distribution of ants and their effects on endemic biota of Haleakala and Hawaii Volcanoes National Parks: a preliminary assessment. *Hawaii Volcanoes National Park sixth conference in natural sciences.* Hawaii Volcanoes National Park, 10-13 June 1986.

Menzies, I.S. & Spooner, B.M., 2000. *Henosepilachna argus* (Geoffroy) (Coccinellidae, Epilachninae), a phytophagous ladybird new to the U.K., breeding at Molesey, Surrey. *The Coleopterist.* 9(1), pp.1-4.

Merkl, O., 2008. First record of the harlequin ladybird (*Harmonia axyridis* Pallas) in Hungary (Coleoptera: Coccinellidae). *Növényvédelem.* 44(5), pp.239-242.

Michaud, J.P., 2002. Invasion of the Florida citrus ecosystem by *Harmonia axyridis* (Coleoptera : Coccinellidae) and asymmetric competition with a native species, *Cycloneda sanguinea*. *Environmental Entomology.* 31(5), pp.827-835.

Miller, N., Estoup, A., Toepfer, S., Bourguet, D., Lapchin, L., Derridj, S., Kim, K.S., Reynaud, P., Furlan, L. & Guillemaud, T., 2005. Multiple transatlantic introductions of the western corn rootworm. *Science.* 310(5750), pp.992-992.

Miura, T. & Nishimura, S., 1980. The larval period and predacious activity of an aphidophagous coccinellid, *Harmonia axyridis* PALLAS. *Bulletin of the Faculty of Agriculture, Shimane University.* 14, pp.144-148.

Murchie, A.K., Moore, J.P., Moore, G.A. & Roy, H.E., 2008. The harlequin ladybird (*Harmonia axyridis* (Pallas)) (Coleoptera: Coccinellidae), found in Ireland. *Irish Naturalists' Journal.* 29(1), pp.25-26.

Nakamuta, K., 1987. Diel rhythmicity of prey-search activity and its predominance over starvation in the Lady Beetle, *Coccinella septempunctata bruckii*. *Physiological Entomology.* 12(1), pp.91-98.

Nalepa, C.A., Kidd, K.A. & Ahlstrom, K.R., 1996. Biology of *Harmonia axyridis* (Coleoptera: Coccinellidae) in winter aggregations. *Annals of the Entomological Society of America.* 89(5), pp.681-685.

Nedved, O., Steenberg, T. & Harding, S. 2008. Spread of the invasive ladybird *Harmonia axyridis* in the Czech Republic and Denmark. *XXIII International Congress of Entomology.* Durban, South Africa, 6-12 July 2008.

Niemela, J., Spence, J.R. & Carcamo, H., 1997. Establishment and interactions of carabid populations: an experiment with native and introduced species. *Ecography.* 20(6), pp.643-652.

Obata, S., 1986. Mechanisms of prey finding in the aphidophagous ladybird beetle, *Harmonia axyridis* (Coleoptera, Coccinellidae). *Entomophaga.* 31(3), pp.303-311.

Obrycki, J.J. & Kring, T.J., 1998. Predaceous Coccinellidae in biological control. *Annual Review of Entomology.* 43, pp.295-321.

Odegaard, F. & Tommeras, B.A., 2000. Compost heaps - refuges and stepping-stones for alien arthropod species in northern Europe. *Diversity and Distributions.* 6(1), pp.45-59.

Ongagna, P., Giuge, L., Iperti, G. & Ferran, A., 1993. Life cycle of *Harmonia axyridis* (Col, Coccinellidae) in its area of introduction - south-eastern France. *Entomophaga.* 38(1), pp.125-128.

Osawa, N., 2000. Population field studies on the aphidophagous ladybird beetle *Harmonia axyridis* (Coleoptera : Coccinellidae): resource tracking and population characteristics. *Population Ecology.* 42(2), pp.115-127.

Osawa, N., 1992. Sibling cannibalism in the ladybird beetle *Harmonia axyridis* - fitness consequences for mother and offspring. *Researches on Population Ecology.* 34(1), pp.45-55.

Osawa, N. & Ohashi, K., 2008. Sympatric coexistence of sibling species *Harmonia yedoensis* and *H. axyridis* (Coleoptera : Coccinellidae) and the roles of maternal investment through egg and sibling cannibalism. *European Journal of Entomology.* 105(3), pp.445-454.

Parrott, D., Roy, S., Baker, R., Cannon, R. & Eyre, D. 2009. *Horizon scanning for new invasive non-native animal species in England*, Natural England, Sheffield.

Payne, R.W. & members of the Genstat 6 committee, 2002. *Genstat 6 release 1 reference manual.* Oxford: Oxford University Press.

Pearman, D. & Walker, K., 2009. Alien plants in Britain - a real or imagined problem? *British Wildlife.* 21(1), pp.22-27.

Pell, J.K., Baverstock, J., Roy, H.E., Ware, R.L. & Majerus, M.E.N., 2008. Intraguild predation involving *Harmonia axyridis*: a review of current knowledge and future perspectives. *Biocontrol.* 53(1), pp.147-168.

Phoofolo, M.W. & Obrycki, J.J., 1998. Potential for intraguild predation and competition among predatory Coccinellidae and Chrysopidae. *Entomologia Experimentalis et Applicata.* 89(1), pp.47-55.

Polis, G.A. & Holt, R.D., 1992. Intraguild predation - the dynamics of complex trophic interactions. *Trends in Ecology & Evolution.* 7(5), pp.151-154.

Polis, G.A., Myers, C.A. & Holt, R.D., 1989. The ecology and evolution of intraguild predation - potential competitors that eat each other. *Annual Review of Ecology and Systematics.* 20, pp.297-330.

Pons, J., 2006. DNA-based identification of preys from non-destructive, total DNA extractions of predators using arthropod universal primers. *Molecular Ecology Notes.* 6(3), pp.623-626.

Poutsma, J., Loomans, A.J.M., Aukema, B. & Heijerman, T., 2008. Predicting the potential geographical distribution of the harlequin ladybird, *Harmonia axyridis*, using the CLIMEX model. *Biocontrol.* 53(1), pp.103-125.

Przewozny, M., Barłozek, T. & Bunalski, M., 2007. *Harmonia axyridis* (PALLAS, 1773) (Coleoptera: Coccinellidae) new species of ladybird beetle for Polish fauna. *Polish Journal of Entomology.* 76, pp.177-182.

Rabitsch, W. & Schuh, R., 2006. First record of the multicoloured Asian ladybird *Harmonia axyridis* (Pallas, 1773) in Austria. *Beiträge zur Entomofaunistik.* 7, pp.161-164.

Rhule, E.L., Majerus, M.E.N., Jiggins, F.M. & Ware, R.L., 2010. Potential role of the sexually transmitted mite *Coccipolipus hippodamiae* in controlling populations of the invasive ladybird *Harmonia axyridis*. *Biological Control.* 53, pp.243-247.

Ribbands, B., Brown, P.M.J., Roy, H.E. & Majerus, M.E.N., 2009. The most northerly record of the harlequin ladybird (Col., Coccinellidae) in the British Isles. *Entomologist's Monthly Magazine.* 145, pp.43-44.

Riley, J.R., Chapman, J.W., Reynolds, D.R. & Smith, A.D., 2007. Recent applications of radar to entomology. *Outlooks on pest management.* (February), pp.1-7.

Roques, A., Rabitsch, W., Rasplus, J.Y., Lopez-Vaamonde, C., Nentwig, W. & Kenis, M. 2009. Alien terrestrial invertebrates of Europe. In: DAISIE ed. *Handbook of Alien Species in Europe.* Dordrecht: Springer.

Roy, H.E., Baverstock, J., Ware, R.L., Clark, S.J., Majerus, M.E.N., Baverstock, K.E. & Pell, J.K., 2008a. Intraguild predation of the aphid pathogenic fungus *Pandora neoaphidis* by the invasive coccinellid *Harmonia axyridis*. *Ecological Entomology.* 33(2), pp.175-182.

Roy, H.E., Brown, P. & Majerus, M.E.N. 2006. *Harmonia axyridis*: a successful biocontrol agent or an invasive threat? In: J. Eilenberg & H. Hokkanen eds. *An ecological and societal approach to biological control.* Netherlands: Kluwer Academic Publishers.

Roy, H.E., Brown, P.M.J., Rothery, P., Ware, R.L. & Majerus, M.E.N., 2008b. Interactions between the fungal pathogen *Beauveria bassiana* and three species of coccinellid: *Harmonia axyridis*, *Coccinella septempunctata* and *Adalia bipunctata*. *Biocontrol.* 53(1), pp.265-276.

Roy, H.E. & Migeon, A. in press. Coccinellidae. In: A. Roques, et al., eds. *Arthropod invasions in Europe.* Dordrecht: Springer.

Roy, H.E., Rowland, F., Brown, P.M.J. & Majerus, M.E.N., 2005. Ecology of the harlequin ladybird: a new invasive species. *British Wildlife.* 16(6), pp.403-407.

Saini, E.D., 2004. Presencia de *Harmonia axyridis* (Pallas) (Coleoptera: Coccinelidae) en la provincia de Buenos Aires: Aspectos biológicos y morfológicos. *RIA.* 33(1), pp.151-160.

Sakurai, H., Kawai, T. & Takeda, S., 1992. Physiological changes related to diapause of the lady beetle, *Harmonia axyridis* (Coleoptera, Coccinellidae). *Applied Entomology and Zoology.* 27(4), pp.479-487.

Sakurai, H., Kumada, Y. & Takeda, S., 1993. Seasonal prevalence and hibernating-diapause behavior in the lady beetle, *Harmonia axyridis*. *Research Bulletin of the Faculty College of Agriculture Gifu University.* 58, pp.51-55.

Sato, S. & Dixon, A.F.G., 2004. Effect of intraguild predation on the survival and development of three species of aphidophagous ladybirds: consequences for invasive species. *Agricultural and Forest Entomology.* 6(1), pp.21-24.

Sato, S., Jimbo, R., Yasuda, H. & Dixon, A.F.G., 2008. Cost of being an intraguild predator in predatory ladybirds. *Applied Entomology and Zoology.* 43(1), pp.143-147.

Schellhorn, N.A. & Andow, D.A., 1999. Cannibalism and interspecific predation: Role of oviposition behavior. *Ecological Applications.* 9(2), pp.418-428.

Schmidt, J.E.U., Almeida, J.R.M., Rosati, C. & Arpaia, S., 2009. Identification of trophic interactions between *Macrolophus caliginosus* (Heteroptera: Miridae) and *Myzus persicae* (Homoptera: Aphididae) using real time PCR. *Biocontrol.* 54(3), pp.383-391.

Schneider, N. & Loomans, A.J.M., 2006. Sur la présence au Luxembourg de la coccinelle arlequin *Harmonia axyridis* (Pallas, 1773) (Insecta, Coleoptera, Coccinellidae). *Bulletin de la Société des naturalistes luxembourgeois.* 106, pp.71-74.

Sebolt, D.C. & Landis, D.A., 2004. Arthropod predators of *Galerucella calmariensis* L. (Coleoptera : Chrysomelidae): An assessment of biotic interference. *Environmental Entomology.* 33(2), pp.356-361.

Servicio de Información Fitosanitaria de Almería, 2004. *Organismos de control biológico.*

Sheppard, S.K., Bell, J., Sunderland, K.D., Fenlon, J., Skervin, D. & Symondson, W.O.C., 2005. Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. *Molecular ecology.* 14(14), pp.4461-4468.

Sheppard, S.K., Henneman, M.L., Memmott, J. & Symondson, W.O.C., 2004. Infiltration by alien predators into invertebrate food webs in Hawaii: a molecular approach. *Molecular ecology.* 13(7), pp.2077-2088.

Sidlyarevich, V.I. & Voronin, K.E., 1973. Trials on using *Leis axyridis* under glass. *Zashchita Rastenii.* 6, pp.24.

Sloggett, J.J., Obrycki, J.J. & Haynes, K.F., 2009. Identification and quantification of predation: novel use of gas chromatography-mass spectrometric analysis of prey alkaloid markers. *Functional Ecology.* 23(2), pp.416-426.

Smith, B., 2008. Biosecurity risk - Asian lady beetle. *Wine Industry Newsletter (Government of Western Australia Department of Agriculture & Food).* 78(June), pp.3.

Smith, W.M., Arnold, D.C., Eikenbary, R.D., Rice, N.R., Shiferaw, A., Cheary, B.S. & Carroll, B.L., 1996. Influence of ground cover on beneficial arthropods in pecan. *Biological Control.* 6, pp.164-176.

Soares, A.O. & Serpa, A., 2007. Interference competition between ladybird beetle adults (Coleoptera : Coccinellidae): effects on growth and reproductive capacity. *Population Ecology*. 49(1), pp.37-43.

Soares, A.O., Borges, I., Borges, P.A.V., Labrie, G. & Lucas, E., 2008. *Harmonia axyridis*: What will stop the invader? *Biocontrol*. 53(1), pp.127-145.

Stals, R. 2009. Tracking two southern African ladybird beetle invasions: into the past for one, into the future for the other (Coleoptera: Coccinellidae). *Harmonia axyridis and other invasive ladybirds (IOBC/WPRS Study Group).* Engelberg, Switzerland, 6-9 September 2009.

Stals, R., 2008. Invasive ladybird spreading in South Africa. *Biocontrol News and Information*. 29(2), pp.24-26.

Staverloekk, A., Saethre, M.G. & Haagvar, E., 2007. A review of the biology of the invasive harlequin ladybird *Harmonia axyridis* (Pallas, 1773) (Coleoptera, Coccinellidae). *Norwegian Journal of Entomology*. 54, pp.97-104.

Steenberg, T. & Harding, S., 2009a. Entomopathogenic fungi recorded from the harlequin ladybird, *Harmonia axyridis*. *Journal of invertebrate pathology*. 102(1), pp.88-89.

Steenberg, T. & Harding, S., 2009b. The harlequin ladybird (*Harmonia axyridis* Pallas) in Denmark: spread and phenology during the initial phase of invasion. *Ent. Meddr*. 77, pp.27-39.

Stewart, R., 2008. A new butterfly predator. *Surrey Skipper*. 42, pp.11.

Suarez, A.V., Holway, D.A. & Case, T.J., 2001. Patterns of spread in biological invasions dominated by long-distance jump dispersal: Insights from Argentine ants. *Proceedings of the National Academy of Sciences of the United States of America*. 98(3), pp.1095-1100.

Sunderland, K.D., 1988. Quantitative methods for detecting invertebrate predation occurring in the field. *Annals of Applied Biology*. 112(1), pp.201-224.

Takahashi, K., 1989. Intra- and inter-specific predations of lady beetles in spring alfalfa fields. *Japanese journal of entomology*. 57(1), pp.199-203.

Tedders, W.L. & Schaefer, P.W., 1994. Release and establishment of *Harmonia axyridis* (Coleoptera, Coccinellidae) in the southeastern United States. *Entomological news*. 105(4), pp.228-243.

Thalji, R. & Stojanovic, D., 2008. First sighting of the invasive ladybird *Harmonia axyridis* Pallas (Coleoptera, Coccinellidae) in Serbia. *Biljni Lekar*. 36, pp.389-393.

Thomas, C., Lombaert, E., Ware, R.L., Estoup, A., Majerus, M.E.N. & Lawson-Handley, L., in press. Investigating global invasion routes of the harlequin ladybird (*Harmonia axyridis*) using mtDNA. *IOBC Bulletin*.

Tolasch, T., 2002. *Harmonia axyridis* (Pallas) (Col., Coccinellidea) breitet sich in Hamburg aus – ausgangspunkt für en besiedlung Mitteleuropas? *Entomologische Nachrichten und Berichte.* 46(3), pp.185-188.

Tomov, R. & Kenis, M. 2009. Review of ladybirds (Coleoptera: Coccinellidae) of Bulgaria. *Harmonia axyridis and other invasive ladybirds (IOBC/WPRS Study Group).* Engelberg, Switzerland, 6-9 September 2009.

Trouvé, C. 1995. Essais de lutte biologique contre le puceron du houblon (*Phorodon humuli*) dans le nord de la France: résultats 1993 et 1994. *47th International Symposium on Crop Protection.* Ghent, Belgium, 9 May 1995.

Trouvé, C., Ledee, S., Ferran, A. & Brun, J., 1997. Biological control of the damson-hop aphid, *Phorodon humuli* (Hom.: Aphididae), using the ladybeetle *Harmonia axyridis* (Col.: Coccinellidae). *Entomophaga.* 42(1-2), pp.57-62.

Turnock, W.J., Wise, I.L. & Matheson, F.O., 2003. Abundance of some native coccinellines (Coleoptera : Coccinellidae) before and after the appearance of *Coccinella septempunctata*. *Canadian Entomologist.* 135(3), pp.391-404.

van Lenteren, J.C., Babendreier, D., Bigler, F., Burgio, G., Hokkanen, H., Kuske, S., Loomans, A., Menzler-Hokkanen, I., Rijn, P.V., Thomas, M., Tommassini, M. & Zeng, Q., 2003. Environmental risk assessment of exotic natural enemies used in inundative biological control. *Biocontrol.* 48, pp.3-38.

van Lenteren, J.C., Bale, J., Bigler, E., Hokkanen, H.M.T. & Loomans, A.M., 2006. Assessing risks of releasing exotic biological control agents of arthropod pests. *Annual Review of Entomology.* 51, pp.609-634.

van Lenteren, J.C., Loomans, A.J.M., Babendreier, D. & Bigler, F., 2008. *Harmonia axyridis*: an environmental risk assessment for Northwest Europe. *Biocontrol.* 53(1), pp.37-54.

Wang, L.Y., 1986. Mass rearing and utilization in biological control of the lady beetle *Leis axyridis* (Pallas). *Acta Entomologica Sinica.* 29, pp.104.

Ware, R.L., Evans, N., O’Farrell, K., Malpas, L., Michie, L.J. & Majerus, M.E.N., 2008a. Intraguild predation of British and Japanese coccinellid eggs by the invasive ladybird *Harmonia axyridis*. *Neobiota.* 7, pp.263-275.

Ware, R.L. & Majerus, M.E.N., 2008. Intraguild predation of immature stages of British and Japanese coccinellids by the invasive ladybird *Harmonia axyridis*. *Biocontrol.* 53(1), pp.169-188.

Ware, R.L., Majerus, M.E.N., Roy, H.E. & Symington, F., 2005. The harlequin ladybird arrives in Britain: a threat to our native species? *Bulletin of the Amateur Entomologists' Society.* 64, pp.175-186.

Ware, R.L., Yguel, B. & Majerus, M.E.N., 2008b. Effects of larval diet on female reproductive output of the European coccinellid *Adalia bipunctata* and the invasive species *Harmonia axyridis* (Coleoptera : Coccinellidae). *European Journal of Entomology.* 105(3), pp.437-443.

Watanabe, M., 2002. Cold tolerance and myo-inositol accumulation in overwintering adults of a lady beetle, *Harmonia axyridis* (Coleoptera : Coccinellidae). *European Journal of Entomology.* 99(1), pp.5-9.

Weber, D.C. & Lundgren, J.G., 2009. Detection of predation using qPCR: Effect of prey quantity, elapsed time, chaser diet, and sample preservation on detectable quantity of prey DNA. *Journal of Insect Science.* 41, pp.1-12.

Wells, P., Baverstock, J., Roy, H.E., Jiggins, F., Majerus, M.E.N. & Pell, J., in press. Intraguild predation of non-coccinellid aphid natural enemies by *Harmonia axyridis*: prey range and factors influencing intraguild predation. *IOBC Bulletin.*

Westgarth-Smith, A.R., Leroy, S.A.G., Collins, P.E.F. & Harrington, R., 2007. Temporal variations in English populations of a forest insect pest, the green spruce aphid (*Elatobium abietinum*), associated with the North Atlantic Oscillation and global warming. *Quaternary International.* 173, pp.153-160.

Wetterer, J.K., Wild, A.L., Suarez, A.V., Roura-Pascual, N. & Espadaler, X., 2009. Worldwide spread of the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). *Myrmecological News.* 12, pp.187-194.

Williamson, M., 1996. *Biological Invasions.* London: Chapman and Hall.

Yarbrough, J.A., Armstrong, J.L., Blumberg, M.Z., Phillips, A.E., McGahee, E. & Dolen, W.K., 1999. Allergic rhinoconjunctivitis caused by *Harmonia axyridis* (Asian lady beetle, Japanese lady beetle, or lady bug). *Journal of Allergy and Clinical Immunology.* 104(3), pp.704-705.

Yasuda, H., Evans, E.W., Kajita, Y., Urakawa, K. & Takizawa, T., 2004. Asymmetric larval interactions between introduced and indigenous ladybirds in North America. *Oecologia.* 141(4), pp.722-731.

Yasuda, H., Takagi, T. & Kogi, K., 2000. Effects of conspecific and heterospecific larval tracks on the oviposition behaviour of the predatory ladybird, *Harmonia axyridis* (Coleoptera : Coccinellidae). *European Journal of Entomology.* 97(4), pp.551-553.

Zaidi, R.H., Jaal, Z., Hawkes, N.J., Hemingway, J. & Symondson, W.O.C., 1999. Can multiple-copy sequences of prey DNA be detected amongst the gut contents of invertebrate predators? *Molecular ecology.* 8(12), pp.2081-2087.

Zhang, G.F., Lu, Z.C. & Wan, F.H., 2007a. Detection of *Bemisia tabaci* remains in predator guts using a sequence-characterized amplified region marker. *Entomologia Experimentalis et Applicata.* 123(1), pp.81-90.

Zhang, G.F., Lu, Z.C., Wan, F.H. & Lovei, G.L., 2007b. Real-time PCR quantification of *Bemisia tabaci* (Homoptera : Aleyrodidae) B-biotype remains in predator guts. *Molecular Ecology Notes.* 7, pp.947-954.

Zhou, X., Carter, N. & Powell, W., 1994. Seasonal distribution and aerial movement of adult coccinellids on farmland. *Biocontrol Science and Technology.* 4(2), pp.167-175.

Zhou, X., Perry, J.N., Woiwod, I.P., Harrington, R., Bale, J.S. & Clark, S.J., 1997. Temperature change and complex dynamics. *Oecologia*. 112(4), pp.543-550.

Appendices

Appendix 1. Summary of ladybird data from eastern England field surveys

Appendix 1 (part a to d) relates to Chapter 5.

Appendix 1a. Total number of ladybirds (adults and larvae) of each species recorded per year, per site, for all sites (A to K). Data for sites E to G time-adjusted for direct comparison with other sites, but not adjusted for missing surveys. Integer values shown (# used to indicate value < 0.5). Blank cells indicate nil values.

Site code	Year	*Adalia 10-punctata*	*Adalia 2-punctata*	*Adalia spp.* larvae	*Anatis ocellata*	*Anisosticta 19-punctata*	*Aphidecta obliterata*	*Calvia 14-guttata*	*Chilocorus renipustulatus*	*Coccinella 7-punctata*	*Exochomus 4-pustulatus*	*Halyzia 16-guttata*	*Harmonia 4-punctata*	*Harmonia axyridis*	*Harmonia spp.* larvae	*Hippodamia variegata*	*Myrrha 18-guttata*	*Propylea 14-punctata*	*Subcoccinella 24-punctata*	*Thea 22-punctata*	*Tytthaspis 16-punctata*	Total
A	1	112	50	55				25		8	3	2						13		1		269
	2	72	34	31				11			1			105	17			2				273
	3	59	21	38				27			3	2		361	28			4				543
B	1	58	105	61				16		22	4	2		1				41				310
	2	115	63	32	1			22			4	5		278	41			9		1		571
	3	113	22	23	1			11			6	2		154	2			5				339
C	1	1	1	1						40	10		14	1			11	2				81
	2	52	6	20	6			2		25	125		41	19	8		9		1	1		315
	3				1					34	47		22	11	3		26			1		145
D	1	1								35	56		4					1				97
	2	5		2						3	40		2					6				58
	3	3								10	1	1						1				16
E	1	3	5		1			#		49	11		38	#			30	3	52		2	195
	2	4	7		3			1		75	16	#	49	21			12	4	90			281
	3	4			2		1	1		1	11		32	20			15	#	28	#		116
F	1	68	41		1		36	9	1	38	36	1	61				33	11				335
	2	56	37		1		125	6	3	3	47	#	42	35			16	6		#		376
	3	30	8		1		134	6		1	27		23	46			10	#				286
G	1	71	47					4		33	1	8						33	19	2		219
	2	63	35					4		7	1	1		9				13	5	1	#	140
	3	33	13					1		2	#			18				#				69
H	1	2	15	7				1		55								42	6	7	2	137
	2		9							30				2	1			9	9	5	3	68
	3	1	2					1		30				7	6			2	5	1		55
I	1									96								11	4	4	42	157
	2		15	2						44						1		23	20	2	7	114
	3									4								1	7	1	3	16
J	1		1							19								19			1	40
	2							1		1		1		1				1				5
	3							1		3				4								8
K	1					1				5								5		1	1	13
	2									1								2			1	4
	3									1				2					1	1		5
Total		926	538	272	17	1	295	149	4	676	451	25	328	1095	106	1	161	269	247	30	63	5655

Appendix 1b. Mean number of ladybird adults of each species recorded per year, per survey, at all arboreal sites (A to G) combined. Includes adjusted data for sites E to G, to allow for missing surveys. Surveys labelled by month. May and June surveys also numbered (1 or 2) to indicate early and late month surveys. Blank cells indicate nil values.

Year	Survey	*Adalia 2-punctata*	*Adalia 10-punctata*	*Anatis ocellata*	*Aphidecta obliterata*	*Calvia 14-punctata*	*Chilocorus renipustulatus*	*Coccinella 7-punctata*	*Exochomus 4-pustulatus*	*Halyzia 16-guttata*	*Harmonia axyridis*	*Harmonia 4-punctata*	*Myrrha 18-guttata*	*Propylea 14-punctata*	*Subcoccinella 24-punctata*	*Thea 22-punctata*	*Tytthaspis 16-punctata*
1	Apr	1.71	3.73	0.04	0.57	0.11		10.47	1.58		0.08	2.06	0.55	0.23	0.38		
	May 1	4.87	7.52	0.13	0.15	0.08	0.04	3.03	1.57	0.18		3.76	1.49	4.26	1.32	0.14	
	May 2	3.53	5.99		0.26	0.08		1.87	0.77	0.16		1.87	1.42	4.86	0.74		
	Jun 1	7.67	5.45	0.04	0.08	0.43		0.69	1.02	0.63		1.00	1.12	0.33	0.41	0.19	
	Jun 2	6.41	7.84		1.85	1.25		0.14	0.72	0.46		1.35	1.03	0.14			
	Jul	4.44	6.62		1.39	1.28		0.57	1.58			2.00	1.20	3.49	0.22	0.06	
	Aug	3.18	3.37		0.11	1.33		4.43	3.77			1.01	1.80	0.64	1.60		0.32
	Sep	2.40	2.42		0.30	0.89		3.92	1.95			0.99	1.50	0.39	2.09		
	Oct	1.38	1.64		0.38	1.45		4.72	2.20	0.21		1.08	0.48	0.14	2.15		
2	Apr	1.60	2.95		0.72	0.15		0.84	1.96	0.05		4.31	0.54	0.99	0.18		
	May 1	4.24	5.02		0.49	0.20		0.05	1.86	0.06		1.78	0.72	2.47	0.05	0.14	
	May 2	4.99	6.28	0.05	0.15	1.42		0.50	1.62	0.04	0.04	1.00	0.64	0.81	0.05	0.06	0.06
	Jun 1	3.39	7.87		4.82	1.10		4.26	1.25	0.14	0.14	0.84	0.44	0.14		0.04	
	Jun 2	4.74	15.88	0.16	3.62	0.49		3.58	1.25	0.43	1.87	0.82	0.23	0.08	0.41		
	Jul	2.59	6.26	0.33	3.09	0.73		0.95	2.30		1.21	1.46	0.51	0.44	1.09	0.13	
	Aug	1.44	3.47	0.05	1.62	0.65		0.84	6.55	0.14	1.08	1.60	0.96	0.52	2.38	0.14	
	Sep	2.15	3.37	0.08	1.32	0.84		0.58	5.84	0.06	19.20	1.41	1.02	0.05	4.90		
	Oct	0.47	0.88		0.87			0.68	3.11		21.58	2.24			4.28		
3	Apr	0.88	2.64	0.06	1.64	0.70			2.95		2.42	2.58	1.01				
	May 1	3.31	4.81	0.28	4.20	0.81			1.70	0.14	5.03	1.94	0.91	0.43	0.55		
	May 2	1.36	7.60		1.92	0.29			0.92		1.54	1.77	0.74		0.14		
	Jun 1	0.19	3.61		1.36				1.83		1.08	0.28	0.39		0.09		
	Jun 2	0.99	6.24	0.14	4.90	0.65			1.76	0.14	3.98	0.76	0.70	0.05	0.05		
	Jul	1.28	4.72	0.12	2.37	0.80		0.14	1.40		5.59	0.67	1.68	0.47	0.46		
	Aug	1.06	3.36	0.03	1.64	2.12		2.35	1.15		12.22	1.31	1.18	0.05	0.77	0.19	0.01
	Sep	0.47	1.87	0.05	2.94	0.68		1.93	1.44		14.52	1.33	0.85	0.14	0.92	0.05	
	Oct			0.02	1.58	0.20		2.73	0.54	0.15	22.66	1.14	0.62		0.81		0.01

Appendix 1c. Mean number of ladybird larvae of each species recorded per year, per survey, at all arboreal sites (A to G) combined. Includes adjusted data for sites E to G, to allow for missing surveys. Surveys labelled by month. May and June surveys also numbered (1 or 2) to indicate early and late month surveys. Blank cells indicate nil values.

Year	Survey	*Adalia* spp.	*Anatis ocellata*	*Aphidecta obliterata*	*Calvia 14-punctata*	*Chilocorus renipustulatus*	*Coccinella 7-punctata*	*Exochomus 4-pustulatus*	*Halyzia 16-guttata*	*Harmonia* spp.	*Harmonia axyridis*	*Harmonia 4-punctata*	*Myrrha 18-guttata*	*Propylea 14-punctata*	*Subcoccinella 24-punctata*	*Thea 22-punctata*	*Tytthaspis 16-punctata*
1	Apr																
	May 1																
	May 2																
	Jun 1	7.14										0.49					
	Jun 2	8.29	0.04		0.71	0.11	0.55	0.86				0.65		0.29			
	Jul	1.29			0.14			0.14			0.14	0.33			1.19	0.06	
	Aug																
	Sep																
	Oct																
2	Apr																
	May 1	0.29					0.09			0.29		0.11					
	May 2	2.05		1.09		0.15	1.85	1.00		0.86	0.08	0.75			0.27		
	Jun 1	1.62	0.32	0.04	0.05	0.19	1.78	1.86			0.70	1.22		0.14			
	Jun 2	2.43	0.52		0.29	0.08	0.05	1.86			0.49	0.58			0.05		
	Jul	2.29	0.09		0.57			1.14			0.43	0.12	0.14				
	Aug	3.86						0.14	0.06	6.86	10.32						
	Sep									0.29	2.65	0.27					
	Oct									1.14	4.36						
3	Apr			0.05		0.02						0.11					
	May 1																
	May 2	0.18								0.14	1.71	0.45		0.06	0.41		
	Jun 1	3.71			0.43			0.14		1.29	2.26	0.51			1.37		
	Jun 2	3.29			0.05			0.71		0.43	5.80	0.29	0.14	0.14	0.05		
	Jul	1.14			0.29			0.18		0.43	1.23	0.50	0.14				
	Aug	0.02		0.04		0.02	0.16			0.38	1.38	0.12		0.19	0.13		
	Sep	0.14								1.71	8.71						
	Oct						0.16			0.24	0.60				0.13		

Appendix 1d. Means and standard errors of numbers of ladybirds per repeat survey, for each year, grouped by habitat, as shown in Figure 5.1.

Associated letter from Fig. 5.1, site codes and ladybird life stage	Species	Year 1 mean	Year 2 mean	Year 3 mean	Year 1 standard error	Year 2 standard error	Year 3 standard error
(a) Sites A & B adults	*Adalia 10-punctata*	9.33	10.28	9.44	3.00	2.50	3.00
	Adalia 2-punctata	8.61	5.39	2.39	3.06	1.61	0.06
	Coccinella 7-punctata	1.61	0.00	0.00	0.83	0.00	0.00
	Exochomus 4-pustulatus	0.39	0.28	0.39	0.06	0.17	0.17
	Harmonia axyridis	0.00	13.67	20.83	0.00	5.56	5.06
	Other	5.11	2.44	2.39	1.00	1.33	0.50
(b) Sites C & D adults	*Adalia 10-punctata*	0.11	3.17	0.17	0.00	2.61	0.17
	Adalia 2-punctata	0.06	0.33	0.00	0.06	0.33	0.00
	Coccinella 7-punctata	3.50	1.50	1.94	0.50	1.17	1.06
	Exochomus 4-pustulatus	2.83	6.17	2.33	1.83	5.50	2.22
	Harmonia axyridis	0.00	0.72	0.56	0.00	0.72	0.56
	Other	1.39	2.89	2.33	0.94	2.11	2.22
(c) Sites E, F & G adults	*Adalia 10-punctata*	5.26	4.51	2.87	2.49	2.07	1.12
	Adalia 2-punctata	3.45	2.83	0.90	1.44	1.02	0.44
	Aphidecta obliterata	1.32	4.33	6.43	1.32	4.33	6.38
	Coccinella 7-punctata	4.33	2.19	0.15	0.39	1.71	0.05
	Exochomus 4-pustulatus	1.78	2.38	1.78	1.14	1.52	1.12
	Harmonia 4-punctata	3.47	2.79	2.29	1.83	1.47	1.30
	Harmonia axyridis	0.02	2.11	2.50	0.01	0.85	0.72
	Subcoccinella 24-punctata	2.31	3.42	0.73	1.39	3.15	1.10
	Other	5.06	2.45	1.68	0.57	0.37	0.80
(d) Sites A to G adults	*Adalia 10-punctata*	4.95	5.78	3.98	1.83	1.62	1.82
	Adalia 2-punctata	3.96	2.85	1.07	1.59	0.94	0.43
	Coccinella 7-punctata	3.31	1.37	0.62	0.53	1.02	0.55
	Exochomus 4-pustulatus	1.68	2.86	1.54	0.70	1.62	0.72
	Harmonia axyridis	0.01	5.01	7.18	0.01	2.58	3.72
	Other	7.08	7.09	6.12	2.15	2.92	3.20
(e) Sites A to G larvae	*Adalia* spp.	1.86	1.39	0.99	1.19	0.62	0.66
	Coccinella 7-punctata	0.06	0.42	0.00	0.06	0.39	0.00
	Exochomus 4-pustulatus	0.11	0.67	0.12	0.09	0.53	0.09
	Harmonia axyridis	0.02	2.11	2.51	0.02	1.56	1.69
	Other	0.45	1.84	1.17	0.12	0.65	0.49

Appendix 2. Relevant published papers

Brown, P.M.J., 2007. In love with ladybirds. *BBC Wildlife* 25(7): 58-63.

Brown, P.M.J., Adriaens, T., Bathon, H., Cuppen, J., Goldarazena, A., Hägg, T., Kenis, M., Klausnitzer, B.E.M., Kovar, I., Loomans, A.J.M., Majerus, M.E.N., Nedved, O., Pedersen, J., Rabitsch, W., Roy, H.E., Ternois, V., Zakharov, I.A. and Roy, D.B., 2008. *Harmonia axyridis* in Europe: spread and distribution of a non-native coccinellid. *Biocontrol* 53: 5-21.

Brown, P.M.J., James, T.J., Stewart, A. and Roy, H.E., 2008. Mapping insect distributions in the 21st century: a role for the Royal Entomological Society in partnership with the Biological Records Centre and National Biodiversity Network? *Antenna* 32: 149-151.

Brown, P.M.J. and Roy, H.E., 2007. The spread of the harlequin ladybird *Harmonia axyridis* in late 2006. *Atropos* 31: 32-33.

Brown, P.M.J., Roy, H.E. and Majerus, M.E.N., 2006. Ladybird recording in Cambridgeshire and Huntingdonshire. *Nature in Cambridgeshire* 48: 12-16.

Brown, P.M.J., Roy, H.E. and Majerus, M.E.N., 2007. The spread of the harlequin ladybird in Cambridgeshire and Huntingdonshire. *Nature in Cambridgeshire* 49: 55-57.

Brown, P.M.J., Roy, H.E. and Majerus, M.E.N., 2007. Update on the spread of an invasive ladybird. *Biocontrol News and Information* 28(1): 16-17.

Brown, P.M.J., Roy, H.E. and Majerus, M.E.N., 2008. The rise and rise of the harlequin ladybird in Cambridgeshire. *Nature in Cambridgeshire* 50: 91-92.

Brown, P.M.J., Roy, H.E., Rothery, P., Roy, D.B., Ware, R.L. and Majerus, M.E.N., 2008. *Harmonia axyridis* in Great Britain: analysis of the spread and distribution of a non-native coccinellid. *Biocontrol* 53: 55-67.

Brown, P.M.J., Roy, H.E. and Ware, R.L., 2010. Wildlife reports: Ladybirds. *British Wildlife* 21: 208-209.

Brown, P.M.J., Roy, H.E., Ware, R.L. and Majerus, M.E.N., 2006. Distribution and identification of the harlequin ladybird *Harmonia axyridis*. *Atropos* 28: 63-69.

Brown, P.M.J., Ware, R.L. and Roy, H.E., in press. Looking beyond the spots: inspiring the public to record ladybirds. *IOBC Bulletin.*

CAB International, 2006. *Harmonia axyridis* [original text by H.E. Roy, P.M.J. Brown and M.E.N. Majerus]. *Crop Protection Compendium*, 2006 edition. CAB International, Wallingford, UK.

Frost, R. and Brown, P.M.J., 2008. Huntingdonshire ladybirds. *Huntingdonshire Flora and Fauna* 60: 20-28.

Frost, R. and Brown, P.M.J., 2009. Cambridgeshire and Huntingdonshire ladybirds. *Nature in Cambridgeshire* 51: 20-27.

Hall, R., Ware, R.L., Michie, L.J., Brown, P.M.J. and Majerus, M.E.N., 2009. First occurrence of *Rhyzobius chrysomeloides* (Herbst, 1792) (Coleoptera: Coccinellidae) in Cambridgeshire. *Entomologist's Record and Journal of Variation* 121(2): 78.

Holroyd, O., Brown, P.M.J., Roy, H.E. and Majerus, M.E.N., 2008. The harlequin ladybird, *Harmonia axyridis*, reaches Scotland. *Entomologist's Record and Journal of Variation* 120(1): 42-43.

Kenis, M., Adriaens, T., Brown, P., Katsanis, A., Van Vlaenderen, J., Eschen, R., Golaz, L., Zindel, R., San Martin y Gomez, G., Babendreier, D., Ware, R., in press. Impact of *Harmonia axyridis* on European ladybirds: which species are most at risk? *IOBC Bulletin.*

Kenis, M., Brown, P.M.J., Ware, R.L. and Roy, D.B., in press. Invasion of the harlequin ladybird, *Harmonia axyridis*, in Europe: when beauty becomes the beast. In: *Atlas of Biodiversity Risk*, Eds. Josef Settele et al. Pensoft, Sofia and Moscow.

Majerus, M.E.N., Brown, P.M.J., Roy, H.E. and Ware, R.L., 2009. Wildlife reports: Ladybirds. *British Wildlife* 20: 210-212.

Majerus, M.E.N., Roy, H.E., Brown, P.M.J., Ware, R.L. and Shields, C., 2006. *A Guide to the Ladybirds of the British Isles* (fold-out chart). Field Studies Council, Shrewsbury.

Pascoe, S., Brown, P.M.J. and Majerus, M.E.N., 2007. Hybrid mating between *Harmonia axyridis* and *Adalia bipunctata*. *Bulletin of Amateur Entomologists' Society* 66:156-159.

Ribbands, B., Brown, P.M.J., Roy, H.E. and Majerus, M.E.N., 2009. The most northerly record of the harlequin ladybird in the British isles. *Entomologist's Monthly Magazine* 145: 43-44.

Roy, H.E., Brown, P.M.J., James, T.J., Munford, J. and Majerus, M.E.N., 2005. Monitoring an alien: *Harmonia axyridis*. *Journal of Practical Ecology and Conservation Special Series* 4: 77-82.

Roy, H.E., Brown, P.M.J. and Majerus, M.E.N., 2006. *Harmonia axyridis*: A successful biocontrol agent or an invasive threat? In: *An ecological and societal approach to biological control* (eds.: J. Eilenberg and H. Hokkanen). Kluwer Academic Publishers, Netherlands.

Roy, H.E., Brown, P.M.J., Rothery, P., Ware, R.L. and Majerus, M.E.N., 2008. Interactions between the fungal pathogen *B. bassiana* and three species of ladybird: *Harmonia axyridis*, *Coccinella septempunctata* and *Adalia bipunctata*. *Biocontrol* 53: 265-276.

Roy, H.E., Brown, P.M.J. and Ware, R.L., 2010. Encounters with an alien: a European perspective. *Biocontrol News and Information* 30(4): 74-76.

Roy, H.E., Brown, P.M.J., Ware, R.L. and Majerus, M.E.N., 2006. Potential impact of *Harmonia axyridis* on functional biodiversity. Proceedings of the European meeting IOBC/WPRS, Zurich, Switzerland, 16-19 May 2006. *IOBC Bulletin.*

Roy, H.E., Brown, P.M.J., Ware, R.L., Michie, L.J., Beckmann, B. and Majerus, M.E.N., 2008. The harlequin ladybird marches on. *British Wildlife* 19: 182-186.

Roy, H.E., Rowland, F., Brown, P.M.J., and Majerus, M.E.N., 2005. Ecology of the harlequin ladybird: a new invasive species. *British Wildlife* 16: 403-407.

Thomas, A., Philippou, S., Ware, R.L., Kitson, H. and Brown, P.M.J., in press. Is *Harmonia axyridis* really eating *Adalia bipunctata* in the wild? *IOBC Bulletin*.

Thomas, R.J., Majerus, M.E.N., Brown, P.M.J. and Roy, H.E., 2008. A first British record of *Cynegetis impunctata* (Coleoptera: Coccinellidae). *British Journal of Entomology and Natural History* 21.

Appendix 3. Relevant conference presentations

Only those conference presentations for which PMJB was the presenting author are listed (i.e. other presentations for which PMJB was a non-presenting author are not shown). Presentations shown in reverse chronological order.

Brown, P.M.J., Henshall, A. and Roy, H.E., 2009. Watch out for that alien! Engaging the public in recording non-natives. *National Biodiversity Network Conference.* Royal Institution of Great Britain, London, 20 November 2009.

Brown, P.M.J., Frost, R., Sparks, T., Doberski, J. and Roy, H.E., 2009. Temporal changes in ladybird species assemblages coinciding with the arrival of *Harmonia axyridis* in the UK. *Harmonia axyridis and other invasive ladybirds (IOBC/WPRS Study Group)*. Engelberg, Switzerland, 6-9 September 2009.

Brown, P.M.J., Ware, R.L. and Roy, H.E., 2009. Looking beyond the spots: inspiring the public to record ladybirds. *Harmonia axyridis and other invasive ladybirds (IOBC/WPRS Study Group).* Engelberg, Switzerland, 6-9 September 2009.

Brown, P.M.J., Kitson, H., Philippou, S., Ware, R.L. and Thomas, A., 2009. Are harlequin ladybirds really eating our native species? *Amateur Entomologists' Society AGM and Members' Day*. University of Cambridge, 18 April 2009.

Brown, P.M.J., Roy, H.E. and Majerus, M.E.N., 2009. The Harlequin Ladybird in Scotland. *Scottish Biodiversity Forum*. Royal Botanical Gardens, Edinburgh, 6 March 2009.

Brown, P.M.J., Roy, H.E., Ware, R.L., Roy, D.B., Bacon, J. and Majerus, M.E.N., 2008. Tracking an alien: the value of public involvement in the UK Harlequin Ladybird Survey. *British Ecological Society Annual Meeting*. Imperial College, London, 3-5 September 2008.

Brown, P.M.J., Majerus, M.E.N., Roy, H.E., and Roy, D.B., 2008. The spread and distribution in Europe of *Harmonia axyridis*, a non-native coccinellid. *XXIII International Congress of Entomology*. Durban, South Africa, 6-12 July 2008.

Brown, P.M.J., Roy, H.E., Roy, D.B. and Majerus, M.E.N., 2008. The value of public involvement in an entomological survey: a model from the UK. *XXIII International Congress of Entomology*. Durban, South Africa, 6-12 July 2008.

Brown, P.M.J., 2008. The spread of an invasive ladybird, *Harmonia axyridis*, in Europe. *Second Annual Research Students' Conference*. Anglia Ruskin University, 2 April 2008.

Brown, P.M.J., Roy, H.E., Majerus, M.E.N. and Roy, D.B., 2008. The spread of an invasive ladybird, *Harmonia axyridis*, in Europe. *Royal Entomological Society Postgraduate Forum 2008*. Rothamsted Research, Harpenden, 6 Februrary 2008.

Brown, P.M.J., Majerus, M.E.N., Roy, H.E. and Roy, D.B., 2007. The spread of *Harmonia axyridis* in Europe. *Ecology of Aphidophaga 10*. Agricultural University of Athens, 5-10 September 2007.

Brown, P.M.J., Roy, H.E., Ware, R.L. and Majerus, M.E.N., 2007. The spread of the harlequin ladybird *Harmonia axyridis* in the UK and Europe. *ENTO '07 – Royal Entomological Society Annual National Meeting*. University of Edinburgh, 16-18 July 2007.

Brown, P.M.J., Roy, H.E., Roy, D.B., Hill, M.O. and Majerus, M.E.N., 2007. The spread of the harlequin ladybird in the UK. *4th Non-Native Species Stakeholder Forum*. Edinburgh, 3 May 2007.

Brown, P.M.J., 2007. The spread of an invasive ladybird, *Harmonia axyridis*, in the UK. *First Annual Research Students' Conference*. Anglia Ruskin University, 18 April 2007.

Brown, P.M.J., Roy, H.E. and Majerus, M.E.N., 2007. The spread of an invasive ladybird, *Harmonia axyridis*, in the UK. *ESRC Conference*. Anglia Ruskin University, 31 January 2007.

Brown, P.M.J., 2006. Using Ladybird Recording Scheme data for research. *National Biodiversity Network Conference*. Natural History Museum, London, 17 November 2006.

Brown, P.M.J., Roy, H.E., Ware, R.L. and Majerus, M.E.N., 2006. Recording the spread of an alien ladybird, *Harmonia axyridis*, in the UK. *Neobiota Conference on Biological Invasions*. Vienna, 27-29 September 2006.

Brown, P.M.J., Roy, H.E., Ware, R.L. and Majerus, M.E.N., 2006. The growing threat of *Harmonia axyridis*, the harlequin ladybird. *British Ecological Society Annual Meeting*. University of Oxford, 5-7 September 2006.

Brown, P.M.J., Roy, H.E., James, T.J. and Majerus, M.E.N., 2005. *Harmonia axyridis*: a model system for monitoring invasive species. *ENTO '05 – Royal Entomological Society Annual National Meeting and Symposium on Insect Conservation Biology*. University of Sussex, 12-14 September 2005.