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Populations of aphid natural enemies in agroecosystems, with special emphasis on the Multicoloured Asian Ladybird *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae)

Axel VANDEREYCKEN

Essai présenté en vue de l'obtention du grade de docteur en sciences agronomiques et ingénierie biologique

Promoteurs: Prof. Eric Haubruge
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Vandereycken Axel (2014), Populations of aphid natural enemies in agroecosystems, with special emphasis on the Multicoloured Asian Ladybird *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) (PhD thesis). Gembloux, Belgium, University of Liege, Gembloux Agro-Bio Tech, 171p., 27 tabl., 44 fig.

Abstract – The international trade of goods and food can lead to the introduction of alien species in the importing countries. Even if in the most cases there is no negative impact induced by alien species, in some cases the new imported species can spread out of control. These species can cause economical and ecological damages. The Multicoloured Asian ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is one of these species which was intentionally introduced for biological control and has spread from its native range in Central and Eastern Asia to large parts of North and South America, Europe and Africa. The decline of native species is linked to the spread and the aggressive behaviour of *H. axyridis*. The objective of this thesis is the evaluation of aphid's predator population densities and predator diversities, with a special focus on *H. axyridis* in agroecosystems in Wallonia, in the South of Belgium. First, field crops preferences of *H. axyridis* were determined. Later aphid's predator population changes during time were evaluated. Finally *H. axyridis* population changes between two crop farming were evaluated. Our main findings were as follows. Results, based on insects collected since 2001, highlighted that the first collected *H. axyridis* was in 2002 and the population of *H. axyridis* is increasing until 2008. In the same time population of *Adalia bipunctata, Propylea quatuordecimpunctata* and *Psyllobora vigintiduopunctata*, three native species, are decreasing. Samplings in agroecosystems highlighted that aphid's predator community is composed of few dominant species: three coccinellids *Coccinella septempunctata, P. quatuordecimpunctata* and *H. axyridis,* one syrphid *Episyrphus balteatus* and one chrysopid *Chrysoperla carnea*. We have shown that *H. axyridis* do not invade all Wallonia crops at the same rate because corn and broad bean are more invaded that wheat and potato at both larval and adult stages. Moreover in corn, *H. axyridis* populations has strongly increased from 2009 to 2011. Finally, organic farming do not enhance abundance of *H. axyridis* but lead to increase the total abundance of aphid natural enemies.

Vandereycken Axel (2014), Evaluation des populations d'ennemis naturels de pucerons dans les agroécosystèmes, avec une attention particulière pour la coccinelle asiatique *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) (Thèse de doctorat). Gembloux, Belgium, Université de Liège, Gembloux Agro-Bio Tech, 171p., 27 tabl., 44 fig.

Les échanges internationnaux de marchandises peuvent conduire à l'importation d'espèces exotiques sur le territoire du pays importateur. Si de nombreuses introductions d'espèces exotiques n'engendrent peu ou pas de conséquences négatives pour l'environnement, dans certain cas ces espèces prospèrent et deviennent incontrôlables. Elles peuvent de ce fait occasionner des dégats écologiques et économiques. La coccinelle asiatique *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) est une espèce volontairement introduite pour lutter de manière biologique contre les pucerons. Originaire d'Asie, cette espèce a colonisé les continents européen, américain et africain. Cette colonisation rapide et son comportement agressif ont causé le déclin de plusieurs espèces de coccinelles natives. Le principal objectif de cette thèse est de caractériser de manière quantitative et qualitative les populations de prédateurs de pucerons et plus particulièrement des populations de coccinelles asiatiques au sein des agroécosystèmes wallons, situés dans le Sud de la Belgique. Nous avons déterminé les habitats préférentiels d'*H. axyridis* et évalué les densités de populations de prédateurs de pucerons dans ces habitats au cours des années. Nous avons également étudié l'influence que peuvent avoir les pratiques liées à l'agriculture biologique sur les densités de ces populations. Sur base des collectes d'insectes réalisées depuis 2001, il ressort qu'*H. axyridis* est présente en Belgique depuis 2002 et que ses populations n'ont cessé d'augmenter jusqu'en 2008. Parallèlement à cela, les populations d'*Adalia bipunctata, Propylea quatuordecimpunctata* et *Psyllobora vigintiduopunctata*, coccinelles natives, n'ont cessé de diminuer. Les inventaires réalisés en agroécosystèmes nous permettent de conclure que les communautés de prédateurs de pucerons sont composées de peu d'espèces abondantes, à savoir, trois coccinelles, *Coccinella septempunctata, P. quatuordecimpunctata* et *H. axyridis,* un syrphe, *Episyrphus balteatus* et une chrysope, *Chrysoperla carnea*. *H. axyridis* présente une variabilité de densités de populations au sein des cultures étudiées. En effet, les cultures de maïs et de fève présentent des densités de populations d'*H. axyridis* plus élevées qu'en froment et pomme de terre, que ce soit aux stades larvaires ou au stade adulte. De plus, en culture de maïs, les populations d'*H. axyridis* ont fortement augmenté de 2009 à 2011. Finalement, les pratiques liées à la culture biologique n'augmentent pas l'abondance de *H. axyridis* mais augmentent l'abondance totale des prédateurs de pucerons.

REMERCIEMENTS

Au terme de ce travail, je tiens à remercier toutes les personnes qui ont contribué de près ou de loin à l’élaboration de cette thèse. Un tout grand merci :

- au professeur Eric Haubruge, qui m’a permis de réaliser ma thèse au sein de son unité d'Entomologie. Ses idées, conseils et remarques m’ont guidé tout au long de ces quatre années. Les souvenirs de son humour décapant et de nos "interactions" (poisson rouge, rugby à la mer, repas et activités de service, aspirateur,…) seront à jamais associés à ma thèse.
- au Dr François Verheggen, mon co-promoteur et surtout mon "conseiller", qui m’a suivi et guidé dans les bonnes directions afin de mener à bien cette thèse.
- au professeur Frédéric Francis, pour ses indications scientifiques "warémiennes" qui ne laissent jamais perplexe mais qui nécessitent une période de réflexion.
- au professeur Yves Brostaux qui m’a guidé dans le labyrinthe des statistiques. Merci Papy.
- à l’Harmonia Team : Emilie Joie, qui m’a accompagné sur le terrain par tous les temps et parfois même douloureusement. Sa motivation et sa pugnacité font d’elle un élément clé dans ce projet. Delphine Durieux et Bérénice Fassotte qui m’ont aidé dans tous mes protocoles et rédactions. Les stagiaires : Virginie Sibret et Alice-Marie Buset pour leur contribution technique.
- à toute l’unité d’Entomologie et plus particulièrement: Béru, Ammar, Kacem, Ludo, Christine, Gil, Vianney, Alabi, Fanny, Rudy, Nico, Jean-Yves, Kim, Yattara, Maud, Sandrine, Jessica, Lara X2, Thomas X2, Emilie, Sophie, Slimane, René-Noel, Raki, Jacques, Fara, Laurent, Antoine et tous les autres pour leur bon soutien et leur joie de vivre.
- au Dr Pascal Leroy pour ses conseils éclairés aussi bien scientifiques que personnels.
- Didier, Marcelline et Jeannine pour leur travail et leur motivation indispensables à la cohésion d’une unité et d’une famille.
- à Charles Gaspar pour ses remarques et électrochocs gratuits.
- aux Dalton: Coraline, Quanah et Violaine pour le travail de précision.
- à mes amis et ma famille pour leur écoute, leur présence et leurs encouragements.
- à ma compagne, Caroline De Clerck, pour sa disponibilité, son écoute, ses encouragements, son aide, son soutien et les desserts qu'elle fait tous les jeudis.

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Chapter I : GENERAL INTRODUCTION

Agricultural pests such as phytophagous insects, viruses, bacteria, and fungi induce a lot of damages leading to economical losses due to yield decreasing. Aphids, known to be a major agronomical pest, cause plant weakening by feeding on the phloem sap that contains nutrients for plant. Aphids may be present worldwide and on all plant organs. These phytophagous species are controlled by several taxa as predatory insects (Coccinellidae, Syrphidae, Chrysopidae, Miridae, Anthocoridae) and parasitic insects (Braconidae, Chalcicidae, Aphelinidae). The composition of the aphidophagous community differs from one crop to another and is influenced by several abiotic (Honěk 1985) and biotic factors (Wright and Laing 1980, Honěk 1982, Alhmedi et al. 2009). Some of these natural enemies, known for their high voracity and fecundity, are commonly used in biological control: *Episyrphus balteatus* De Geer, *Adalia bipunctata* L., *Chrysoperla carnea* Stephens and *Aphidius ervi* Haliday.

Biological control practices are used to manage agricultural pests and reduce chemical applications leading to resistances in target species and causing health and environmental issues. Not all "biological practices" are safe for the environment (Louda et al. 2003): the introduction of an exotic species in a new environment can lead to an ecological disequilibrium (invasion of this exotic species and decrease of native species). The introduction of exotic species is the second cause of biodiversity erosion (Pascal et al. 2000). Focusing on arthropods, only 14% of the introduced species were intentional and most of them were meant to be used in biological control (Rabitsch 2010). One of the most famous example with a coccinellid is the introduction in the USA of *Coccinella septempunctata* L. to control aphids. After the introduction of *C. septempunctata* the local coccinellid community was modified and the decrease of two native coccinellid species (*A. bipunctata* and *C. transversoguttata* Faldermann) was observed (Elliott et al. 1996).

Introduction of exotic species - also named alien species or non native species - in a new area can either be intentional (e.g. for biological control strategies) or unintentional (e.g. mediated by trades of food and goods or travelling peoples). These human activities are increasing the spread of alien species, including plants, animals and microbes worldwide (Pascal et al. 2000). It was estimated that 480.000 alien species have been introduced into varied ecosystems on earth (Pimentel et al. 2001). Many of intentional introduced species, like corn, wheat, rice, plantation forests, domestic chicken, cattle, and others are beneficial and provide the majority of world food.

However, alien species are also known to cause major economical losses in both agriculture and forestry (Mack and D'Antonio 1998). The ecological consequences of these invasions could lead to homogenisation of the flora and fauna, extinction of species or modifications of the biocoenosis (Samways 1997). There are many examples of environmental damages and control costs due to alien species. The first example concerns plants with the invasion of water hyacinth (*Eichhornia crassipes* Mart. Solms). This invasion causes alteration of the habitats of fish and other aquatic species, choke waterways, alteration of nutrient cycles, and reduction of recreational use of rivers and lakes (Pimentel et al. 2001). The second example concerns burrowing activity and crop/forest destruction by muskrat (*Ondatra zibethicus* L.) (Gosling and Baker 1989). A third example concerns numerous damages (US$ 1.1 billion/year) caused by pigeon (*Columba livia* Gmelin). There are many other examples of damages caused by introduced species, among others amphibians and reptiles: cane toad (*Bufo marinus* L.) and fishes: rainbow trout (*Oncorhynchus mykiss* Walbaum) (Pimentel et al. 2001).

Threatening of biodiversity is the main consequence of the introduction of alien species. This great problem concerns all countries and many specialist working groups have been created including Invasive Species in Belgium (Harmonia), IUCN Invasive Species Specialist Group (ISSG), SEBI2010 Invasive Species Expert Group, European and Mediterranean Plant Protection Organization (EPPO) and European Research Network on Aquatic Invasive Species (ERNAIS). There are also many databases on the web that inform on the invasive and exotic species: Delivering Alien Invasive Species Inventories for Europe (DAISIE), European Information System on Invasive Alien Species (RBIC), Global Invasive Species Database (GISD).

Harmonia axyridis Pallas (Coleoptera: Coccinellidae) was imported from Asia into Western Europe and other parts of the world to control aphid and coccid populations. First importations to the USA started in 1916 until 1990 (Chapin and Brou 1991). The goal of *H. axyridis* use was to control aphids in several crops in agroecosystems as pecans (Tedders and Schaefer 1994), sweet corn (Musser and Shelton 2003), alfalfa (Buntin and Bouton 1997), cotton (Wells et al. 2001) or winter wheat (Colunga-Garcia and Gage 1998). To date, this alien species is one of the most abundant ladybird in the USA and it impacts negatively other coccinellid species especially native species such as *Brachiacantha ursina* Hatch and *Cycloneda munda* Say, and alien species such as *C. septempunctata* (Colunga-Garcia and Gage 1998, Brown 2003).

In mainland Europe, *H. axyridis* has been sold for biological control strategies since 1982 (Iperti and Bertrand 2001). In Belgium, *H. axyridis* was used as a biological control agent since 1997 and was observed in the wild for the first time in 2001. Since then, *H. axyridis* populations have increased and gradually expanded into Belgium (Adriaens et al. 2008, Brown et al. 2008). It is now considered as an invasive species because *H. axyridis* is an exotic species with high widespread potentialities and is a danger for the native species (Fig. 1).

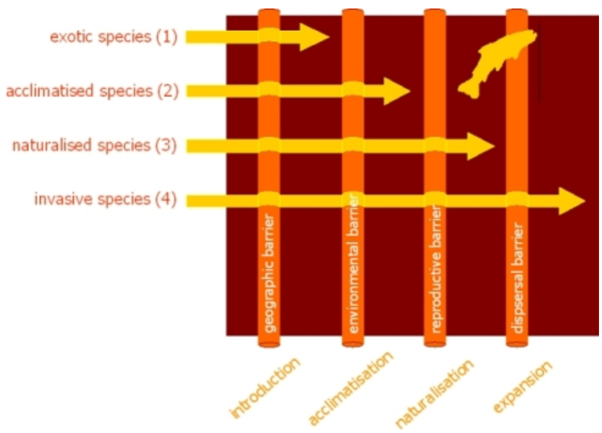

Figure 1: Alien species have to overcome geographic, environmental, reproductive and dispersal barriers before becoming invasive (http://ias.biodiversity.be).

H. axyridis intrinsec characteristics that make it a successful invader are multiple (Sloggett 2012) : large body size, high voracity and predation efficiency (Soares et al. 2001, Labrie et al. 2006), good colonization efficiency (With et al. 2002), ubiquity (Tedders and Schaefer 1994), overwintering behaviour (Berkvens et al. 2010) and strong chemical defence (Nedvěd et al. 2010). Roy et al. (2012) highlighted that the biodiversity erosion and the decrease of five coccinellid species in Belgium and seven in UK are linked to the invasion of *H. axyridis*. One of the most impacted species is *A. bipunctata,* which declined by 30% in Belgium and 44% in UK over 5 years after the arrival of *H. axyridis*. Competition for mutual food resources, intraguild predation and superior physical and chemical defence strategies are keys to dominate native aphidophagous species.

Now, *H. axyridis* is one of the 10.822 exotic species in Europe and is considered by Delivering Alien Invasive Species Inventories Europe (DAISIE) as one of the 100 worst alien invasive species (Européenne 2008). Many European initiatives lead to a better understanding of this problematic and try to find some strategies to decrease the erosion of the biodiversity: LIFE, SEBI2010.

Legal restrictions on imports and trade of exotic species in Belgium and/or Europe are currently very scarce. They mainly affect pests for plant and animal health according to EC Directives or their implementation in the federal legislation. In terms of biodiversity harmful exotic species, only the European regulation on the protection of wild fauna and flora by regulating trade (CITES implementation, EC N ° 338/97) prohibits the importation of a small number of organisms such as the gray squirrel or bullfrog. New regulatory tools are however in preparation for Europe, Belgium and Wallonia based on the proposed list of EPPO.

In Belgium species management and introduction are lightly regulated. In Wallonia, the laws about Nature Conservation (2001) say that the introduction of exotic species is forbidden except species for agriculture and sylviculture. The introduction of *H. axyridis* seems to be permitted for the biological control. Another law named "Habitat Directive" (Natura2000) highlights that the introduced species does not damage natural habitat and native species. In Flander, the introduction of exotic species is forbidden (San Martin et al. 2004).

Most studies focus on the impact of *H. axyridis* on biodiversity in urban or arboreal habitat and not in agroecosystems. In this context, the overall objective of this thesis is to determine the extent of the invasion of *H. axyridis* in agroecosystems of the south of Belgium. We aimed at increasing our understanding of the effects of the predatory behaviour of *H. axyridis* on aphids predator community. This thesis is presented in three sections. Chapter II is a review of the habitat preferences of *H. axyridis*. Chapter III is a study concerning coccinellid sampling by students since 2001. Finally, the chapter IV and subsequent focus on field observations conducted eight years after the first observation of this alien species in nature and lasted during three years.

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Chapter II : HABITAT DIVERSITY OF *HARMONIA AXYRIDIS*

II.1 General introduction to chapter II

What are *H. axyridis* habitats? Where do they reproduce? Where do they feed? And where do they overwinter? One factor that influences the habitat selection is the food specialisation: generalist species breed on a wide range of habitats and specialist species used to breed on a specific habitat or plant species. *H. axyridis* is a generalist species and all the literature agree with the fact that *H. axyridis* is an ubiquist species, generally regarded as a semi-arboreal species, occurring mostly on deciduous trees (*Acer* sp. and *Salix* sp.) (Hodek 1973) but also in various herbaceous habitats (Koch et al. 2006). But, *H. axyridis* is not only found in a broad range of semi-natural biotopes but also frequently in more urbanised and anthropogenic landscapes (Adriaens et al. 2008). In fact, *H. axyridis* is more frequent in these last habitats than in landscapes with forests and other natural elements.
Sampling of aphidophagous species, studies of intraguild predation in natural habitat and basic observations contribute to increase the knowledge on the *H. axyridis* habitat diversity. All these studies have referenced *H. axyridis* on more than 100 habitats including arboreal, crop, herbaceous, ornamental and orchard species. Following Lucas et al. (2007) *H. axyridis* seems to prefer *Urtica* sp. as herbs, *Acer* sp. as arboreal species and *Zea* sp. as crop fields. Various and complex factors (host plant, microclimate, prey abundance, change habitat, influence of the adjacent habitats) are implicated in the habitat selection (Hodek and Honěk 1996). Due to the complexity of this topic, factors which can explain this species distribution, are not considered in this section.

In chapter II.2, the review focuses on the habitat and plant species where *H. axyridis* has been observed during sampling or as part of integrated pest management. This chapter emphasis the diversity of the plants where the eurytopic *H. axyridis* can live and where this exotic species could negatively act as an intraguild predator. The review highlights that the Asian Multicoloured Ladybird is predominantly a generalist predator occupying arboreal species but that it also breeds on herbaceous or orchards species.

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II.2 Habitat diversity of the Multicolored Asian ladybeetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in agricultural and arboreal ecosystems: a review

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Reference - Vandereycken A., Durieux D., Joie E., Haubruge E., Verheggen F.J. 2012. Habitat diversity of the Multicolored Asian ladybeetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in agricultural and arboreal ecosystems: a review. *Biotechnologie Agronomie Société et Environnement* 16(4): 553-563

Abstract - The Multicolored Asian ladybeetle, *Harmonia axyridis* (Pallas), native to Asia, is an invasive species in many European and American countries. Initially introduced as a biological control agent against aphids and coccids in greenhouses, this alien species rapidly invaded many habitats such as forests, meadows, wetlands, and agricultural crops. This paper reviews the habitats (forests, crops, herbs, gardens and orchards) where *H. axyridis* has been observed, either during insect samplings or as part of Integrated Pest Management (IPM) programs. Studies have referenced *H. axyridis* on 106 plant taxa (35 arboreal species, 21 crop species, 27 herbaceous species, 11 ornamental species, and 12 orchard species) and have identified 89 plant-prey relationships (34 arboreal species, 16 crop species, 13 herbaceous species, 10 ornamental species, and 16 orchard species) in different countries. *Harmonia axyridis* is more abundant in forest areas, principally on *Acer*, *Salix*, *Tilia* and *Quercus*, than in agroecosystems. Some plant species, such as *Urtica dioica* L., which surround crops, contain large numbers of *H. axyridis* and could constitute important reserves of this alien species in advance of aphid invasions into crops. This review highlights the polyphagia and eurytopic aspect of *H. axyridis*.

Keywords - Coccinellidae, biological control agents, habitats, integrated pest management, predatory insects, introduced species, invasive species, forests, agroecosystems, censuses.

Résumé - Diversité des habitats de la coccinelle asiatique *Harmonia axyridis* Pallas (Coleoptera : Coccinellidae) au sein des différents écosystèmes agricoles et forestiers (synthèse bibliographique). La coccinelle asiatique, *Harmonia axyridis* (Pallas), originaire d'Asie, est une espèce invasive dans de nombreux pays européens et américains. Introduite comme agent de contrôle biologique afin de lutter contre les pucerons et les cochenilles dans les serres, cette espèce a rapidement envahi différents habitats tels que les forêts, les cultures agricoles, les prairies, les jardins et les vergers. Cet article présente une synthèse des habitats où *H. axyridis* a été observée lors d'inventaires et dans lesquels elle a été utilisée dans la gestion de lutte contre les ravageurs. Cent-six taxons de plantes sur lesquelles *H. axyridis* a été observée (35 espèces arborescentes, 21 espèces de culture, 27 espèces herbacées, 11 espèces ornementales et 12 espèces de verger) et 89 relations plantes-proies (34 espèces arborescentes, 16 espèces de culture, 13 espèces herbacées, 10 espèces ornementales et 16 espèces de verger) ont été dénombrés dans différents pays. *Harmonia axyridis* est plus abondante en milieu forestier et plus précisément sur *Acer*, *Salix*, *Tilia* et *Quercus* qu'en milieu agricole. Certaines espèces végétales, telles que l'ortie *Urtica dioica* L., présentes à proximité des habitats agricoles, contiennent de grandes quantités d'*H. axyridis* et peuvent donc servir de réserve de prédateurs avant les invasions de pucerons en grandes cultures. Cette synthèse bibliographique souligne la polyphagie et le comportement eurytopique de cette coccinelle exotique.

Mots-clés: Coccinellidae, agent de lutte biologique, habitat, agriculture, insecte prédateur, espèce envahissante, espèce introduite, forêt, agroécosystème, recensement.

Introduction

The Multicolored Asian ladybeetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), is native to south-east Asia, between Siberia and China (Chapin, 1965). This species has long been used as a biological control agent against aphids and coccids on both sides of the Atlantic Ocean. Since 1988, *H. axyridis* has become established in at least 38 countries around the world: 9 in America, 26 in Europe and 3 in Africa (Brown et al., 2011).

In America, the first introduction of *H. axyridis* was conducted in California in 1916, but the first established populations were referenced 72 years later (Gordon, 1985; Chapin et al., 1991). In this area, *H. axyridis* has been commonly used for biological control in diverse crops such as pecans (Tedders et al., 1994), red pines (McClure, 1987), apple orchards (Brown et al., 1998), soybeans (Fox et al., 2004), sweet corn (Musser et al., 2003), alfalfa (Buntin et al., 1997; Colunga-Garcia et al., 1998), cotton (Wells et al., 2001), tobacco (Wells et al., 1999) and winter wheat (Colunga-Garcia et al., 1998). *Harmonia axyridis* was introduced into Europe in 1964 and has been commercialized as a biological control agent since 1982 (Iperti, 1991; Katsoyannos et al., 1997). Thanks to a rapid reproductive cycle allowing this beetle to achieve two or three generations per year, *H. axyridis* has become the dominant coccinellid in many ecosystems (Brown et al., 2008).

Harmonia axyridis has been released as a biocontrol agent into numerous crops, arboreal habitats, and orchards (Tables 1 to 5). These releases partly explain the rapid expansion of this alien ladybeetle, favored by its notable dispersal abilities (Koch et al., 2006).

This review will focus on the natural and semi-natural habitats of the multicolored Asian ladybeetle *H. axyridis* both inside and outside of its native range, and will highlight its associated plant species and prey species.

Habitats of *Harmonia axyridis*

Arboreal habitats

In both native and invaded areas, arboreal habitats are commonly colonized by *H. axyridis* for growth and reproduction. The most common trees on which *H. axyridis* is observed are *Acer*, *Salix*, *Tilia*, *Quercus* and *Pinus* (LaMana et al., 1996; Osawa, 2000; Adriaens et al., 2008; Brown et al., 2008) (Table 1). In Oregon, this species represents 70% of the coccinellid community in forest stands, where it is often considered as a dominant generalist aphidophagous predator (LaMana et al., 1996). In western Europe, Adriaens et al. (2008) inventoried several habitats and found that *H. axyridis* was present on more than 100 plant species. In that study, 52% of *H. axyridis* observations were made on trees and 14% on shrubs. The plants on which *H. axyridis* was most frequently observed were *Acer*, *Salix*, *Tilia*, *Quercus*, *Betula*, *Pinus*, and *Crataegus*. In eastern Europe, the first detection of *H. axyridis* was recorded on the same arboreal species, including *Tilia*, *Quercus* and *Acer*, respectively infested by the following aphid species: *Eucallipterus tiliae* L. (Hemiptera:

Aphididae), *Myzocallis walshii* Monell (Hemiptera: Aphididae) and *Drepanosiphum* sp. (Hemiptera: Aphididae) (Tomov et al., 2009) (Table 1).

The presence of forest ecosystems increases the occurrence of *H. axyridis* in the surrounding habitats such as agroecosystems (Gardiner et al., 2009). A consequence of a higher density of the species in landscapes in close proximity to forests is that predation on native species is higher than that observed in fields surrounded by other croplands (Gardiner et al., 2011).

Table 1: List of arboreal species where *Harmonia axyridis* was observed. The column "prey species" contains phytophagous species observed with *H. axyridis*.

Plant species	Prey species	References
Abies procera Rehder	*Cinara* sp.	LaMana et al., 1996
Acacia sp.	*Cicadellidae*	Saini, 2004
Acer negundo L.	*Perihpyllus negundinis* (Thomas)	Koch et al., 2003
Acer pseudoplatanus L.	*Drepanosiphum* sp.	Tomov et al., 2009
Acer saccharum Marsh	*Drepanaphis idahoensis* Smith & Dilley; *Drepanosiphum platanoides* Schrank; *Periphyllus testudinaceae* Fernie	LaMana et al., 1996
Acer sp.		San Martin, 2003; Adriaens et al., 2008; Brown et al., 2008
Alnus spp.		Adriaens et al., 2008
Betula pendula L.	*Callipterinella calipterus* Hartig; *Euceraphis betulae* Kalterbach	LaMana et al., 1996
Betula sp.		Adriaens et al., 2008
Corylus spp.		Adriaens et al., 2008
Crataegus sp.		Adriaens et al., 2008
Fagus sylvatica L.	*Phyllaphis fagi* L.	LaMana et al., 1996
Juniperus sp.	*Cinara juniperi* De Geer	Saini, 2004
Lafoensia pacari L.	Psyllidae	Martins et al., 2009
Liriodendron tulipifera L.	*Illinoia liriodendri* Monell	LaMana et al., 1996
Magnolia macrophylla Michaux		Tedders et al., 1994
Myrciaria cauliflora Mart. O. Berg	Curculionidae	Martins et al., 2009
Nicotiana tabacum L.	*Myzus nicotianae* Blackman	Wells et al., 1999
Nicotiana tabacum L.	*Helicoverpa armigera* Hübner; *Spodoptera exigua* Hübner	Knutson et al., 1996
Picea spp.		Adriaens et al., 2008
Pinus resinosa Aiton	*Matsucoccus resinosae* Bean& Goodwin	McClure, 1987
Pinus sp.		San Martin, 2003; Adriaens et al., 2008
Pinus sp.	*Cinara atlantica* Wilson; *Cinara pinovora* Wilson	de Almeida et al., 2002; Martins et al., 2009
Pinus sp.	*Essigella californica* Essig	Martins et al., 2009
Pinus sylvestris L.		Brown et al., 2008
Pinus taeda L.	*Eulachnus agilis* (Kaltenbach)	Tedders et al., 1994

Podocarpus sp.	*Neophyllaphis podocarpi* Takahashi	Tedders et al., 1994
Podocarpus sp.	*Neophyllaphis podocarpini* Carrilo	Martins et al., 2009
Populus sp.		Colunga-Garcia et al., 1998
Quercus rubra L.	*Myzocallus occultus* Richards	LaMana et al., 1996
Quercus rubra L.	*Myzocallis walshii* Monell	Tomov et al., 2009
Quercus sp.		Adriaens et al., 2008
Salix koriyanagi Kimura	*Chaitophorus horii horii* Takahashi	Osawa, 2000
Salix sieboldiana Bi.	*Aphis farinosa yanagicola* Matsumura; *Tuberolachnus salignus* Gmellin	Osawa, 2000
Salix sp.	*Tuberolachnus salignus* Gmellin	LaMana et al., 1996
Salix sp.		Adriaens et al., 2008
Sambucus sieboldiana (Miq.)	*Aulacorthum magnoliae* Essig & Kuwana	Osawa, 2000
Tilia americana L.	*Eucallipterus tiliae* L.	LaMana et al., 1996
Tilia cordata Mill.	*Eucallipterus tiliae* L.	Tomov et al., 2009
Tilia sp.		San Martin, 2003; Adriaens et al., 2008; Brown et al., 2008
Tipuana tipu (Benth.) Kuntze	Psyllidae	Martins et al., 2009

Trees are not the only arboreal areas where *H. axyridis* are able to find food. In addition, *H. axyridis* has been observed on shrubs, feeding on *Tinocallis kahawaluokalani* Kirkaldy (Hemiptera: Aphididae) and has also been observed on crape myrtle, *Lagerstroemia indica* L. (de Almeida et al., 2002).

Most of these studies have reported observations of *H. axyridis* mainly in arboreal habitats and in high density. Two reasons may explain the occurrence of this alien coccinellid in arboreal habitats: first, the arboreal taxa cited are affected by high aphid populations in spring and are thus attractive to the coccinellid; second, these arboreal taxa are the most popular taxa in Europe. Further information on studies concerning *H. axyridis* in arboreal ecosystems can be seen in table 1. Thirty-five plant species and 34 plant-prey-predator relationships have been observed on diverse continents.

Agroecosystems

Agroecosystems may be infested by large quantities of prey and so can constitute habitats where ladybeetles are able to thrive and reach their adult stage. Nevertheless, in these particular ecosystems, the numbers of the most abundant coccinellid species are generally low (three or four dominant species) (Hodek et al., 1996). The composition of the aphidophagous community differs from one crop to another and is influenced by several abiotic factors, such as insolation and humidity (Honěk, 1985) and by biotic factors including the quantity and quality of host plants (Alhmedi et al., 2009), aphid density and diversity (Wright et al., 1980; Honěk, 1982; Thalji, 2006) and adjacent habitats (Colignon et al., 2000; Alhmedi et al., 2009).

Many studies highlight the dominance of *H. axyridis* in crop areas, *e.g.*: in Minnesota, 10 years after its initial detection in 1994 (Koch et al., 2003), *H. axyridis* rapidly became the most abundant generalist predator in corn, reaching 77.4% of Coccinellidae on the crop (Koch et al., 2006). In other cases, *H. axyridis* can quickly become the dominant species, as was observed in Michigan crops (alfalfa, soybean, corn and winter wheat), where four years after its arrival, this ladybeetle became dominant, with its proportions varying from 2.8% to 32.3% between 1994 and 1998 on all inventoried crops (Colunga-Garcia et al., 1998). However, some reports claim contrary conclusions. Nault et al. (2003) showed that three years after the arrival of *H. axyridis* in North Carolina agroecosystems, the most abundant species were not *H. axyridis* but *Coccinella septempunctata* L., *Coleomegilla maculata* De Geer and *Hippodamia convergens* Guérin-Méneville. In this area, *H. axyridis* colonized wheat, corn, and potato but reproduced only in wheat and potato (Kidd et al., 1995; Nault et al., 2003). In tobacco plants in Georgia, during 1997 and 1998 (15 years after initial release), *H. axyridis* was dominated by *H. convergens* and *C. septempunctata* (Wells et al., 1999). Finally, in alfalfa in Japan, adult and larval proportions of *H. axyridis* were found to respectively form 24.6% and 15.5% of coccinellids, figures lower than those of *C. septempunctata* and *Propylea japonica* (Thunberg) (Takahashi et al., 1984).

Variations in coccinellid dominance from year to year or between crops are dependent on multiple factors, but these variations are complex and misunderstood. For example, in agricultural habitats, population densities can be influenced by margin strips of herb species situated in close proximity. Nettle, *Urtica dioica* L., is known to be a plant species hosting many *H. axyridis* (Adriaens et al., 2008); the presence of nettle among margin field crops (wheat, green pea) therefore significantly increases the density of *H. axyridis* (Alhmedi et al., 2007) in these crops. These surrounding areas (nettle, forest) may provide a population of *H. axyridis* before aphid invasion in the adjacent field crops. The prey species is also an important factor determining the abundance and reproduction rate of *H. axyridis*. Evans et al. (2005) highlighted that in alfalfa, *H. axyridis* is an efficient predator, consuming both pea aphids *Acyrthosiphon pisum* Scop. (Hemiptera: Aphididae) and larvae of alfalfa weevils *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), but that the consumption rates of aphids were greater than those for the weevil. Moreover, when alfalfa weevil is the available food source, *H. axyridis* does not reproduce, and both larval survival and development of

H. axyridis are low (Evans et al., 2005). A second example highlights the problem of prey quality: *Uroleucon nigrotuberculatum* (Olive) (Hemiptera: Aphididae) on *Solidago canadensis* L. represent unsuitable prey for *H. axyridis* during its life cycle (Kamo et al., 2011). These prey species are considered by Hodek et al. (1996) to be "acceptable but inadequate prey" for *H. axyridis*.

At the beginning of the 1990s, LaMana et al. (1996) highlighted that *H. axyridis* was more abundant in arboreal habitats than in agricultural areas. Their results showed that only 4% of *H. axyridis* were observed in agroecosystems such as alfalfa *Medicago sativa* L., clover *Trifolium* sp. L. and peppermint *Mentha piperita* L. In these ecosystems, *H. axyridis* was observed in association with 17 aphid species on 17 host plants. While *H. axyridis* has been more observed in arboreal habitats, it can still thrive and dominate the aphidophagous guild in agroecosystems. In agricultural systems of eastern Canada (pome fruit, grape, field corn, sweet corn, sweet pepper, lettuce, and soybean), *H. axyridis* was clearly one of the dominant coccinellid species from 1999 to 2003 (Lucas et al., 2007). Unlike in American and Asian studies, *H. axyridis* presented in Belgium high population levels only one year after its original detection in potato fields (2004-2005) and was able to complete its larval development even with low aphid population, or with no aphids at all (Jansen et al., 2008). In this invaded area, *H. axyridis* was found to be one of the most abundant coccinellids along with *C. septempunctata* and *Propylea quatuordecimpunctata* L. (Jansen et al., 2008).

One of the most important pests of American soybean is the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae). In 2000, *H. axyridis* was found to constitute more than 25% of the aphidophagous species in this crop; other such species present included *Orius insidiosus* (Says) (Hemiptera: Anthocoridae) and *Leucopis* spp. (Diptera: Chamaemyiidae), which are able to control *A. glycines* (Fox et al., 2004). *Harmonia axyridis* was found to cause a reduction in aphid populations by 21-56% in the early season and by 54-95% in midseason (Landis et al., 2004). Five years later, *H. axyridis* was found to occur at levels of 45 to 62% of the total coccinellid community (Gardiner et al., 2009).

Despite the fact that *H. axyridis* is an arboreal species, a high diversity of crops (21) is subject to invasion by this coccinellid (Table 2). *Harmonia axyridis* invades selected crops according to the aphid species present and to the microclimate inside the field. The high density and high voracity of *H. axyridis* make it a highly efficient beneficial species. It would be interesting to use *H. axyridis* as part of a pest control approach through a push-pull strategy in organic crops to control aphid populations. This technique, which could be an alternative to the use of chemical insecticides, consist to manipulate the distribution of pest population with the use of repellent and attractive stimuli (Cook et al. 2007). The pests or in this case *H. axyridis* are repelled away from a specific crop (push) by using stimuli that mask host apparency or are repellent or deterrent. The pests are simultaneously attracted (pull), using highly apparent and attractive stimuli, to other areas such as traps where they are concentrated, facilitating their elimination.

Herbs, domestic and ornamental gardens

Harmonia axyridis also occurs in herbaceous habitats such as heathland, meadows, and wetlands (Adriaens et al., 2008). Among these habitats, *H. axyridis* has been most frequently observed on *U. dioica* (Adriaens et al., 2008), but also on *Bidens pilosa* L. (Martins et al., 2009), *Phragmites* spp. (Adriaens et al., 2008), *Artemisia vulgaris* L. (Agarwala et al., 2003), and *Typha angustifolia* L. (Osawa, 2000). The alien species has also been observed in urban habitats, such as in domestic gardens, on *Hibiscus rosa sinensis* L. (Martins et al., 2009), *Lagerstroemia indica* L. (Chapin et al., 1991), and *Rosa* sp. (de Almeida et al., 2002).

Harmonia axyridis can be used as a biocontrol agent in gardens, but it is not common *e.g.*: *H. axyridis* can be used to control *Chaetosiphon fragaefolii* (Cockerell) (Hemiptera: Aphididae) on strawberry (Sun et al., 1996) and *Macrosiphum rosae* L. (Hemiptera: Aphididae) on roses (Finlayson et al., 2010). *Harmonia axyridis* can also be used to control pests on ornamental plant species *e.g.*: on greenhouse roses (*Rosa hybrida* L.), the aphid, *Macrosiphum euphorbiae* Thomas (Hemiptera: Aphididae), can be controlled by *H. axyridis* and a wasp, *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae). *Harmonia axyridis* can be used to complement aphid biocontrol, without disrupting control despite intraguild predation (Snyder et al., 2004).

The presence of *H. axyridis* in gardens is more anecdotal. The use of ladybeetles as beneficial species needs to be focused on native species such as *A. bipunctata*. Further examples of *H. axyridis* habitats, including herbs and ornamental species, are presented in tables 3 and 4. *Harmonia axyridis* individuals have been observed on herbs and ornamental habitats containing respectively 27 and 11 plant taxa.

Table 2: List of crop species where *Harmonia axyridis* was observed. The column "prey species" contains phytophagous species observed with *H. axyridis*.

Plant species	Prey species	References
Allium schoenoprasum L.	*Neotoxoptera formosana Takahashi*	Martins et al., 2009
Apium graveolens L.		Lucas et al., 2007
Brassica napus L.		Lucas et al., 2007
Brassica oleracea L.		de Almeida et al., 2002
Brassica oleracea L. *var. italica*	*Myzus persicae* Sulzer	Martins et al., 2009
Brassica oleracea L. *var. italica*	*Lipaphis erysimi* Kaltenbach	Martins et al., 2009
Brassica oleraceae L. *var. capitata*	*Brevicoryne brassicae* L.	Martins et al., 2009
Brassica oleraceae L. *var. leucocephala*	*Brevicoryne brassicae* L.	Martins et al., 2009
Cucurbita sp.		Koch et al., 2004
Fragaria sp.	*Chaetosiphon fragaefolii* Cockerell	Sun et al., 1996
Glycine max L.		Colunga-Garcia et al., 1998; Koch et al., 2004; Saini, 2004
Glycine max L.	*Pseudoplusia includens* Walker	Knutson et al., 1996
Glycine max L.	*Aphis glycines* Matsumura	Fox et al., 2004; Landis et al., 2004; Gardiner et al., 2007; Gardiner et al., 2009; Xue et al., 2012; Rutledge CE, personal communication
Gossypium hirsutum L.	*Aphis gossypii* Glover; *Helicoverpa zea* Boddie	Knutson et al., 1996
Gossypium hirsutum L.		Wells et al., 2001
Hordeum vulgare L.		Colunga-Garcia et al., 1998
Humulus lupulus L.		LaMana et al., 1996
Lactuca sativa L.	*Uroleucon ambrosiae* Thomas; *Uroleucom sonchi* L.	Martins et al., 2009
Lolium perenne L.		Agarwala et al., 2003
Medicago sativa L.		Takahashi et al., 1984; Buntin et al., 1997
Medicago sativa L.	*Acyrtosiphum pisum* Scop.	Saini, 2004; Evans et al., 2005
Medicago sativa L.	*Hypera postica* Gyllenhal	Evans et al., 2005
Medicago sativa L.		LaMana et al., 1996; Colunga-Garcia et al., 1998
Mentha piperita L.		LaMana et al., 1996
Pisum sativum L.		Alhmedi et al., 2007
Solanum tuberosum L.		Nault et al., 2003; Alyokhin et al., 2004; Jansen et al., 2008
Solanum tuberosum L.	*Macrosiphum euphorbiae* Thomas; *Myzus persicae* Sulzer	Finlayson et al., 2010
Triticum aestivum L.		Nault et al., 2003; Alhmedi et al., 2007
Zea mays L.		Lucas et al., 2007
Zea mays L.		Colunga-Garcia et al., 1998; Musser et al., 2003; Nault et al., 2003

Table 3: List of herbaceous species where *Harmonia axyridis* was observed. The column "prey species" contains phytophagous species observed with *H. axyridis*.

Plant species	Prey species	References
Achillea Millefolium L.		Lucas et al., 2007
Artemisia vulgaris L.		Agarwala et al., 2003
Baccharis sp.	*Aphis spiraecola Patch; Coccidae; Aphis coreopsidis Thomas*	Martins et al., 2009
Bidens pilosa L.	*Uroleucom sonchi* L.	Martins et al., 2009
Bidens pilosa L.	*Hyperomyzus lactucae* L.	Martins et al., 2009
Bidens sulphurea Cav. Sch. Bip.	*Macrosiphoniella yomogifoliae* Shinji	Martins et al., 2009
Capsicum annuum var. angulosum	*Aphis gossypii* Glover*; Myzus persicae* (Sulzer)	Iguchi et al., 2012
Capsicum sp.		Lucas et al., 2007
Chrysanthemum leucanthemum L.	*Brachycaudus helichrysi* Kaltenbach	Martins et al., 2009
Cirsium arvense L.		LaMana & Miller, 1996
Cirsium spp.		Adriaens et al., 2008
Dipsacus sylvestris Huds		LaMana & Miller, 1996
Duranta repens L.	Coccidae	Martins et al., 2009
Echinacea purpurea (L.) Moench		Lucas et al., 2007
Foeniculum vulgare Miller	*Aphis fabae* Scopoli	Martins et al., 2009
Helianthus annuus L.	*Aphis fabae* Scopoli	Martins et al., 2009
Hipochoeris radicata L.	*Uroleucon ambrosiae* Thomas	Martins et al., 2009
Hypericum perforatum L.		Lucas et al., 2007
Nasturtium sp.		LaMana & Miller, 1996
Phragmites spp.		Adriaens et al., 2008
Rubus sp.		Koch et al., 2004; Lucas et al., 2007
Schefflera arboricola (Hayata) Merr.	*Aphis* sp.	Martins et al., 2009
Sonchus oleraceus L.	*Uroleucom sonchi* L.	Martins et al., 2009
Spartium junceum L.	*Aphis craccivora* Koch	Martins et al., 2009
Tanacetum spp.		Adriaens et al., 2008
Typha angustifolia L.	*Schizaphis acori* Theobald	Osawa, 2000
Urtica dioca L.		Adriaens et al., 2008
Valeriana officinalis L.		Lucas et al., 2007

Table 4: List of ornamental species where *Harmonia axyridis* was observed. The column "prey species" contains phytophagous species observed with *H. axyridis*.

Plant species	Prey species	References
Hibiscus rosa sinensis L.	*Toxoptera* sp.; *Aphis* sp.	Martins et al., 2009
Hibiscus sp.		Agarwala et al., 2003
Lagerstroemia indica L.	*Tinocallis kahawaluokalani* Kirkaldy	de Almeida & da Silva, 2002
Lagerstroemia indica L.	*Toxoptera aurantii* Boyer de Fonscolombe	Martins et al., 2009
Lagerstroemia sp.	*Tinocallis kahawaluokalani* Kirkaldy	Chapin & Brou, 1991
Leucaena leucocephala (Lam.) de Wit	*Heteropsylla cubana* Crawford	de Almeida & da Silva, 2002
Rosa multiflora Thumb.	*Macrosiphum pseudorosae* Patch	Finlayson et al., 2010
Rosa sp.		LaMana & Miller, 1996; de Almeida & da Silva, 2002
Rosa sp.	*Macrosiphum rosae* L.	Saini, 2004; Martins et al., 2009
Spirea blumei L.	*Aphis spiraecola* Patch	Osawa, 2000
Spirea douglasii Hook		LaMana & Miller, 1996
Spirea thunbergii Sieb.	*Aphis spiraecola* Patch	Osawa, 2000
Tabebuia sp.	Psyllidae	Martins et al., 2009

Orchards

Before winter, coccinellids also thrive in orchards, for example, on apples (*Malus* spp.) and citrus (*Citrus* spp.). In these habitats, they accumulate fat content and glycogen reserves (polyol myo-inositol) in response to a decline in food resources (Hodek, 1986; Watanabe, 2002; Hodek, 2011). A consequence of insects feeding on apples and pears is that it causes blemishing of the fruits and reduces the value of the crop (Majerus et al., 2006). In Virginian orchards, *H. axyridis* has become the most abundant coccinellid species (representing between 40.9% and 90.7% of those species) and it sometimes replaces the dominant native species (Brown, 2003). In citrus orchards (Florida), *H. axyridis* was also found to be the most abundant coccinellid species between 1997 and 2001. During this period, *H. axyridis* replaced *Cycloneda sanguine* (L.), which had been the numerically dominant species before 1997 (Michaud, 2002). In citrus, *H. axyridis* has also been tested successfully for the control of the root weevil *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae), a major pest in citrus orchards (Stuart et al., 2002). Similar results have been obtained with the citrus aphid, *Aphis spiraecola* Patch (Hemiptera: Aphididae) and with *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae) in Florida (Michaud, 2000). In apple orchards in West Virginia, *H. axyridis* has also provided good biological control of *A. spiraecola*. However, the use of this alien species as a biocontrol agent has displaced *C. septempunctata* (Brown et al., 1998). *Harmonia axyridis* has been used effectively many times in pecan orchards, *Carya illinoinensis* Wangenh., to control the pecan aphid complex comprising *Melanocallis caryaefolia* (Davis) (Hemiptera: Aphididae), *Monellia caryella* Fitch (Hemiptera: Aphididae) and *Monelliopsis pecanis* Bissell (Hemiptera: Aphididae) (Tedders et al., 1994; LaRock et al., 1996). In Georgia, *H. axyridis* was released in pecan with legume cover between 1978 and 1981. Nine years after its release, the species had spread and was recorded at a distance of 174 km from the release point. In 1994, *H. axyridis* was found to be the dominant species at the release site, comprising 54% of coccinellids (Tedders et al., 1994). In Argentina, *H. axyridis* was also found to be the most abundant coccinellid in *Carya* sp., with its proportion among coccinellids increasing from 51% in 2001 to 74% in 2003 (Saini, 2004).

Table 5: List of orchard species where *Harmonia axyridis* was observed. The column "prey species" contains phytophagous species observed with *H. axyridis*.

Plant species	Prey species	References
Carya illinoinensis (Wangenh.) K.Koch	*Melanocallis caryaefolia* Davis	Tedders & Schaefer, 1994; LaRock & Ellington, 1996
Carya illinoinensis (Wangenh.) K.Koch	*Monellia caryella* Fitch	Tedders & Schaefer, 1994; LaRock & Ellington, 1996; Saini, 2004
Carya illinoinensis (Wangenh.) K.Koch	*Monelliopsis pecanis* Bissell	Tedders & Schaefer, 1994; LaRock & Ellington, 1996
Citrus aurantiifolia (Christm.) Swingle		de Almeida & da Silva, 2002
Citrus limon L.	*Toxoptera citricida* Kirkaldy	Martins et al., 2009
Citrus reticulata L.	Aphididae	Martins et al., 2009
Citrus sinensis L.	Aphididae	Martins et al., 2009
Citrus spp.	*Diaprepes abbreviatus* L.	Stuart et al., 2002
Citrus spp.	*Toxoptera aurantii* Boyer de Fonscolombe	Katsoyannos et al., 1997
Citrus spp.	*Aphis spiraecola* Patch	Katsoyannos et al., 1997; Michaud, 2000
Citrus spp.	*Aphis gossypii* Glover	Katsoyannos et al., 1997
Malus domesticas Borkh.		Brown, 2003
Malus sp.	*Aphis spiraecola* Patch	Chapin & Brou, 1991; Brown & Miller, 1998; Brown, 2011
Malus sp.		Koch et al., 2004; Kovach, 2004; Lucas et al., 2007
Malus sp.	*Aphis pomi* DeGeer	Coderre et al., 1995
Prunus persica (L.) Batsch	*Hyalopterus pruni* Geoffroy; *Myzus varians* Davidson Passerini	Osawa, 2000
Prunus sp.	*Hyalopterus pruni* Geoffroy	LaMana & Miller, 1996
Prunus sp.		Adriaens et al., 2008; Burgio et al., 2008
Psidium guajava L.	*Triozoida* sp.	Martins et al., 2009
Vitis sp.		Koch et al., 2004; Lucas et al., 2007

The presence of *H. axyridis* in orchard crops is more controversial than in other crops. While *H. axyridis* acts as efficient biocontrol agent, in the fall season, the species causes damage to fruits. The solution might be to catch *H. axyridis* adults once the presence of aphids has reached its peak, in order to avoid yield loss through fruit damage. For more examples, see table 5 for a list of studies of *H. axyridis* in orchard ecosystems. Twelve plant species and 16 plant-prey relationships have been observed on diverse continents.

Conclusion

This review focused on the diversity of natural and semi-natural habitats where *H. axyridis* has been observed or introduced as a biocontrol agent. The following topics were considered: evaluation of the efficiency of *H. axyridis* for biological control, the impact of *H. axyridis* on native species, its occurrence among aphidophagous species, and the evolution of its invasion. Sixty-six studies have referenced *H. axyridis* on 106 plant taxa (35 arboreal species, 21 crop species, 27 herbaceous species, 11 ornamental species, and 12 orchard species) and have identified 89 plant-prey relationships (34 arboreal species, 16 crop species, 13 herbaceous species, 10 ornamental species, and 16 orchard species) in different countries. This diversity of plants and habitats where *H. axyridis* has been observed has been used to justify the classification of this alien species as an eurytopic species. The high abundance of *H. axyridis* within the aphidophagous guild in the majority of the habitats where it is found has had an impact on native species. This highlights the ecological problems provoked by *H. axyridis*: its presence causes a decline in biodiversity and the displacement of native species. *Harmonia axyridis* is one of the most abundant species in all natural and semi-natural ecosystems. Its ability to spread across ecosystems raises the proposition of using individuals of the species in nature to control aphid populations. A push-pull strategy could be implemented to control alien species populations in order to decrease their impact on native aphidophagous species.

Acknowledgements

This research was funded by the Service Public de Wallonie (SPW – DGO3, project No. D31-1247). Delphine Durieux was financially supported by a PhD grant from the Fonds pour la Formation à la Recherche dans l'Industrie et l'Agriculture (FRIA), Belgium.

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Chapter III : Coccinellid community in Belgium

III.1 General introduction to chapter III

Coccinellids belonging to Coleoptera order is divided into seven sub-families based on the morphology of the larvae and the adult: Coccidulinae, Scymninae, Chilocorinae, Coccinellinae, Hyperaspidinae, Sticholotidinae and Epilachninae (Baugnée and Branquart 2000). The diversity of coccinellid species is important with more than 5200 coccinellid species in the world (Majerus 1994). More than interspecific diversity, there is also intraspecific diversity, concerning colour (red, black, yellow, orange), size and form. For example, both *H. axyridis* and *A. bipunctata* have several morphotypes, red colour with black spots or black colors with red smears. Phenotypic variability based on polymorphism is one of factors that improve the colonisation efficiency. In this context, by phenotypic variability we mean melanism of *H. axyridis*. The melanism is an advantage when according to the weather, melanic or succinic forms preferentially increase. One advantage of melanic form is that it present a lower thermal optimum to feed aphids and that would presumably extend diurnal foraging periods when temperatures are cool (Soares et al. 2003). During hot summer weather, melanic forms become disadvantaged relative to succinics, due to their lower cuticular reflectance and greater temperature excess (De Jong et al. 1996).

More than 60 species have been observed in Belgium (Baugnée and Branquart 2000). We know that the invasive ladybird was imported in Belgium and was first observed in 2001. Previous studies highlighted that this species is able to threaten biodiversity by native population change. In order to investigate changes in species diversity occurring in Belgium the chapter III.2 exposes the results of coccinellid sampling realised by students of Gembloux Agro-Bio Tech few years after the introduction of *H. axyridis*, the invasive coccinellid. Species diversity and relative densities of the coccinellid community were studied during nine years from 2001 to 2009. More than 3300 coccinellids were identified and analysed according to their geographical repartition and the year of their trapping.

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III.2 Evolution des populations de coccinelles indigènes et de l'espèce exotique, *Harmonia axyridis* (Pallas 1773), en Wallonie et en région de Bruxelles-Capitale

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Reference – Durieux D., Vandereycken A., Joie E., Haubruge E., Verheggen F.J. 2012. Evolution des populations de coccinelles indigène et de l'espèce exotique, *Harmonia axyridis* (Pallas 1773), en Wallonie et en région de Bruxelles-Capitale. *Entomologie Faunistique - Faunistic Entomology* 65: 81-92.

Résumé - Un inventaire des Coccinellidae présents au sein de la collection de l'unité d'Entomologie fonctionnelle et évolutive (ULg - Gembloux Agro-Bio Tech) a été réalisé sur la période s'étendant de 2001 à 2009. Cette collection est essentiellement constituée par les récoltes des étudiants de première Bachelier de Gembloux Agro-Bio Tech. A travers ce recensement, nous avons étudié l'évolution des espèces récoltées au sein de cette famille en termes d'effectif relatif et de richesse spécifique. Notre étude s'est focalisée sur les données issues de Wallonie et de la Région de Bruxelles-Capitale, trop peu de collectes ayant été réalisées en Flandres. Parmi les individus identifiés, 27 espèces ont été observées, dont 21 appartiennent à la sous-famille des Coccinellinae, 2 à celle des Epilachninae et 4 à celle des Chilocorinae. La plupart des espèces sont communes à la Belgique. Cependant, *Harmonia axyridis* (Pallas 1773), espèce originaire du sud-est de l'Asie, a été introduite sur le territoire belge en 1997. Cette coccinelle exotique fait son apparition dans les récoltes de 2002 et son effectif ne cesse d'augmenter au fil des années prospectées. En parallèle à cette augmentation, une diminution de la richesse spécifique, ainsi que de l'effectif relatif d'*Adalia bipunctata* (L. 1758), de *Propylea quatuordecimpunctata* (L. 1758) et de *Psyllobora vigintiduopunctata* (L. 1758), est observée. Le lien possible entre ces évolutions est discuté.

Mots-clés - Coccinellidae, coccinelle asiatique, compétition intraguilde, espèce invasive, Belgique

Abstract - An inventory of the Coccinellidae present in the collection of the Department of functional and evolutionary Entomology (ULg - Gembloux Agro-Bio Tech) was performed on the period extending from 2001 to 2009. This collection is essentially made up of insects collected by first year students. The aim of this work is to assess the evolution of the species belonging to this family with special interest in their relative amount and the species richness. This study was focused on the data from Wallonia and Brussels-Capital Region (Belgium), not enough insects having been collected in Flanders. Among the identified individuals, 27 species were observed, including 21 belonging to the subfamily of Coccinellinae, 2 to Epilachninae and 4 to Chilocorinae. Most of the species are native to Belgium. However, *Harmonia axyridis* (Pallas 1773), an exotic species coming from the South-East of Asia, was introduced in Belgium in 1997. This alien ladybird was firstly collected by the students in 2002 and the amount of collected individuals does not cease increasing until 2009. By contrast, a decrease of species richness, as well as the relative amount of *Adalia bipunctata* (L. 1758), *Propylea quatuordecimpunctata* (L. 1758) and *Psyllobora vigintiduopunctata* (L. 1758), is highlighted. The link between these evolutions is discussed.

Keywords - Coccinellidae, multicoloured Asian ladybird, intraguild competition, invasive species, Belgium

Introduction

La famille des Coccinellidae appartient à la super-famille des Cucujoidea au sein de l'ordre des Coléoptères. La subdivision de cette famille se base sur la morphologie des larves et des adultes et aboutit ainsi à la formation de sept sous-familles (Hodek, 1973). Parmi celles-ci, cinq sont rencontrées en Belgique: les Coccidulinae, Scymninae, Chilocorinae, Coccinellinae et Epilachninae (Baugnée & Branquart, 2000).

Plus de 5200 espèces de coccinelles sont répertoriées à travers le monde (Majerus, 1994) et près de soixante espèces indigènes sont présentes en Wallonie (Baugnée & Branquart, 2000). Ces dernières années, une espèce exotique s'est également établie sur le territoire belge: la coccinelle asiatique, *Harmonia axyridis* (Pallas 1773). Les larves très voraces, polyphages et faciles à élever de cette espèce (Adriaens *et al.*, 2008), ont conduit à l'introduction de cette coccinelle, originaire du sud-est de l'Asie, en Belgique à partir de 1997 pour lutter biologiquement contre les pucerons et cochenilles dans les serres, cultures et jardins (Adriaens *et al*., 2003). Ce n'est qu'en 2001 que la première population sauvage d'*H. axyridis* a été observée aux environs de Gand. Cependant, fin 2006, il semble qu'elle ait colonisé tout le pays (Adriaens *et al*., 2008).

Harmonia axyridis s'est révélée être un agent de lutte très efficace contre les populations de pucerons (Koch, 2003). Mais des impacts négatifs sur des espèces non ciblées, telles que d'autres prédateurs de ces ravageurs phytophages, ont rapidement été observés (Koch & Galvan, 2008). Elle constitue donc une importante menace pour la biodiversité et est actuellement considérée comme une espèce exotique envahissante en Europe (Brown *et al*., 2008). De plus, elle s'agrège au sein des habitations en hiver afin de survivre aux rigueurs du climat (Huelsman *et al*., 2002). Ces amas, pouvant comporter des centaines voire des milliers d'individus, créent des nuisances aux particuliers de par le nombre important de coccinelles mais également par la sécrétion de substances pouvant être responsables de réactions allergiques (Goetz, 2006; Nakazawa *et al*., 2007).

L'objectif de cet inventaire est d'étudier l'évolution des espèces de Coccinellidae présentes dans les collections de Gembloux Agro-Bio Tech sur la période s'étendant de 2001 à 2009, en termes d'effectif relatif et de richesse spécifique. Une attention particulière sera portée aux liens possibles entre l'évolution des espèces indigènes et celle de la coccinelle asiatique.

Matériel et méthodes

Les Coccinellidae identifiés proviennent des récoltes effectuées par les étudiants de première Bachelier de Gembloux Agro-Bio Tech (GxABT) - Université de Liège, entre 2001 et 2009. Ceux-ci avaient pour consigne de collecter de 50 à 100 insectes en attachant une importance particulière à la diversité de ceux-ci. Aucun protocole ne leur avait été imposé quand à la méthode d'échantillonnage (méthode de piégeage, milieux visités ou période de récolte). Ces récoltes constituent en partie les collections entomologiques de GxABT. Notre étude de la diversité des coccinelles ne prend en considération que les individus provenant de la Wallonie et de la Région de Bruxelles-Capitale. Le faible nombre de récoltes ayant été effectuées en Flandres ne nous permet pas d'avoir un échantillon représentatif de la diversité des Coccinellidae sur ce territoire. L'identification des espèces récoltées a été réalisée grâce à l'utilisation d'une clé spécifique aux coccinelles rencontrées en Wallonie (Baugnée &

Branquart, 2000). Les cartes représentant la répartition géographique des différentes espèces en Wallonie et en région de Bruxelles-Capitale ont été réalisées grâce à l'utilisation du logiciel ArcGIS® version 9.1. Les localités de récolte utilisées pour l'illustration de cette répartition étaient indiquées par les étudiants sur une étiquette accompagnant chaque individu.

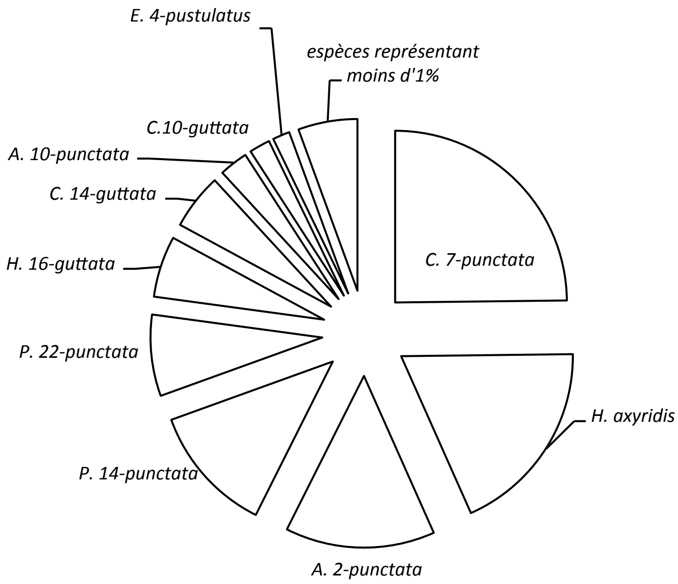


Figure 1: Effectif relatif des espèces de Coccinellidae récoltées par les étudiants de GxABT au cours de la période 2001-2009. 3369 individus récoltés.

Résultats

Parmi les 3369 individus récoltés, 27 espèces, réparties dans 3 sous-familles, ont été identifiées. On distingue 21 espèces de Coccinellinae (*Adalia bipunctata* (L. 1758), *Adalia decempunctata* (L. 1758), *Anatis ocellata* (L. 1758), *Anisosticta novemdecimpunctata* (L. 1758), *Aphidecta obliterata* (L. 1758), *Calvia decemguttata* (L. 1758), *Calvia quatuordecimguttata* (L. 1758), *Coccinella undecimpunctata* L. 1758, *Coccinella quinquepunctata* L. 1758, *Coccinella hieroglyphica* L. 1758, *Coccinella septempunctata* L. 1758, *Halyzia sedecimguttata* (L. 1758), *Harmonia quadripunctata* (Pontoppidan 1763), *H. axyridis*, *Hippodamia tredecimpunctata* (L. 1758), *Hippodamia variegata* (Goeze 1777), *Myrrha octodecimguttata* (L. 1758), *Oenopia conglobata* (L. 1758), *Propylea quatuordecimpunctata* (L. 1758), *Psyllobora vigintiduopunctata* (L. 1758), *Tytthaspis sedecimpunctata* (L. 1758)), 2 espèces d'Epilachninae (*Epilachna argus* (Geoffroy 1758), *Subcoccinella vigintiquatuorpunctata* (L. 1758)) et 4 espèces de Chilocorinae (*Chilocorus bipustulatus* (L. 1758), *Chilocorus renipustulatus* (Scriba 1790), *Exochomus quadripustulatus*

(L. 1758), *Platynaspis luteorubra* (Goeze 1777)). Les cinq espèces les plus abondantes sont *C. septempunctata*, *H. axyridis*, *A. bipunctata*, *P. quatuordecimpunctata* et *P. vigintiduopunctata*, représentant respectivement 24,8%, 18,5%, 14,1%, 12,1% et 7,7% de l'ensemble de la récolte (Figure 1 et Tableau 1).

Tableau 1: Liste des espèces de coccinelles les plus collectées.

Espèces	Adultes	Larves
Coccinella septempunctata		
Harmonia axyridis		
Adalia bipunctata		
Propylea quatuordecimpunctata		
Psyllobora vigintiduopunctata		

Le Tableau 2 représente l'effectif relatif des espèces de Coccinellidae collectées, ainsi que la richesse spécifique de ces récoltes en fonction de l'année considérée. Une réduction de la richesse spécifique s'opère au cours de la période s'étendant de 2002 à 2009. De plus, une diminution de l'effectif relatif d'*A. bipunctata* est observée au cours de ces années de récolte. Une diminution plus légère est également observée pour *P. quatuordecimpunctata* et *P. vigintiduopunctata*. En revanche, l'effectif relatif d'*H. axyridis*, lui, ne cesse d'augmenter. Les mêmes évolutions sont observées en ce qui concerne le nombre absolu de récoltes (Figure 2).

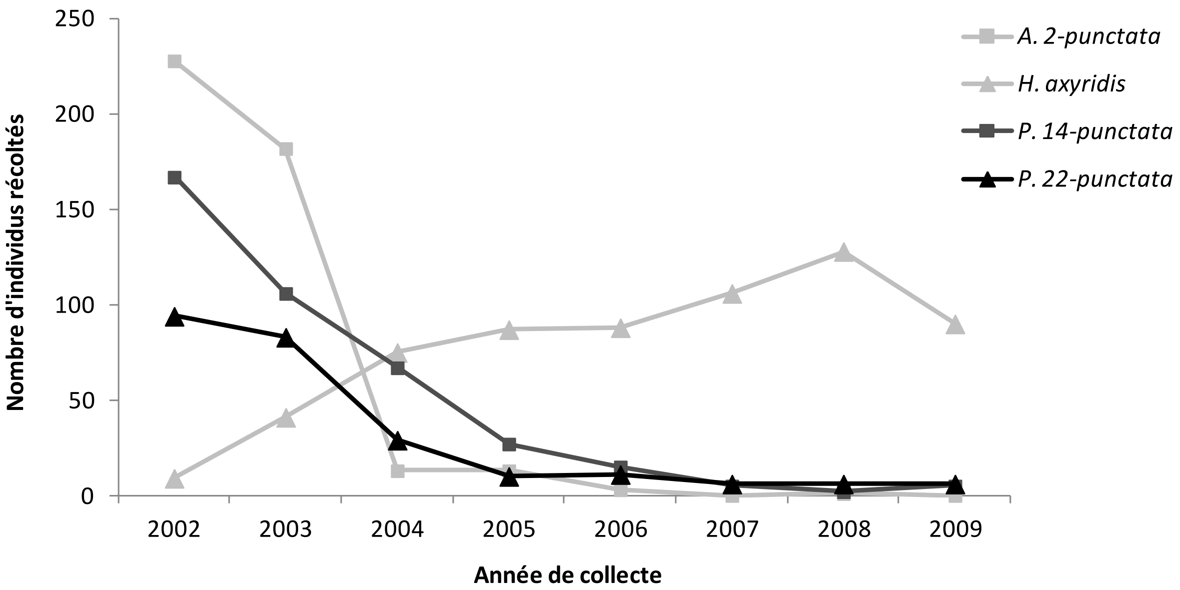


Figure 2: Nombre d'individus récoltés par les étudiants de GxABT au cours de la période 2002-2009.

Les Figures 3-29 reprennent la répartition géographique des captures de Coccinellidae réalisées par les étudiants. 3197 et 172 récoltes ont été recensées respectivement en Wallonie et en Région de Bruxelles-Capitale.

Tableau 2: Effectif relatif des espèces (exprimé en %) et richesse spécifique (nombre d'espèces) des Coccinellidae capturés par les étudiants de GxABT au cours des diverses années de récolte

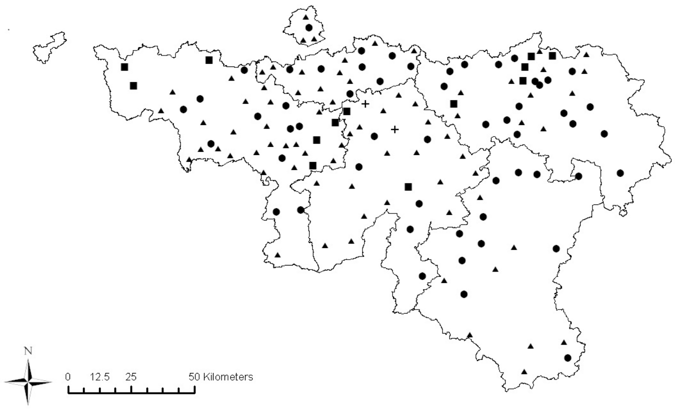
Espèces de Coccinellidae	Années de récolte									Effectif relatif
	2001	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	
Adalia bipunctata	29.3	22.0	23.9	3.1	4.8	1.4		0.5		14.1
Adalia decempunctata	2.6	4.9	3.1	2.8	0.4					2.7
Anatis ocellata	0.9	0.8	0.4	0.2	0.4	0.5	2.0	1.5	2.1	0.7
Anisosticta novemdecimpunctata		0.1								0.0
Aphidecta obliterata	2.6	0.7	0.5	0.5						0.5
Calvia decemguttata	1.7	1.9	3.8	2.1	1.5				1.4	2.0
Calvia quatuordecimguttata	4.3	7.7	5.8	4.0	1.8	1.4	2.5	5.5	4.9	5.3
Chilocorus bipustulatus			0.1	0.2						0.1
Chilocorus renipustulatus	0.9	0.5	0.9	0.5	0.7					0.5
Coccinella hieroglyphica				0.2						0.0
Coccinella quinquepunctata	0.9	0.3	0.7	0.2	0.4					0.3
Coccinella septempunctata	17.2	21.9	21.7	30.6	35.2	39.3	28.1	17.9	15.5	24.8
Coccinella undecimpunctata		0.3	0.3	0.2						0.2
Epilachna argus	1.7	1.3	0.5	1.2			0.5			0.7
Exochomus quadripustulatus	1.7	2.3	0.9	2.8	1.5	0.9	0.5	0.5	0.7	1.6
Halyzia sedecimguttata	6.0	6.4	4.9	7.1	7.0	2.8	6.5	6.0	2.8	5.8
Harmonia axyridis		0.9	5.4	17.6	31.9	41.1	53.3	63.7	63.4	18.5
Harmonia quadripunctata	0.9	0.7	0.1	0.7		0.5			0.7	0.4
Hippodamia tredecimpunctata			0.1							0.0
Hippodamia variegata	0.9	0.2	0.5	0.7					0.7	0.3
Myrrha octodecimguttata								0.5		0.0
Oenopia conglobata	5.2	1.5	0.9	0.2						0.9
Platynaspis luteorubra		0.1								0.0
Propylea quatuordecimpunctata	11.2	16.1	13.9	15.8	9.9	7.0	2.5	1.0	3.5	12.1
Psyllobora vigintiduopunctata	11.2	9.1	10.9	6.8	3.7	5.1	3.0	3.0	4.2	7.7
Subcoccinella vigintiquatuorpunctata		0.1	0.3	0.9						0.2
Tytthaspis sedecimpunctata	0.9	0.4	0.4	1.4	1.1		1.0			0.6
Richesse spécifique	**18**	**23**	**23**	**23**	**14**	**10**	**10**	**10**	**11**	

Discussion et conclusion

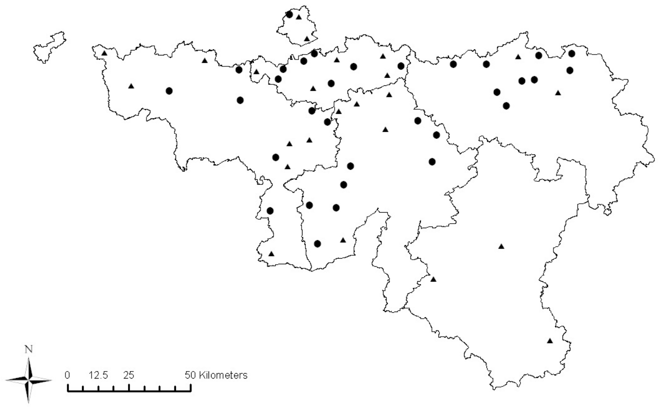
L'étude de la diversité des coccinelles au sein des collections de GxABT a mis en évidence cinq espèces majoritaires: *C. septempunctata, H. axyridis, A. bipunctata, P. quatuordecimpunctata* et *P. vigintiduopunctata*. Ces résultats sont similaires à ceux présentés par Francis en 2001, si ce n'est l'apparition de l'espèce exotique en deuxième position. On remarque également que l'effectif relatif d'*H. axyridis* ne cesse d'augmenter de 2002 à 2009. En parallèle à cette augmentation, une diminution de la richesse spécifique, ainsi que de l'effectif relatif de coccinelles à deux points, *A. bipunctata*, de coccinelles à damier, *P.*

quatuordecimpunctata, et de coccinelles à 22 points, *P. vigintiduopunctata,* au fil des ans est mise en évidence. Les mêmes évolutions sont observées quant au nombre de récoltes, aussi bien pour les espèces natives que pour la coccinelle asiatique. Ceci est en accord avec plusieurs études belges soulignant le déclin des populations de coccinelles indigènes suite à l'introduction d'*H. axyridis* (Adriaens *et al*., 2008; Adriaens *et al*., 2010). Les résultats recensés dans la littérature suggèrent que la décroissance des espèces natives est, entre autres, liée à l'expansion de l'espèce exotique. En effet, il a largement été démontré que la coccinelle asiatique entre en compétition avec les espèces indigènes présentes sur les territoires où elle a été introduite (Colunga-Garcia & Gage, 1998 ; Majerus, 2008 ; Brown *et al*., 2011). Premièrement, *H. axyridis* s'est révélée être un super-prédateur au sein de la guilde des insectes aphidiphages (Dixon, 2000). En d'autres termes, cette espèce exotique est capable de se nourrir d'autres prédateurs de pucerons. De nombreuses études, réalisées en laboratoire, ont mis en évidence la consommation de plusieurs espèces de coccinelles par *H. axyridis* (Koch, 2003; Ware & Majerus, 2008 ; Ware *et al*., 2009). Qui plus est, la preuve de la consommation d'*A. bipunctata* et de *P. quatuordecimpunctata* sur le terrain a été apportée par Hautier *et al*. (2008, 2011) grâce à une méthode analytique basée sur la détection d'alcaloïdes exogènes chez la coccinelle asiatique. D'autre part, la réduction du nombre de coccinelles natives récoltées peut également résulter d'un déplacement de ces dernières suite à des mécanismes indirects, telle que la compétition pour la nourriture et l'espace. En effet, certaines études ont démontré qu'*H. axyridis* était un prédateur plus vorace et qu'il présentait une fécondité plus élevée que les espèces natives (Labrie *et al*., 2006; Mignault *et al*., 2006). Il a également été rapporté par Adriaens *et al*. (2008) qu'*H. axyridis* occupe les mêmes niches écologiques que les espèces indigènes belges.

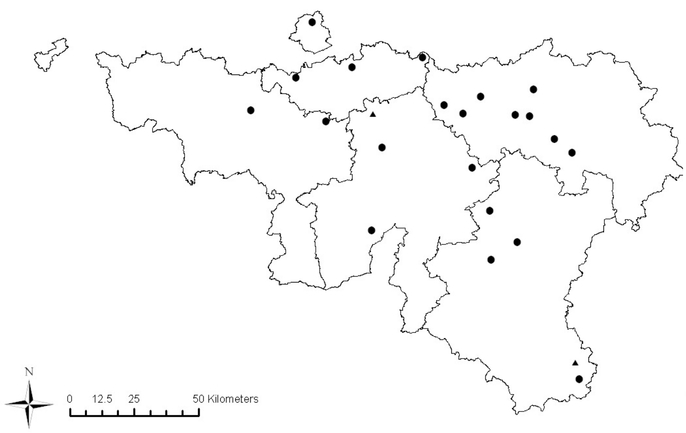
Les échantillonnages pris en considération dans cette étude n'étant pas standardisés, il va de soit que les résultats présentés dans cet article ne correspondent pas à la phénologie réelle des Coccinellidae. Cependant, bien que les milieux visités par les étudiants ne couvrent certainement pas tous les habitats colonisés par les Coccinelles, les résultats de cet inventaire semblent représenter l'évolution globale des espèces de cette famille ces dernières années. En effet, les résultats de cette étude concordent avec les recensements de Coccinellidae réalisés sur le territoire belge depuis l'introduction de la coccinelle asiatique (Adriaens *et al*., 2008; Adriaens *et al*., 2010).



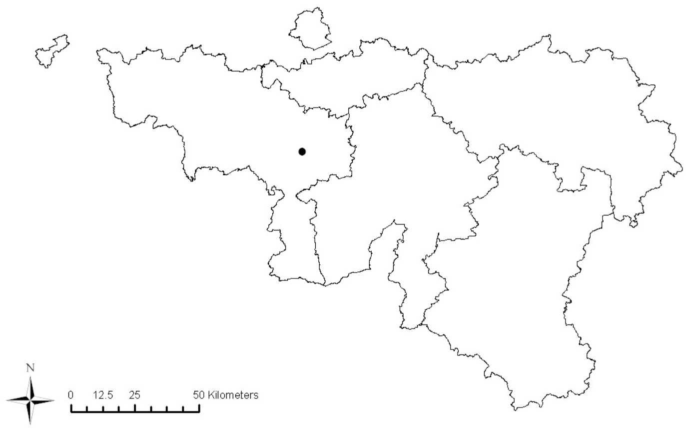
Carte 1: Répartition géographique des captures d'*Adalia bipunctata* par les étudiants de GxABT (● représente 1 individu récolté par localité, ■ représente de 2 à 5 individus récoltés, ▲ représente de 6 à 20 individus récoltés et + représente plus de 20 individus récoltés).



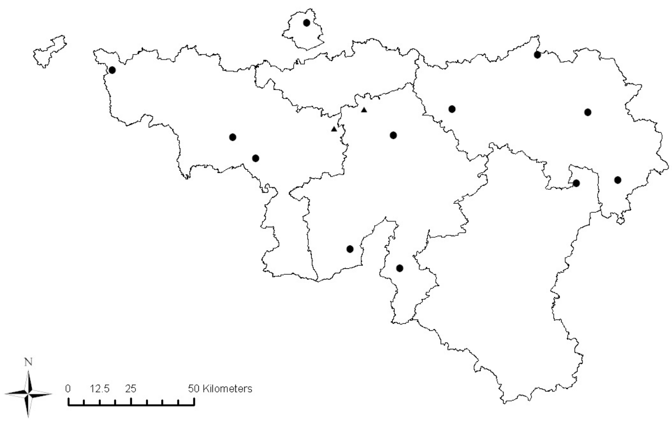
Carte 2: Répartition géographique des captures d'*Adalia decempunctata* par les étudiants de GxABT (● représente 1 individu récolté par localité et ▲ représente de 6 à 20 individus récoltés).



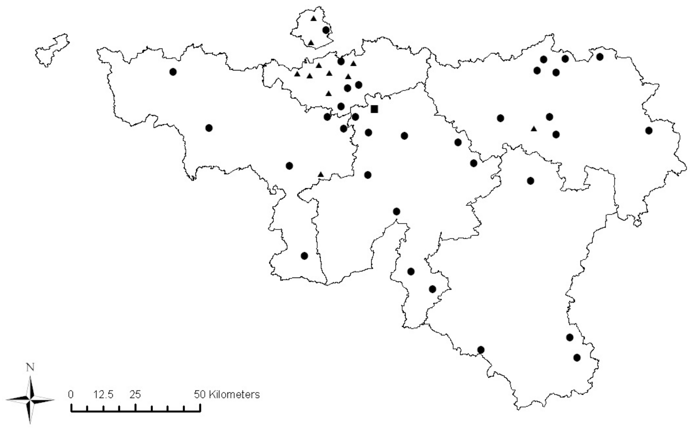
Carte 3: Répartition géographique des captures d'*Anatis ocellata* par les étudiants de GxABT (● représente 1 individu récolté par localité et ▲ représente de 6 à 20 individus récoltés).



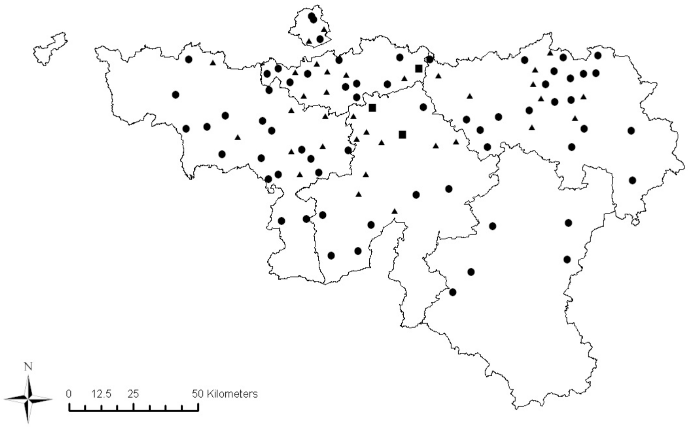
Carte 4: Répartition géographique des captures d'*Anisosticta novemdecimpuctata* par les étudiants de GxABT (● représente 1 individu récolté par localité).



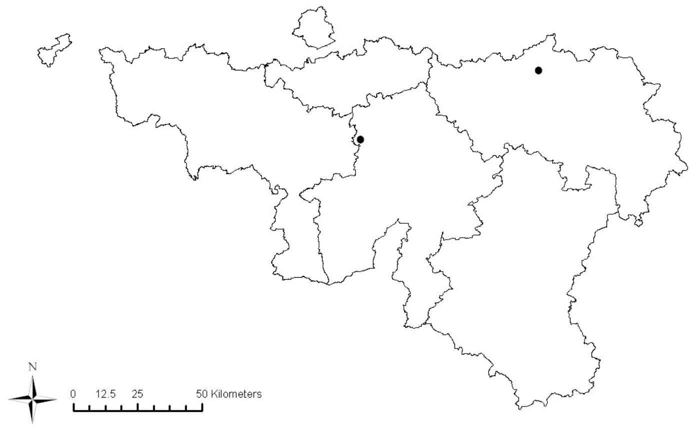
Carte 5: Répartition géographique des captures d'*Aphidecta obliterata* par les étudiants de GxABT (● représente 1 individu récolté par localité et ▲ représente de 6 à 20 individus récoltés).



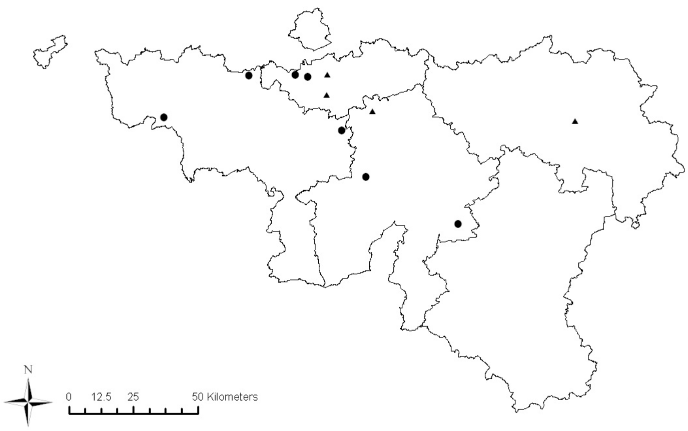
Carte 6: Répartition géographique des captures de *Calvia decemguttata* par les étudiants de GxABT (● représente 1 individu récolté par localité et ▲ représente de 6 à 20 individus récoltés).



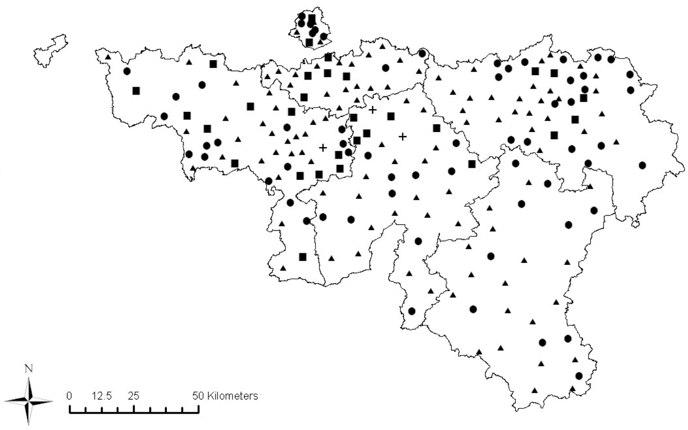
Carte 7: Répartition géographique des captures de *Calvia quatuordecimguttata* par les étudiants de GxABT (● représente 1 individu récolté par localité, ■ représente de 2 à 5 individus récoltés et ▲ représente de 6 à 20 individus récoltés).



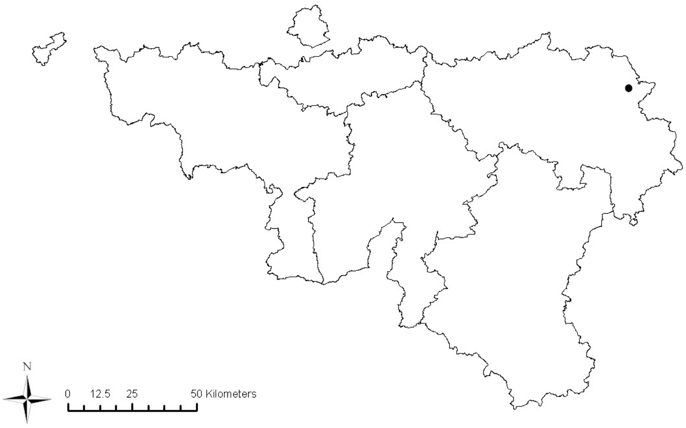
Carte 8: Répartition géographique des captures de *Chilocorus bipustulatus* par les étudiants de GxABT (● représente 1 individu récolté par localité).



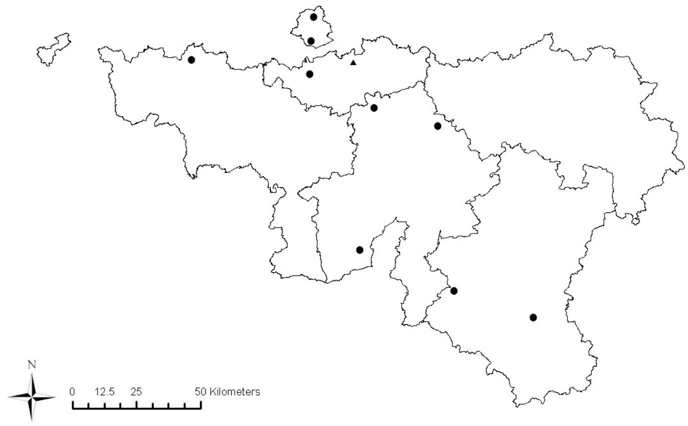
Carte 9: Répartition géographique des captures de *Chilocorus renipustulatus* par les étudiants de GxABT (● représente 1 individu récolté par localité et ▲ représente de 6 à 20 individus récoltés).



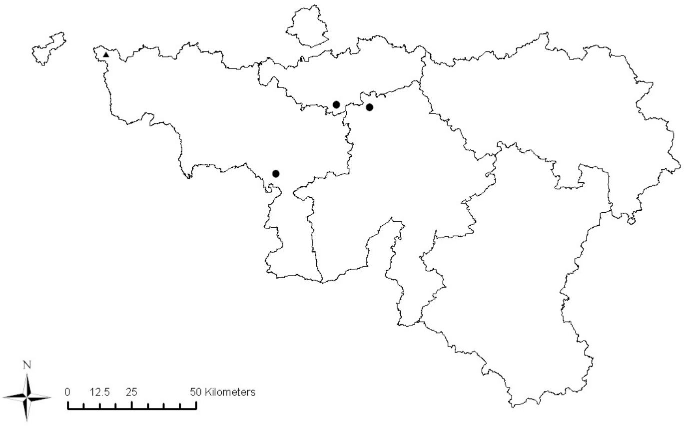
Carte 10: Répartition géographique des captures de *Coccinella septempunctata* par les étudiants de GxABT (● représente 1 individu récolté par localité, ■ représente de 2 à 5 individus récoltés, ▲ représente de 6 à 20 individus récoltés et + représente plus de 20 individus



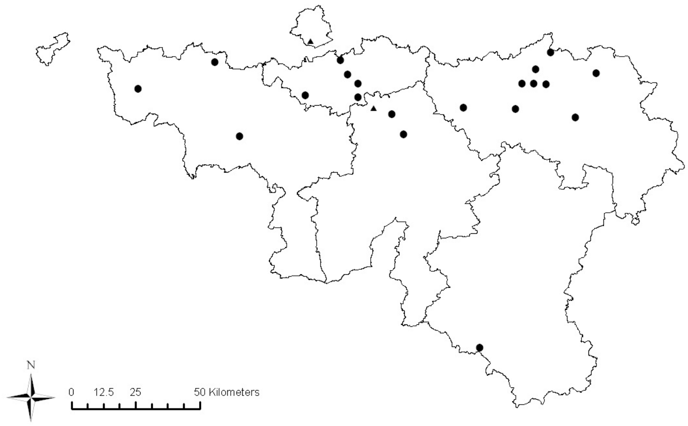
Carte 11: Répartition géographique des captures de *Coccinella hieroglyphica* par les étudiants de GxABT (● représente 1 individu récolté par localité).



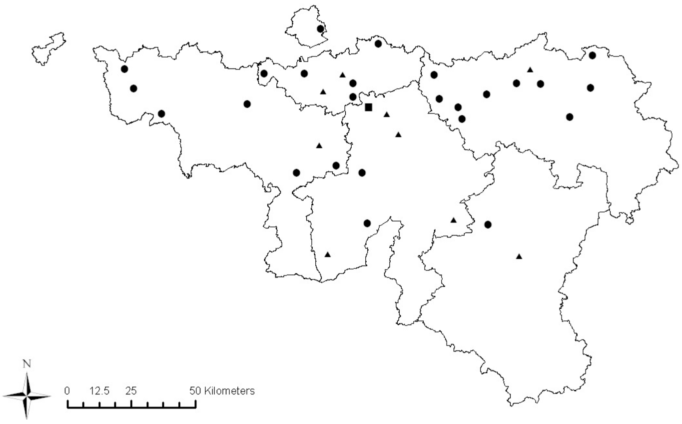
Carte 12: Répartition géographique des captures de *Coccinella quinquepunctata* par les étudiants de GxABT (● représente 1 individu récolté par localité et ▲ représente de 6 à 20 individus récoltés).



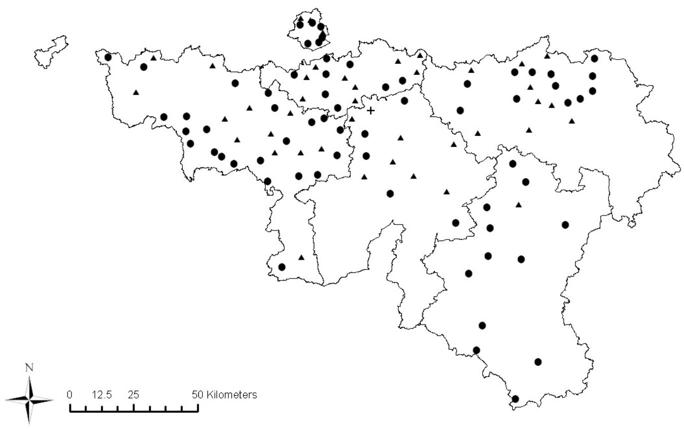
Carte 13: Répartition géographique des captures de *Coccinella undecimpunctata* par les étudiants de GxABT (● représente 1 individu récolté par localité et ▲ représente de 6 à 20 individus récoltés).



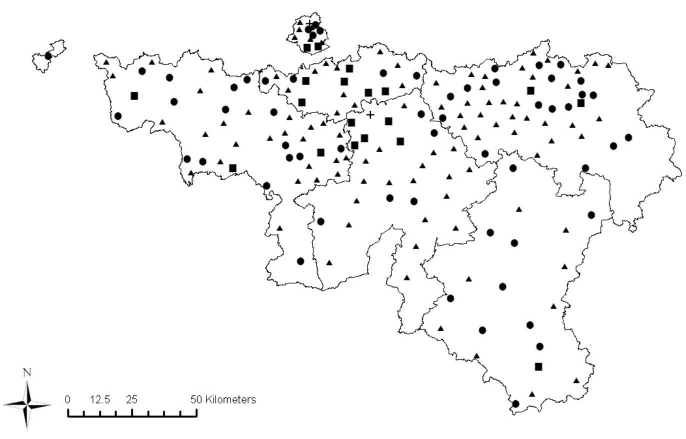
Carte 14: Répartition géographique des captures d'*Epilachna argus* par les étudiants de GxABT (● représente 1 individu récolté par localité et ▲ représente de 6 à 20 individus récoltés).



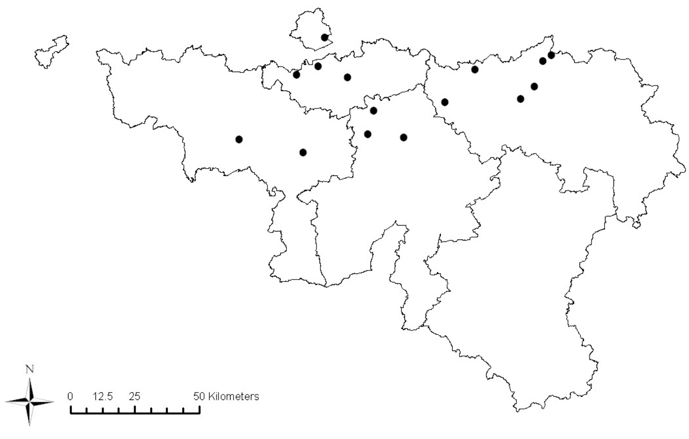
Carte 15: Répartition géographique des captures d'*Exochomus quadripustulatus* par les étudiants de GxABT (● représente 1 individu récolté par localité, ■ représente de 2 à 5 individus récoltés et ▲ représente de 6 à 20 individus récoltés).



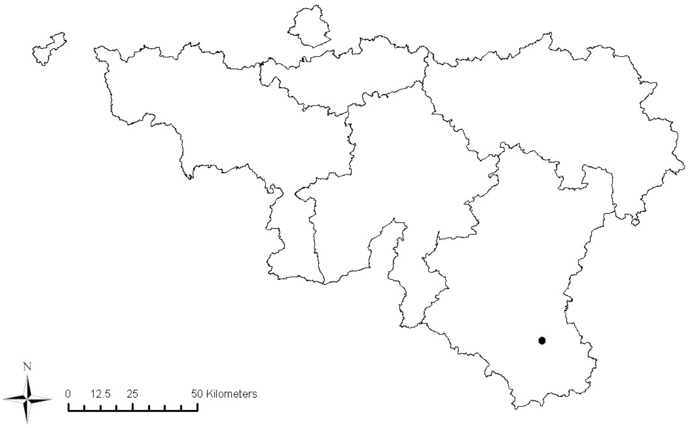
Carte 16: Répartition géographique des captures d'*Halyzia sedecimguttata* par les étudiants de GxABT (● représente 1 individu récolté par localité, ▲ représente de 6 à 20 individus récoltés et + représente plus de 20 individus récoltés).



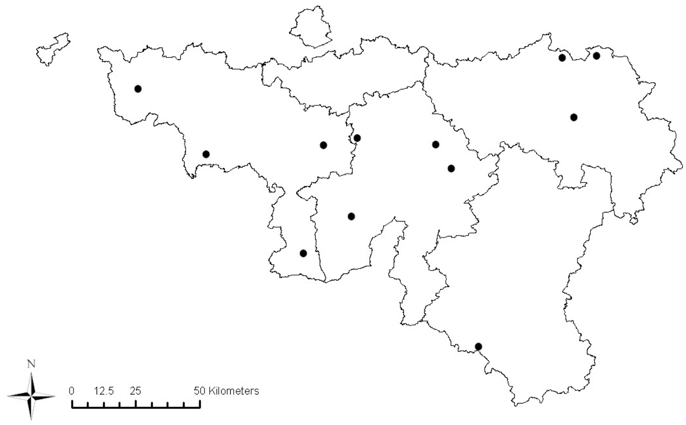
Carte 17: Répartition géographique des captures d'*Harmonia axyridis* par les étudiants de GxABT (● représente 1 individu récolté par localité, ■ représente de 2 à 5 individus récoltés, ▲ représente de 6 à 20 individus récoltés et + représente plus de 20 individus récoltés).



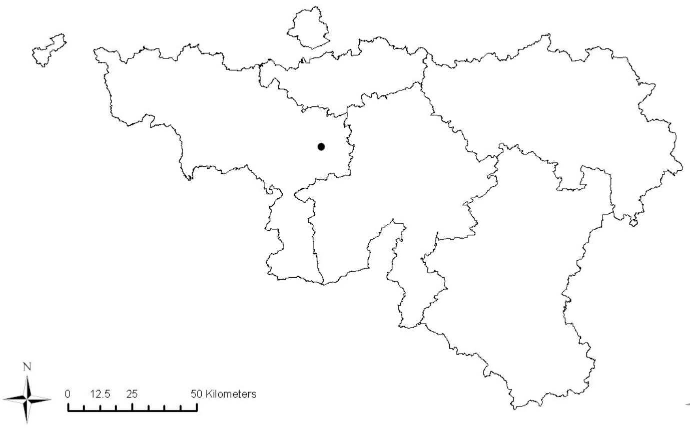
Carte 18: Répartition géographique des captures d'*Harmonia quadripunctata* par les étudiants de GxABT (● représente 1 individu récolté par localité).



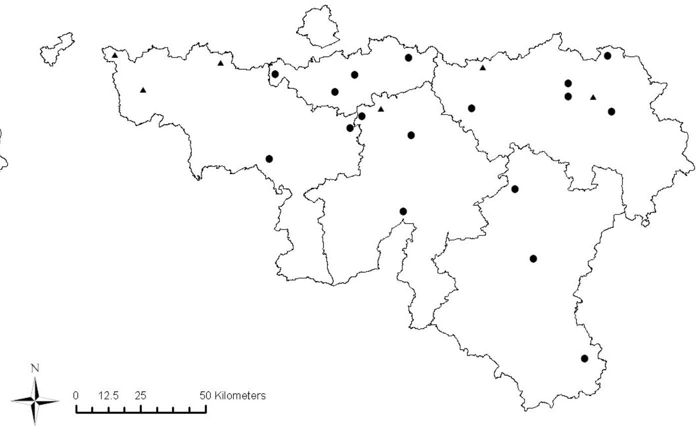
Carte 19: Répartition géographique des captures d'*Hippodamia tredecimpunctata* par les étudiants de GxABT (● représente 1 individu récolté par localité).



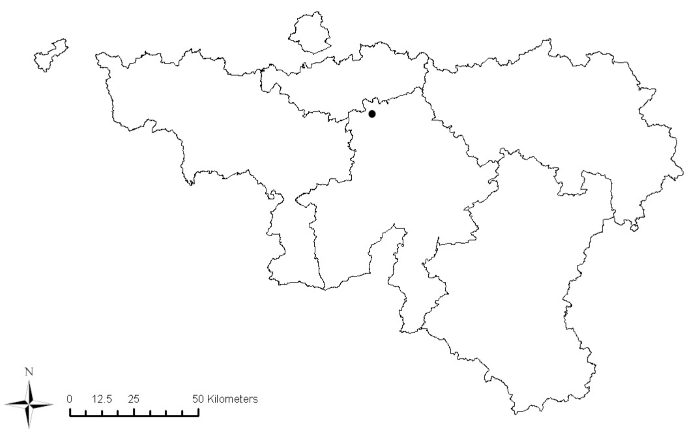
Carte 20: Répartition géographique des captures d'*Hippodamia variegata* par les étudiants de GxABT (● représente 1 individu récolté par localité).



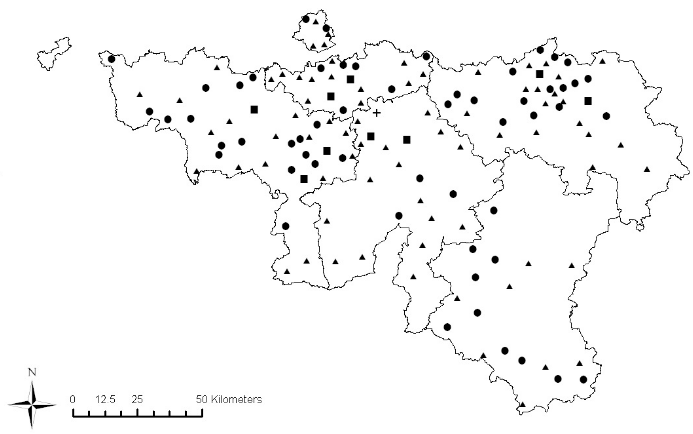
Carte 21: Répartition géographique des captures de *Myrrha octodecimpunctata* par les étudiants de GxABT (● représente 1 individu récolté par localité).



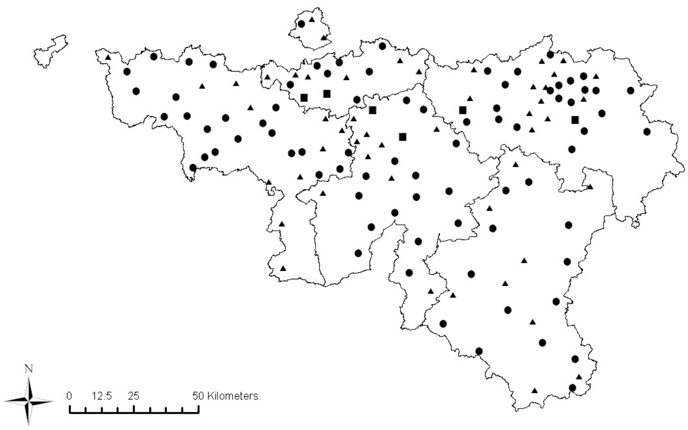
Carte 22: Répartition géographique des captures d'*Oenopia conglobata* par les étudiants de GxABT (● représente 1 individu récolté par localité et ▲ représente de 6 à 20 individus).



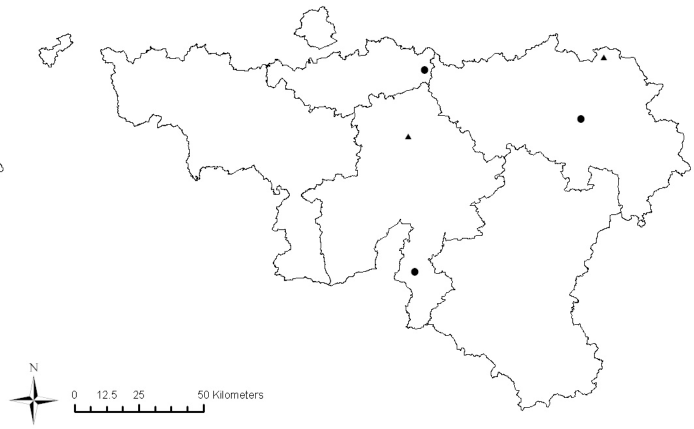
Carte 23: Répartition géographique des captures de *Platynaspis luteorubra* par les étudiants de GxABT (● représente 1 individu récolté par localité).



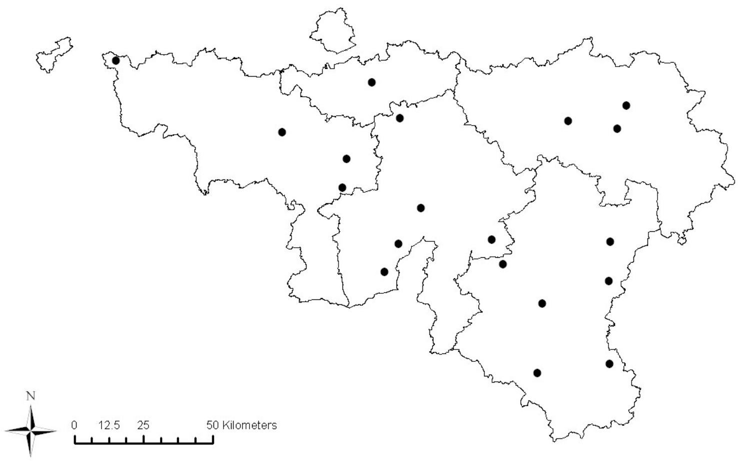
Carte 24: Répartition géographique des captures de *Propylea quatuordecimpunctata* par les étudiants de GxABT (● représente 1 individu récolté par localité, ■ représente de 2 à 5 individus récoltés, ▲ représente de 6 à 20 individus récoltés et + représente plus de 20 individus récoltés).



Carte 25: Répartition géographique des captures de *Psyllobora vingintiduopunctata* par les étudiants de GxABT (● représente 1 individu récolté par localité, ■ représente de 2 à 5 individus récoltés et ▲ représente de 6 à 20 individus récoltés).



Carte 26: Répartition géographique des captures de *Subcoccinella vigintiquatuorpunctata* par les étudiants de GxABT (● représente 1 individu récolté par localité et ▲ représente de 6 à 20 individus récoltés).



Carte 27: Répartition géographique des captures de *Tytthaspis sedecimpunctata* par les étudiants de GxABT (● représente 1 individu récolté par localité).

Remerciements

Delphine Durieux est financée par le Fonds pour la Formation à la Recherche dans l'Industrie et l'Agriculture (F.R.I.A.). Axel Vandereycken et Emilie Joie sont financés par le Service Public de Wallonie (SPW – DGO3, projet n°D31-1197).

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Chapter IV : OBJECTIVES

The Multicoloured Asian ladybird was imported from Asia to America and Europe during the twentieth century in the aim to control aphids and coccid's populations. Few years after its introduction, this exotic insect was well adapted to temperate climate conditions and has spread out all over ecosystems leading to negative impacts on biodiversity. For example, *H. axyridis* is well known to be a predator of native coccinellids and other aphid's predators. This behaviour leads to a decline of these native species. Increasing number of studies show that the decline started and could increase in the future.

This thesis focuses on evaluating the population (habitats, population size changes, phenology) of the Multicoloured Asian ladybird in agroecosystems of Wallonia (Belgium). The first specific objective is to describe the interactions between aphids and their predators, in agricultural landscapes. The second objective is to describe the changes in aphidophagous predator community present in agroecosystems, over a three-year period. A third objective is to determine whether *H. axyridis* feed and reproduce in field crops. And finally, we aimed at comparing aphid predator communities between biological and conventional farming processes.

In chapter V, we evaluated, through several samplings in agroecosystems, natural enemies population density and diversity during a three years period (2009-2011). The aim of these samplings was to investigate whether *H. axyridis* was one of the most abundant aphidophagous predator in Belgian agroecosystems like in other parts of the world (Colunga-Garcia and Gage 1998, Lucas et al. 2007). We know that in Belgium this species has started to invade mainly shrubby and forest habitats but we do not have information about its invasion in crops fields. In this chapter we answer two questions: first "Is *Harmonia axyridis* present in belgian agroecosystems and does its occurrence change between 2009 and 2011?" then "Is *Harmonia axyridis* the most aphid predator in agroecosystems?". The answers to these questions allow to get an idea of the situation of the invasion between 2009 and 2011.

In the chapter VI we compare the community of aphid predator guild between field crops with conventional farming and field crops with organic farming. Many studies highlight that organic farming (eg: use of crop residues, reduction of the chemical pesticide) could be a solution to enhance biodiversity (Hole et al. 2005). For example, the integration of hedgerow structures in the agricultural landscape or the use of organic manures, compost and crop residues may contribute to favour biodiversity in agricultural areas (Kromp 1999, Lampkin

2000). Samplings of aphid predators species in both organic and conventional farming were realised. Question asked in this chapter is "Does organic farming increase the biodiversity of natural enemies population?".

Finally, in the last chapter of this thesis, we present a general discussion and a conclusion about the results obtained during our four years of research on Multicoloured Asian Ladybird. Perspectives about a general collaboration between scientists for a better control of this species conclude this thesis.

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Chapter V : ABUNDANCE OF *HARMONIA AXYRIDIS* AND OTHER APHID PREDATORS IN AGROECOSYSTEMS

V.1 General introduction to chapter V

The introductive chapter highlights that *H. axyridis* is present in the major parts of the world including America, Europe and Africa (Brown et al. 2011). In these areas it is an ubiquist species living and breeding in broad scale of habitats such as herbaceous, crops and forests (Colunga-Garcia and Gage 1998, Lucas et al. 2007, Burgio et al. 2008).

Main studies focuses on the occurrence and density of *H. axyridis* in general habitats apart on agroecosystems. In Belgium, *H. axyridis* was first observed in field crops (potatoes) in 2003 (Jansen and Warnier 2004) and since this time there are relatively little information about the occurrence of *H. axyridis* in Belgian agroecosystems. It has only been studied in a few field crops, e.g. potato (Jansen and Hautier 2008), green pea, wheat and stinging nettle (Alhmedi et al. 2009). This chapter aims to evaluate the density of the population of *H. axyridis* and other aphid predators in several agro-habitats from 2009 to 2011. Changes its density and phenology are also highlighted. All these data allowed a better understanding of the population change of both native and exotic species.

In the chapter V.2 we focus (1) on the adult abundance obtained by trapping and (2) diversity of predator species belonging to coccinellids, syrphids and chrysopids in several crops. This study aims to highlighted wich species of aphid predators are the most abundant in wheat, corn, broad bean and potato. Are there occurrences the same in diverse crops? The chapter V.3 include species diversity index of adult aphidophages in agroecosystems. The change in abundance of larvae and adult stages of the five most abundant aphid predators in agroecosystems was also studied. The population densities change is explained by the influence of the aphid population density on the abundance of aphid predators. Finally in the chapter V.4 we focus on the *H. axyridis* phenology and we realise the modelling of the cumulated abundance in agro-ecosystems of *H. axyridis* at both adult and larvae stages according to the cumulated temperature recorded in the agroecosystems.

Samplings are used to estimate the density of *H. axyridis*. Species density relates to an estimation of the real number of individuals per area. This estimation could be realised by several sampling methods such as hand-picking, sweeping, visual counting, vacuum sampling, traps (Hodek et al. 2012). Each sampling method is used to estimate the relative or absolute abundance of coccinellids but provides a differently biased estimation of abundance (Hodek et al. 2012). Among these sampling methods, observations within 1m^2 quadrat and the use of yellow sticky traps lead to the highest estimates of numbers per unit area (Michels et al. 1997, Udayagiri et al. 1997). These two methods are not well correlated but unlike quadrats method, traps are useful in detecting long-terms changes in abundance. Bias could also be due to the environment (fluctuations of weather, changes of light density) or to the species specificity (inhomogeneous distribution of species in vegetation, different behaviour according to the daily changes of light) (Hodek et al. 2012). The evaluation of the presence of a species in a particular habitat is also function of many interconnected factors as prey availability, host plant species, microclimate changes or landscape heterogeneity (Evans 2003). All these factors make it difficult to estimate the density.

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V.2 Aphid species and associated natural enemies in field crops: what about *Harmonia axyridis* (Coleoptera: Coccinellidae)?

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Reference - Vandereycken A., Durieux D., Joie E., Francis D., Haubruge E., Verheggen F.J. Aphid species and associated natural enemies in field crops: what about the *Harmonia axyridis* (Pallas) ?. *Applied Entomology and Zoology* (Under revision)

Abstract - Following the introduction in the 80's of the invasive coccinellid species *Harmonia axyridis* (Pallas) in Europe, several studies have begun to quantify how it directly and indirectly impacts the biodiversity of invaded areas. Yet, there remain a paucity of field studies on this species in agricultural areas. In this study, aphids and associated predators were sampled to determine their relative distributions in four agricultural crop types (broad bean, corn, potato, and wheat) in Belgium during 2010 and 2011. The Moerick trap was used to quantify the mean number of aphids and aphid predators (aphidophagous species) from May to September in both years. A total of 28 aphid species and 21 aphidophagous species were observed. In both years, *H. axyridis* was among the most abundant aphidophagous predators in all four crop types, and was the second most abundant coccinellid species after *Coccinella septempunctata* L. The community of aphidophagous species associated with *H. axyridis* was similar across all four inventoried crop types. However, the highest population densities of this alien species were recorded in broad bean and potato crops, which also hosted the highest aphid population densities. In conclusion, this study demonstrates that the communities of aphid predators are highly diversified in the agroecosystems of Belgium, despite the high abundance of *H. axyridis*, an introduced predator that has become well-established to this environment.

Keywords - *Harmonia axyridis*, Coccinellidae, agrosystems, alien species, aphid predator, culture, invasive

Introduction

An alien species that is deliberately or accidentally introduced to a given site will likely interact with the community of native species, potentially causing a decline of the number of native species. When assessing the impact of an invasive species on native communities, both direct (competition for food and intraguild predation) and indirect (apparent competition) interactions should be assessed; however, this requirement is often difficult to fulfill under field conditions (Holt and Lawton 1994).

When determining how the invasion of an alien species affects local biodiversity, the abundance of native species must first be quantified through field surveys. Because alien species exhibit habitat selectivity, such surveys should be realized in representative habitats. For instance, the habitat selectivity of alien species may be identified through the use of graphical food webs (Bersier et al. 2002). Food webs have been widely used to identify links between species from different trophic levels, such as parasitoids and their hosts (e.g., Gagic et al. 2012) or leafminers and their hosts (Morris et al. 2005). A food web provides a graphical illustration of such trophic relationships, including both the food resources and natural enemies of target organisms (Cohen 1978; Rott and Godfray 2000). Classical food webs only show the absence or presence of data regarding interactions (Polis and Winemiller 1996). In contrast, quantitative food webs contain sets of binary links between trophic species, in addition to information about species abundance (Alhmedi et al. 2011). Several studies have constructed quantitative food webs of various prey species, including aphids (Alhmedi et al. 2011; Muller et al. 1999), leaf mining insects (Valladares et al. 2001), Lepidoptera (Henneman and Memmott 2001), and gall-forming insects (Schönrogge and Crawley 2000).

The multicolored Asian ladybeetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) has been introduced from 80's to many European countries, including Belgium, to control aphid and coccid populations (Adriaens et al. 2008; Brown et al. 2008; Durieux et al. 2012; Gordon 1985). Following the introduction in 1997 of this species to Belgium, *H. axyridis* was observed to have a negative impact on native species, which generated particular concern (Roy and Wajnberg 2008). As a result, many studies focused on the intraguild predation by this species (Pell et al. 2008; Roy et al. 2012). Ten years after this species was first observed in the wild in Belgium, intraguild predation by *H. axyridis* has induced the decline of five native coccinellid species; namely, *Adalia bipunctata* L., *A.*

decempunctata L., *Calvia quatuordecimguttata* L., *Exochomus quadripustulatus* L., and *Propylea quatuordecimpunctata* L. (Roy et al. 2012; Ware et al. 2009).

This study aimed to compare the communities of aphids and associated insect predator species in four different crop types (i.e., wheat, broad bean, corn, and potato), with a primary focus on *H. axyridis*, an alien coccinellid species. Samples were collected from the four agrosystems over a 5 month period in 2010 and 2011. The diversity and abundance of aphids and associated predator species were used to determine the effectiveness of aphid biological control by predator species in these four agrosystems, and to identify the most economically beneficial strategy to promote in this region.

Material and methods

Study site

The study was conducted during 2010 and 2011 in Hesbaye (50° 34' N; 5° 15' E), which is an intensive agricultural production area in the southern region of Belgium. The climate in this area is temperate, with an average annual temperature of 10.5 °C (min. 3 °C, max 18 °C) during the two study years. The mean annual rainfall was 850 mm during the two study years, and was uniformly distributed across both years. Four crop types were selected for their agronomic importance; namely, wheat, *Triticum aestivum* (L.); corn, *Zea mays* (L.); potato, *Solanum tuberosum* (L.); and broad bean, *Vicia faba* (L.). Three fields (surface area >1 ha) were selected for each crop, and inventoried. All 12 fields were surrounded by conventional crops (intensive farming area).

Sampling methods

The sampling period for wheat and broad bean extended from mid-May to late August, while the sampling period for corn and potato extended from mid-May to late September. The fields of each crop type were visited once weekly. Three Moerick traps were set per field (yellow water traps, 30 cm diameter) to assess the diversity and abundance of aphids and their predators (Fig. 1). Mean species densities are expressed as the numbers of specimens per trap. Predatory hoverfly and ladybird species were identified using the keys of Verlinden (1994) and Baugnée and Branquart (2000), respectively. Aphid species were identified using two keys developed by Leclant (1999a,b).

Fig. 1: Moerick trap in potato

Statistical analysis

The mean number of aphid and aphidophagous species was calculated per crop per year. Insect abundance data were calculated per trap per week. Aphid abundance was compared among crop types, and analyzed by Analysis of Variance (ANOVA: General Linear Model), using crop type ($q = 4$) and month ($n = 5$) as factors ($\alpha = 0.05$). The factor "month" was used to reduce any natural variability of insect populations across the sampling period. ANOVA was also used to compare abundance between years ($q = 2$), with months ($n = 5$) being used as the factor ($\alpha = 0.05$). Within crops, predator abundance was compared by the Least Square Difference (LSD; $\alpha = 0.05$). Ryan-Joiner test was used to assess the population normality. Data were $\log_{10}(x+1)$ transformed before performing the statistical analyses on Minitab® 15.1.30.0 (State College, Pennsylvania, USA).

Graphical representation

The graphical results were drawn following the same conventions as described in earlier works on the natural enemy communities of aphids (Alhmedi et al. 2011; Muller et al. 1999). Aphid species (Fig. 2) and natural enemies (Fig. 3) were organized as a series of bars in an upper register, with the width of each bar being proportional to the cumulative abundance of each species across each year. Crops were arranged as a series of bars in a lower register, with either the width of each bar being equal (Fig. 2) either proportional to the cumulative abundance of aphids (Fig. 3). Natural enemies and crops were linked by triangular wedges, the relative widths of which represented the proportion of the natural enemies observed for each crop type.

Results

In 2010 and 2011, 16 and 20 aphid species were observed in the broad bean crops, respectively, 15 and 19 species in the potato crops, 17 species for both years in the wheat crops, and 15 and 17 species in the corn crops. These aphid species were numbered, with their corresponding identities being provided in Table 1. Total aphid density varied between years ($F_{1,954}$ = 37.21 ; P < 0.001) and among crop types in 2011 only (2010: $F_{3,468}$ = 1.02 ; P = 0.385 ; 2011: $F_{3,463}$ = 10.02; P < 0.001) (Fig. 1). Sixteen aphid species were common to all four crop types.

Table 1: Identity of predators and aphids caught in broad bean, corn, wheat and potato crops during 2010 and 2011. (Code numbers represent the species shown in Figure 2 and 3).

Code	Aphid name	Code	Predator name
1	*Metopolophium dirhodum* Walker	Coccinellidae	
2	*Sitobion avenae* Fabricius	1	*Coccinella quinquepunctata* L.
3	*Sitobion fragariae* Walker	2	*Coccinella septempunctata* L.
4	*Aphis fabae* Scopoli	3	*Coccinella undecimpunctata* L.
5	*Aphis craccivora* Koch	4	*Harmonia axyridis* Pallas
6	*Aphis nasturtii* Kaltenbach	5	*Hippodamia undecimnotata* Schneider
7	*Megoura viciae* Buckton	6	*Hippodamia variegata* Goeze
8	*Acyrthosiphon pisum* Harris	7	*Propylea quatuordecimpunctata* L.
9	*Myzus persicae* Sulzer	Syrphidae	
10	*Rhopalosiphum padi* L.	8	*Episyrphus balteatus* De Geer
11	*Rhopalosiphum maidis* Fitch	9	*Melanostoma mellinum* L.
12	*Nasonovia ribisnigri* Mosley	10	*Metasyrphus corollae* Fabricius
13	*Rhopalosiphum insertum* Walker	11	*Metasyrphus latifasciatus* Macquart
14	*Hyperomyzus lactucae* L.	12	*Metasyrphus luniger* Meigen
15	*Capitophorus horni* Börner	13	*Metasyrphus nitens* Zetterstedt
16	*Cavariella pastinacea* L.	14	*Parasyrphus macularis* Zetterstedt
17	*Cavariella aegopodii* Scopoli	15	*Platycheirus clypeatus* Meigen
18	*Macrosiphum rosae* L.	16	*Scaeva pyrastri* L.
19	*Macrosiphum euphorbiae* Thomas	17	*Sphaerophoria menthastri* L.
20	*Aulacorthum solani* Kaltenbach	18	*Sphaerophoria scripta* L.
21	*Sarucallis kahawaluokalani* Kirkaldy	19	*Syrphus ribesii* L.
22	*Cinara sp.*	20	*Syrphus vitripennis* Meigen
23	*Tetraneura sp.*	Chrysopidae	
24	*Metopolophium festucae* Theobald	21	*Chrysoperla carnea* Stephens
25	*Brevicoryne brassicae* L.		
26	*Schizaphis graminum* Rondani		
27	*Phyllaphis fagi* L.		
28	*Phorodon humuli Schrank*		

We also observed 21 predator species in the four studied crop types (Table 1 and 3, Fig. 3). The community of predator species differed among crops and between years in 2010 and 2011. A total of 12 and 16 predator species were recorded in the broad bean crops, respectively, 12 and 14 species in both the wheat and corn crops, and 11 and 12 species in the potato crops. Of these 21 observed predators, 7 were coccinellid species, 12 were syrphid species, and one was a chrysopid species (Table 3 and Table 4). Out of the 21 observed predators, 10 species were recorded in all four crop types; namely, *H. axyridis*, *Coccinella septempunctata* L., *P. quatuordecimpunctata*, *Episyrphus balteatus* De Geer, *Melanostoma mellinum* L., *Metasyrphus corollae* Fabricius, *M. latifasciatus* Masquart, *Sphaerophora menthastri* L., *S. scripta* L., and *Chrysoperla carnea* Stephen (Table 3 and 4). Five of these 10 species were abundant (more than 99% of the total observed individuals) on all four crop types in both years; namely, *H. axyridis*, *C. septempunctata*, *P. quatuordecimpunctata*, *E. balteatus*, and *C. carnea* (Table 2).

Table 2: Hoverfly and lacewing species mostly observed during this study.

Species	Adults	Larvae
Episyrphus balteatus		
Chrysoperla carnea		

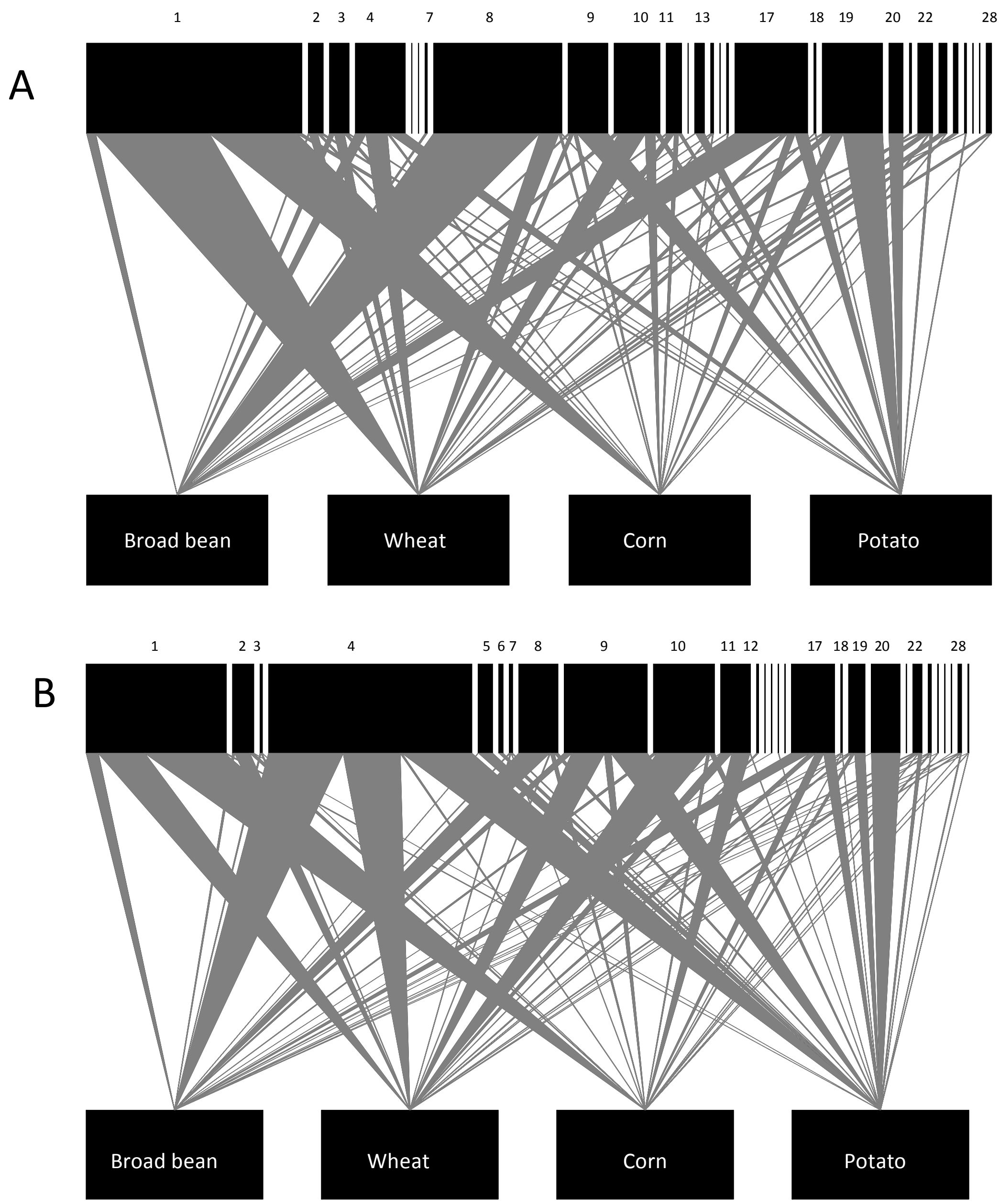

Fig. 2 Graphical presentation of aphid abundance on broad bean, wheat, corn, and potato crops during 2010 (A) and 2011 (B). The top bars represent aphid abundance. Species are represented by numbers, which are presented in Table 1.

The density of the five most abundant species varied across the four crop types (2010: $F_{3,2421} = 9.68$; $P < 0.001$; 2011: $F_{3,2391} = 14.06$; $P < 0.001$) (Fig. 4) and between the two years ($F_{1,4810} = 7.45$; $P = 0.006$). In 2010, *C. septempunctata* was the most abundant species in broad bean, corn, and potato crops. In contrast, three species dominated the predator guild in wheat crops; namely, *E. balteatus, C. septempunctata*, and *C. carnea* (Fig. 4A). In 2011, *C. septempunctata* and *C. carnea* were the most abundant species in broad bean crops, whereas *E. balteatus* dominated the wheat crops (Fig. 3B). In corn, no difference in predator density was observed among the species of the predator guild (Fig. 4B).

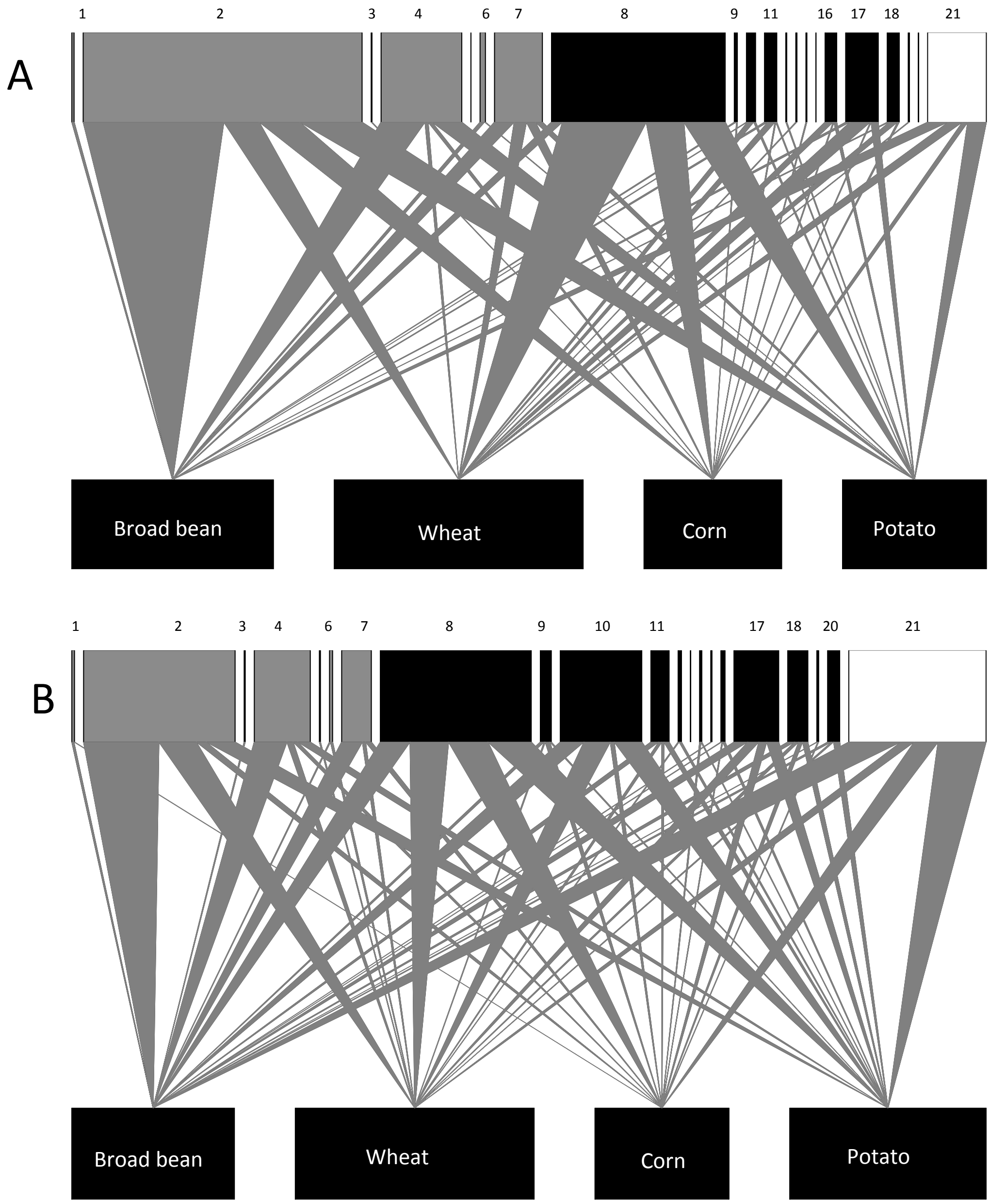

Fig. 3. Graphical presentation of aphid predator abundance on broad bean, wheat, corn, and potato crops during 2010 (A) and 2011 (B). The top bars represent predator abundance. Species are represented by numbers, which are presented in Table 1. (Upper bars: grey = coccinellids; black = syrphids; white = chrysopids)

Discussion

This study identified 28 aphid species and 21 associated predators in communities sampled from four of the most important crop types in Belgian. In 2011 only, aphid abundance significantly differed across the four crop types. This variation might be explained by the intrinsic proprieties of crop species, including architecture, blends of volatile organic components, and sap composition (Webster et al. 2008). Alternatively, this variation might be explained by different plot management practices used for the four crop types, including differences in mechanical field preparation and pesticide treatment. Plant density could be also a factor involved in the aphid abundance ($12m^{-2}$ in corn and $100–150m^{-2}$ in wheat and broad bean). Another reason for the variation in aphid abundance might be the positioning of the yellow traps in the cornfield. For instance, the traps were not placed at the upper level of plants throughout the cultivation season, unlike the other three crops (corn final height reached 2.5m). Moreover, many factors that regulate the size of insect populations might influence aphid abundance, including abiotic environmental conditions (micro-climate), plant volatiles (Park and Hardie 2004), plant structure (Goffreda et al. 1988; Powell et al. 1999), and host plant diversity around the crops (Alhmedi et al. 2007).

Table 3: Mean number and SE of aphidophagous species per trap per week during 2010. Species are sorted by family (Coccinellidae, Syrphidae, Chrysopidae).

	Mean number (SE) of aphidophagous species per trap and per week in 2010							
	Broad bean		Wheat		Corn		Potato	
Coccinella quinquepunctata	0.020	(0.014)	0		0		0	
Coccinella septempunctata	1.267	(0.248)	0.318	(0.062)	0.365	(0.089)	0.563	(0.099)
Coccinella undecimpunctata	0		0		0.007	(0.007)	0	
Harmonia axyridis	0.396	(0.087)	0.027	(0.016)	0.034	(0.015)	0.270	(0.054)
Hippodamia variegata	0.040	(0.024)	0		0		0.008	(0.008)
Propylea quatuordecimpunctata	0.178	(0.055)	0.109	(0.035)	0.081	(0.026)	0.063	(0.025)
Episyrphus balteatus	0.099	(0.033)	0.755	(0.359)	0.338	(0.087)	0.373	(0.177)
Melanostoma mellinum	0.020	(0.014)	0		0.007	(0.007)	0	
Metasyrphus corollae	0.010	(0.009)	0.055	(0.028)	0		0.016	(0.011)
Metasyrphus latifasciatus	0.030	(0.022)	0.064	(0.030)	0.007	(0.007)	0.008	(0.008)
Metasyrphus luniger	0		0		0		0.008	(0.008)
Metasyrphus nitens	0		0.009	(0.009)	0		0	
Parasyrphus macularis	0		0		0.007	(0.007)	0	
Scaeva pyrastri	0		0.073	(0.046)	0.007	(0.007)	0.024	(0.014)
Spaerophoria scripta	0.040	(0.019)	0.182	(0.058)	0.007	(0.007)	0.063	(0.025)
Sphaerophoria menthastri	0.020	(0.014)	0.073	(0.042)	0.014	(0.009)	0	
Syprhus ribesii	0		0.009	(0.009)	0		0	
Chrysoperla carnea	0.188	(0.053)	0.127	(0.039)	0.047	(0.024)	0.167	(0.057)

Although 10 predator species were observed in the four inventoried crops, five species were consistently dominant; namely, *H. axyridis*, *C. septempunctata*, *P. quatuordecimpunctata*, *E. balteatus*, and *C. carnea*. Species from these five taxa are frequently considered as major aphid predators in agrosystems worldwide (Bode 1980; Chambers et al. 1982; Evans 2000; Honěk 1979; Thalji 2006). For instance, *C. septempunctata*, *H. axyridis*, and *E. balteatus* have been documented as dominant predator species in previous works conducted in Belgium (Adriaens et al. 2008; Alhmedi et al. 2009; Derume et al. 2007).

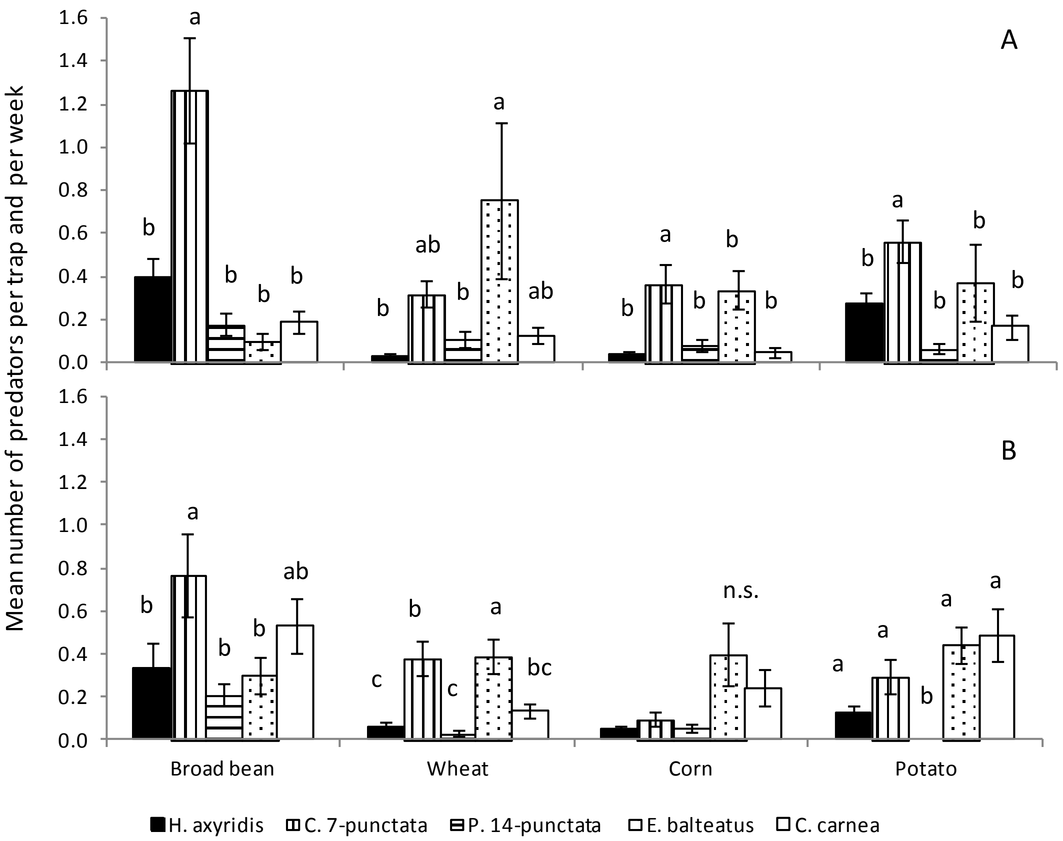

Fig. 4 Abundance (mean and SE) of aphidophagous species trapped in the four agrosystems during 2010 (A) and 2011 (B). Means within a crop followed by the same letter are not significantly different (P > 0.05; LSD test), (*C. 7-punctata* represents *C. septempunctata; P. 14-punctata represents P. quatuordecimpunctata*)

The invasive coccinellid, *H. axyridis,* is known to be uniformly distributed and invasive among various crop types (Brown et al. 2011b; Lombaert et al. 2010), and was one of the five most abundant predatory species recorded in the current study. The high density of this species might affect native species by decreasing the diversity and density of indigenous species, particularly *P. quatuordecimpunctata* (Brown et al. 2011a; Roy et al. 2012; Ware et al. 2009). Furthermore, *H. axyridis* has already been demonstrated to be an efficient intraguild predator (Ingels and De Clercq 2011; Phoofolo and Obrycki 1998; Wells et al. 2010). Despite the behavior of this species, the diversity of aphidophagous species remained high in potato and broad bean crops, despite the relatively high abundance of *H. axyridis*. In both study years, *C. septempunctata* was more abundant compared to *H. axyridis* in broad bean, wheat, potato, and corn crops. This observation was expected, as *H. axyridis* is considered to be an arboreal species, whereas *C. septempunctata* preferentially breed in herbaceous stratum (Hodek 1973).

Table 4: Mean number and SE of aphidophagous species caught per trap per week during 2011. Species are ordered by family (Coccinellidae, Syrphidae, Chrysopidae).

	Mean number (SE) of aphidophagous species per trap and per week in 2011			
	Broad bean	Wheat	Corn	Potato
Coccinella quinquepunctata	0.020 (0.015)	0	0.007 (0.007)	0
Coccinella septempunctata	0.768 (0.198)	0.381 (0.078)	0.099 (0.033)	0.298 (0.080)
Coccinella undecimpunctata	0.010 (0.010)	0	0	0
Harmonia axyridis	0.333 (0.121)	0.062 (0.025)	0.046 (0.019)	0.130 (0.031)
Hippodamia undecimnotata	0	0.010 (0.010)	0	0
Hippodamia variegata	0.010 (0.010)	0.021 (0.015)	0	0
Propylea quatuordecimpunctata	0.212 (0.054)	0.031 (0.018)	0.059 (0.019)	0
Episyrphus balteatus	0.303 (0.087)	0.392 (0.080)	0.401 (0.146)	0.443 (0.088)
Melanostoma mellinum	0.040 (0.025)	0.010 (0.010)	0.046 (0.025)	0.015 (0.011)
Metasyrphus corollae	0.242 (0.064)	0.268 (0.075)	0.039 (0.018)	0.282 (0.069)
Metasyrphus latifasciatus	0.081 (0.042)	0.031 (0.018)	0.020 (0.015)	0.053 (0.029)
Metasyrphus luniger	0	0.010 (0.010)	0	0.023 (0.017)
Parasyrphus macularis	0	0	0.013 (0.009)	0.008 (0.007)
Platycheirus clypeatus	0.010 (0.010)	0	0	0
Scaeva pyrastri	0.010 (0.010)	0	0.007 (0.007)	0.023 (0.013)
Spaerophoria scripta	0.141 (0.047)	0.113 (0.036)	0.079 (0.024)	0.122 (0.039)
Sphaerophoria menthastri	0.071 (0.030)	0.041 (0.025)	0.039 (0.021)	0.053 (0.020)
Syprhus ribesii	0.010 (0.010)	0	0.007 (0.007)	0
Syrphus vitripennis	0.040 (0.025)	0.021 (0.015)	0	0.061 (0.034)
Chrysoperla carnea	0.535 (0.127)	0.134 (0.035)	0.243 (0.086)	0.489 (0.121)

In addition, the density of *H. axyridis* individuals depends on the type of adjacent habitats (such as hibernation sites, feeding areas represented by nettles, specific species with attracted volatile compounds) (Alhmedi et al. 2009; Colignon et al. 2001; Durieux et al. 2010; Hodek 1973), with arboreal habitats, which are the preferred habitat type by this species, being scarce in agroecosystems.

Among the 28 aphid species observed in this study, some occurred at very low densities on certain crop types. These species tended to be winged aphids that just passed through the inventoried fields; including, *Phorodon humuli*, *Hyperomyzus lactucae*, *Macrosiphum rosae*, *Brevicoryne brassicae*, and *Phyllaphis fagi*. In contrast, the six most abundant aphid species observed in this study were uniformly spread across the four crop types. These six species have been observed on more than 200 plant species (Holman 2009).

The current study demonstrated that the community of aphids and aphid predators species is highly diverse on four major crop types in Belgium. Among the aphid predator species, the invasive ladybird, *H. axyridis*, was the second most abundant coccinellid after *C. septempunctata*. Because this invasive species exhibits intraguild predatory behavior, it might threaten the persistence of other native aphidophagous species, by reducing their population densities. Yet, at present, the community of aphidophagous species around *H. axyridis* was similar in all four crops, and contained a large number of species. In conclusion, this type of graphical representation (based on foodwebs) provides a useful approach towards determining the potential effectiveness of biocontrol techniques, based on the identification of habitat diversity ranges of species, which might be applied towards developing efficient biological pest management practices.

Acknowledgments

We thank Ammar Alhmedi and Charles Godfray for their advices on food web analysis. This research was funded by the Service Public de Wallonie (SPW – DGO3, project n°D31-1247).

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V.3 Is the multicolored Asian ladybeetle, *Harmonia axyridis*, the most abundant natural enemy to aphids in agroecosystems?

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Reference - Vandereycken A., Durieux D., Joie E., Sloggett J.J., Haubruge E., Verheggen F.J. 2013. Is the multicolored Asian ladybeetle, *Harmonia axyridis*, the most abundant natural enemy to aphids in agroecosystems? *Journal of Insect Science*. 13(158): 1-14.

Abstract - The multicolored Asian ladybeetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), was introduced into Western Europe in the late 1990s. Since the late 2000s, this species has been commonly considered one of the most abundant aphid predators in most Western European countries. In spite of the large amount of research on *H. axyridis*, information concerning its relative abundance in agroecosystems is lacking. This study aims to evaluate the abundance of *H. axyridis* within the aphidophage community in four crops situated in southern Belgium: wheat, *Triticum aestivum* L. (Poaels: Poaceae), corn, *Zea mays*, potato, *Solanum tuberosum* (Solanales: Solanaceae), and broad bean *Vicia faba* (Fabales: Fabaceae). In order to assess the species diversity, the collected data were analyzed by considering (1) the species richness and (2) the evenness according to the Shannon diversity index. Eleven aphidophages were observed in every inventoried agroecosystem, including five abundant species: three coccinellids, the seven-spotted ladybug, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), the 14-spotted Ladybird, *Propylea quatuordecimpunctata*, and *H. axyridis*; one hoverfly, the marmalade hoverfly, *Episyrphus balteatus* De Geer (Diptera: Syrphidae); and one lacewing, the common green lacewing, *Chrysoperla carnea* Stephens sensu lato (= s.l.) (Neuroptera: Chrysopidae). *Harmonia axyridis* has been observed to thrive, breed, and reproduce on the four studied crops.

Harmonia axyridis is the most abundant predator of aphids in corn followed by *C. septempunctata*, which is the main aphid predator observed in the three other inventoried crops. In wheat and potato fields, *H. axyridis* occurs in low numbers compared to other aphidophages. These observations suggest that *H. axyridis* could be considered an invasive species of agrosystems, and that potato and wheat may intermittently act as refuges for other aphidophages vulnerable to intraguild predation by this invader.

Keywords - Aphidophagous insects, biological control, crop pests, Coccinellidae, invasive species

Introduction

The multicolored Asian ladybeetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), was introduced from Asia into Western Europe and other parts of the world to control aphid and coccid populations (Adriaens et al. 2008; Brown et al. 2008). In Belgium, *H. axyridis* was used as a biological control agent beginning in 1997 and was first observed in the wild in 2001. Since then *H. axyridis* populations have increased and gradually expanded into Belgium (Adriaens et al. 2008).

This species presents all the characteristics shared by an efficient aphid predator: large body size, high voracity, high predation efficiency (Labrie et al. 2006), high colonization aptitude (With et al. 2002), rapid development, high fecundity, and low susceptibility to pathogens or natural enemies (Marco et al. 2002). *Harmonia axyridis* has become ubiquitous in many parts of the world, including America, Europe, and Africa (Lombaert et al. 2010; Brown et al. 2011b), and has been reported in many different habitats, such as agroecosystems, gardens, and arboreal habitats (Majerus et al. 2006).

Due to its large body and efficient physical and chemical defenses, *H. axyridis* has become an intraguild predator (Sato and Dixon 2004; Ware and Majerus 2008). Intraguild predation has been observed among other ladybeetle species (Pell et al. 2008; Ware and Majerus 2008); other aphid natural enemies, including syrphids, chrysopids, and parasitoids (Phoofolo and Obrycki 1998; Wells et al. 2010; Ingels and De Clercq 2011); and aphid pathogenic fungus (Roy et al. 2008). This intraguild predation behavior is thought to have led to a decrease in native species (Brown and Miller 1998; Harmon et al. 2007; Ware et al. 2009; Brown et al. 2011a; Roy et al. 2012). In Belgian urban areas, Adriaens et al. (2010) found a significant decline in native species, including the two-spot ladybird, *Adalia bipunctata* L. (Coleoptera: Coccinellidae), and the 10 spotted ladybird, *Adalia decempunctata* L., on pine, lime, and maple trees following the arrival of *H. axyridis*. The decline of native species can possibly be explained by the decline in number of their principal prey, resulting in reduced survivorship in local habitats and altered dynamics of habitat use and dispersal (Evans 2004).

According to previous reports, the most dominant aphidophage in Belgian agroecosystems appear to be two coccinellids, the seven-spotted ladybug, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), and *H. axyridis*; one hoverfly, the marmalade hoverfly, *Episyrphus balteatus* De Geer (Diptera: Syrphidae); and one braconid, the parasitic wasp *Aphidius ervi* Haliday (Hymenoptera: Braconidae) (Derume et al. 2007; Adriaens et al. 2008; Alhmedi et al. 2009). In arboreal habitats, four coccinellids were reported as abundant species: *A. bipunctata,* the 14-spotted ladybird, *Propylea quatuordecimpunctata* L. (Coleoptera: Coccinellidae), the 22-spot ladybird *Psyllobora vigintiduopunctata* (L.), and *H. axyridis* (Adriaens et al. 2008). In 2001, the same results were observed by Francis (2001), with the exception of *H. axyridis*.

Our study was conducted eight years after the first observation of *H. axyridis* in the wild in Belgium (Adriaens et al. 2003). Following aphidophagous decline highlighted by several studies, the current study was conducted in order to assess the relative abundance of *H. axyridis* through the quantification of the abundance of this exotic species and other aphidophages in four important Belgian crops (wheat, *Triticum aestivum* L. (Poales: Poaceae), corn, *Zea mays*, potato, *Solanum tuberosum* (Solanales: Solanaceae), and broad bean *Vicia faba* (Fabales: Fabaceae)) using a three-year inventory.

Material and methods

Study site

Aphidophagous insect populations were sampled from 2009 to 2011 in Hesbaye, an intensive agricultural production area in Wallonia, the southern region of Belgium (individual sites given in Table 1). Four crops were chosen for their agronomic importance: wheat, corn, potato, and broad bean *Vicia faba*. The sampling period ran from mid-May to late September. Every week, nine fields of each crop were sampled.

Table 1: Sites surveyed for aphidophagous species from 2009 to 2011 in Belgium.

Year	Site	Latitude (°)	Longitude (°)	Crops
2009	Bassenge	5,652	50,768	wheat (2.5ha), corn (1.9ha)
	Bousval	4,505	50,63	wheat (9.3ha), corn (6.9ha), potato (15.2ha)
	Geer	5,19	50,67	wheat (11.6ha), corn (6.4ha)
	Godardville	4,284	50,482	wheat (9.2ha), potato (4.4; 13.9ha)
	Loyers	4,934	50,454	wheat (5.8 ha)
	Perwez	4,813	50,645	broad bean (13.8; 2.4; 1.3; 1.8ha), wheat (13.7ha), corn (0.7; 1.3ha), potato (9.9ha)
	Ramillies	4,866	50,624	wheat (3.5ha), corn (3.8ha)
	Redu	5,158	50,004	corn (5.7; 6.2ha)
	Rhisnes	4,83	50,5	broad bean (8.7; 4.2; 4.5; 1.3; 6.2ha), wheat (9.6ha), corn (2.7ha), potato (5.6; 6.7ha)
	Walhain	4,735	50,616	wheat (7.5ha), corn (0.5ha)
2010	Eben-Emael	5,676	50,789	potato (2.9; 6.7ha), corn (3.5ha)
	Gembloux	4,695	50,563	broad bean (3.2ha)
	Isnes	4,732	50,515	broad bean (1.6ha)
	Nil-St-Vincent	4,689	50,646	broad bean (3.4ha)
	Perwez	4,813	50,645	potato (12.0; 9.4ha), corn (7.5; 3.6ha), broad bean (22.7ha), wheat (10.2; 13.3; 10.7; 25.4; 22.1ha)
	Plancenoit	4,398	50,664	corn (0.6ha), broad bean (3.4ha)
	Ramillies	4,866	50,624	potato (10.0ha), corn (3.6ha), wheat (4.8ha)
	Rhisnes	4,83	50,5	wheat (8.7ha; 6.7; 5.6ha), broad bean (9.7ha)
	Richelle	5,703	50,713	potato (1.5; 1.1ha), corn (4.5; 11.1ha), broad bean (1.3; 6.3ha)
	Walhain	4,735	50,616	potato (7.7; 9.6ha), corn (5.8; 8.7ha), broad bean (2.7ha)
2011	Gembloux	4,695	50,563	potato (4.5; 7.8; 3.7ha), corn (6.9ha), broad bean (1.5ha)
	Grez-Doiceau	4,696	50,741	corn (5.2; 6.2ha), broad bean (2.1ha), wheat (4.9ha)
	Ligny	4,581	50,508	broad bean (0.8ha)
	Perwez	4,813	50,645	potato (19.3; 9.2; 10.4; 8.0ha), corn (17.0ha), broad bean (13.4; 7.6ha), wheat (19; 21.5; 11.8; 9.8ha)
	Plancenoit	4,398	50,664	corn (4.9ha), broad bean (3.4ha)
	Ramillies	4,866	50,624	potato (4.2ha), corn (3.7ha), wheat (6.3ha)
	Rhisnes	4,83	50,5	wheat (9.1ha)
	Richelle	5,703	50,713	corn (3.0; 7.8; 2.8ha), broad bean (4.0; 2.8; 2.7ha)
	Walhain	4,735	50,616	potato (7.8ha), wheat (7.6; 9.5ha)

Sampling methods

The sampling methods used to assess the numbers of aphidophagous predators and aphids consisted of whole-plant visual inspections, using 1 m^2 quadrats distributed randomly throughout the whole field. In order to avoid the influence of surrounding crops, a 20 m buffer zone around the edge of each field was not sampled. Visual sampling was conducted, as it provides an easy and accurate method for the estimation of larval and adult densities of coccinellids in agroecosystems (Michels and Behle 1992).

Forty-eight quadrats were examined in each crop every week. Quadrats were located along transect lines across each field and spaced 20 m apart. All leaves and stems within the quadrat were examined, and all aphidophagous stages were recorded. Aphid species were also quantified on all leaves and stems. Larvae and pupae were brought to the laboratory to develop under laboratory conditions (24 ± 1° C, 75 ± 5% RH) for identification at the species level. All aphid predators were identified, with the exception of members of the common green lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) species complex, which were pooled together. This group comprises three cryptic species, *C. kolthoffi* Navas, *C. lucasina* Lacroix, and *C. carnea* Stephens, which can only be differentiated using molecular techniques (Bozsik et al. 2003; Lourenco et al. 2006).

Statistical analysis

In order to assess the species diversity, the collected data were analyzed by considering (1) species richness and (2) evenness according to the Shannon diversity index (H) (Magurran 1988), which considers both the number of species and the distribution of individuals among species. This index include p_i, the proportion of individual belonging to the species i. H is minimal if all individuals belong to only one species or if all species are represented by one individual; H is maximal if all individuals are evenly distributed. The maximum diversity (Hmax) is the maximal diversity which could occur if all species collected were equally abundant. This value includes k that is the species richness. Evenness (J) varies from 0, if only one species dominates, to 1, if all species show similar abundance. Evenness (J) and the Shannon diversity index (H) were calculated as followed:

$$H = -\sum(p_i \ \ln(p_i)) \qquad \text{Diversity}$$

$$H_{max} = \ln k \qquad \text{Max diversity}$$

$$J = \frac{H}{H_{max}} \qquad \text{Evenness}$$

Because mean densities per m² were low, these values are presented per 100 m². The mean abundance per species was analyzed with an analysis of variance (ANOVA: general linear models) with crops (q = 4) and years (n = 3) used as factors ($\alpha = 0.05$). Within crops, densities of species were compared using the least square difference (LSD; $\alpha = 0.05$). To account for the variations of predator abundances, the abundance per species was analyzed using an analysis of covariance (ANCOVA: general linear models) with crops (q = 4) and years (n = 3) used as factors ($\alpha = 0.05$) and aphid densities used as the covariable. Ryan-Joiner test was used to assess the population normality. Prior to analyses, a $\log_{10}(x + 1)$ was used to transform the data distribution (counting) due to its asymmetry (Dagnelie 2011). Although statistical analyses were performed on transformed data, untransformed data are presented in Tables 4 and 5. Statistical analyses were performed using Minitab® release 1.5 (www.minitab.com).

Results

Diversity of aphidophages

During the three years (2009–2011), 11 aphidophagous taxa were observed on the four different cultures: the hoverfly *E. balteatus,* the coccinellids *C. septempunctata, C. quinquepunctata* L. (Coleoptera: Coccinellidae), *C. undecimpunctata* (L.), *H. axyridis, P. quatuordecimpunctata, A. bipunctata, A. decempunctata,* the cream-spot ladybird, *Calvia quatuordecimguttata* (L.), *Hippodamia variegata* Goeze; and the *C. carnea* species group (Table 2).

From 2009 to 2011, species richness increased in broad bean, corn, and wheat, reaching 6, 8, and 7 species respectively (Table 3). Species richness did not evolve in potato crops, remaining at five species during the entire period. Overall, five aphidophages were continually observed during the three-year period in each crop and represented 95% of all the aphidophage observed in 2009 and 99% in both 2010 and 2011: *E. balteatus, C. carnea* s.l., *C. septempunctata*, *P. quatuordecimpunctata*, and *H. axyridis*.

Table 2: Aphidophagous species diversity in four crops (broad bean, wheat, corn and potato) from 2009 to 2011 (+: presence of aphidophages; *A. 10-punctata: Adalia decempunctata; A. 2-punctata: Adalia bipunctata; C. 7-punctata: Coccinella septempunctata; C. 14-guttata: Calvia quatuordecimguttata; C.11-punctata: Coccinella undecimpunctata; C. 5-punctata: Coccinella quinquepunctata; P. 14-punctata: Propylea quatuordecimpunctata*).

	Broad bean			Wheat			Corn			Potato		
	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011
Coccinellidae												
A. 10-punctata L.		+										
A. 2-punctata L.							+		+			
***C. 7-punctata* L.**	+	+	+	+	+	+	+	+	+	+	+	+
C. 14-guttata L.						+						
C.11-punctata L.									+			
C. 5-punctata L.			+				+	+	+			
***H. axyridis* Pallas**	+	+	+	+		+	+	+	+	+	+	+
H. variegata Goeze						+						
***P. 14-punctata* L.**	+	+	+	+	+	+	+	+	+	+	+	+
Syrphidae												
***E. balteatus* De Geer**	+	+	+	+	+	+	+	+	+	+	+	+
Chrysopidae												
C. carnea s.l.	+	+	+	+	+	+	+	+	+	+	+	+

The evenness value was low in wheat with J = 0.35, 0.25, and 0.38 in 2009, 2010, and 2011, respectively, with the numerical dominance of two species: *C. septempunctata* and *E. balteatus* (Tables 3, 4, and 5). In broad bean and potato, evenness varied from year to year. In broad bean, *C. septempunctata* was the most abundant in 2010 (J = 0.45) and 2011 (J = 0.59). In potato, *C. carnea* s.l. and *C. septempunctata* numerically dominated the aphidophagous guild in all three years (J = 0.65, 0.79, 0.74) (Table 3). In corn, the evenness during the three years decreased from 0.82 (2009) to 0.61 (2010).

Table 3: Species richness and diversity index (H= Shannon-Weiner Diversity Index, where absolute diversity = 1.00; J= Evenness or relative Diversity (H/ Hmax), where absolute evenness=1.00).

	Broad bean			Wheat			Corn			Potato		
	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011
Species Richness	5	6	6	5	4	7	7	6	8	5	5	5
Diversity (H)	1,42	0,8	1,05	0,56	0,35	0,74	1,6	1,47	1,27	1,05	1,27	1,19
Max. Diversity (Hmax)	1,61	1,79	1,79	1,61	1,39	1,95	1,95	1,79	2,08	1,61	1,61	1,61
Evenness (J)	0,88	0,45	0,59	0,35	0,25	0,38	0,82	0,82	0,61	0,65	0,79	0,74

Relative abundance of aphidophage in four crops

The relative abundance of both adult and larval stages of the five aphidophage within each crop showed significant differences (Tables 4 and 5), with the exception of potato in 2010, in which densities of larvae populations of different predators were not significantly different ($F_{4,3570}$ = 1.4 ; p = 0.25) (Table 5).

a) Corn

The most abundant adult species in 2009 and in 2010 was *C. carnea* and *C. septempunctata* (2009: $F_{4,1065}$ =10.7 ; P < 0.001 ; 2010: $F_{4,3425}$ = 14.3 ; P < 0.001) (P < 0.05; LSD) (Table 4). In 2011, *H. axyridis* and *C. septempunctata* densities were both significantly higher than those of other species (P < 0.05; LSD) ($F_{4,4055}$ = 24.2 ; P < 0.001) (Table 4). *H. axyridis* larvae densities were in 2009 and 2011 significantly higher (2009: $F_{4,1065}$ = 5.4 ; P < 0.001 ; 2010: $F_{4,3425}$ =25.5 ; P < 0.001 ; 2011: $F_{4,4055}$ = 57.0 ; P < 0.001) than those observed for the other species (Table 5).

b) Wheat

Adult populations of *H. axyridis* remained lower than other species during the three-year sampling (2009: $F_{4,1765}$ = 5.4, p < 0.001 ; 2010: $F_{4,2925}$ = 24.1, p < 0.001; 2011: $F_{4,2625}$ = 38.0, p < 0.001) (p < 0.05; LSD) and did not exceed 1.1 ± 0.6 adults per 100 m^2 (Table 4). *Episyrphus balteatus* was the most frequently encountered adult species in 2009, whereas in 2010 and 2011 *C. septempunctata* was the most abundant species (p < 0.05; LSD) (Table 4). Larvae of *E. balteatus* were the most abundant (p < 0.05; LSD) of the aphidophage during the three years (2009: $F_{4,1765}$ =11.7, p < 0.001 ; 2010: $F_{4,2925}$ = 91.1, p < 0.001; 2011: $F_{4,2625}$ = 273.6, p < 0.001) (Table 5).

Table 4: Abundance (means and SE) of aphidophagous species (adults) in four agroecosystems from 2009 to 2011. Means within a crop followed by the same letter are not significantly different for the same field (P> 0.05; LSD test), (*C. 7-punctata: Coccinella septempunctata; P. 14-punctata: Propylea quatuordecimpunctata*).

		Mean number of adults aphidophages/100m²		
Crops	Aphidophages	2009	2010	2011
Corn	***H. axyridis***	7.80 ± 2.04 *a*	3.91 ± 0.96 *ab*	19.98 ± 1.80 *c*
	C. 7-punctata	10.09 ± 2.42 *a*	12.03 ± 1.49 *c*	22.30 ± 2.00 *c*
	P. 14-punctata	0.46 ± 0.46 *a*	5.65 ± 0.93 *b*	3.68 ± 0.90 *ab*
	E. balteatus	8.26 ± 1.87 *a*	1.16 ± 0.54 *a*	0.74 ± 0.30 *a*
	C. carnea s.l.	29.82 ± 5.66 *b*	4.06 ± 0.86 *ab*	12.25 ± 1.90 *b*
Wheat	***H. axyridis***	1.12 ± 0.56 *b*	0 *a*	0.19 ± 0.20 *a*
	C. 7-punctata	7.28 ± 1.95 *bc*	5.01 ± 1.14 *c*	36.93 ± 4.30 *c*
	P. 14-punctata	0 *a*	0.17 ± 0.17 *b*	0.95 ± 0.40 *a*
	E. balteatus	15.97 ± 3.10 *c*	0.33 ± 0.24 *b*	14.02 ± 2.40 *b*
	C. carnea s.l.	2.52 ± 0.92 *bc*	1.00 ± 0.41 *b*	11.17 ± 1.80 *b*
Potato	***H. axyridis***	1.90 ± 1.34 *b*	2.50 ± 0.73 *a*	1.98 ± 0.70 *a*
	C. 7-punctata	1.90 ± 1.34 *b*	14.19 ± 1.80 *b*	8.70 ± 1.30 *b*
	P. 14-punctata	0 *a*	0.70 ± 0.31 *a*	0.31 ± 0.20 *a*
	E. balteatus	0.95 ± 0.95 *b*	0.28 ± 0.20 *a*	3.21 ± 1.00 *a*
	C. carnea s.l.	10.48 ± 4.04 *c*	1.53 ± 0.46 *a*	14.05 ± 1.90 *b*
Broad bean	***H. axyridis***	5.71 ± 3.98 *b*	2.02 ± 0.63 *b*	5.81 ± 1.50 *a*
	C. 7-punctata	5.71 ± 5.71 *b*	29.96 ± 4.79 *d*	35.27 ± 6.10 *b*
	P. 14-punctata	0 *a*	2.23 ± 0.78 *b*	3.41 ± 0.90 *a*
	E. balteatus	0 *a*	0 *a*	5.01 ± 1.20 *a*
	C. carnea s.l.	0 *a*	4.66 ± 0.99 *c*	50.70 ± 5.70 *b*

c) Potato

Trends for *H. axyridis* were the same in potato as in wheat: *H. axyridis* was not the most abundant species, and its density did not exceed 2.5 ± 0.7 adults per 100 m² (Table 4). Two species were more abundant than others: *C. carnea* s.l. in 2009 and 2011 (2009: $F_{4,500} = 7.7$, $p < 0.001$; 2011: $F_{4,3250} = 10.8$, $p < 0.001$), and *C. septempunctata* in 2010 ($F_{4,3570} = 12.8$, $p < 0.001$) and 2011 ($p > 0.05$; LSD). In 2009, larvae of *C. carnea* s.l., *P. quatuordecimpunctata*, and *H. axyridis* were the most abundant species but densities remained low (Table 5). In 2010, larvae densities of the five above-mentioned species were not significantly different from each other ($F_{4,3570} = 1.4$, $p = 0.25$) (Table 5). In 2011, only *C.*

carnea s.l. numerically dominated the aphidophages community ($F_{4,3250} = 5.5$, $p < 0.001$) ($p > 0.05$; LSD) (Table 5).

Table 5 : Abundance (means and SE) of aphidophagous species (larvae) in four agroecosystems from 2009 to 2011. Means within a crop followed by the same letter are not significantly different for the same field (P> 0.05; LSD test), (*C. 7-punctata: Coccinella septempunctata; P. 14-punctata: Propylea quatuordecimpunctata*).

		Mean number of larvae aphidophages/100m^2											
Crops	Aphidophages	2009				2010				2011			
Corn	***H. axyridis***	11.93	±	4.19	*ab*	13.33	±	2.89	*b*	70.83	±	6.60	*c*
	C. 7-punctata	7.34	±	2.47	*a*	6.38	±	1.57	*ab*	25.00	±	3.90	*b*
	P. 14-punctata	25.23	±	5.61	*b*	2.46	±	0.72	*a*	2.57	±	0.70	*a*
	E. balteatus	4.59	±	1.93	*a*	36.67	±	5.35	*c*	19.24	±	2.10	*b*
	C. carnea s.l.	3.21	±	1.20	*a*	5.51	±	0.96	*ab*	0.61	±	0.30	*a*
Wheat	***H. axyridis***	2.24	±	1.48	*a*	0			*a*	0.19	±	0.20	*b*
	C. 7-punctata	3.08	±	1.39	*a*	1.50	±	0.80	*b*	8.71	±	1.80	*b*
	P. 14-punctata	13.45	±	3.92	*a*	0			*a*	0			*a*
	E. balteatus	194.40	±	16.21	*b*	79.30	±	10.42	*c*	160.98	±	10.20	*c*
	C. carnea s.l.	2.52	±	1.00	*a*	0.17	±	0.17	*b*	1.33	±	0.50	*b*
Potato	***H. axyridis***	0.95	±	0.95	*b*	0.70	±	0.37	*a*	0.46	±	0.30	*a*
	C. 7-punctata	0			*a*	2.50	±	1.26	*a*	0.31	±	0.20	*a*
	P. 14-punctata	1.90	±	1.34	*b*	0.14	±	0.14	*a*	0.31	±	0.20	*a*
	E. balteatus	0			*a*	5.42	±	1.85	*a*	0.61	±	0.40	*a*
	C. carnea s.l.	5.71	±	2.65	*b*	3.34	±	0.75	*a*	4.43	±	1.00	*b*
Broad bean	***H. axyridis***	0			*a*	6.68	±	2.09	*a*	21.84	±	6.30	*a*
	C. 7-punctata	0			*a*	42.92	±	9.33	*b*	114.23	±	35.70	*b*
	P. 14-punctata	5.71	±	5.71	*b*	0.20	±	0.20	*a*	0.20	±	0.20	*a*
	E. balteatus	17.14	±	11.94	*b*	5.06	±	1.64	*a*	2.20	±	0.80	*a*
	C. carnea s.l.	2.86	±	2.86	*b*	0.61	±	0.35	*a*	2.00	±	0.60	*a*

d) Broad bean

In 2009, *H. axyridis* and *C. septempunctata* adults were the only adult species observed in broad bean ($F_{4,160} = 0.7$, $p > 0.05$). *Coccinella septempunctata* was the most abundant species in 2010 ($F_{4,2415} = 22.1$, $p < 0.001$), while in 2011 both *C. septempunctata* and *C. carnea* s.l. were profusely observed ($F_{4,2480} = 37.5$, $p < 0.001$) ($p < 0.05$; LSD) (Table 4). In

2009, three species were present at the larval stage: *P. quatuordecimpunctata, C. carnea* s.l., and *E. balteatus* ($F_{4,160}$ = 1.0 , p = 0.43) (p < 0.05; LSD). In 2010 ($F_{4,2415}$ = 8.35, p < 0.001) and 2011 ($F_{4,2480}$ = 27.2, p < 0.001), all species were observed, and *C. septempunctata* was the most abundant (Table 5).

Table 6: ANOVA and ANCOVA summary of effect of aphid abundance and year sampling (2009, 2010, 2011) on five predators abundance at the adults stage in four crops (corn, wheat, potato and broad bean) (P values come from GLM *** P < 0.001, ** P < 0.01, * P < 0.05, ns = not significant P > 0.05) ([a]Analyze of ANOVA, [b] analyze of ANCOVA).

	[a]*Years*		[b]*Years/Aphids*		[b]*Aphids*	
Aphidophages	*F*	*P*	*F*	*P*	*F*	*P*
Corn						
H. axyridis	14.53	***	15.36	***	6.62	*
C. 7-punctata	5.21	**	6.40	**	12.35	***
P. 14-punctata	4.04	*	4.18	*	0.61	*ns*
E. balteatus	19.71	***	16.93	***	15.74	***
C. carnea s.l.	20.96	***	17.87	***	38.98	***
Wheat						
H. axyridis	5.78	**	5.62	**	0.23	*ns*
C. 7-punctata	32.52	***	36.18	***	5.40	*
P. 14-punctata	1.63	*ns*	1.54	*ns*	0.04	*ns*
E. balteatus	10.47	***	13.30	***	5.39	*
C. carnea s.l.	13.59	***	16.51	***	4.58	*
Potato						
H. axyridis	2.02	*n.s*	2.54	*ns*	6.81	**
C. 7-punctata	1.72	*n.s*	2.22	*ns*	9.49	**
P. 14-punctata	0.18	*n.s*	0.19	*ns*	0.01	*ns*
E. balteatus	0.86	*n.s*	0.71	*ns*	0.65	*ns*
C. carnea s.l.	10.62	***	9.63	***	23.04	***
Broad bean						
H. axyridis	0.41	*n.s*	0.20	*ns*	0.96	*ns*
C. 7-punctata	2.34	*n.s*	2.78	*ns*	0.65	*ns*
P. 14-punctata	0.98	*n.s*	0.82	*ns*	1.25	*ns*
E. balteatus	3.94	*	5.14	**	7.98	**
C. carnea s.l.	17.97	***	22.38	***	0.06	*ns*

Effect of aphid densities and sampling year on relative abundance of aphidophage

Abundances of *H. axyridis* in wheat and potatoes were not interpreted, due to very low numbers of individuals observed during the three-year inventory. Over the three-year sampling, adult populations of *H. axyridis* in corn significantly increased ($F_{2,1709} = 14.5$, $p < 0.001$) (Table 6) from 7.8 ± 2.0 in 2009 to 19.9 ± 1.8 individuals per 100 m² in 2011 (Table 4). Larval populations in the same crop also increased statistically ($F_{2,1709} = 39.9$, $p < 0.001$) (Table 6), rising from 11.9 ± 4.2 to 70.8 ± 6.6 larvae per 100 m² (Table 5). In broad bean, relative abundance of *H. axyridis* was not significantly different among the three years, neither at the adult (Table 6) nor larval (Table 7) stages.

Coccinella septempunctata larvae declined in broad bean ($F_{2,1011} = 4.7$, $p = 0.009$), wheat ($F_{2,1463} = 14.4$, $p < 0.001$), and corn ($F_{2,1709} = 31.9$, $p < 0.001$) (Table 7); densities decreased by 10 and 29.6 times in corn and broad bean respectively. In wehat, no larvae were observed in 2011, while 13.4 ± 3.9 larvae per 100 m² were observed in 2009.

The abundances of three other aphidophage showed variable changes (Tables 6 and 7).

Aphid populations and correlation with aphidophage densities

In 2009, 2010, and 2011, seven, nine, and 10 species of aphids were identified, respectively: the pea aphid, *Acyrthosiphon pisum* Scopoli (Hemiptera: Aphididae); the cowpea aphid, *Aphis craccivora* Koch; the black bean aphid, *Aphis fabae* Scopoli; *Aphis frangulae* Walker; the buckthorn aphid, *Aphis nasturtii* Kaltenbach; the potato aphid, *Macrosiphon euphorbiae* Thomas; the vetch aphid, *Megoura viciae* Buckton; the rose grain aphid, *Metopolophium dirhodum* Walker (Figure 1); the green peach aphid, *Mizus persicae* Sulzer; *Rhopalosiphum* sp.; and *Sitobion* sp. (Table 8). The mean number of observed aphids increased in corn ($F_{2,2589} = 39.$, $p < 0.001$) and potato ($F_{2,1410} = 17.11$, $p < 0.001$) from 2009 to 2011. Aphid densities also statistically varied in broad bean ($F_{2,974} = 8.7$, $p < 0.001$) and wheat ($F_{2,1392} = 102.7$, $p < 0.001$) from 2009 to 2011, but without any general evolution (Table 8).

Figure 1: *Metopolophium dirhodum* on wheat

The ANCOVA analyses showed a linear relationship between aphid and predator populations in 55% of adult populations (Table 6) and 35% of larvae populations (Table 7) ($p_{\text{aphids}} < 0.05$, ANCOVA). In these cases, aphid densities influenced the predator abundance. Results (p_{years}) comparison between ANOVA and ANCOVA showed that the influence of aphid populations on predator abundance variations between years was not statistically significant.; p_{years} of the two statistic analyses showed the same results.

Table 7: ANOVA and ANCOVA summary of effect of aphid abundance and year sampling (2009, 2010, 2011) on five predators abundance at the larvae stage in four crops (corn, wheat, potato and broad bean) (P values come from GLM *** P < 0.001, ** P < 0.01, * P < 0.05, ns = not significant) ([a]Analyze of ANOVA, [b] analyze of ANCOVA).

	[a]*Years*		[b]*Years/Aphids*		[b]*Aphids*	
Aphidophages	*F*	*P*	*F*	*P*	*F*	*P*
Corn						
H. axyridis	39.99	***	41.24	***	2.65	*ns*
C. 7-punctata	4.69	**	5.02	**	1.06	*ns*
P. 14-punctata	31.86	***	33.62	***	5.06	*
E. balteatus	9.82	***	8.28	***	7.11	**
C. carnea s.l.	9.72	***	9.67	***	0.14	*ns*
Wheat						
H. axyridis	1.30	*n.s*	1.19	*ns*	0.88	*ns*
C. 7-punctata	2.54	**	5.30	**	0.18	*ns*
P. 14-punctata	14.42	***	15.13	***	1.91	*ns*
E. balteatus	38.69	***	48.75	***	10.74	**
C. carnea s.l.	1.87	*n.s*	2.99	*ns*	2.30	*ns*
Potato						
H. axyridis	0.05	*n.s*	0.08	*ns*	0.77	*ns*
C. 7-punctata	0.61	*n.s*	0.54	*ns*	3.85	*
P. 14-punctata	1.11	*n.s*	1.11	*ns*	0.03	*ns*
E. balteatus	1.29	*n.s*	2.41	*ns*	29.79	***
C. carnea s.l.	0.28	*n.s*	0.21	*ns*	0.25	*ns*
Broad bean						
H. axyridis	1.72	*n.s*	1.59	*ns*	0.65	*ns*
C. 7-punctata	4.14	*	3.01	*	102.38	***
P. 14-punctata	4.69	**	4.33	*	9.77	**
E. balteatus	2.59	*n.s*	2.55	*ns*	0.01	*ns*
C. carnea s.l.	2.11	*n.s*	1.43	*ns*	2.69	*ns*

Discussion

Since the invasive coccinellid *H. axyridis* spread over Europe (Brown et al. 2008), imposing negative impacts on native aphidophage and affecting composition of several guilds (Soares et al. 2008; Roy et al. 2012), studies have evaluated the population spread of this coccinellid. The current sampling of aphid predators in Belgian agroecosystems from 2009 to 2011 showed that *H. axyridis* lives and reproduces more efficiently in corn and broad bean than in wheat and potato. In corn, the evenness during the three years decreased when *H. axyridis* population increased strongly and was higher than the population of other species.

During the three-year sampling, 11 aphidophage were observed in these agroecosystems, but five of them predominated: *E. balteatus*, *C. septempunctata, P. quatuordecimpunctata*, *H. axyridis*, and *C. carnea* s.l. Five dominant species in agroecosystems is a common observation (Hodek and Honěk 1996). Observations on predator densities highlight that *H. axyridis* was not the numerically dominant species in every crop: in wheat and potato, *C. septempunctata* was more abundant than *H. axyridis*. In many European agricultural crops, both *C. septempunctata* and *P. quatuordecimpunctata* were dominant prior to the arrival of *H. axyridis* (Honěk 1979; Bode 1980; Chambers et al. 1982), and it appears that these two species have maintained their dominance in spite of being prone to intraguild predation by *H. axyridis* in the field (Hautier et al. 2008).

In our study, larvae of *E. balteatus* were the most abundant observed predators in wheat, which has already been reported by Tenhumberg and Poehling (1995) prior to the arrival of *H. axyridis*. *Episyrphus balteatus* has also been previously reported as one of the most abundant aphidophage in vegetable crops, such as broad beans (Colignon et al. 2001; Colignon et al. 2002). This could be explained by abiotic conditions (high density cereal crop, with high humidity and low temperature) that are more favorable to the larvae of *E. balteatus* (Honěk 1983).

The fact that *H. axyridis* is not the most abundant aphidophage in agrosystems is probably due to its generalist behavior and arboreal habitat selection (Hodek 1973; Chapin and Brou 1991; LaMana and Miller 1996; Brown and Miller 1998; Labrie 2007). However, it has been reported that *H. axyridis* can also thrive in agrosystems such as wheat, corn, and potato (LaMana and Miller 1996; Buntin and Bouton 1997; Colunga-Garcia and Gage 1998; Michaud 2002; Brown 2003; Nault and Kennedy 2003; Snyder et al. 2004; Jansen and Hautier

2008), as well as in herbaceous habitats (LaMana and Miller 1996; Koch et al. 2006; Alhmedi et al. 2007).

There were evident obvious changes in the abundance of aphidophages in crops through the years, but this study does not propose to identify a global evolution (increase or decline) in any of the species that were observed in this study. The causes of such fluctuations are diverse and may include factors such as the diversity and abundance of aphid species (Wright and Laing 1980; Honěk 1982; Thalji 2006). The results of the ANCOVA showed that there was a linear relationship between prey and predator populations, but additional biotic and abiotic factors contribute to the annual variability of predator abundance. Climate could be one such factor, due to its influence on natural enemies, overwintering mortality, and aphid populations (Hodek and Honěk 1996; Szentkirályi 2001; Rotheray and Gilbert 2011). Several other factors could also explain the variation between crops: insolation, humidity (Honěk 1985), quantity and quality of host plants (Alhmedi et al. 2009), and adjacent habitats (Colignon et al. 2001; Alhmedi et al. 2009).

A particularly interesting finding is that although *H. axyridis* breeding occurred in all four inventoried crops to some extent, adults of this species are not ubiquitous; few immature individuals were recovered from potato and wheat. Assuming that declines in native species are caused by *H. axyridis* (Roy et al. 2012), this suggests that certain crops, such as wheat and potato, could act as refuges from *H. axyridis* at certain times, while native species, such as *E. balteatus* and *C. septempunctata*, are able to breed with a lower risk of intraguild predation or other forms of competition from the invaders. Such habitats could become even more important as native species adapt to the invader by evolving to avoid habitats where *H. axyridis* occurs in high numbers, as has been seen in co-occurring aphidophages in their native habitats (Sloggett 2012).

In conclusion, our study indicates that *H. axyridis* was not the most frequently observed aphidophage in the four most important Belgian agronomical crops. In future studies, longer samplings would be preferable in order to eventually identify quantitative changes in the native fauna, as suggested in other studies. Agroecosystems may even constitute an ecological reservoir for certain native aphidophages.

Table 8: Mean numbers and SE of aphids/100 m² observed in four fields (wheat, broad bean, corn, potato) from 2009 to 2011.

		Mean number of aphids per 100 m²												
		A. pisum	*A. craccivora*	*A. fabae*	*A. frangulae*	*A. nasturtii*	*M. euphorbiae*	*M. viciae*	*M. dirhodum*	*M. persicae*	*Rhopalosiphum sp.*	*Sitobion sp.*	TOTAL	
Wheat	2009			3.4 ± 1.9					2892.2 ± 272.0			18.8 ± 12.4	2914.3 ± 272.1	a
	2010								244.1 ± 38.8			11.3 ± 5.2	255.4 ± 0.4	b
	2011								3491.5 ± 284.9			43.6 ± 14.1	3535.0 ± 287.2	a
Broad bean	2009	42.9 ± 22.2		845.7 ± 337.7				305.7 ± 217.6					1194.3 ± 381.2	a
	2010	454.6 ± 77.2		39.2 ± 31.8					6.8 ± 6.2				500.6 ± 0.8	b
	2011	303.6 ± 43.4		4325.7 ± 1859.5				8.4 ± 4.6					4637.7 ± 1859.2	a
Corn	2009			56.0 ± 16.4					698.6 ± 188.7		45.9 ± 45.8	2.3 ± 2.3	802.8 ± 192.9	b
	2010								2045.2 ± 230.4		300.9 ± 119.2	118.0 ± 14.3	2464.1 ± 2.6	b
	2011								12366.8 ± 1073.4		49.0 ± 16.1	278.9 ± 51.2	12694.7 ± 1098.1	a
Potato	2009			5.7 ± 2.6			1.0 ± 0.9	2.9 ± 1.6					9.5 ± 3.2	c
	2010			10.8 ± 2.9	0.1 ± 0.1	1.9 ± 1.1	14.6 ± 7.7			228.1 ± 62.4			255.6 ± 0.7	b
	2011	0.6 ± 0.4	259.1 ± 33.9	3.1 ± 0.8		458.0 ± 78.3	3.7 ± 1.4			15.4 ± 4.2			739.8 ± 92.1	a

Acknowledgments

We thank V. Sibret and A.M. Buset for their technical assistance, Dr Y. Brostaux for his advice on statistical analysis, and P. Leroy and C. De Clerck for their helpful comments on previous versions of the manuscript. The authors are grateful to Pr B. Bodson and F. Vancutsem of the Unité de Phytotechnie des Régions Tempérées (ULg, GxABT) for the availability of fields. This research was funded by the Service Public de Wallonie (SPW – DGO3, project n°D31-1197). Delphine Durieux was financially supported by a PhD grant from the Fonds pour la formation à la Recherche dans l'Industrie et l'Agriculture (FRIA), Belgium.

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V.4 Occurrence of *Harmonia axyridis* (Coleoptera: Coccinellidae) in field crops

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Reference - Vandereycken A., Brostaux Y., Joie E., Haubruge E., Verheggen F.J. 2013. Occurrence of *Harmonia axyridis* (Coleoptera: Coccinellidae) in field crops. *European Journal of Entomology* 110(2): 285-292

Abstract - The Multicoloured Asian Ladybird, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) is known to thrive principally in shrubby and arboreal habitats. This study focuses on the occurrence of this exotic species and its seasonal abundance in various field crops. The abundance of adults, larvae and pupae of *H. axyridis* was evaluated over a three-year period, from 2009 to 2011, in four important agronomical crops (wheat, corn, broad bean and potato) in Belgium. From May to September, 48 1m² quadrats were visually inspected in each of the fields sampled on several farms every seven days. *H. axyridis* colonized and reproduced in all of the four crops studied, with the largest numbers recorded in corn and broad bean crops. Larvae and adults of *H. axyridis* were recorded mainly in corn and to a much less extent in wheat and potato crops. From 2009 to 2011, the mean weekly abundance of *H. ayxridis* remained constant except in corn crops, where the recorded densities of all the immature stages and adults were higher in 2011 than in 2009. The population dynamics of aphids and *H. axyridis* are well described by a symmetric logistic function (S-shape) of cumulative population size. *H. axyridis* was not always recorded where aphids were abundant, e.g. aphids were abundant on wheat where no *H. axyridis* were recorded. *H. axyridis* start reproducing after the peak in aphid population, suggesting that *H. axyridis* is able to complete its development by feeding on alternative prey such as larvae and pupae of the same and other species of ladybird and other aphidophagous species. *H. axyridis* is often considered to be

bivoltine but it only completes one generation per year in field crops. The second generation generally develops late in the season in other habitats.

Keywords - *Harmonia axyridis*, habitat, crops, agroecosystems, ladybird, exotic species.

Introduction

At the end of the 20th century, the Multicoloured Asian ladybird *Harmonia axyridis* Pallas (Coleoptera; Coccinellidae), a native species of Asia, was introduced into North America and Europe as a biological control agent of aphids and coccids (Adriaens et al. 2008, Brown et al. 2008a, Brown et al. 2011) because its larvae are both voracious and polyphagous (Ferran & Dixon 1993). It has successfully invaded 26 European, nine American and three African countries (Brown et al. 2011). In Belgium, *H. axyridis* was first observed in field crops (potatoes) in 2003 (Jansen & Warnier 2004). *H. axyridis* is often considered to be semi-arboreal (Hodek 1973) but it has become ubiquitous in many parts of the world including America, Europe and Africa (Brown et al. 2011) where it occurs in agricultural areas (Colunga-Garcia & Gage 1998, Lucas et al. 2007), riparian zones (Adriaens et al. 2008) and orchards (Michaud 2002, Burgio et al. 2008).

H. axyridis has been introduced as a biological control agent of various pests on crops such as pecan (Tedders & Schaefer 1994), red pine (McClure 1987), apple (Brown & Miller 1998), soybeans, sweet corn (Musser & Shelton 2003), alfalfa (Buntin & Bouton 1997, Colunga-Garcia & Gage 1998), cotton (Wells et al. 2001), tobacco (Wells & McPherson 1999) and winter wheat (Colunga-Garcia & Gage 1998). Its great dispersal ability has enabled it to rapidly colonize large areas (Tedders & Schaefer 1994, Koch 2003).

In Belgium, *H. axyridis* is one of the five most abundant aphidophagous species in field crops such as corn, wheat, potato and broad bean. There is relatively little information on its phenology as it has only been studied in a few field crops in Belgium, e.g. potato (Jansen & Hautier 2008), green pea and wheat, and in stands of stinging nettle (Alhmedi et al. 2009).

H. axyridis has the ability to colonize new habitats and is a very competitive species, being frequently reported as a predator of native species (Phoofolo & Obrycki 1998, Sloggett et al. 2009, Wells et al. 2010, Ingels & De Clercq 2011). With the increase in the abundance of *H. axyridis* there have been reports of a decline in the abundance of native aphidophagous ladybirds (*Adalia bipunctata* Linné) (Ottart 2005, Roy et al. 2012).

Predators and prey species are naturally synchronised (Tenhumberg & Poehling 1995) but field observations indicate that *H. axyridis* arrive later than other ladybirds and the peak in the number of its larvae occurs after the aphid peak (Jansen & Hautier 2008).

There are many European (Adriaens et al. 2008) and American (Colunga-Garcia & Gage 1998) studies on the ecology of *H. axyridis*, but its colonization of field crops is poorly understood. In this study, we quantified and compared the abundance of *H. axyridis* in four important crops: wheat, corn, potato and broad bean, over a period of three-years. We further studied the changes in abundance of each developmental stage during one year in the different habitats. A phenological model was used to describe population growth in the year 2011. Finally, we discuss the potential effect of *H. axyridis* on native species during periods when food is scarce.

Material and methods

Field crops sampled and sampling method

Populations of *H. axyridis* were sampled from 2009 to 2011 in a Belgian agricultural production area called Hesbaye. In this area, nine fields of four crops were chosen for their agronomic importance: wheat *Triticum aestivum* (L.); corn *Zea mays* (L.); potato *Solanum tuberosum*; (L.) and broad bean *Vicia faba* (L.). All crop fields received conventional treatments to control pests. The sampling period for wheat and broad bean was from mid-May to late August, and for corn and potato from mid-May to late September.

The method used to monitor and assess the numbers of predators and aphids consisted of visual whole-plant inspections of the plants in forty-eight 1m² quadrats per crop. The number of quadrats sampled in a field was a function of the area of the field. The quadrats were distributed randomly throughout the fields. In order to limit the influence of other nearby crops quadrats were not placed within 20 meters of the border of each field. All quadrats were examined every week. Visual sampling was conducted as it provides an easy and accurate method of estimating the numbers of larvae and adults of coccinellids in field crops (Michels & Behle 1992). Quadrats were located along transect lines across each field and spaced 20 meters apart. All leaves and stems within the quadrat were examined and all aphids and aphidophagous insects were recorded. Eggs were counted individually rather than per clutch. Eggs, first instar larvae and pupae were brought back in order to let them develop under laboratory conditions (T = 24 ± 1°C; HR= 75 ± 5%) for identification to species level. Aphid species were also determined and their population densities evaluated: aphids were counted on all leaves and stems at the same time as the aphidophagous insects were sampled.

Statistical analyses

The mean numbers of *H. axyridis* per crop and per period of observation were calculated per quadrat. As mean densities recorded per $1m^2$ were low, values per 100 m^2 are presented. This mean number was also determined within each crop for each season, and analysed using an Analysis of Variance (ANOVA) with crop (q = 4) and years (n = 3) as factors ($\alpha = 0.05$). Mean numbers of aphid predators were compared by Least Square Differences (LSD). To explain variations in predator abundances, the mean abundance per species was then analysed using Analysis of Covariance (ANCOVA: General Linear Models) with years (n = 3) as factors ($\alpha = 0.05$) and aphid densities as a co-variable. Ryan-Joiner test was used to assess the population normality. The distribution of data (counting) was asymmetric and had to be $\log_{10}(x+1)$ transformed before analysis. Although statistical analyses were performed on the transformed data, the untransformed data are presented in Fig. 1, Fig. 2, Fig. 3, Fig. 4 and Fig. 5. Statistical analyses were carried out using Minitab® 15.1.30.0 (State College, Pennsylvania, USA).

Phenological model

Prey and predator species specialized in the exploitation of ephemeral resources can be modelled using Pearl-Verhulst models (Matis et al. 2009). From this model, logistic and Gompertz curves are commonly used to describe phenology and population growth in fish, birds and mammals (López et al. 2000, Darmani Kuhi et al. 2003, Phoofolo et al. 2009). The phenological observations are distributed following roughly bell-shaped curves. Instead of adjusting these models, we chose to work with cumulative population sizes and then integrated distributional functions, which are easier and more robust to fit. The resulting sigmoid functions (S-shapes) provide population parameters corresponding to charateristic phenological points, e.g. the inflection point, which gives the maximum specific population peak (Debouche 1979). The parameters permit a description and comparison over time of population phenology.

For 2011, we described the *H. axyridis* and aphid cumulative population sizes using logistic and Gompertz growth curves (Debouche 1979). These two equations are composed of three mathematical constants: M, maximum cumulated densities; a, abscise of the inflection point; and b, measure of population spread (Table 1). The choice of the best adjusted model was based on the lowest residual sum of square (RSS) (e.g. Zwietering et al. 1990). Factors used to compare growth trajectories are coordinates: cumulative temperature (Temperatures were measured each hour and a mean daily temperature was calculated. The cumulative temperature is the sum of these daily temperatures.); cumulative densities, values of maximum growth rate (Vmax) and mean growth rate (Vm) and time (T) (Winsor 1932). The temperatures were first recorded on May 17th, 2011.

Table 1: Logistic and Gompertz equations with their parameters (See text for explanation of parameters M, a and b).

Model	Equation	Growth rate		Coordinate of inflection point		T
		Max	Mean	x	y	
Logistic	$y = \frac{M}{1 + e^{-\left(\frac{x-a}{b}\right)}}$	$\frac{M}{4b}$	$\frac{M}{6b}$	a	$\frac{M}{2}$	6b
Gompertz	$y = Me^{-e^{-\left(\frac{x-a}{b}\right)}}$	$\frac{M}{eb}$	$\frac{M}{4b}$	a	$\frac{M}{e}$	4b

Results

Population changes and habitat preferences

Very few individuals of *H. axyridis* were recorded in wheat and potato, but some larvae were recorded indicating that reproduction occurred in these crops (Fig. 1and Fig. 2). However, there are too few data to support statistical analyses such as ANOVA and LSD. Corn and broad bean were the two crops with the most *H. axyridis*. Over the three years of the study more larvae of *H. axyridis* were recorded on broad bean than corn (Table 2). In 2009, no *H.axyridis* were recorded on broad bean (t_{217} = 2.85; pvalue=0.0024). In corn crops, the numbers of larvae varied significantly from 2009 to 2011 ($F_{2,1709}$ = 39.99, P < 0.001) with an increase in 2011 (70.8 ± 6.6 larvae per 100 m^2) compared to 2010 (13.3 ± 2.8 larvae per 100 m^2) (P < 0.05; LSD) (Fig. 1). Similarly in 2011 there were more adults in corn than broad bean crops (Table 2). The density in 2011 was 19.98 ± 1.80 individuals per 100 m^2, which is significantly higher than the 7.8 ± 2.0 and 3.9 ± 0.9, respectively, recorded in 2009 and 2010 ($F_{2,1709}$ = 14.53, P < 0.001) (Fig. 2).

Table 2: Summary of the results of the ANOVA of the density of *H. axyridis* recorded in corn and broad bean crops over a period of three years (2009, 2010 and 2011). The Crop column records the crop where *H.axyridis* was the most abundant (C: corn). (P values obtained using the GLM ns: P > 0.05, * P<0.05, ***: P<0.001).

		F		P	Crops
Larvae					
	2009	1.43	(1,245)	ns	
	2010	4.43	(1,1168)	*	C
	2011	44.54	(1,1307)	**	C
Adults					
	2009	0.11	(1,245)	ns	
	2010	0.15	(1,1168)	ns	
	2011	16.74	(1,1307)	***	C

The ANCOVA analyses highlighted two things: (1) a linear relationship between abundance of aphids and number of adults of *H. axyridis* recorded on corn ($P_{aphids}<0.05$, ANCOVA) and (2) annual changes in the abundance of larvae ($F_{2,1708} = 41.24$; $P_{Years/aphids} < 0.001$) and adult predators on corn ($F_{2,1708} = 15.36$; $P_{Years/aphids} < 0.001$). The linear relation between the abundance of aphids and *H. axyridis* indicates that on average the higher the abundance of aphids the more *H. axyridis* are recorded. The exact relation can be more complex, with for example a shift or a nonlinear tendency.

The phenology of *H. axyridis* was studied only in corn (Fig. 3) and broad bean crops (Fig. 4), where sufficient numbers of this ladybird were recorded (with densities higher than 5 individuals per 100 m^2).

In corn, depending on the year, adults appeared between mid-June and early July and there were two peaks in abundance, the first in July and the second in August. Adults were recorded up to the end of August in 2010 and 2011 and mid-September in 2009. Larvae were recorded from end of June to early September, except in 2009 when no larvae were recorded after mid-August. There was one peak in the numbers of larvae, which was recorded each year at the beginning of July. Depending on the year, adult emergence occurred from mid-August until early September.

Phenology

In broad bean crops, adults were recorded between June 1 and June 23, depending on the year (Fig. 4). There were two peaks in the numbers of adults, the first at the end of June and second at the end of July. Larvae were recorded in mid-June in 2011 (Fig. 4C) and mid-

July in 2010 (Fig. 4,B). The larvae were recorded over a period of two weeks in 2010 and five weeks in 2011.

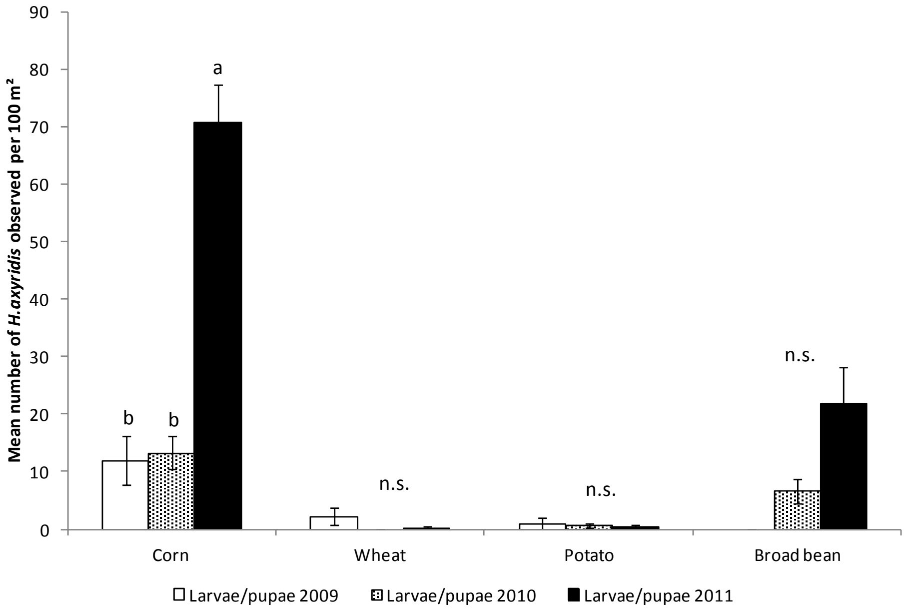

Figure 1: Mean number (±SE) of *H.axyridis* larvae recorded in corn, wheat, potato and broad bean crops in 2009, 2010 and 2011. Means for a crop followed by the same letter do not differ significantly, P> 0.05, LSD test n.s.= not significant.

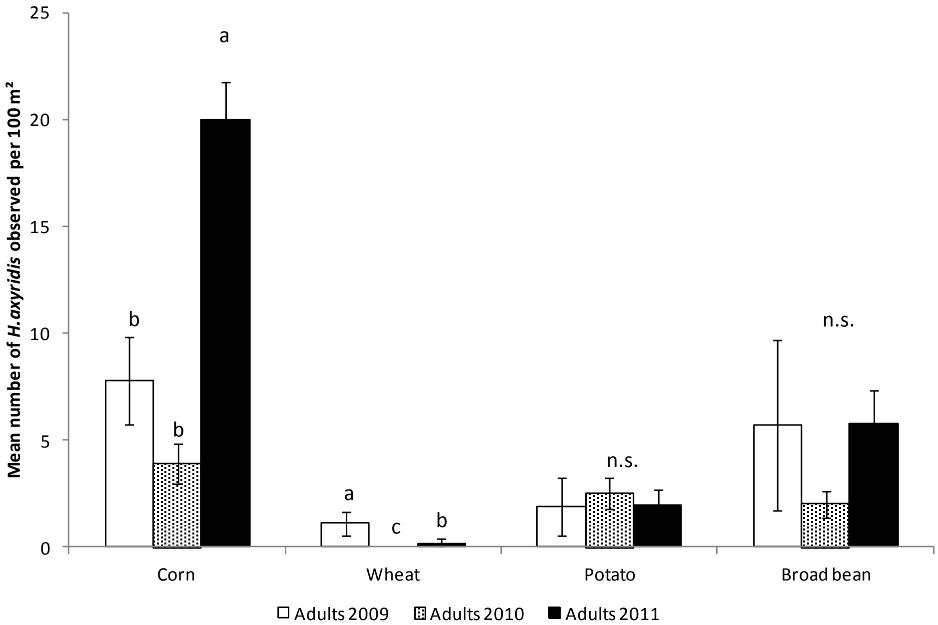

Figure 2: Mean number (±SE) of adult *H.axyridis* recorded in corn, wheat, potato and broad bean crops in 2009, 2010 and 2011. Means for a crop followed by the same letter do not differ significantly, P> 0.05, LSD test; n.s.= not significant.

Aphids were recorded earlier on corn in 2011 (18^{th} May) (Fig. 3C) than 2009 (27th June) (Fig. 3A) and 2010 (16th June) (Fig. 3B). There was a decrease in aphid numbers and an increase in the numbers of larvae at the end of June. Similar trends were recorded on broad bean crops but aphids were recorded earlier on beans in 2011 (before 19^{th} May) (Fig. 4C). The numbers of aphids started to decrease in June before *H. axyridis* started reproducing (Fig. 4B,C).

Phenological model

The change in the numbers of both aphids and predators on broad bean and corn was linked to cumulative temperature. Two models (logistic and Gompertz) were compared using the values of their residual sum of squares (RSS). Both of these models fit the data well as their RSSs are low. Nevertheless, we decided to use the logistic model to analyze the growth trajectory because it has the lowest RSS for the aphid and predator data (Table 3). The logistic curves are sigmoidal in shape and the inflection point occurs earlier in the data recorded for aphids, larvae and adults of *H. axyridis* on broad bean than on corn (Table 4 and Fig. 5). This reflects the earlier development of both prey and predator on broad bean than on corn. The peak larval populations were recorded at 426 and 466 degree-days after those of aphids on broad bean and corn, respectively. The peak larval population growth rate recorded on corn was 0.052 and twice the 0.025 predators/m^2/degree-day recorded on broad bean. The length of larval activities was similar in the two cultures despite differences in length of aphid activities, which were two times higher in broad bean than in corn (Table 4).

Discussion

Over a period of three years the numbers of *H. axyridis* in four crops (broad bean, corn, wheat and potato) in the southern part of Belgium were recorded. Our data show that this exotic ladybird regularly occurs in these field crops. Previous studies on *H. axyridis* indicate that this species is more frequently found in urban and arboreal habitats (65.6%) than on herbaceous plants (34.4%) (LaMana & Miller 1996, Adriaens et al. 2008). In addition to arboreal habitats, it is also recorded in field crops such as wheat, corn and potato (LaMana & Miller 1996, Buntin & Bouton 1997, Colunga-Garcia & Gage 1998, Michaud 2002, Brown 2003, Nault & Kennedy 2003, Snyder et al. 2004, Jansen & Hautier 2008), and also in various natural or semi-natural herbaceous habitats, such as nettle beds, clover or peppermint (LaMana & Miller 1996, Koch et al. 2006, Alhmedi et al. 2007).

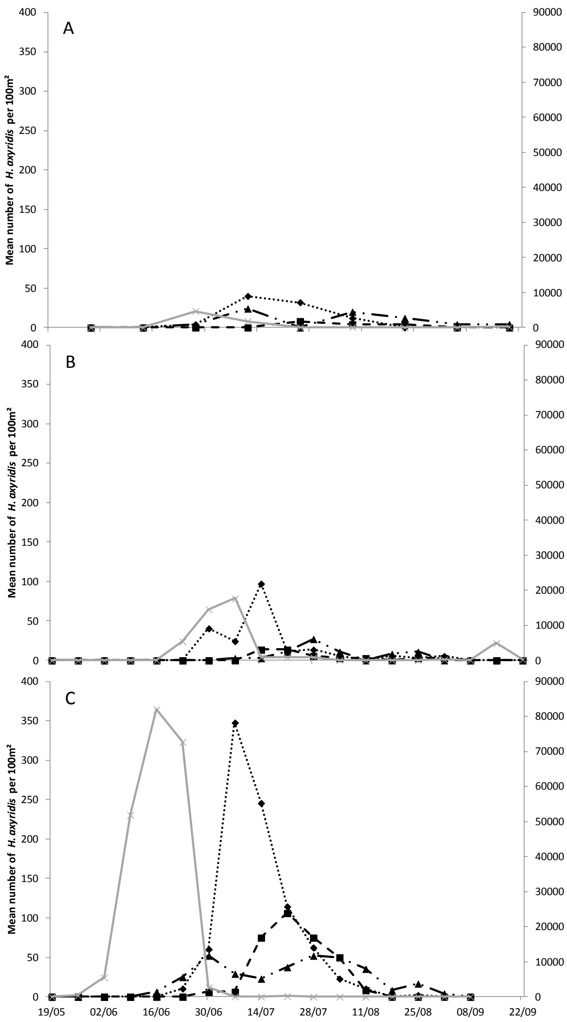

Figure 3: Trends in time in the numbers of *H. axyridis* recorded in corn crops in 2009 (A), 2010 (B) and 2011 (C) (grey plain line: aphids, dotted: larvae, dashed: pupae, dotted/dashed: adults).

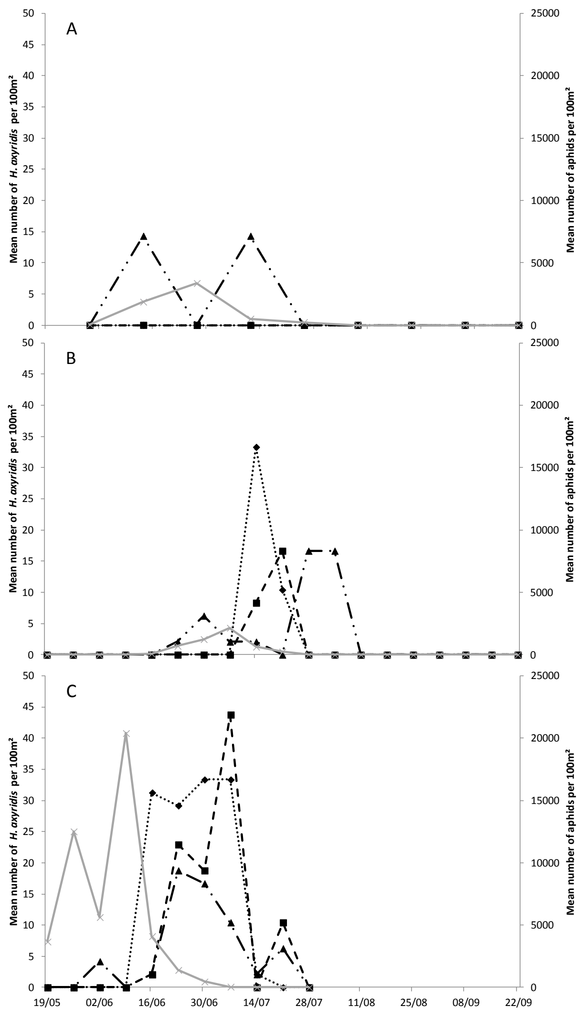

Figure 4: Trends in time in the numbers of *H. axyridis* recorded in broad bean crops in 2009 (A), 2010 (B) and 2011 (C) (grey plain line: aphids, dotted: larvae, dashed: pupae, dotted/dashed: adults).

H. axyridis larvae and adults were recorded in all of the four crops sampled but were less prevalent in potato and wheat crops, where less than one individual per 100 m² were recorded. Their reproduction in these two crops was limited and did not vary during the three years of this study. Other studies have shown that they tend to reproduce where aphids are abundant (Evans & Gunther 2005, Jansen & Hautier 2008). However, even when aphids were present in significant numbers on wheat, we recorded very few *H. axyridis*. The use of insecticides to control aphid populations on wheat is unlikely to be the cause of the low numbers of *H. axyridis* because after the insecticide application there were many individuals of other species. It is likely that the abiotic conditions in wheat, associated with high plant density (low brightness, high humidity), are not suitable for *H. axyridis*. The numbers of *H. axyridis* recorded on potato were also low, which was possibly due to the low availability of food on potato and some of the plant's characteristics. Indeed, the distribution of predators and prey and their interactions can be influenced by the trichomes on potato plants (Lucas 2005). Other factors may account for the choice of habitat by *H. axyridis*: abiotic ones such as landscape structure (Gardiner et al. 2009), insolation and humidity (Honěk 1985), quantity and quality of host plants (Alhmedi et al. 2009), aphid species and their abundance (Wright & Laing 1980, Honěk 1982, Thalji 2006) and adjacent habitats (Colignon et al. 2001, Alhmedi et al. 2009).

Table 3: Fit and RSS values of the phenological model of *H.axyridis* abundance recorded in broad bean and corn crops.

	Larvae		Adults	
	Gompertz	Logistic	Gompertz	Logistic
Broad bean	$y = 9.08e^{-=e^{-\left(\frac{x-693.77}{132.72}\right)}}$	$y = \frac{9.08}{1+e^{-\left(\frac{x-758.47}{90.51}\right)}}$	$y = 2.23e^{-e^{-\left(\frac{x-679.16}{128.38}\right)}}$	$y = \frac{2.23}{1+e^{-\left(\frac{x-736.44}{93.42}\right)}}$
	RSS: 1.39	RSS: 0.76	RSS: 0.05	RSS: 0.06
Corn	$y = 20.20e^{-e^{-\left(\frac{x-957.19}{138.23}\right)}}$	$y = \frac{20.20}{1+e^{-\left(\frac{x-1020.98}{97.07}\right)}}$	$y = 8.71e^{-e^{-\left(\frac{x-938.03}{274.95}\right)}}$	$y = \frac{8.71}{1+e^{-\left(\frac{x-1069.60}{187.40}\right)}}$
	RSS: 0.83	RSS: 0.93	RSS: 1.46	RSS: 1.06

Table 4: The phelonogical factors determining the trajectories of *H. axyridis* and aphid abundance recorded in broad bean and corn crops. Logistic model was used to determine these data.

	Broad bean			Corn		
	Larvae	Adults	Aphids	Larvae	Adults	Aphids
a	758,47	736,44	332,62	1020,98	1069,60	554,90
b	90,51	93,42	100,29	97,07	187,40	52,72
M	9,08	2,23	1895,21	20,20	8,71	5917,17
Vmax coordinates	(758.47, 4.54)	(736.44, 1.12)	(332.62, 947.61)	(1020.9, 10.1)	(1069.6, 4.35)	(554.89, 2958.58)
Vmax (aphidophages/m²)	0,025	0,006	4,724	0,052	0,012	28,059
Vm (aphidophages/m²)	0,017	0,004	3,150	0,035	0,008	18,706
T (degree-day)	543,06	560,52	601,74	582,40	1124,40	316,33

In this study the species of aphids on the different crops were not the same. There were four species on wheat and broad bean, five and eight on corn and potato, respectively (Table 5). Nevertheless, it is not possible to draw any conclusions about the influence of these differences on the habitat preferences of *H. axyridis* as in our study and that of Alhmedi et al. (2007) no *H. axyridis* were recorded on wheat, a crop heavily infested with aphids whereas *H. axyridis* colonizes and reproduces in potato crops that are not infested with aphids (Nault & Kennedy 2003). Other studies record *H. axyridis* in the same crops as we studied but at lower densities: 0.02, 0.03 and 0.01 individuals per m² in wheat, potato and corn crops, respectively (Nault & Kennedy 2003).

Recording the numbers of *H. axyridis* over long periods of time is of interest if we want to confirm that this species is continuing to increase in abundance. Studies on habitats other than crops indicate that since 2001 the numbers of *H. axyridis* in Belgium have increased (Adriaens et al. 2008). The variations in annual abundance of larvae in corn crops revealed by the ANCOVA analysis seem to be firstly linked to aphid abundance: larval numbers reflect number of eggs laid, which could reflect aphid abundance when adults are present early in the year. Secondly, larval abundance could also be due to a natural increase in the number of individuals in the landscape with continuing reproduction from year to year. Annual changes in *H. axyridis* abundance are also recorded in field crops in Quebec, where the percentage of *H. axyridis* was 55.4% in 2002 and 16.7% in 2003 (Lucas et al. 2007) and absent in potato crops in 2006, which followed a year when this species was abundant on this crop.

Table 5: Aphid species diversity recorded in the four crops sampled.

	Wheat	Corn	Potato	Broad bean
Acyrthosyphon pisum Harris			X	X
Aphis craccivora Koch			X	
Aphis fabae Scopoli	X	X	X	X
Aphis frangulae Kaltenbach			X	
Aphis nasturtii Kaltenbach			X	
Macrosiphum euphorbiae Thomas			X	
Megoura viciae Buckton			X	X
Metopolophium dirhodum Walker	X	X		X
Myzus persicae Sulzer			X	
*Rhopalosiphum maidis*Fitch		X		
Rhopalosiphum padi L.		X		
*Sitobion avanae*Fabricius	X			
Sitobion fragariae Walker	X	X		

Regarding the phenology of the occurrence of adults of *H. axyridis* in corn and broad bean crops there are two peaks in abundance between their arrival in June and departure in

September. The first peak consists of individuals that emerged from overwintering sites and were searching for food. The second peak consists of individuals that are the offspring of the overwintering adults. That is in the crops sampled *H. axyridis* only completed a single generation per year. However, in Europe, *H. axyridis* is known to be multivoltine, with two generations per year in Great Britain (Brown et al. 2008b) and France (Ongagna et al. 1993) and up to four generations in Greece (Katsoyannos et al. 1997). It is likely that Belgian *H. axyridis* are bivoltine as there two peaks in the abundance of larvae, one in July and one in October (Adriaens et al. 2008). The second peak in the numbers of larvae is not recorded in field crops because the growing season only lasts until September.

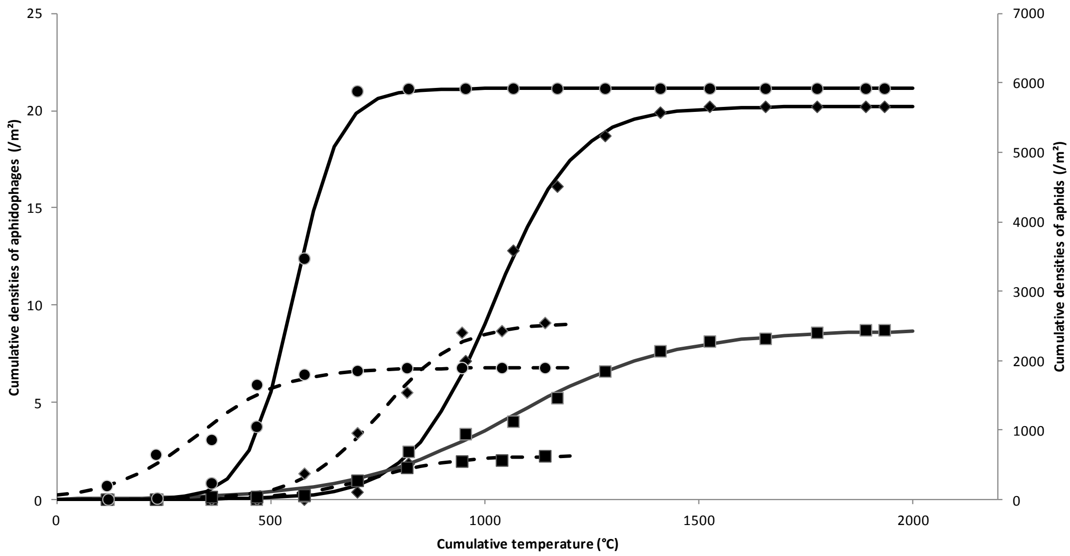

Figure 5: Relationships between cumulative temperature (°C) and cumulative abundance of larvae and adults of *H. axyridis* and aphids recorded in broad bean and corn crops. (line: corn, dote: broad bean, circles: aphids, squares: *H.axyridis* adult, diamonds: *H. axyridis* larvae).

The first peak in the numbers of larvae occurred 21 days after the aphid population peaked. That is, the numbers of *H. axyridis* were not synchronised with aphid abundance, as previously reported by Jansen & Hautier (2008) in potato crops. *H. axyridis* larvae are able to reach the adult stage in the absence of aphids by feeding on alternative prey. The alternative food can be pollen (Berkvens et al. 2008) or other aphidophagous predators such as *C. septempunctata, A. bipunctata*, *P. quatuordecimpunctata* or *E. balteatus* (Phoofolo & Obrycki 1998, Wells et al. 2010, Hautier et al. 2011, Ingels & De Clercq 2011). We observed *H. axyridis* feeding on syrphid and coccinellid pupae, and Colorado beetle larvae in potato crops.

H. axyridis adults arrived in and left broad bean crops earlier than corn crops. The difference in its phenology in these two crops might be due, among other aspects, to differences in the phenology of these two crops and characteristics of the surrounding environment (Colignon et al. 2001, Alhmedi et al. 2009). It is likely that the *H. axyridis* adults that leave broad bean early (20/7) colonize other crops such as corn. Indeed, *H. axyridis* is a good flyer (Hodek et al. 1993), with a high dispersal capacity (With et al. 2002).

Logistic and Gompertz curves adequately describe the changes in the numbers of aphids and aphidophagous predators in corn and broad bean crops. These equations are based on the Pearl-Verhulst logistic equation, widely used to model density dependent population growth (Matis et al. 2009). Despite the fact that the RSS value of the fit of the Gompertz model to the numbers of adult *H. axyridis* on broad bean and of larvae on corn was lower than the value for the logistic model we used the later for two reasons: (1) the RSS values of the fit of the logistic model were not different from those of the Gompertz model and (2) the rapid decrease in aphid abundance implies a rapid increase in the abundance predatory larvae. This growth trajectory corresponds to a symmetric population characterised by logistic curves.

H. axyridis abundance and phenology in the field crops sampled reveal that (1) *H. axyridis* is able to complete its development in corn and broad bean crops; (2) its phenology in corn and in broad bean crops differ and it is likely this is due to several factors including crop phenology and nature of the surrounding habitats; (3) larvae of *H. axyridis* can complete their development even when aphids are scarce, which indicates its larvae are able to feed on alternative prey such as other aphidophagous predators or pollen. This study appears to strengthen the hypothesis that *H. axyridis* can also inhabit field crops.

Acknowledgements

We thank V. Sibret and A.M. Buset for their technical assistance and D. Durieux for her helpful comments on previous versions of the manuscript. The authors are grateful to Pr B. Bodson and F. Vancutsem of the "Unité de Phytotechnie des Régions Tempérées" (ULg, GxABT) for allowing us to sample the fields included in this study. This research was funded by the "Service Public de Wallonie" (SPW – DGO3, project n°D31-1197 and D31-1247).

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Chapter VI : Crop farming influence on the abundance of *Harmonia axyridis*

VI.1 General introduction to chapter VI

The previous chapter of this thesis focused on the repartition of aphid predator species through four crops in Belgium. The high level of occurrence of *H. axyridis* observed in two crop fields: broad bean and corn, could generate some negative impacts on native species, owing to its agressive behaviour.

This chapter is based on the hypothesis that organic farming increases aphid natural enemies biodiversity, which could affect negatively the relative abundance of *H. axyridis* (Hole et al. 2005). Organic farming actions include compost and crop residues, minimum tillage, reduction of chemical pesticides, as well as hedgerow structures (Kromp 1999, Lampkin 2000, Hole et al. 2005).

This chapter is dedicated to the comparison of aphid predator communities between field crops using conventional farming and field crops using organic farming. Samplings focused on aphid predators were realised in 2010 and 2011 and species abundance of both larvae and adults were studied in broad bean and corn.

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VI.2 Occurrence of aphid predator species in both organic and conventional corn and broad bean

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Reference - Vandereycken A., Joie E., Françis F., Haubruge E., Verheggen F.J. 2013. Occurrence of aphid predator species in both organic and conventional corn and broad bean. *Entomologie Faunistique-Faunistic Entomology* 66: 77-87.

Keywords - Multi-coloured Asian ladybird, *Harmonia axyridis*, aphids, invasive species, interspecific interactions, biological control, Coccinellidae

Abstract - Organic farming has been suggested to enhance beneficial species abundance and diversity in agrosystem habitats. In this study, the abundance of aphid predators was compared in organic and conventional corn and broad bean fields during a two-year inventory. In both farming strategies, there were no differences between species diversity. Five aphid predator species were mainly observed: *Coccinella septempunctata* L. 1758 (Coleoptera: Coccinellidae), *Propylea quatuordecimpunctata* (L. 1758) (Coleoptera: Coccinellidae), *Harmonia axyridis* Pallas 1773 (Coleoptera: Coccinellidae), *Chrysoperla carnea* (Stephens 1836) (Neuroptera: Chrysopidae) and *Episyrphus balteatus* (De Geer 1776) (Diptera: Syrphidae). The abundance of the five above-mentioned aphidophagous species varied for the most part according to almost all the observed parameters, including sampled year, crop and agricultural practices. Differences in abundance of all aphid predators between conventional and organic crop fields were observed in broad bean. In conclusion, our findings do organic practices in broad bean as key options to increase the abundance of aphid natural enemies.

Mot-clés - Coccinelle asiatique, *Harmonia axyridis*, pucerons, espèce invasive, interaction interspécifique, contrôle biologique, Coccinellidae

Résumé - L'agriculture biologique permettrait d'augmenter l'abondance des espèces auxiliaires et d'accroitre la biodiversité dans les habitats agricoles. Dans cette étude, l'abondance des prédateurs de pucerons a été étudiée en cultures biologiques et conventionnelles pendant une période de deux ans. Une différence de diversité d'espèces n'a pu être mise en évidence entre les deux méthodes culturales. Cinq espèces prédatrices de pucerons ont été abondamment observées dans les cultures cultivées indépendament des pratiques culturales: *Coccinella septempunctata* L. 1758 (Coleoptera: Coccinellidae), *Propylea quatuordecimpunctata* (L. 1758) (Coleoptera: Coccinellidae), *Harmonia axyridis* Pallas 1773 (Coleoptera: Coccinellidae), *Chrysoperla carnea* (Stephens 1836) (Neuroptera: Chrysopidae) et *Episyrphus balteatus* (De Geer 1776) (Diptera: Syrphidae). Des différences d'abondances de prédateurs ont été observées entre les cultures conventionnelles et biologiques mais les densités observées n'étaient cependant pas majoritairement en faveur des cultures biologiques. L'abondance de ces 5 espèces variait selon différents paramètres tels que l'année d'inventaire, la culture inventoriée ou la pratique culturale utilisée. En conclusion, nous ne pouvons affirmer que les pratiques de l'agriculture biologique exercées en maïs et fève augmentent la diversité et l'abondance des auxiliaires.

Introduction

Most cultivated crops are situated in intensive agricultural area, where chemical biocides are used to control pest and plant diseases, with serious environmental consequences including loss of biodiversity (Ghorbani *et al.*, 2008). Organic farming has been proposed as an alternative in order to increase biodiversity in agricultural landscapes (Hole *et al.*, 2005). The use of organic manures, compost and crop residues and the set-up of mechanical weeding, minimum tillage, a prohibition/reduction of chemical pesticides, as well as hedgerow structures and ploughing modifications may contribute to favour biodiversity in agricultural areas (Kromp, 1999; Lampkin, 2000; Hole *et al.*, 2005). Worldwide organic production continues to rise, reaching 37.5 million hectares in 2009 and valued at more than 60 billon US dollars (Leu *et al.*, 2011; Raducuta & Doroftei, 2012). These specific management practices are either absent or rarely utilized in the majority of conventional farming (Gardner & Brown, 1998). The increase in abundance and/or species richness thanks to organic farming practices can touch a large range of taxa including mammals, invertebrates, flora, and birds (Hole *et al.*, 2005; Rundlöf *et al.*, 2010; Smith, 2010). Nevertheless, as shown in Hole *et al.* (2005), 32% of studies have either highlighted no difference between the two farming systems or a negative impact of organic farming on biodiversity, e.g., a negative impact of mechanical weeding on eggs and chicks of ground nesting bird species.

One of the most important components that could influence insect abundance in agroecosystems, and more particularly aphid natural enemies, is the use of insecticides in conventional farming that have shown different impacts on ladybirds: spinosad or indoxacarb induce stronger declines than chlorpyrifos, carbaryl, bifenthrin, and A-cyhalothrin (Galvan *et al*., 2005). Pyrethrins are often used in organic farming (Isman, 2006) and although they leave no persistent toxins, their toxicity for several beneficial arthropods has been confirmed in many previous works (e.g. Kraiss & Cullen, 2008). To reduce the impact on beneficial species, insecticide soaps are also used to control aphid populations (Karagounis *et al*., 2006) as these products show less toxicity to important aphid predators, including *Harmonia axyridis* Pallas 1773 (Coleoptera: Coccinellidae)¸ *Chrysoperla carnea* (Stephens 1836) (Neuroptera: Chrysopidae) and *Episyrphus balteatus* (De Geer 1776) (Diptera: Syrphidae) (Bigler & Waldburger, 1994; Pineda *et al*., 2008).

In this study, the abundance of aphidophagous species in both broad bean and corn cultivated under organic and conventional farming systems was considered. We focused our attention on the multi-coloured Asian ladybird, *H. axyridis*, an invasive species (Brown *et al*., 2008) and intraguild predator of native aphidophagous species (Brown *et al*., 2011; Roy *et al*., 2012).

Material and methods

Study sites and sampling method

Aphidophagous insect populations were sampled in 2010 and 2011 in the southern region of Belgium, in an area of agricultural production named Hesbaye (individual sites are given in Table 1). Samplings were performed on two crops known for their abundance of aphidophagous predators: corn, *Zea mays* L. 1753 and broad bean, *Vicia faba* L. 1753 (Vandereycken *et al*., 2010). The sampling period ran from mid-May to late September. The total surface of all conventional fields and organic fields was of 40 ha and 10 ha respectively. This difference of area was due to weak availability of organic fields compared to conventional ones. All fields were sampled once per week. Two different treatments were applied on each crop: a conventional treatment and an organic one. Fields of both farming systems were located in the same agricultural area. Fields of organic crops were separated from others by hedgerows composed of several tree species including *Acer* sp., *Crataegus* sp., *Viburnum* sp., *Ligustrum* sp., *Quercus* sp., *Betula* sp. and several herbs. The organic crops did not receive any synthetic manure, mechanically weeded, and received a minimum of tillage. A grass strip with flowers five meters wide was present around each crop field. Conventional farming fields were not surrounded by tree structures and were situated in an agro-intensive area in Hesbaye. In conventional cornfields, to prevent aphid damages, weeds were coated with insecticide (Thiamethoxam). In conventional broad bean, insecticides including pirimicarb and lambda-cyhalotrin were sprayed at the end of the flowering period to control aphids, thrips and bruchids. Fungicides including azoxystronbine and chlorothalonil were sprayed against anthracnose and botrytis.

Table1: Experimental sites (Belgium) where aphidophagous predators were surveyed from 2010 to 2011

Year	Site	Latitude	Longitude	Crops
2010	Walhain	4.735	50.616	Corn, broad bean, organic corn
	Perwez	4.813	50.645	Corn, broad bean
	Ramillies	4.866	50.624	Corn
	Rhisnes	4.830	50.500	Broad bean, organic corn, organic broad bean
	Gembloux	4.695	50.563	Broad bean
	Plancenoit	4.398	50.664	Corn, broad bean
	Nil-St-Vincent	4.689	50.646	Broad-bean
	Isnes	4.732	50.515	Broad bean
2011	Perwez	4.813	50.645	Corn, broad bean
	Gembloux	4.695	50.563	Corn, broad bean
	Plancenoit	4.398	50.664	Corn, broad bean
	Ligny	4.581	50.508	Broad bean
	Ramillies	4.866	50.624	Corn
	Rhisnes	4.830	50.500	Organic corn
	Walhain	4.735	50.616	Organic broad bean

The sampling method used to assess the numbers of aphidophagous predators and aphids consisted of visual whole-plant inspections using 1 m^2 quadrats. Visual sampling was conducted as it provides an easy and accurate method for the estimation of larval and adult densities of coccinellids in agroecosystems (Michels & Behle, 1992). Thirty-five quadrats for each crop were examined once per week in conventional and organic crops. Quadrats were located along transect lines across each field and spaced 20 meters apart. All leaves and stems within the quadrat were observed and all aphidophagous species at any stage were recorded. Aphid populations were also quantified on all leaves and stems. First instar and pupae were brought to the laboratory for rearing under laboratory conditions (T=24±1°C; HR=75±5%) for identification to the species level.

Statistical analyses

As mean densities observed for 1 m^2 were low, these values were presented per 100 m^2 for better understanding. The evaluation of the most abundant species was realised for a specific developmental stage (larvae or adults), within crops and within treatments with an Analysis of Variance (ANOVA: General Linear Model, GLM) with species (n = 5) and month (n = 5) used as factors ($\alpha = 0.05$). The factor "month" was used in the GLM to decrease the impact of natural annual variations in predator densities. After this analysis, mean numbers of predators were compared using the Least Square Difference (LSD).

The mean numbers of aphidophagous species were compared between two treatments by an ANOVA: GLM with treatment (n = 2) and month (n = 5) used as factors ($\alpha = 0.05$). To explain the variations in predator abundances between two treatments, the mean species abundances within each crop were analysed by an Analysis of Covariance (ANCOVA: General Linear Model), with treatment (n = 2) and month (n = 5) used as factors ($\alpha = 0.05$) and aphid densities as a co-variable.

Aphid densities were compared between two treatments by ANOVA: GLM with treatment (n = 2) and month (n = 5) used as factors ($\alpha = 0.05$).

Ryan-Joiner test was used to assess the population normality. The distribution of data (counting) was asymmetric and had to be $\log_{10}(x+1)$ transformed before analysis. Although the statistical analysis were performed on transformed data, untransformed data were presented in Table 2. Statistical analyses were performed using Minitab® 15.1.30.0 (State College, Pennsylvania, USA). When presenting the results, the "data point" term was used to refer to the density of one particular species at one developmental stage within one of the two crops during one specific year (Table 3).

Results

Five aphidophagous species were mainly observed in both crops and under both organic and conventional treatments: *C. septempunctata*, *Propylea quatuordecimpunctata* (L. 1758) (Coleoptera: Coccinellidae), *H. axyridis*, *C. carnea s.l.* and *E. balteatus* (Table 2). These five species represented 100% and 99% of all aphidophagous species observed in 2010 and 2011, respectively.

Table 2: Abundance (mean and SE) of aphidophagous species (larvae and adults) in two crops (corn and broad bean) and two treatments (conventional and organic) in 2010 and 2011. Means within a developmental stage followed by the same letter are not significantly different. (P > 0.05; LSD test). *C. 7-punctata: Coccinella septempunctata; P. 14-punctata: Propylea quatuordecimpunctata*. "/" there is no significant difference of mean number of aphidophages.

		Mean number of aphidophages /100 m²							
2010		Corn				Broad bean			
Larvae		Conventional		Organic		Conventional		Organic	
	H. axyridis	14.2 ± 3.1	*b*	13.6 ± 5.8	*ab*	5.9 ± 2.4	/	15.7 ± 8.1	*b*
	C. 7-punctata	8.7 ± 2.2	*b*	3.0 ± 1.5	*b*	15.8 ± 4.8	/	48.6 ± 23.0	*a*
	P. 14-punctata	0.8 ± 0.5	*c*	0.5 ± 0.6	*b*	0	/	0	*b*
	E. balteatus	42.0 ± 8.3	*a*	17.7 ± 4.1	*a*	5.2 ± 1.9	/	0	*b*
	C. carnea s.l.	5.2 ± 1.3	*b*	0.5 ± 0.6	*b*	0.2 ± 0.3	/	4.3 ± 2.5	*b*
Adults									
	H. axyridis	5.2 ± 1.6	*b*	10.1 ± 2.4	*b*	2.4 ± 0.8	*b*	2.9 ± 2.1	*b*
	C. 7-punctata	13.9 ± 2.2	*a*	32.3 ± 5.2	*a*	12.0 ± 1.8	*a*	17.1 ± 5.8	*a*
	P. 14-punctata	2.5 ± 0.9	*b*	8.6 ± 2.4	*bc*	0.7 ± 0.5	*b*	1.4 ± 1.5	*b*
	E. balteatus	0	*b*	0	*c*	0	*b*	1.4± 1.5	*b*
	C. carnea s.l.	5.7 ± 1.4	*b*	5.1 ± 1.9	*bc*	5.0 ± 1.2	*b*	1.4 ± 1.5	*b*
2011									
Larvae									
	H. axyridis	37.4 ± 5.7	*a*	1.3 ± 1.3	/	33.1 ± 9.5	*b*	84.0 ± 34.3	*ab*
	C. 7-punctata	20.9 ± 5.3	*b*	6.3 ± 3.8	/	171.1 ± 53.9	*a*	126.0 ± 34.5	a
	P. 14-punctata	0.5 ± 0.4	*c*	0	/	0.3 ± 0.3	*b*	2.0 ± 2.0	*c*
	E. balteatus	10.7 ± 1.9	*b*	0	/	3.0 ± 1.2	*b*	12.0 ± 4.7	*bc*
	C. carnea s.l.	0.5 ± 0.4	*c*	3.8 ± 2.2	/	1.8 ± 0.8	*b*	0	*c*
Adults									
	H. axyridis	18.7 ± 2.6	*ab*	7.5 ± 3.0	*b*	8.8 ± 2.3	*b*	12.0 ± 6.8	*b*
	C. 7-punctata	23.0 ± 3.1	*a*	35.0 ± 7.4	*a*	45.9 ± 9.0	*a*	74.0 ± 22.1	*a*
	P. 14-punctata	3.5 ± 1.3	*c*	3.8 ± 2.2	*b*	2.7 ± 0.9	*b*	2.0 ± 2.0	*b*
	E. balteatus	1.1 ± 0.7	*c*	0	*b*	7.0 ± 1.8	*b*	48.0 ± 22.0	*b*
	C. carnea s.l.	16.8 ± 2.6	*b*	6.3 ± 3.3	*b*	49.9 ± 6.8	*a*	82.0 ± 27.6	*a*

All aphid predator species were identified with the exception of the members of the *C. carnea* species complex, which were grouped together even if comprising three cryptic species: *Chrysoperla kolthoffi* Navas, *Chrysoperla lucasina* Lacroix and *C. carnea*, which can only be differentiated using molecular techniques (Bozsik *et al.*, 2003; Lourenço *et al.*, 2006).

Aphidophagous species abundance in conventional and organic farming

In 2010, the most abundant adult aphidophagous observed on either conventional or organic corn was *Coccinella septempunctata* L. 1758 (P < 0.05; LSD) (Table 2). In 2011, both *C. septempunctata* and *H. axyridis* numerically dominated the aphidophagous inventory on conventional corn (P < 0.05; LSD) while on organic corn, only *C. septempunctata* dominated (P < 0.05; LSD) (Table 2).

The most abundant larvae were, in 2010, *E. balteatus* on conventional corn and both *E. balteatus* and *H. axyridis* on organic corn. In 2011, only the larvae of *H. axyridis* dominated the aphidophagous guild on conventional corn (P < 0.05; LSD). No significant differences in larval densities were observed on organic corn.

In broad bean, *C. septempunctata* in 2010 and both *C. septempunctata* and *C. carnea s.l.* in 2011 were the two most frequently observed adult predators in both conventional and organic fields (P < 0.05; LSD) (Table 2).

There was no significant difference in larval densities observed on conventional broad bean in 2010, while *C. septempunctata* dominated on organic beans. In 2011, the most abundant larvae were those of *C. septempunctata* on conventional broad bean, while both *C. septempunctata* and *H. axyridis* were the most abundant on its organic counterpart (P < 0.05; LSD) (Table 2).

Organic versus conventional farming

The dominant aphidophagous species were mostly similar in both conventional and organic crops: in corn, *E. balteatus*, *H. axyridis* and *C. septempunctata* and in broad bean, *C. septempunctata*, *H. axyridis* and *C. carnea* (Table 2). However, densities differed between both kinds of treatment as well as from one year to another. Across the two crops and the two sampling years, 8 out of 20 data point (40%) and 11 of 20 data (55%) in 2010 and in 2011, respectively, showed a significant difference in predator abundance between the two treatments ($P_{treat.} < 0.05$; ANOVA) (Table 3). More precisely, a higher abundance in organic crops was observed than in conventional ones in 6 out of 20 data (30%) in 2010 and 5 out of 20 data point (25%) in 2011 (Table 3) ($P_{treat.} < 0.05$; ANOVA). In contrast, during these two years, 8 out of 40 data (20%) showed a lower abundance with organic farming than in conventional farming (Table 3).

Table 3: ANOVA and ANCOVA summary of effects of aphid abundance and treatments (conventional, organic) on predator abundances at the adult and larval stages in corn and broad bean crops in 2010 and 2011 (P values come from GLM, *** P < 0.001, ** P < 0.01, * P < 0.05, ns = not significant P > 0.05; [a]analyzed by ANOVA, [b]analyzd by ANCOVA, [1] more predators in conventional crops, [2] more predators in organic crops; *H. a : Harmonia axyridis, C. 7: Coccinella septempunctata, P. 14: Propylea quatuordecimpunctata, E. b: Episyrphus balteatus, C.c: Chrysoperla carnea s.l.*)

	2010						2011					
	[a]*Treat.*		[b]*Treat./Aphids*		[b]*Aphids*		[a]*Treat.*		[b]*Treat/Aphids*		[b]*Aphids*	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Corn												
Larvae												
H. a	0.03	*ns*	0.02	*ns*	0.00	*ns*	27.84	***[1]	23.72	***	0.06	*ns*
C. 7	1.95	*ns*	1.77	*ns*	0.05	*ns*	4.97	*[1]	4.87	*	0.11	*ns*
P. 14	0.00	*ns*	0.01	*ns*	0.58	*ns*	0.33	*ns*	0.29	*ns*	0.00	*ns*
E. b	4.60	*[1]	1.86	*ns*	18.20	***	11.42	**[1]	12.44	***	1.05	*ns*
C. c	5.23	*[1]	5.22	*	0.04	*ns*	7.37	**[2]	5.78	*	0.18	*ns*
Adults												
H. a	6.85	**[2]	6.02	*	0.41	*ns*	6.70	*[1]	2.36	*ns*	7.16	**
C. 7	12.16	**[2]	11.31	**	0.12	*ns*	1.99	*ns*	3.67	*ns*	2.93	*ns*
P. 14	6.11	*[2]	6.13	*	0.07	*ns*	0.18	*ns*	0.12	*ns*	0.02	*ns*
E. b	/	/	/	/	/	/	0.70	*ns*	1.85	*ns*	2.76	*ns*
C. c	0.16	*ns*	0.21	*ns*	0.15	*ns*	8.06	**[1]	5.37	*	1.05	*ns*
Broad bean												
Larvae												
H. a	3.38	*ns*	2.74	*ns*	2.25	*ns*	10.11	**[2]	10.43	**	0.35	*ns*
C. 7	18.58	***[2]	15.76	***	11.29	**	6.60	*[1]	4.92	*	2.26	*ns*
P. 14	/	/	/	/	/	/	2.23	*ns*	2.40	*ns*	0.19	*ns*
E. b	1.47	*ns*	1.17	*ns*	1.31	*ns*	5.74	*[2]	4.78	*	0.62	*ns*
C. c	12.74	***[2]	12.03	**	0.51	*ns*	0.75	*ns*	0.74	*ns*	0.01	*ns*
Adults												
H. a	0.02	*ns*	0.10	*ns*	2.79	*ns*	0.07	*ns*	0.10	*ns*	0.07	*ns*
C. 7	0.84	*ns*	0.65	*ns*	0.89	*ns*	14.27	***[2]	12.77	***	0.37	*ns*
P. 14	1.40	*ns*	1.72	*ns*	1.48	*ns*	0.01	*ns*	0.03	*ns*	0.17	*ns*
E. b	5.58	*[2]	5.50	*	0.00	*ns*	33.87	***[2]	26.32	***	4.57	*
C. c	2.96	*ns*	2.55	*ns*	1.06	*ns*	2.16	*ns*	3.14	*ns*	2.66	*ns*

A linear relation between aphid abundance and predator abundance was identified in 4 out of 40 data (10%) during the two sampling years ($P_{aphids} < 0.05$; ANCOVA). This correlation was highlighted for *C. septempunctata* larvae ($F_{1.404} = 11.29$) and *E. balteatus* adults ($F_{1.307} = 4.57$) in broad bean and for *E. balteatus* larvae ($F_{1.489} = 18.20$) and *H. axyridis* adults ($F_{1.339} = 7.16$) in corn (Table 3).

Differences in density between both two farming systems for *E. balteatus* larvae in corn in 2010 and *H. axyridis* adults in corn in 2011were shown ($P_{treat.} < 0.05$; ANOVA and $P_{treat./}$aphids > 0.05; ANCOVA).

No general conclusion can be made for the distribution of *H. axyridis*, the only alien aphidophagous species observed during our inventory. While adult densities were higher in organic corn than in conventional corn in 2010 ($F_{1,490}$ = 6.85 ; P = 0.009), the opposite findings were recorded in 2011 ($F_{1,340}$ = 6.70 ; P = 0.010) (Figure 1). Results of larval density in corn showed no difference in 2010, while in 2011, higher densities of larvae were observed in conventional corn ($F_{1,340}$ = 27.8 ; P < 0.001).

In broad bean, a difference in *H. axyridis* density between the two treatments was only found in 2011, where the numbers of predatory larvae in conventional broad bean were lower ($F_{1,308}$ = 10.1; P = 0.002) (Figure 1).

The study of the occurrence of the all aphid predator species in the two different traitements show that results are different in broad bean and in corn. In corn results are different according years and stages. In broad bean, no significant difference were observed in 2010 unlike in 2011 (Figure 2). In 2001, there was more individuals of the all aphid predator species in organic crops than in conventional at the larvae stages ($F_{1,2739}$ = 12.50 ; P < 0.001) and at the adult stage ($F_{1,2739}$ = 11.62 ; P = 0.001).

Aphid abundances were also studied in both conventional and organic farming during the two years. No significant difference in aphid abundance was observed between conventional and organic treatments (Figure 3).

Discussion

The present study explored differences in densities of aphidophagous species in relation to aphid abundance, between conventional and organic crop management, in both broad bean and corn crops. Both in 2010 and 2011, only five beneficial species were observed on the two crops, whether organically or conventionally farmed: *C. septempunctata*, *P. quatuordecimpunctata*, *H. axyridis*, *C. carnea s.l.* and *E. balteatus*. These five species have also been reported as predominant in previous works conducted in agroecosystems in Western Europe (Hodek & Honěk, 1996; Alhmedi *et al*., 2007; Vandereycken *et al*., 2010) and in other countries (Colunga-Garcia & Gage, 1998; Lucas *et al*., 2007).

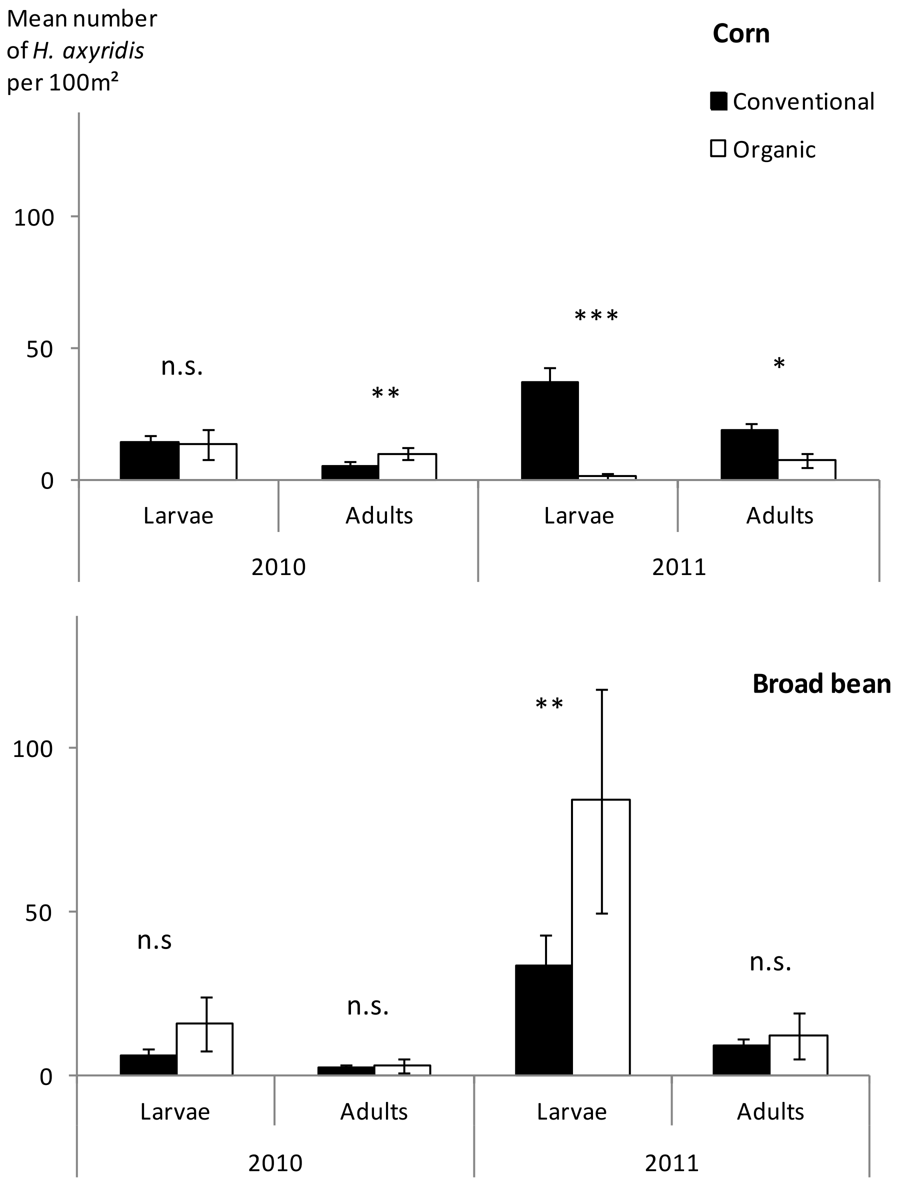

Figure 1: Mean numbers and SE of *H. axyridis* observed per 100 m² on corn and broad bean with conventional and organic treatment in 2010 and 2011. (P values come from GLM, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns = not significant $P > 0.05$)

The impact of the organic treatment on the abundance of a specific aphid predator species was not highlighted. However, the total amount of aphid predator seems te be higher in organic than in conventional broad bean. Organic farming with an absence of chemical treatments and a more open crop structure lead to increase the total abundance of aphid predators. Our findings are in accordance with previous ones stating that densities of aphidophagous species are higher in organic farming than in conventional farming (Belfrage *et al*., 2005; Wu *et al*., 2006). In these studies, several parameters were proposed to increase biodiversity and abundance in organic farming, including management practices (mechanical weeding, minimum tillage, intercropping) (Sunderland & Samu, 2000; Hole et al., 2005), hedgerow structures, and maintenance of nearby vegetation or plant corridors (Chamberlain & Wilson, 1999; Kromp, 1999). In corn, no difference of predator abundance was observed between two crop farming. It is probably because chemical treatments such as herbicide are realised during the first weeks of the plant growth. No other treatments are realised because corn plants are too high. The structure of the corn crops are the same in both two crop farmings.

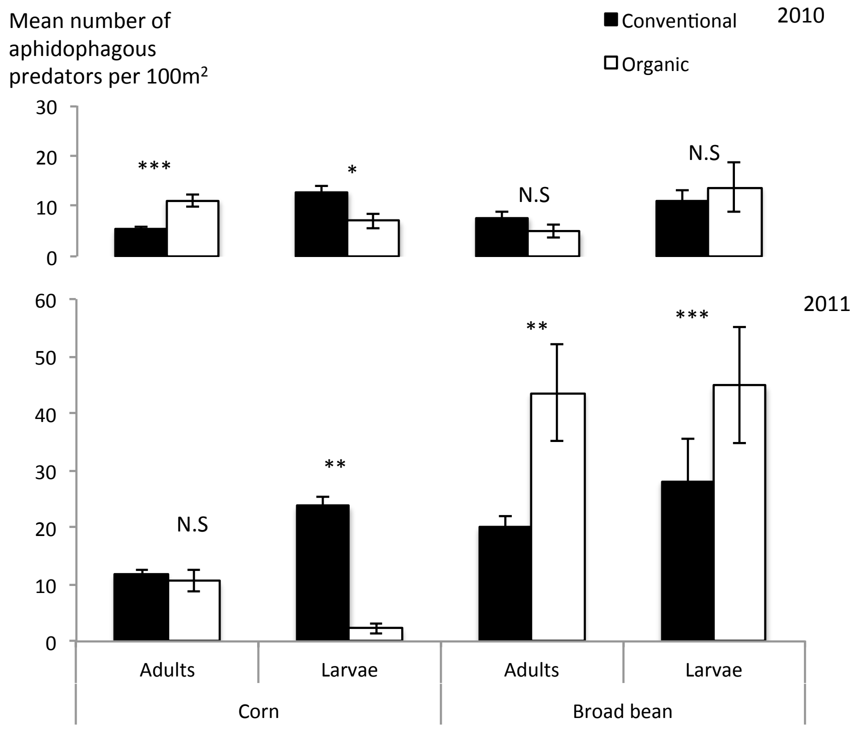

Figure 2: Mean numbers and SE of aphid predators observed per 100 m² on corn and broad bean with conventional and organic treatment in 2010 and 2011. (P values come from GLM, *** P < 0.001, ** P < 0.01, * P < 0.05, ns = not significant P > 0.05)

The low abundance and diversity of aphid predators observed on organic corn could be explained by the low growth of corn in 2011 due to the association of two factors: the drought during spring and the absence of chemical fertilizer. The drought affects more corn than broad bean because corn needs more water than the other. The corn growth deficiency, associated with a low aphid density, could have contributed to the low density of predators observed in 2011.

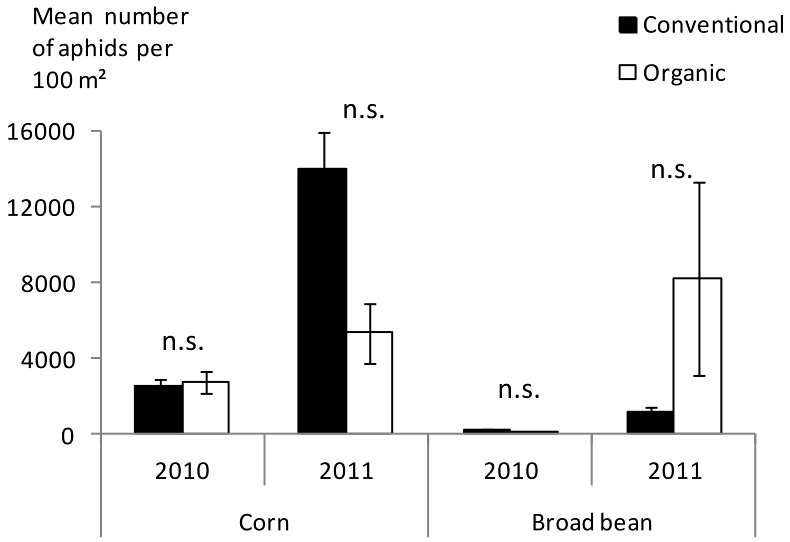

Figure 3: Mean numbers and SE of aphids observed per 100 m² on corn and on broad bean with conventional treatment and organic treatment in 2010 and 2011. (P values come from GLM, ns = not significant P > 0.05)

Secondly, the absence of insecticide use in both conventional and organic corn farming decreases the variability between the two farming practices. Factors such as landscape structure and organic practices obviously did not increase the abundance of aphid predators. Additionally, aphid densities were found to directly impact the abundance of some aphid predators on corn, as suggested for *E. balteatus* (Leroy *et al.*, 2011a) and *H. axyridis* (Leroy *et al.*, 2011b).

Because of its status as an invasive and intraguild predator (Brown *et al.*, 2011; Roy *et al.*, 2012), our analysis focussed on *H. axyridis*. Excepting in organic farming in 2011, *H. axyridis* at the adult stage was found to be more abundant on corn than on broad bean, as suggested in previous works (Colunga-Garcia & Gage, 1998; Koch *et al.*, 2006; Lucas *et al.*, 2007). The architectural structure of a corn plant is comparable to that of a tree, i.e., a stiff trunk with many branches. *H. axyridis* is known to be a semiarboreal species (Hodek, 1973; LaMana & Miller, 1996). On the other hand, *C. septempunctata* prefers agroecosystem habitats (Maredia *et al.*, 1992; Alhmedi *et al.*, 2009; Gardiner *et al.*, 2009).

In conclusion, our findings support organic practices in broad bean as key options to increase the abundance of aphid natural enemies.

Acknowledgments

We thank Dr Y. Brostaux for his advice on statistical analyses and D. Durieux for her helpful comments on previous versions of the manuscript. This research was funded by the Service Public de Wallonie (SPW – DGO3, project n°D31-1247).

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Chapter VII : CONCLUSIONS, DISCUSSIONS AND PERSPECTIVES

1. Conclusions and discussions

Harmonia axyridis is an alien species causing important ecological and societal inconveniencies. Amongst them, competition with native species, which has been widely studied in various habitats. A lack of information was however noticed in agricultural lands. This thesis focused on the diversity of aphid predators in agricultural ecosystems, focusing especially on *Harmonia axyridis*.

Using field samplings, the population changes of *H. axyridis* as well as native aphidophagous species were assessed in several crops during three years. The evaluation of the impact of *H. axyridis* on native species was hardly feasible in this work, because this study began in 2009, eight years after the first observation of *H. axyridis* in Belgium. No data of aphid predator sampling in agricultural crops in Belgium were available before the introduction in 1997, to allow comparisons. However, coccinellids collected in Belgium from 2001 to 2009 allow us to assess the situation during the early years of its invasion.

Before measuring the occurrence of *H. axyridis* in agroecosystems, we have analysed coccinellids from samplings realised in Belgium since 2001. Our first detection of *H. axyridis* occurred in 2002 (5 years after its introduction) and from this time has increased continuously. Its population then rose to reach more than 60% of the coccinellid community. From 2006, this species is the most abundant coccinellid amongst the 27 species of Coccinellidae that we observed.

This is in accordance with trends observed in other Belgian studies, with the exception that they observed *H. axyridis* for the first time in 2001(Adriaens et al. 2003). *H. axyridis* was observed in 2001 on trees (*Tilia* spp., *Acer* spp. and *Pinus* spp.) in Ghent, situated in the north of Belgium. It seems that the spread of *H. axyridis* began in the north of Belgium from two cities, Brussels and Ghent. Meanwhile a decrease of native species (*A. bipunctata*, *P. quatuordecimpunctata* and *P. vigintiduopunctata*) has been noticed. The decline of native species in additional habitats in Belgium was also observed by several authors (Ottart 2005, Adriaens et al. 2010).

Previous studies reported that *H. axyridis* thrives more on arboreal habitats with deciduous or resinous trees. The first question was to determine the habitats preferences of *H.*

axyridis. We studied the habitat diversity of *H. axyridis* and we highlighted that *H. axyridis* is an ubiquist species that lives in a broad range of habitats including crops (*Solanum* spp., *Triticum* spp. and *Zea mays*) . This ability to adapt to different habitats gives *H. axyridis* an advantage on native species and explains its rapid invasion in many countries on different continents.

We have conducted most our inventories in agricultural lands because *H. axyridis* was mainly studied in urban habitat with arboreal areas. After three years of samplings (2009-2011) in agroecosystems, visual observations allowed the identifications of the community of aphid predators. This community is highly diverse in the four crop types we screened in Belgium. 21 species of aphid predators were observed, mainly composed of ladybirds and hoverflies. Five of them were particularly abundant (representing more than 99% of the aphid predators community): three coccinellids (*C. septempunctata*, *P. quatuordecimpunctata*, *H. axyridis*), one hoverfly (*E. balteatus*) and one lacewing (*C. carnea*).

The alien coccinellid, *H. axyridis*, was observed in the four studied crops but its abundance varied according to these crops. Its abundance was higher in corn and broad bean than in potato and wheat. This species seems mainly to select its habitat according to prey availability (aphid species diversity and abundance) (Sloggett and Majerus 2000, Evans and Gunther 2005). *H. axyridis* is attracted by aphids and associated aphid semiochemicals (pheromones and honeydew odors), so they are considered as attractant and/or arrestant for immigrating adults of *H. axyridis* (Leroy et al. 2011a). Plant volatiles (Park and Hardie 2004), plant structure (Goffreda et al. 1988) and climate (Hodek and Honěk 1996, Szentkirályi 2001) could be others factors involved in the habitat selection. However, abundance of aphids does not necessarily mean large populations of *H. axyridis*: in our work, wheat fields were heavily infested (mainly by *Metopolophium dirhodum* Walker) but few *H. axyridis* at the adult and larval stages were observed.

The concept of biological invasion is generally used to refer to the arrival or introduction, establishment, geographical expansion and integration of a species into a region where it has never been before (Shigesada and Kawasaki 1997). We highlighted the increase of *H. axyridis* population in corn until the end of the study in 2011, reaching 34% of the aphid predators, while in 2009 it represented only 14%. This confirms, if necessary, the invasive status of *H. axyridis*. Its exceptional capacities linked to several morphological (large body, spines), physiological (alkaloid component) and behavioural traits (aggressiveness, polyphagia) allow *H. axyridis* to be successful in any new environment (Soares et al. 2008).

The phenology of *H. axyridis* followed the aphid phenology but they were shifted in time. This suggests that larvae of *H. axyridis* should be able to complete their development without any aphids by feeding on alternative preys. *H. axyridis* is known to be polyphagous and able to feed on larvae of hoverflies, lepidoptera and even spiders (Yasuda and Katsuhiro 1997); and to feed indifferently on aphids, larvae of heterospecific species (Katsanis et al. 2012, Thomas et al. 2013) or conspecific species (Yasuda and Ohnuma 1999). At the end of the season, when there is no aphids left in corn fields, *H. axyridis* is also able to feed on corn fruit (personal observations) and pollen (Berkvens et al. 2007). This feeding diversity is an asset for the invasiveness of this alien species.

Is the biocontrol agent, *Harmonia axyridis,* an invader in Belgian agroecosystems? The importation of an exotic species to control agricultural pests according to biological control strategy is not new and has already worked in the past. However the lack of studies and watchfulness contributed to the actual situation: the invasion of an exotic ladybird. After many years of investigation, there is no doubt that *H. axyridis* is an invader in agroecosystems and could be a threat for biodiversity. Our study provides a better understanding of the invasion of *H. axyridis* in Belgian agroecosystems. It is time and necessary to react in order to find a solution to control this alien species before the extinction of native species.

2. Perspectives

H. axyridis is currently established in 38 countries (Brown et al. 2011) and its expansion is likely to continue in the coming years. It is therefore necessary to survey *H. axyridis* population density and to study population changes in native coccinellid communities. By population changes we are meaning species displacement and decrease of non target species by intraguild predation. For example, in the USA, the abundance of three coccinellids *B. ursina*, *C. munda*, and *Chilocorus stigma* decreased after the establishment of *H. axyridis* in agricultural landscape (Colunga-Garcia and Gage 1998).

Additionnal inventories of *H. axyridis* were realised until 2012 and we observed that the amount of *H. axyridis* population increased to reach 86% of the aphid predators.

The impact of *H. axyridis* on native species is currently evaluated by molecular techniques: Polymerase Chain Reaction (PCR). Analysis of the gut contents of *H. axyridis* larvae allows detecting traces of DNA intraguild prey. Species selected as intraguild prey are

C. septempunctata, *P. quatuordecimpunctata*, *C. carnea* and *E. balteatus*. First results based on larvae caught in corn in 2012 revealed that 0.3% (2 of 708) of the samples were positive. The low abundance of intraguild preys and high abundance of extraguild preys can explain this. A second analysis of the larvae collected in 2013 is underway.

After all these considerations it is time to act and find a solution to control this alien species. We have to try to control *H. axyridis* instead of eradicating it because this species is well acclimated to all habitats where it was introduced and it is to late to hope exterminate this species completely. On the other hand this species is also a great beneficial species as aphid predator. If this species is eradicated it could be result in a rapid decline in pest suppression services if the remaining community is unbable to respond (Bahlai et al. 2013).

The use of pesticides is unsuitable in fields to control *H. axyridis* because all non-target species (other coccinellids, hoverflies, lacewings) could be affected. Moreover, insecticide treatments could lead to resistance issues. The use of semiochemical compounds in traps as attractant, aggregative or arrestant for *H. axyridis* would be more appropriate to control its populations. A push-pull strategy with these semiochemicals as attractants and repellents might help to manage *H. axyridis* populations. This approach is using i) chemical repellents to push beetles away from fields, and ii) chemical attractants or aggregation pheromone components to pull *H. axyridis* into collecting vessels or traps (Riddick et al. 2000, Sloggett et al. 2011). This action could handle populations of *H. axyridis* in an Integrated Pest Management program. Semiochemicals from aphids and volatile compounds from damaged or attacked plants are largely known to act as kairomones for many predatory and parasitic insects, inducing specific behaviors like active search for prey or egg-laying (Han and Chen 2002, Francis et al. 2004, Zhu and Park 2005, Verheggen et al. 2008).

The first group of compounds used to manage *H. axyridis* populations consisted of repellents. Some trials involving the use of plant derived natural products such as menthol and camphor as repellents, have show promising results, although the persistence of formulations available is not adequate for the moment (Riddick et al. 2000, Mannix 2001).

The organic compounds playing a role in the overwintering aggregation in house could be also used to manage outdoor *H. axyridis* populations. Two different blends of non-volatiles compounds were identified in aggregation sites: the first leads conspecifics toward aggregation sites and the second ensures the cohesion of the aggregation (Durieux et al. 2012). These compounds could be used in a trap in crop fields as well as in infested houses (Kenis et al. 2008). However, the hydrocarbons identified seem only to be perceived by the ladybirds upon direct contact. In order to render a trap efficient, i.e. attracting *H. axyridis* ladybirds at long distances, volatile compounds (VC) would be required (Durieux et al. 2012). VC such as sex pheromones could be also used in such a trap.

Additional semiochemicals act as attractants for *H. axyridis*: aphid alarm pheromone (E)-β-farnesene (Verheggen et al. 2007, Leroy et al. 2011b), aphid sex pheromone Z,E-nepetalactone (Leroy et al. 2011b) or a component of the aggregation pheromone of coccinellids (-)-β-caryophyllene (Verheggen et al. 2007). However these compounds are not specific enough because they can also act as attractant for *C. septempunctata* (Nakamuta 1991), *A. bipunctata* (Francis et al. 2004) or *Hippodamia convergens* (Zhu et al. 1999). Z,E-nepetalactone was tested in natural conditions and seems to be the most attractive compound. It could be used in fields to attract *H. axyridis* for an efficient biological control approach against this invasive species (Leroy et al. 2011b).

Volatile compounds from aphid honeydew (3-hydroxy-2-butanone, 3-methyl-butanal, 3-methyl-1-butanol and limonene) were also highlighted to attract *H. axyridis* (Leroy et al. 2011a). These compounds could also be used in an IPM program to manage *H. axyridis* populations. Currently, natural aphid honeydew has only been studied a few times as a contact kairomone and an arrestant for coccinellid larvae (Carter and Dixon 1984, Ide et al. 2007). The use of artificial honeydews (sucrose and yeast product solutions) for the control of coccinellids has however been studied, showing that sugary products could be helpful in managing the Coccinellidae dispersal in field crops (Evans and Richards 1997).

Another way to control *H. axyridis* could be the use of entomopathogenic species of this alien species as bacteria, fungi (Garcés and Williams 2004) (Figure 1), nematodes (Shapiro-Ilan and Cottrell 2005), protozoa (Saito and Bjørnson 2008), wasp or flies with the species selectivity as the main focus idea .

Figure 1: Parasitic laboulbeniales of the genus *Hesperomyces virescens* on *H. axyridis*

Theses biological control strategies proposed to manage *H. axyridis* in the future could be very efficient but with the inconvenience of non-selectivity. More research is needed to find an efficient way to control this invasive species. The solution to decrease the impact of this alien species on the other aphidophagous species is maybe an interaction between several mechanical and chemical practices produce by an international scientific collaboration.

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Chapter VIII : LIST OF PUBLICATIONS, ORAL COMUNICATIONS AND POSTERS

VIII.1 Publications

a) Articles in the thesis:

Vandereycken A, Brostaux Y, Joie E, Haubruge E, Verheggen FJ. 2013. Occurrence of *Harmonia axyridis* (Coleoptera: Coccinellidae) in field crops. *Eur. J. Entomo.* 110(2): 285-292.

Vandereycken A, Durieux D, Joie E, Sloggett JJ, Haubruge E, Verheggen FJ. 2013. Is the multicolored Asian ladybeetle, *Harmonia axyridis*, the most abundant natural enemy to aphids in agroecosystems?. *J. Insect Sci.* 13(158): 1-14.

Vandereycken A, Joie E, Francis F, Haubruge E, Verheggen FJ. 2013. Occurrence of aphid predator species in both organic and conventional corn and broad bean. *Entomol. faun. – Faun. Entomol.* 66: 77-87.

Vandereycken A, Durieux D, Joie E, Haubruge E, Verheggen FJ. 2012. Habitat diversity of the Multicolored Asian ladybeetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in agricultural and arboreal ecosystems: a review. *Biotechnol. Agron. Soc.* 16(4): 553-563.

Durieux D, **Vandereycken A**, Joie E, Haubruge E, Verheggen FJ. 2012. Evolution des populations de coccinelles indigènes et de l'espèce exotique, *Harmonia axyridis* (Pallas 1773), en Wallonie et en Région de Bruxelles- Capitale. *Entomol. faun. – Faun. Entomol.* 65: 81-92.

Vandereycken A, Durieux D, Joie E, Francis F, Haubruge E, Verheggen FJ. Aphid species and associated natural enemies in field crops: what about ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae)? *Appl. Entomol Zool.* (In revision)

b) Articles out of the thesis

Vandereycken A, Durieux D, Joie E, Haubruge E, Verheggen FJ. 2011. Impact of *Harmonia axyridis* (Coleoptera: Coccinellidae) on the survival rates of other aphidophagous species in semi-field conditions. *Communication in agricultural and applied biological sciences.* 76(2): 219-226.

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VIII.2 Oral communications

Vandereycken, A, Durieux, D, Joie, E, Haubruge, E, & Verheggen, F. (2010, May 05). Occurrence of the multicolored ladybird, *Harmonia axyridis* PALLAS in Walloon agro-ecosystems. Paper presented at One-Day Symposium on Chemical Entomology, Gembloux, Belgique.

Vandereycken, A, Durieux, D, Haubruge, E, & Verheggen, F. (2010, May 18). The occurrence of multicolored ladybird, *Harmonia axyridis* Pallas, a biological control agent in agroecosystems in Wallonia. Paper presented at 62nd International Symposium on Crop Protection, Ghent, Belgium.

Vandereycken, A, Durieux, D, Joie, E, Haubruge, E, & Verheggen, F. (2010, July 06). Occurrence de la coccinelle asiatique, *Harmonia axyridis* Pallas, dans les agro-écosystèmes wallons. Paper presented at 7ème Conférence Internationale Francophone d'Entomologie, Louvain-la-Neuve, Belgium.

Vandereycken, A, Durieux, D, Joie, E, Haubruge, E, & Verheggen, F. (2010, September 23). Impact of the Asian ladybeetles' invasions on agro-ecosystems (Belgium). Paper presented at International Symposium: Ecology of Aphidophaga 11, Perugia, Italie.

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Durieux, D, Fischer, C, Deneubourg, JL, Brostaux, Y, Lognay, G, **Vandereycken, A**, Joie, E, Haubruge, E, & Verheggen, F. (2011, July 27). Chemical and physical factors involved in the aggregation behaviour of *Harmonia axyridis* Pallas. Paper presented at 27th meeting of the International Society of Chemical Ecology, Burnaby, Canada.

Vandereycken, A, Durieux, D, Joie, E, Haubruge, E, & Verheggen, F. (2011, October 26). La coccinelle asiatique est-elle l'aphidiphage dominant dans les agro-écosystemes ? 9ème Conférence Internationale sur les Ravageurs en Agriculture, Montpellier, France.

Durieux, D, Fischer, C, Deneubourg, JL, Brostaux, Y, Lognay, G, **Vandereycken, A**, Joie, E, Haubruge, E, Verheggen, F. (2012, February 10). Study of the factors involved in the aggregation of *Harmonia axyridis* Pallas (Coleoptera : Coccinellidae). Paper presented at 17th PhD Symposium on Applied and Biological Sciences, Leuven, Belgium.

Durieux, D, Fischer, C, Deneubourg, JL, Brostaux, Y, Lognay, G, **Vandereycken, A**, Joie, E, Haubruge, E, & Verheggen, F. (2012, May 22). Factors involved in the aggregation of *Harmonia axyridis* Pallas. Paper presented at 64th International Symposium on Crop Protection, Ghent, Belgium

Vandereycken, A, Durieux, D, Joie, E, Francis, F, Haubruge, E, and Verheggen, F, (2013, October 25). A four-year inventory of the invasive ladybeetle *Harmonia axyridis* in agricultural ecosystems. Paper presented at the International Congres of Biological Invasion Qingdao, China

VIII.3 Posters

Vandereycken, A, Durieux, D, Joie, E, Haubruge, E, & Verheggen, F. (2010, November 06). Occurrence de la coccinelle asiatique (*Harmonia axyridis* Pallas), la coccinelle à 7 points (*Coccinella septempunctata* L.), le syrphe ceinturé (*Episyrphus balteatus* De Geer) et la chrysope (*Chrysoperla* sp.) au sein d'agro-écosystèmes wallons. Paper presented at 10ème Journée entomologique de Gembloux, Gembloux, Belgium.

Vandereycken, A, Durieux, D, Joie, E, Leroy, P, Haubruge, E, & Verheggen, F. (2011, March 06). Occurrence of aphid predators in Belgian agro-ecosystems. Paper presented at Global Conference on Entomology, Chaing Mai, Thailand.

Vandereycken, A, Durieux, D, Joie, E, Haubruge, E, & Verheggen, F. (2011, May 24). Field evaluation of survival rates of *Harmonia axyridis* (Coleoptera: Coccinellidae) and other aphidophagous species. Paper presented at 63rd International Symposium on Crop Protection, Ghent, Belgium.

Vandereycken, A, Durieux, D, Joie, E, Haubruge, E, & Verheggen, F. (2012, February 10). Aphid predators sampling in agrosystems in Belgium between 2009 and 2011. Paper presented at 17th PhD Symposium on Applied Biological Sciences, Leuven, Belgium.

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