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PRÉDATION INTRAGUILDE CHEZ LES COCCINELLIDAE :
Développement d'un nouvel outil moléculaire

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Résumé

La prédation intraguilde (IGP) constitue une interaction qui suscite beaucoup d'intérêt chez les écologistes et les utilisateurs de la lutte biologique puisqu'elle engendre, dans certains cas, des impacts négatifs sur le contrôle des populations d'organismes nuisibles. Les études réalisées jusqu'à présent sont en grande majorité effectuées dans des milieux artificiels, pouvant interférer avec les interactions intraguildes. Ce projet visait à étudier l'IGP en milieu ouvert dans les champs de soya, au sein de quatre espèces de coccinelles: *Harmonia axyridis*, *Coccinella septempunctata*, *Coleomegilla maculata lengi* et *Propylea quatuordecimpunctata*. Les populations de ces prédateurs subissent fréquemment des pertes d'effectifs liées à l'IGP. Les principaux objectifs de cette étude étaient de: i) développer des outils moléculaires permettant de détecter et de quantifier *in situ* les interactions intraguildes; ii) établir des relations entre l'intensité de l'IGP et certains paramètres écologiques; et iii) évaluer l'impact de la densité des proies extraguildes et de la structure de la plante sur l'IGP entre les coccinelles.

Des amorces PCR, élaborées pour chacune des quatre espèces de coccinelles, ont servi à détecter les proies intraguildes du contenu gastrique de près de 1000 prédateurs récoltés sur trois années, permettant ainsi d'établir des taux d'IGP mutuelle d'en moyenne 35% entre les espèces de Coccinellidae. Nous avons appliqué à ces taux une correction pour compenser les différences de temps de digestion entre prédateurs et proies de différentes espèces. Les facteurs favorisant l'IGP étaient : la densité des proies extraguildes, le ratio prédateur:proie, le stade de développement du prédateur ainsi que la période d'échantillonnage. Nous avons de plus évalué l'impact de la densité des proies extraguildes et de la structure de la plante sur l'IGP entre *H. axyridis* et *P. quatuordecimpunctata*. L'IGP était modulée principalement par la densité des proies extraguildes. Ces travaux démontrent l'omniprésence de l'IGP dans les interactions entre les coccinelles et identifient les principaux facteurs écologiques modulant son intensité. Cette thèse soutient donc que l'IGP, bien qu'extrêmement fréquente, n'a pas toujours un impact concret sur la lutte biologique et qu'il importe de considérer les principaux facteurs régulant son intensité.

Abstract

Understanding intraguild predation (IGP) between predators is of great interest for ecologists and biological control practitioners because its presence can, in some cases, impede biological control. Studies on IGP are usually realized under artificial environments, which may interfere with intraguild predation. This project focused on the study of IGP, in open field in soybean crop, between four species of ladybirds: *Harmonia axyridis*, *Coccinella septempunctata*, *Coleomegilla maculata lengi* and *Propylea quatuordecimpunctata*. Those predator populations frequently engage in IGP and it can be an important cause of mortality. Principal goals of this study were to: i) develop molecular tools to detect and quantify *in situ* IGP; ii) establish relations between IGP and ecological factors; and iii) evaluate the impact of extraguild prey density and plant structure on IGP between ladybirds.

DNA markers have been developed for four species of ladybirds to detect intraguild prey in the gut-content of near 1000 predators, sampled in three years. We established a mean rate of IGP of 35% between ladybird species. We applied a correction to those rates to compensate for differences in digestion rate between predators and prey of different species. Factors increasing the prevalence of IGP were: extraguild prey density, the ratio of predator:prey, developmental stage of the predator and seasonality. Finally, we evaluated the impact of extraguild prey density and plant structural complexity on IGP between *H. axyridis* and *P. quatuordecimpunctata*. IGP was principally modulated by extraguild prey density. This study shows the ubiquity of IGP among ladybird interactions and the understanding of principal factors regulating the intensity of IGP. This thesis supports the hypothesis that IGP, even if extremely frequent, did not always have a measurable impact on biological control and consideration of principal ecological factors modulating its intensity is important.

Avant-propos

Cette thèse renferme le manuscrit d'un article soumis dans le journal scientifique ***Molecular Ecology Resources*** (Chapitre II de la thèse). L'article, intitulé 'Prey DNA detection success following digestion by intraguild predators: influence of prey and predator species' fut rédigé par Annie-Ève Gagnon. Les co-auteurs de cet article sont Josée Doyon, George Heimpel et Jacques Brodeur. Josée Doyon a contribué de façon significative à cet article en réalisant une partie des travaux au laboratoire alors que J. Brodeur et G. Heimpel ont supervisé les travaux et participé à la rédaction de l'article. Les autres chapitres de cette thèse sont des manuscrits qui seront soumis dans des journaux scientifiques. L'étudiante Annie-Ève Gagnon est l'auteure principale de tous ces manuscrits et a réalisé l'ensemble des travaux. Le directeur de thèse Jacques Brodeur ainsi que le co-directeur George Heimpel ont supervisé l'ensemble des travaux et ont participé à la rédaction de tous ces articles. Au début de chaque chapitre, les noms des co-auteurs sont indiqués, de même que le nom du journal de la soumission à venir.

Remerciements

Ce projet de doctorat a été réalisé sous la direction du Dr. Jacques Brodeur et la co-direction du Dr. George Heimpel. J'aimerais tout d'abord remercier Jacques pour son dévouement, sa grande disponibilité et son professionnalisme. Merci aussi de m'avoir permis d'assister et de m'avoir accompagné à de nombreux congrès, me permettant ainsi de créer de plusieurs contacts précieux. Merci également pour ton humanisme et ta compréhension pour tout ce qui était hors du travail. A special thank to my co-advisor George for his precious collaboration and help all along this project. Thank you also for all the learning in molecular ecology, I really enjoy being in your team!

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Un grand merci à Sophie Rochefort et Renée Lalancette, deux amies très chères qui m'ont aidé et encouragé tout au long de mon doctorat.

Merci à toi Benjamin, mon compagnon de vie, mon ami et même mon collègue, pour m'avoir épaulé, conseillé, écouté, encouragé…je ne saurais mettre assez de mots ici pour exprimer toute ma gratitude envers toi.

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À vous trois…

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Introduction générale

Introduction

Cette thèse porte sur la prédation intraguilde (IGP) entre quatre espèces de coccinelles. Les travaux ont été réalisés dans un contexte de lutte biologique contre le puceron du soya, la proie commune de ces coccinelles dans les champs de soya. Cette étude tente d'apporter une nouvelle méthode de détection de l'IGP afin de mieux comprendre son occurrence en milieu naturel ainsi que son impact sur le contrôle des organismes nuisibles.

Cette introduction se divise en cinq principales parties. Dans un premier temps, je dresserai un portrait du système à l'étude, c'est-à-dire la culture du soya et ses ravageurs ainsi que les principaux ennemis naturels reliés. Par la suite, je décrirai le phénomène de l'IGP ainsi que des principales connaissances que nous possédons jusqu'à présent sur ce sujet. Je poursuivrai en énonçant les principales problématiques quant à l'étude de l'IGP en milieu naturel, pour enchaîner en proposant une nouvelle méthode de détection de l'IGP : l'utilisation d'outils moléculaires. Je terminerai en énonçant les hypothèses de recherche ainsi que les objectifs à atteindre au cours des travaux présentés dans cette thèse.

1.1 Le système à l'étude

1.1.1 L'établissement du puceron du soya au Québec

L'arrivée en 2000 en Amérique du Nord du puceron du soya, *Aphis glycines* Matsumura, un ravageur d'origine asiatique, a alerté les producteurs de soya (Venette et Ragsdale, 2004). À l'été 2002, ce puceron avait déjà colonisé la totalité des régions agricoles du Québec où la culture du soya est importante (Brodeur *et al.*, 2003). Le puceron du soya peut altérer la physiologie de la plante (diminution de la taille des plants, des gousses ou du nombre de graines par gousse) entraînant des pertes de rendement (Wu *et al.*, 2004). Au Québec, les pertes de rendement associées au puceron du soya en 2004 peuvent excéder 10% (Rhainds et al., 2007a). Des dommages indirects peuvent aussi être causés par la transmission de virus phytopathogènes (Domier *et al.*, 2003), et par le développement de la fumagine, une affection fongique se manifestant en présence de miellat (Lenné et

Trutmann, 1994; Hirano *et al.*, 1996). Au Québec, la mosaïque du soya (SMV) représente le principal virus rencontré et le plus problématique dans la culture du soya, bien que son incidence soit faible, mais variable d'une année à l'autre (Rioux *et al.*, 2008). Les infestations massives du puceron du soya ont déjà enclenché l'utilisation de traitements insecticides coûteux et nuisibles pour l'environnement et la santé humaine au Québec. Les coûts d'application d'insecticides à grande échelle peuvent être très importants. En 2007, plus de 75 000 ha ont été traités au lambda-cyhalothrine, ce qui équivaut à 3,5 M$ (Brodeur et Roy, 2008).

La culture du soya au Québec est en nette progression depuis quelques années avec des superficies de culture avoisinant les 178 000 hectares, représentant ainsi une hausse d'environ 125% depuis les 25 dernières années (Statistique Canada, 2006). Compte tenu des grandes superficies en culture, les traitements insecticides ont donc inévitablement un impact important sur le milieu agricole ainsi que les écosystèmes environnants. Avant l'arrivée du puceron du soya, l'impact des quelques ravageurs présents (ex : altises, pentatomidés, tétranyques, légionnaires) dans la culture du soya était minime et il était donc inutile de traiter cette culture, sauf à l'occasion. La culture du soya aurait donc joué un rôle important comme réservoir d'ennemis naturels pour les cultures adjacentes, dont le maïs (Heimpel et Shelly, 2004). Afin de maintenir ces services écologiques et de préserver la qualité de notre environnement, il importe d'évaluer l'impact des prédateurs présents sur la dynamique des populations du puceron du soya et de promouvoir l'utilisation de méthodes de lutte biologique ou intégrée. Présentement, les prédateurs généralistes, tel que les coccinelles, dominent la communauté d'ennemis naturels au Canada, avec l'observation rarissime de parasitoïdes (Fox *et al.*, 2004; Rutledge *et al.*, 2004; Mignault *et al.*, 2006).

1.1.2 Les coccinelles en lutte biologique

Les coccinelles sont bien connues en lutte biologique depuis le succès de *Rodolia cardinalis* (Mulsant) en Californie pour le contrôle de la cochenille *Icerya purchasi* Maskell dans les vergers d'agrumes (Dixon *et al.*, 1997). Malheureusement, les coccinelles aphidiphages n'ont pas très bonne figure en ce 21^{e} siècle. Alors qu'elles étaient les reines

de la lutte biologique suite au succès foudroyant de *Rodolia*, les coccinelles sont aujourd'hui considérées par plusieurs comme des agents de lutte peu efficaces (Hemptinne et Dixon, 1991; Kindlmann et Dixon, 1993; Dixon *et al.*, 1997). On déplore leur manque de spécificité (plusieurs cas de lutte biologique classique ont engendré des impacts indirects négatifs dus à l'habitude alimentaire généraliste de l'ennemi naturel), leur satiété rapide lorsque la densité des pucerons est élevée et le taux d'accroissement de leurs population très lent par rapport à celui des pucerons (Mills, 1982). Certaines études ont même affirmé que leur présence ne réduit pas de façon significative les populations de pucerons. Ainsi, les populations du puceron *Aphis gossypii* Glover atteignent des densités considérables sur des hibiscus, et ce, même en présence de nombreuses coccinelles prédatrices (Kindlmann *et al.*, 2005).

Par contre, les agents de lutte généralistes comme les coccinelles sont vu par d'autres comme des ennemis naturels contribuant efficacement au contrôle des populations de ravageurs (Symondson *et al.*, 2002; Obrycki *et al.*, 2009). Leur intérêt réside dans le fait qu'elles sont en mesure de consommer d'autres types de proies lorsque le ravageur ciblé est absent. Ceci permet de maintenir des populations de prédateurs dans l'habitat, prêtes à réagir s'il y a résurgence de l'espèce ciblée (Dixon, 2000). Lors de programmes de lutte biologique de conservation ou d'augmentation, les coccinelles peuvent permettre de diminuer les infestations de pucerons (van Emden et Harrington, 2007). Récemment, deux études ont démontré que les coccinelles *Harmonia axyridis* (Pallas) et *Coccinella septempunctata* L. étaient les principales responsables du contrôle biologique du puceron du soya et parvenaient à maintenir les populations de ce ravageur sous le seuil économique (Costamagna *et al.*, 2007; Rhainds *et al.*, 2007b).

1.1.3 Les coccinelles dans la culture du soya

Les coccinelles représentent les prédateurs aphidiphages dominants dans la culture du soya au Québec (Mignault *et al.*, 2006). Les principales espèces recensées sont: la coccinelle asiatique, la coccinelle à sept points, la coccinelle maculée et la coccinelle à quatorze points.

1.1.3.1 La coccinelle asiatique

La coccinelle asiatique, *H. axyridis*, est une espèce polymorphe de grande taille (4,9 à 8,2 mm) (Iablokoff-Khzorian, 1982), et originaire de l'Asie du Centre et de l'Est (Hodek, 1973). Son utilisation comme agent de lutte biologique en Amérique du Nord remonte en 1916 aux États-Unis (Gordon, 1985). Après plusieurs années et quelques introductions successives, l'établissement en 1988 d'une première population de coccinelles asiatiques fut signalé par Chapin et Brou (1991). Son aire de répartition n'a cessé de croître depuis pour ainsi atteindre en 1994 le Québec (Coderre *et al.*, 1995) et elle est maintenant répandue à travers le Québec et ce dans toutes les cultures (Lucas et al. 2007)

1.1.3.2 La coccinelle à sept points

La coccinelle à sept points, *C. septempunctata*, est une espèce de grande taille (5 à 8 mm) présente pratiquement dans toute la région Paléarctique (Iablokoff-Khzorian, 1982). Espèce essentiellement aphidiphage, elle consomme néanmoins d'autres insectes (ex: thrips, aleurodes, chrysopes, lépidoptères, etc.) ainsi que du pollen et du nectar (Iablokoff-Khzorian, 1982). Plusieurs tentatives d'introduction en Amérique du Nord, dans le cadre d'une lutte biologique classique, ont été infructueuses entre les années 1956 et 1971 (Angalet *et al.*, 1979). Par contre, depuis la première détection d'une population naturalisée au New Jersey (États-Unis) en 1973 (Schaefer *et al.*, 1987), cette espèce a pris de l'expansion et colonise maintenant toutes les régions de l'Amérique du Nord. Elle est devenue l'une des principales espèces présentes dans les agroécosystèmes (Gordon, 1985).

1.1.3.3 La coccinelle maculée

La coccinelle maculée, *Coleomegilla maculata lengi* (Timberlake), de taille moyenne (4,2 à 6,6 mm), indigène de l'est de l'Amérique du Nord (Gordon, 1985), représente une espèce agricole importante dans plusieurs cultures (Hodek, 1973; Andow et Risch, 1985; Groden *et al.*, 1990). Sa diète se constitue principalement de pucerons, mais elle consomme également de grandes quantités de pollen, pouvant représenter jusqu'à 50% de son alimentation (Cottrell et Yeargan, 1998a).

1.1.3.4 La coccinelle à quatorze points

La coccinelle à quatorze points, *Propylea quatuordecimpunctata* L., est une espèce de petite taille (3,5 à 5,2 mm), principalement aphidiphage (Gordon, 1985). Elle est originaire de toute la région Paléarctique, mais est surtout commune en Europe (Iablokoff-Khzorian, 1982). Introduite accidentellement en Amérique du Nord, elle a été aperçue pour la première fois en 1968 à Montréal, Qc. (Canada) (Gordon, 1985; Day *et al.*, 1994). Elle a toutefois été également introduite aux États-Unis entre les années 70 et 80 pour lutter contre *Schizaphis graminum* (Rondani) sur le sorgho mais son établissement s'est avéré infructueux (Iablokoff-Khzorian, 1982).

1.1.3.5 La dynamique des populations de coccinelles

Les populations de coccinelles présentes dans les champs de soya au Québec sont échantillonnées depuis 2002 (Mignault *et al.*, 2006; A.-È. Gagnon, *non publié*). Nous avons ainsi pu observer une séquence temporelle quant à l'arrivée des diverses espèces de coccinelles dans les champs de soya infestés de pucerons (Figure 1.1A). *Propylea quatuordecimpunctata* arrivait habituellement la première alors que la densité des pucerons était très faible. Suivait ensuite *C. septempunctata*, *H. axyridis* et *C. maculata*. Par ailleurs, au fil des ans, la composition des espèces de coccinelles s'est modifiée (Figure 1.1A; B). Alors qu'en 2002 *P. quatuordecimpunctata* dominait la communauté de prédateurs aphidiphages, elle a graduellement été remplacée par *H. axyridis*, qui domine largement depuis quelques années la communauté de coccinelles dans les champs de soya. De ce fait, la ségrégation temporelle ainsi que le changement dans la composition des espèces laissent présager la présence d'interactions entre ces quatre espèces de coccinelles.

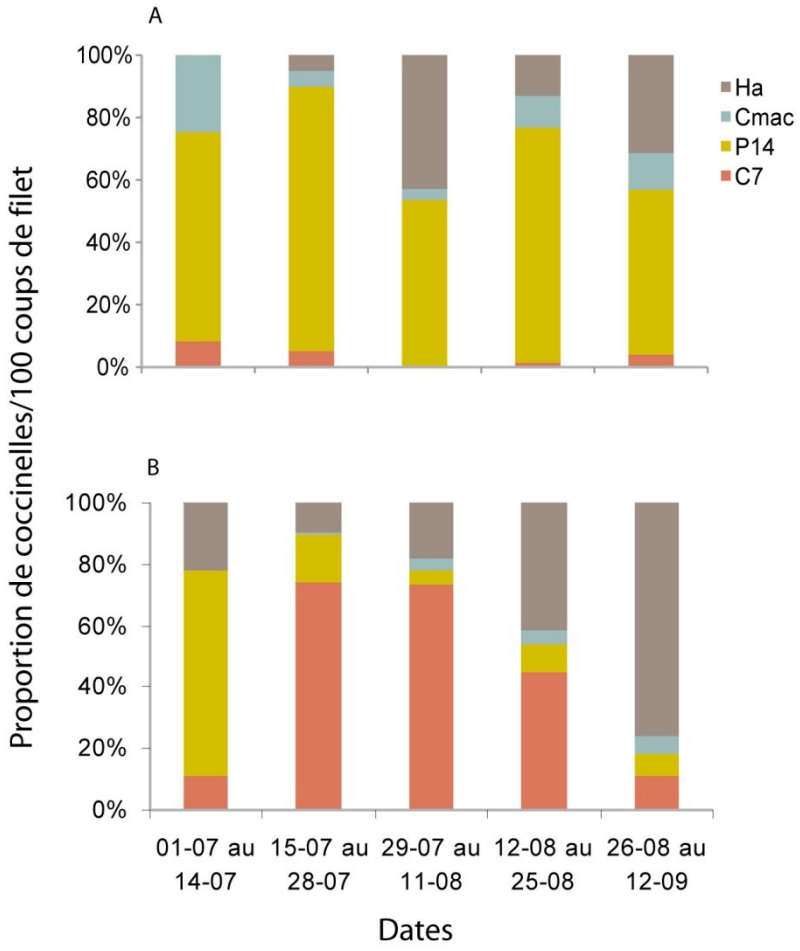

Figure 1.1. Abondance relative de quatre espèces de coccinelles dans les champs de soya au cours de la saison de croissance en 2002 (A) et 2004 (B), au Québec. Ha= *Harmonia axyridis*; Cmac= *Coleomegilla maculata*; P14= *Propylea quatuordecimpunctata* et C7= *Coccinella septempunctata*.

1.2 La prédation intraguilde (IGP)

1.2.1 Définition

Un groupe d'espèces qui exploite une même ressource constitue une guilde (Begon *et al.*, 1996). Il existe de nombreux types d'interactions entre les membres d'une même guilde: symbiose, mutualisme, compétition pour les ressources, prédation. La prédation intraguilde (IGP) est un phénomène où un individu dévore une espèce faisant partie de la même guilde (Polis et McCormick, 1987). Cette interaction a la particularité d'inclure deux processus écologiques majeurs qui structurent les communautés végétales et animales, soit la prédation et la compétition (Polis, 1994). L'IGP peut survenir autant chez les prédateurs, les parasitoïdes que les agents pathogènes (Figure 1.2B) (Rosenheim *et al.*, 1995; Borer *et al.*, 2007). De plus, l'IGP peut être de type mutuel, lorsque les deux protagonistes s'attaquent l'un et l'autre, ou unidirectionnel lorsqu'un des deux protagonistes est systématiquement désavantagé (Rosenheim *et al.*, 1995). Ainsi, l'agresseur constitue le prédateur intraguilde, la victime la proie intraguilde, alors que la ressource commune représente la proie extraguilde (Figure 1.2A) (Lucas *et al.*, 1998). L'IGP apporte comme avantages au prédateur un apport nutritif immédiat et une diminution de la compétition en abaissant le nombre de compétiteurs (Polis et Holt, 1992).

1.2.2 Prépondérance de l'IGP dans diverses communautés

De nombreuses études empiriques en milieu contrôlé ont démontré les possibilités d'IGP entre différentes combinaisons de prédateurs au sein de plusieurs systèmes (e.g. Agarwala et Dixon, 1991; Agarwala et Dixon, 1992; Kester et Jackson, 1996; Mehner *et al.*, 1996; Cottrell et Yeargan, 1998b; Morin, 1999; Burgio *et al.*, 2002). Par contre, peu d'études ont démontré l'importance de l'IGP en milieu naturel. Certains auteurs doutent même de l'importance ou même de l'existence de l'IGP en stipulant qu'elle ne serait qu'un artefact de laboratoire (Pimm et Lawton, 1978; Hemptinne et Dixon, 2005). Néanmoins, l'IGP a été observée en nature au sein de plusieurs groupes taxonomiques animals : insectes, scorpions, poissons, salamandres, oiseaux de proie, mammifères carnivores, etc. (Polis et McCormick,

1987; Palomares et Caro, 1999; Holbrook et Petranka, 2004; Salo *et al.*, 2008; Sergio et Hiraldo, 2008). Pertinemment, une méta-analyse a examiné les liens trophiques entre 763 proies intraguildes potentielles et 599 prédateurs intraguildes potentiels, révélant ainsi la présence d'IGP dans 58,4 à 86,7 % des combinaisons prédateur-proie (Arim et Marquet, 2004).

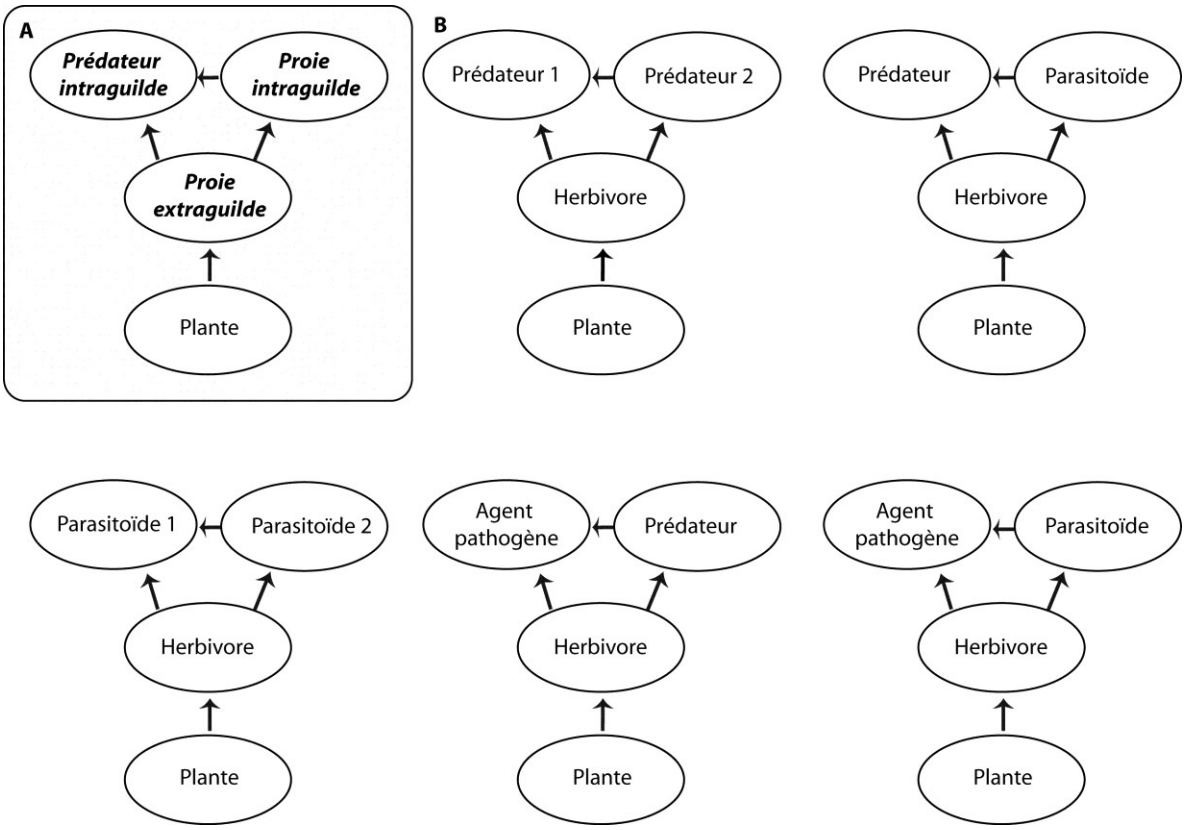

Figure 1.2. Schéma représentatif de la prédation intraguilde : A) nomenclature des différents acteurs de l'IGP; B) Différentes combinaisons potentielles de l'IGP entre prédateurs, parasitoïdes et agents pathogènes. La direction des flèches représente le sens du flux d'énergie entre les niveaux trophiques.

1.2.3 Les modèles théoriques de la prédation intraguilde

Les premiers modèles théoriques sur l'omnivorie (en incluant l'IGP) prédisaient un déséquilibre de la dynamique des populations d'un réseau trophique (Cohen *et al.*, 1986; Schoenly *et al.*, 1991). Puisqu'il est difficile de concevoir qu'il y ait persistance des espèces en condition de déséquilibre, l'omnivorie et l'IGP étaient considérées comme étant des interactions rares. Néanmoins, les études rapportant la présence de l'IGP en milieu naturel ont amené les théoriciens à revoir l'importance et le rôle de cette interaction dans la dynamique d'un écosystème (Polis et Holt, 1992; Holt et Polis, 1997). Les premiers modèles développés spécifiquement sur l'IGP considéraient les interactions entre trois seules espèces : le prédateur intraguilde, la proie intraguilde et la proie extraguilde. Notamment, pour qu'il y ait coexistence et persistance de toutes ces espèces, la proie intraguilde doit représenter l'espèce exploitant le plus efficacement la ressource commune. Il est à noter aussi que ces modèles «simplistes» sous-entendent que l'IGP est unidirectionnelle, *i.e.* que seul le prédateur intraguilde consomme la proie intraguilde et non l'inverse.

Par ailleurs, un modèle a été développé pour décrire les interactions potentielles entre deux prédateurs selon un gradient de productivité (Figure 1.3) (Müller et Brodeur, 2002). Ainsi, à faible productivité, la relation entre deux prédateurs s'explique principalement par une compétition d'exploitation, *i.e.* où deux prédateurs partagent et font compétition pour une même ressource. Or, l'espèce exploitant le plus efficacement la ressource (la proie intraguilde) dominera en abondance la deuxième espèce (le prédateur intraguilde), diminuant ainsi les probabilités d'IGP. Lorsque la productivité du système augmente, l'IGP devient plus importante, faisant en sorte que certains prédateurs sont consommés par leur compétiteur. Une plus forte productivité favorise au contraire une situation de compétition apparente, où une seule espèce prédatrice se nourrit à la fois de la ressource ainsi que du prédateur antagoniste.

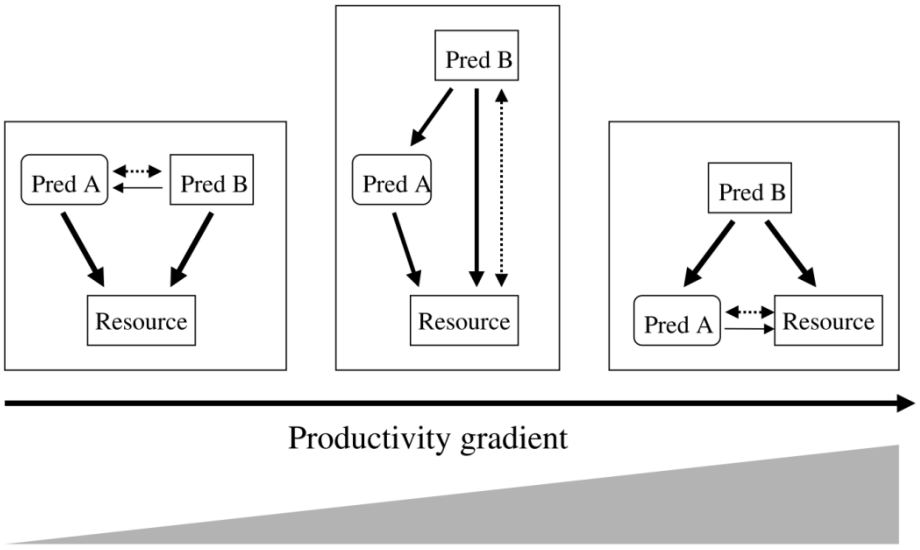

Figure 1.3. Transition entre la compétition d'exploitation, la prédation intraguilde et la compétition apparente selon un gradient de productivité. Les flèches noires et épaisses représentent les liens trophiques, les flèches pointillées les effets indirects et les flèches noires et fines les interactions ayant peu de signification. D'après Müller et Brodeur (2002).

Borer et collaborateurs (2003) ont quant à eux validé, en milieu naturel, un modèle similaire élaboré par Holt et Polis (1997). Les auteurs ont démontré une dominance de la proie intraguilde *Encarsia perniciosi* (Tower) à faible densité de proies extraguildes, *Aonidiella aurantii* (Maskell), et la dominance du prédateur intraguilde *Aphytis melinus* (DeBach) à forte densité. La coexistence entre les deux espèces de parasitoïdes était ainsi possible que lorsque la productivité de la ressource (proie extraguilde) était modérément élevée (Borer *et al.*, 2003).

Les modèles théoriques constituent bien sûr des représentations simplifiées de toute la complexité des interactions directes et indirectes retrouvées en milieu naturel. Néanmoins, plusieurs facteurs ont été intégrés aux modèles afin de mieux prédire l'occurrence de l'IGP, tel que la structure d'âge du prédateur intraguilde et de la proie intraguilde (Mylius *et al.*, 2001), le comportement de changement de préférence alimentaire (*switching*) du prédateur intraguilde selon les densités respectives des proies potentielles (Krivan et Diehl, 2005), le comportement de fuite de la proie intraguilde (Heithaus, 2001),

l'IGP mutuelle (HilleRisLambers et Dieckmann, 2003), la présence d'un niveau trophique supérieur au prédateur intraguilde (McCann *et al.*, 1998) ou le cannibalisme (Schellhorn et Andow, 1999). Toutes ces variantes du modèle de base en IGP tentent de répondre au manque de complémentarité entre les modèles théoriques et les études empiriques (voir section 1.3.2).

1.2.4 Cascade trophique et déplacement des espèces

Les études sur l'IGP se multiplient ces dernières années puisque cette interaction engendre des changements majeurs dans la structure des communautés (Polis, 1994). Malgré des impacts trophiques et intraguildes variables, deux phénomènes impliquant l'IGP ont suscité beaucoup d'intérêt dans la communauté scientifique : la cascade trophique et le déplacement des espèces. La cascade trophique survient lorsque les prédateurs d'un réseau trophique diminuent l'abondance de leur proie, relâchant ainsi le contrôle exercé sur le réseau trophique inférieur (Begon *et al.*, 1996). Dans le cas particulier le l'IGP, le prédateur intraguilde diminue les populations de la proie intraguilde, relâchant ainsi la pression de prédation exercée sur la proie extraguilde. Une telle cascade trophique peut engendrer des augmentations considérables des populations de proies extraguildes (herbivores), pouvant ainsi avoir un impact négatif sur le niveau trophique inférieur, *i.e.* les plantes. Ce phénomène est attentivement étudié en lutte biologique puisqu'un impact néfaste sur le niveau trophique inférieur n'est pas souhaitable en agriculture ou en foresterie. Par ailleurs, l'IGP peut aussi éliminer les populations d'une autre espèce de prédateur. Ce phénomène d'exclusion inquiète particulièrement la communauté scientifique se penchant sur la conservation des espèces menacées (Polis et Holt, 1992; Müller et Brodeur, 2002). Par exemple, l'introduction de nouvelles espèces exotiques et invasives d'ennemis naturels peut affecter négativement les populations indigènes de prédateur suite à l'IGP (Suutari *et al.*, 2004).

1.2.5 La prédation intraguilde et la lutte biologique

L'IGP a souvent été considérée comme ayant des effets antagonistes en lutte biologique. Ceci résulte en partie de la première étude sur ce sujet, produite par Rosenheim et

collaborateurs (1993), qui a démontré que la présence de l'IGP entre les genres C*hrysopa*, *Nabis, Geocoris* et *Zelus* diminuait le contrôle des populations du puceron *A. gossypii*. Cette étude a marqué un point marquant en lutte biologique puisqu'à la suite des travaux de Rosenheim et collaborateurs, le nombre d'études portant sur l'IGP et les ennemis naturels utilisés en lutte biologique n'a cessé de croître (e.g. Cloutier et Johnson, 1993; Kester et Jackson, 1996; Brodeur et Rosenheim, 2000; Lang, 2003; Snyder *et al.*, 2004; Briggs et Borer, 2005; Harvey et Eubanks, 2005; Lucas, 2005). Une revue réalisée par Rosenheim et collaborateurs (1995) expose la grande variabilité quant à l'impact que l'IGP peut engendrer sur le contrôle d'un ravageur. Certaines études rapportent des impacts négatifs sur le contrôle biologique (Markkula et Tiittanen, 1981; Rosenheim *et al.*, 1993), alors que d'autres n'en déclarent aucun (Parrella *et al.*, 1980; Brower et Press, 1992). Rosenheim et collaborateurs concluent en mettant l'emphase sur la nécessité de conduire davantage d'études en milieu naturel, sous des conditions expérimentales les plus similaires à celles rencontrées par les protagonistes, cela afin d'obtenir une meilleure connaissance de l'IGP au sein de différents systèmes. À la suite à de nombreuses études empiriques sur le sujet, Rosenheim et Harmon publient en 2006 une méta-analyse portant sur l'influence que l'IGP peut avoir sur le contrôle des herbivores (Rosenheim et Harmon, 2006). L'analyse révèle que l'IGP ne cause pas *a priori* un effet néfaste sur le contrôle du ravageur en lutte biologique, la moyenne de son impact étant plutôt neutre. Dans le même ordre d'idées, Janssen et collaborateurs (2006) déclarent que l'IGP a rarement un impact négatif sur la lutte biologique et que l'utilisation d'ennemis naturels généralistes semble moins risquée que ce qui avait été prétendue (van Lenteren *et al.*, 2003). Néanmoins, une troisième méta-analyse réalisée cette fois par Vance-Chalcraft et collaborateurs (2007a) apporte une vision différente et recommande de ne pas utiliser une combinaison d'agents de lutte biologique reconnus pour s'engager dans des interactions intraguildes, et ce, que l'IGP soit mutuelle ou unidirectionnelle. Toutefois, Amarasekare (2008) mentionne qu'une prédisposition à l'IGP s'avère une raison insuffisante pour ne pas utiliser un complexe d'ennemis naturels en lutte biologique, sachant que ces ennemis naturels ont développé différentes tactiques pour coexister, diminuant ainsi les risques d'IGP. Bref, cette discussion démontre bien à quel point les impacts de l'IGP en lutte biologique peuvent s'avérer variables d'un système à

l'autre et que les recommandations quant à l'utilisation d'un seul ou de plusieurs agents de lutte varient d'autant.

Dans un autre ordre d'idées, la nature des acteurs de l'IGP, *i.e.* qu'ils soient prédateurs, parasitoïdes ou agents pathogènes a un effet sur le type d'impact qu'ils peuvent engendrer en lutte biologique. Par exemple, deux études ont démontré que le contrôle des ravageurs était plus efficace en présence d'un prédateur généraliste et d'un parasitoïde que lorsque seuls les parasitoïdes assuraient la lutte biologique (Snyder *et al.*, 2004; Harvey et Eubanks, 2005). Dans ces cas, les prédateurs généralistes n'exercent pas une prédation sélective sur les proies parasitées, favorisant ainsi le maintien des populations de parasitoïdes. Il a d'ailleurs été démontré que l'IGP « coïncidente » (interaction entre des ennemis naturels de nature différente, par exemple, entre un prédateur et un parasitoïde) affecte beaucoup moins le contrôle biologique que l'IGP plus classique entre prédateurs (Rosenheim et Harmon, 2006). Cela parce que les interactions impliquant des parasitoïdes ou des agents pathogènes nécessitent la mort de la proie commune lorsqu'ils s'engagent dans une interaction intraguilde. Un parasitoïde s'attaquant à un autre parasitoïde larvaire tuera nécessairement son hôte afin de compléter son développement, et il en va de même pour un agent pathogène. Ces interactions ont donc inévitablement un effet répresseur sur les populations de la proie extraguilde puisque celle-ci est consommée. Il en est tout autrement pour les interactions entre deux prédateurs, pouvant survenir lors de rencontres durant la recherche de proies, et ne nécessitant aucunement la mort de la proie commune.

Bref, l'étude de l'IGP en lutte biologique représente toujours un sujet d'actualité puisque son impact, bien qu'il ne soit pas négatif dans tous les cas, continue d'avoir des effets néfastes sur certains programmes de lutte biologique. La compréhension des facteurs qui régissent l'IGP et des raisons pour lesquelles un prédateur intraguilde a un effet dommageable sur la lutte biologique demeure encore rudimentaire.

1.2.6 Facteurs promouvant la prédation intraguilde

Cette section présente les principales études portant sur les facteurs écologiques régulant l'intensité ou la direction de l'IGP entre deux protagonistes.

1.2.6.1 Caractéristiques intrinsèques des protagonistes

La taille des espèces interagissant entre elles détermine bien souvent la structure d'un réseau trophique (Memmott *et al.*, 2000; Woodward et Hildrew, 2002). Lors d'une interaction intraguilde, un prédateur de petite taille est dans bien des cas plus vulnérable face à un prédateur de plus grande taille (Polis *et al.*, 1989; Wissinger, 1992; Snyder et Hurd, 1995; Lucas *et al.*, 1998). Par exemple, Félix et Soares (2004) ont démontré qu'une différence significative de taille entre les larves de *H. axyridis* et de *Coccinella undecimpunctata* L. déterminait la direction de l'IGP; les individus de taille supérieure gagnant plus fréquemment une interaction intraguilde.

Dans un autre ordre d'idées, le degré de spécialisation des espèces peut faire varier l'occurrence de l'IGP. Un insecte spécialiste se trouve moins bien adapté à s'engager dans une interaction intraguilde sur des proies non-préférées et serait ainsi désavantagé face à un prédateur généraliste (Wissinger, 1989). Lucas et collaborateurs (1998) démontrent une plus grande proportion d'IGP exercée par *Chrysoperla rufilabris* Burmeister et *C. maculata,* deux insectes généralistes, comparativement à la cécidomyie spécialiste *Aphidoletes aphidimyza* Rondani.

La mobilité des protagonistes représente aussi un facteur pouvant influencer l'intensité d'IGP. Les stades sessiles ou les insectes ayant une mobilité réduite se retrouvent bien souvent vulnérables face à l'attaque de prédateurs intraguildes (Lucas *et al.*, 1998; Félix et Soares, 2004). Provost et collaborateurs (2006) ont démontré que *H. axyridis* capture habituellement la proie intraguilde la plus vulnérable, soit une espèce peu mobile, *Tetranychus urticae* Koch, par rapport à la punaise *Hyaliodes vitripennis* (Say), plus active.

1.2.6.2 Densité des proies extraguildes

La densité des proies extraguildes s'avère aussi un facteur important pouvant influencer la fréquence de l'IGP. L'augmentation de la densité des proies extraguildes diminue la compétition entre les prédateurs intraguildes et diminue dans bien les cas la fréquence de l'IGP. Au sein d'une communauté de trois prédateurs aphidiphages, Lucas et collaborateurs (1998) ont observé quatre scénarios différents caractérisant la relation entre la densité des proies extraguildes et la fréquence de l'IGP. Dans un premier cas, l'IGP décroît de façon constante avec l'augmentation des proies extraguildes. Ce scénario pourrait survenir chez deux prédateurs cherchant leurs proies de façon aléatoire. Dans un deuxième scénario, l'IGP diminue exponentiellement avec l'augmentation des proies extraguildes. Dans ce cas, une confrontation présenterait un risque pour les deux individus et en présence de proies extraguildes, ceux-ci éviteraient toute interaction. Dans un troisième scénario, l'IGP demeure constante, et ce, peut importe la densité des proies extraguildes. Cela pourrait s'expliquer selon trois situations différentes : i) le prédateur ne court aucun risque à s'engager dans une interaction intraguilde; ii) le comportement de recherche du prédateur augmente les probabilités de rencontre avec la proie intraguilde et iii) la concentration de la ressource augmente les risques de confrontation entre les prédateurs. Pour le dernier scénario, l'IGP demeure constante et élevée à faible densité de proies extraguildes mais diminue à très forte densité. Un tel scénario pourrait se produire lorsque la compétition entre les prédateurs est forte à faible densité de proies extraguildes et que l'IGP permet de diminuer cette compétition. À plus forte densité, la compétition diminue considérablement et l'effet de dilution réduit d'autant les probabilités de rencontre entre les prédateurs, diminuant ainsi l'IGP.

Par ailleurs, la densité des proies extraguildes peut être directement reliée à la productivité du milieu. Plusieurs modèles théoriques ont prédit une fréquence plus élevée d'IGP lorsque le gradient de productivité augmentait (voir section 1.2.3).

1.2.6.3 Structure de l'habitat

L'intensité de l'IGP en milieu naturel résulte non seulement de l'habileté d'un prédateur à consommer un autre prédateur, mais aussi de la probabilité de rencontre entre les protagonistes lorsqu'ils sont en quête alimentaire (Musser et Shelton, 2003). La probabilité de rencontre peut être fonction, entre autres, de la dynamique temporelle, de la structure de l'habitat ainsi que du mode de prédation (e.g. prédateur actif vs prédateur furtif) (Sih *et al.*, 1998). Plus particulièrement, la structure de l'habitat, en étant plus complexe, peut réduire la fréquence d'une interaction intraguilde en fournissant des refuges, diminuant ainsi le taux de rencontre entre un prédateur et sa proie intraguilde (Finke et Denno, 2002; Janssen *et al.*, 2007). La diversité de la structure de l'habitat s'avère un paramètre important du déterminisme des patrons généraux de l'abondance des arthropodes ainsi que de leur diversité sur une plante, et par conséquent de l'impact que les prédateurs peuvent infliger sur les herbivores (Halaj *et al.*, 2000) ou sur les proies intraguildes. Bien que les proies extraguildes deviennent moins facilement accessibles dans un environnement plus complexe, il a été démontré dans plusieurs systèmes que la diminution du risque d'IGP entre les prédateurs permet un meilleur contrôle de l'herbivore (Finke et Denno, 2002; 2006; Rickers *et al.*, 2006). Dans les agroécosystèmes, la complexité de l'habitat varie selon les pratiques agricoles (Sunderland et Samu, 2000). Par exemple, une culture biologique présente une plus grande diversité végétale (présence de mauvaises herbes) qu'une culture conventionnelle (Roschewitz *et al.*, 2005). De plus, la croissance des végétaux complexifie le milieu en procurant une plus grande superficie foliaire au fur et à mesure que la saison de croissance progresse. Également, il a été démontré que l'augmentation de la complexité végétale de l'habitat, que ce soit à grande échelle (culture intercalaire, semis direct, polyculture) ou à petite échelle (modification de l'architecture de la plante selon la pubescence, le nombre de feuilles ou les ramifications), soutient de plus fortes densités d'ennemis naturels (Kareiva et Sahakian, 1990; Langellotto et Denno, 2004). Ainsi, l'augmentation de la complexité de l'habitat contribue à une meilleure coexistence des ennemis naturels en diminuant le risque d'IGP.

1.2.6.4 Stratégies de défense

Les prédateurs ont développé des tactiques afin de mieux survivre aux interactions intraguildes. Par exemple, *C. septempunctata* pond ses œufs avant *H. axyridis*, une espèce s'engageant fréquemment dans des interactions intraguildes (Yasuda et Shinya, 1997). Ainsi, les larves de *C. septempunctata* se développent plus rapidement et ont un avantage par rapport aux larves de *H. axyridis*. De plus, la défense chimique permet de diminuer les risques d'IGP entre prédateurs hétérospécifiques (Hemptinne *et al.*, 2000). Ainsi, les espèces de petites tailles, telle *Adalia bipunctata* L., sont plus toxiques aux espèces de grande taille comme *C. septempunctata* (Agarwala et Dixon, 1992). La ségrégation spatiale est aussi une tactique permettant de diminuer les rencontres entre prédateurs intraguildes. Par exemple, Hoogendoorn et Heimpel (2004) ont démontré que *C. maculata* se retrouvait plus fréquemment à la base des plants de maïs lorsque *H. axyridis* était présente.

1.2.7 La prédation intraguilde au sein des Coccinellidae

1.2.7.1 Caractéristiques favorisant l'IGP chez les coccinelles

Le cannibalisme et l'IGP sont considérés comme des phénomènes fréquents chez les coccinelles (Hodek, 1973; Iablokoff-Khzorian, 1982; Agarwala, 1991; Hodek et Honěk, 1996; Dixon, 2000; Fox *et al.*, 2004) La nature de ces insectes, généralement polyphages, augmente les probabilités d'IGP (voir aussi 1.2.6.1). Leur habitat et leur comportement de recherche alimentaire augmentent les risques de rencontre entre les espèces de prédateurs aphidiphages. Ainsi, puisque les colonies de pucerons se distribuent en petites taches souvent constituées de plusieurs dizaines d'individus, ces ressources attirent de nombreux prédateurs sur une surface restreinte (Cappuccino, 1988). Une larve de coccinelle cherche des proies en se déplaçant de haut en bas sur des plants, mais aussitôt qu'une proie est trouvée, la recherche s'intensifie en inspectant minutieusement le site (Iablokoff-Khzorian, 1982). De plus, les femelles adultes pondent leurs œufs en périphérie ou au cœur des colonies afin d'assurer des ressources abondantes à leur progéniture (Evans et Dixon, 1986). Or, typiquement, ce sont diverses cohortes de larves d'ennemis naturels qui se retrouveront sur un même plant, à la recherche de proies extraguildes, mais qui auront de fortes probabilités de rencontrer des prédateurs intra- ou hétérospécifiques.

De plus, les coccinelles possèdent de nombreux stades vulnérables à l'IGP (de l'œuf à la pupe), ce qui augmente les risques de prédation (Lucas *et al.*, 2000). Les coccinelles possèdent toutefois divers types de défenses face à l'IGP tel que des comportements antiprédateurs ou de ségrégation spatiale, des défenses chimiques (particulièrement chez les œufs) ou des caractéristiques morphologiques (épines dorsales, cuticules cireuses) (Hemptinne *et al.*, 2000; Michaud et Grant, 2003; Hoogendoorn et Heimpel, 2004).

1.2.7.2 L'IGP et les coccinelles à l'étude

Depuis la naturalisation de *H. axyridis*, un agent de lutte biologique, en Amérique du Nord, plusieurs écologistes se questionnent sur l'impact qu'elle engendre sur les autres espèces d'ennemis naturels indigènes (Cottrell et Yeargan, 1998b). Le choix d'une espèce pour la lutte biologique étant basé sur des critères de performance, cela favorise l'introduction d'espèces compétitives, parfois au détriment d'espèces déjà présentes (Simberloff et Stiling, 1996; Hajek, 2004). L'augmentation des populations de *H. axyridis* dans le milieu, ainsi que des changements dans la composition des communautés de Coccinellidae (Nault et Kennedy, 2003) suscitent un grand intérêt. Plusieurs caractéristiques de *H. axyridis* font d'elle une compétitrice hors pair. Notons par exemple sa grande voracité, sa grande taille, sa fécondité supérieure aux autres espèces de Coccinellidae et son temps de développement relativement court (Iablokoff-Khzorian, 1982; Yasuda et Ohnuma, 1999; Michaud, 2002; Koch, 2003; Labrie *et al*., 2006; Koch et Galvan, 2008). Il a été démontré que cette espèce dominait d'autres coccinelles tel que *C. septempunctata* et *Adalia bipunctata* L. en faisant compétition pour les ressources alimentaires ou directement par l'IGP (Kajita *et al.*, 2000; Yasuda *et al.*, 2001).

L'arrivée de *C. septempunctata* dans le paysage agricole de l'Amérique du Nord a aussi eu un impact néfaste sur les espèces de coccinelles indigènes (Elliott *et al.*, 1996). Par exemple, Evans (2004) rapporte que cette espèce a diminué considérablement l'abondance des ressources locales de puceron du pois, *Acyrthosiphon pisum* (Harris), dans les champs de luzerne, diminuant ainsi la distribution et l'abondance des espèces indigènes, tel que

Hippodamia convergens Guerin et *Coccinella transversoguttata richardsoni* Brown. D'autre part, dans les cultures de soya aux États-Unis, deux espèces de coccinelles, soit *H. axyridis* et *C. septempunctata,* dominent la guilde des prédateurs. Or, *H. axyridis* est un prédateur bien connu de plusieurs espèces de coccinelles, dont *C. septempunctata* (Yasuda *et al.*, 2001). Une étude réalisée par Costamagna et Landis (2006) a observé une plus grande abondance de *C. septempunctata* dans les cultures de soya en semis direct, alors que *H. axyridis* dominait les cultures conventionnelles et les cultures sans intrants. Cette ségrégation pour les systèmes de culture pourrait atténuer les interactions négatives entre ces deux espèces.

De plus, il a été démontré que *C. maculata* était vulnérable à l'IGP exercée par *H. axyridis* (Cottrell et Yeargan, 1998b). Cette dernière étant capable de compléter son cycle de vie à l'aide d'une alimentation basée uniquement sur une diète de *C. maculata.* Néanmoins, Hoogendoorn et Heimpel (2004) et Lucas et collaborateurs. (2000) ont mis en évidence une ségrégation spatiale de *C. maculata* pour éviter la rencontre de l'espèce compétitrice, réduisant ainsi l'IGP entre ces deux espèces en milieu naturel.

Propylea quatuordecimpunctata, quant à elle, serait vulnérable à l'IGP de par sa petite taille (Félix et Soares, 2004). Les coccinelles de petites tailles semblent se doter d'une défense chimique plus importante pour compenser les désavantages liés à leur taille (Sato et Dixon, 2004). Par contre, aucune étude n'identifie une défense chimique chez cette coccinelle. De plus, Jansen et Hautier (2008) ont émis l'hypothèse que dans le système de la pomme de terre, *H. axyridis* doit consommer *P. quatuordecimpunctata* afin de compléter son développement larvaire. Ainsi, la coccinelle à quatorze points représenterait, tout comme la coccinelle maculée, une proie intraguilde potentielle aux autres prédateurs présents, n'excluant pas pour autant leur potentiel comme prédateurs intraguildes.

1.3 Problématiques actuelles dans l'étude de l'IGP

1.3.1 Détection de l'IGP chez les insectes

La détermination précise du régime alimentaire d'un insecte, information indispensable pour l'étude des interactions trophiques et intraguildes au sein d'une communauté, s'avère particulièrement difficile chez les prédateurs. Certaines études ont recours à l'examen du contenu gastrique suite à la dissection de l'insecte afin d'identifier les fragments de proies restants (Sunderland *et al.*, 1987; Triltsch, 1999). Par contre, les proies liquides ou liquéfiées ou celles qui ont été ingurgitées depuis plusieurs heures sont pratiquement impossibles à identifier. D'autres chercheurs observent directement le comportement alimentaire d'un insecte en milieu naturel (Hazzard *et al.*, 1991; Greenstone, 1999), mais la plupart des arthropodes sont petits, cryptiques ou se nourrissent de nuit, ce qui complexifie la tâche. Également, la fréquence des interactions de type IGP peut être très faible. La détection et la quantification de l'IGP sans perturber l'écosystème sont difficiles, voir même impossible à réaliser à l'aide d'expériences réalisées en milieu artificiel, comme les microcosmes. Toutes tentatives d'observer les insectes mènent à une perturbation de l'écosystème, de par l'éclaircissement du couvert végétal, l'éclairage en condition nocturne ou la mise en cage d'une parcelle (Symondson, 2002).

1.3.2 Divergence entre les modèles théoriques et empiriques

Les modèles théoriques sur l'IGP présentent aussi un défi majeur quant à leur validation en milieu naturel ou dans le contexte de la lutte biologique. Alors que la théorie prédit un impact positif de l'IGP sur les densités de proies extraguildes, et donc une perturbation du contrôle biologique d'un ravageur (Polis et Holt, 1992; Holt et Polis, 1997), on observe des patrons très variables en milieu naturel (Janssen *et al.*, 2006; Rosenheim et Harmon, 2006). Dans bien des cas, la densité des proies extraguildes est peu affectée par la présence d'IGP, elle peut même être augmentée (Losey et Denno, 1998). De plus, tel que mentionné ci-haut, les modèles ont été développés sur la base des interactions entre trois acteurs uniquement (le prédateur intraguilde, la proie intraguilde et la proie extraguilde) alors que la réalité repose sur un bien plus grand nombre d'acteurs, tant des ennemis naturels, des herbivores

et voire même des plantes. Ainsi, l'inclusion de proies alternatives dans un modèle se traduit par une réponse bien différente, soit une persistance des proies intraguildes dans le milieu (Holt et Huxel, 2007).

Les aspects temporel et spatial dans les dispositifs expérimentaux présentement utilisés sont aussi des facteurs qui freinent notre compréhension d'interactions complexes. Par exemple, la majorité des études réalisées à ce jour sur l'impact de l'IGP sur la dynamique des populations des protagonistes durent en moyenne 25 jours, ce qui est peu pour l'atteinte d'un état d'équilibre et évaluer les effets à moyen et long termes (Rosenheim et Harmon, 2006). Des études à long terme, couvrant la totalité du cycle de vie d'un insecte, sont nécessaires pour évaluer de façon adéquate les conséquences de l'IGP sur les populations, structure des communautés et la lutte biologique (Rosenheim *et al.*, 1995). Par ailleurs, l'aspect spatial est souvent réduit au minimum dans les études empiriques (voir section 1.2.6.3) (Janssen *et al.*, 2007). Un besoin criant se fait sentir pour de nouvelles méthodes d'analyse des interactions intraguildes permettant d'inclure toute la complexité particulière à un milieu.

1.4 Détection moléculaire du contenu gastrique des insectes

La détection moléculaire des proies du contenu gastrique d'un prédateur permet d'étudier certains phénomènes (parasitisme, prédation, infection pathogénique, symbiose bactérienne) en milieu naturel sans avoir recours à des systèmes simplifiés (études en boîte de pétri, en chambre de croissance ou en cage d'exclusion). Cette approche génère des opportunités nouvelles et complémentaires permettant de formuler des hypothèses autrefois impossibles à tester avec les techniques traditionnelles (Zaidi *et al.*, 1999; Symondson, 2002). L'écologie moléculaire permet donc d'explorer un système biologique avec un tout nouveau niveau de résolution. L'utilisation des méthodes moléculaires pour identifier un organisme ou une proie peut s'avérer avantageuse lorsque qu'ils sont confinés à l'intérieur d'un organisme. Notons par exemple l'identification d'organismes vivants à l'intérieur d'un insecte; de larves de parasitoïdes se nourrissant de son hôte ou tout simplement de proies

contenues dans le tractus digestif des prédateurs (Chen *et al.*, 2000; Hoogendoorn et Heimpel, 2001). Les marqueurs moléculaires à base d'ADN sont très efficaces pour ce genre d'étude puisque qu'une infime quantité d'ADN peut aisément être amplifiée, puis détectée, et ce, même en présence d'ADN hétérospécifique. Certaines bactéries, dont celles du genre *Wolbachia*, ont ainsi été étudiées à l'aide de marqueurs moléculaires afin de déterminer leur présence chez l'hôte (Hoshizaki et Shimada, 1995). De même, la présence et l'identité de larves de parasitoïdes ont facilement pu être décelées chez plusieurs hôtes tels les mouches domestiques (Ratcliffe *et al.*, 2002), les pucerons (Persad *et al.*, 2004; Weathersbee *et al.*, 2004) et certains coléoptères (Aebi *et al.*, 2004). À ce jour, très peu d'études utilisant les méthodes moléculaires ont été réalisées sur l'IGP, et les quelques travaux accomplis dans ce domaine n'ont pas révélé d'interactions intraguildes très fréquentes. Par exemple, Harwood et collaborateurs (2007b) ont analysé le comportement alimentaire de la punaise prédatrice, *Orius insidiosus* (Say), sans toutefois y démontrer la présence de proies intraguildes ou aussi peu que 2,5% envers *H. axyridis* (Harwood *et al.*, 2009). L'application des méthodes de détection moléculaire dans les études sur les interactions intraguildes permet une appréciation précise de la nature et de l'intensité de ce type d'interaction en milieu naturel.

1.5 Hypothèses et objectifs de recherche

La présente thèse a été réalisée dans le but d'approfondir les connaissances théoriques sur la prédation intraguilde. Cela afin de mieux comprendre sa prépondérance dans les écosystèmes terrestres, les facteurs régulant son occurrence ainsi que son rôle écologique, dont l'impact probable en lutte biologique. Pour ce faire, nous avons étudié une communauté de coccinelles associées au puceron du soya, *A. glycines*, dans la culture du soya. Il s'agit d'un système biologique très propice à l'étude de l'IGP puisque plusieurs études suggèrent que les coccinelles sont fréquemment impliquées dans ce type d'interaction. De plus, une ségrégation spatiale et temporelle de diverses espèces de coccinelles a été observée ces dernières années dans les champs de soya du Québec (Lucas *et al.* 2007), ce qui laisse supposer la présence de compétition et de prédation entre les coccinelles. Très peu d'études en milieu naturel, sans perturbation du milieu, ont été

réalisées afin de mieux comprendre le phénomène de l'IGP. La détection moléculaire du contenu gastrique représente une avenue innovatrice et prometteuse afin de caractériser le comportement intraguilde des espèces à l'étude.

Nos hypothèses de recherche sont que : i) l'IGP sera fréquente en milieu naturel au sein des Coccinellidae; ii) l'augmentation de la densité de la proie extraguilde diminuera la fréquence d'IGP; iii) une plus grande complexité de l'habitat aura pour effet d'amoindrir les interactions entre les antagonistes; et iv) une forte fréquence d'IGP entre les coccinelles aura un effet négatif sur la lutte biologique au puceron du soya.

Les principaux objectifs de cette thèse étaient de:

I) Conceptualiser des amorces PCR pour la détection de quatre espèces de coccinelles (proies intraguildes) contenues dans le tractus digestif de coccinelles prédatrices;

II) Mesurer le succès de détection de l'ADN des proies intraguildes dans le temps, suite à la digestion, et ce dans le contenu gastrique de chacune des quatre espèces de prédateurs;

III) Déterminer la fréquence et la symétrie de l'IGP au champ entre les quatre espèces de coccinelles;

IV) Identifier les facteurs écologiques promouvant l'occurrence de l'IGP dans le système à l'étude;

VI) Évaluer l'effet de la densité de la proie extraguilde et de la complexité de l'habitat sur l'IGP entre *H. axyridis* et *P. quatuordecimpunctata* et ses conséquences en lutte biologique.

1.6 Description des chapitres

Le chapitre II présente une technique moléculaire que nous avons développée, puis validée pour quantifier les interactions intraguildes au sein de la communauté de coccinelles. Nous introduisons aussi une méthode originale de correction, basée sur les vitesses de digestion des proies intraguildes pour chacune des combinaisons prédateur-proie, qui doit être adoptée lors de comparaisons des taux d'IGP entre espèces. Le chapitre III, à la suite de

l'utilisation des méthodes moléculaires décrites au chapitre précédent, rapporte une première évidence expérimentale en milieu naturel de fréquences très élevée d'IGP chez des insectes. Le chapitre IV analyse les principaux facteurs écologiques qui favorisent l'IGP par les prédateurs *H. axyridis* et *C. septempunctata*. Le cinquième chapitre présente les résultats d'une expérience réalisée en champ, en milieu ouvert, sur l'effet de la complexité de la structure de la plante et de la densité des herbivores (pucerons) sur la fréquence de l'IGP et, par conséquence, l'impact sur la lutte biologique au puceron du soya. Pour terminer, le chapitre VI résume l'ensemble des résultats obtenus, discute leur signification et suggère de nouvelles avenues de recherche.

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CHAPITRE II

Prey DNA detection success following digestion by intraguild predators: influence of prey and predator species*

*Ce chapitre a été soumis à la revue scientifique ***Molecular Ecology Resources***. Les auteurs sont Annie-Ève Gagnon (Centre de Recherche en Horticulture, Université Laval), Josée Doyon (Institut de recherche en biologie végétale, Université de Montréal), George Heimpel (University of Minnesota) et Jacques Brodeur (Institut de recherche en biologie végétale, Université de Montréal).

2.1 Résumé

Depuis les deux dernières décennies, la prédation intraguilde (IGP) est de plus en plus reconnue comme une interaction d'importance dans les écosystèmes. De nombreuses avancées remarquables ont permis de caractériser sa nature et son intensité. Nous avons mis au point une technique moléculaire utilisant l'analyse du contenu gastrique des prédateurs pour comparer le taux d'IGP entre des espèces de coccinelles. Nous avons premièrement développé des amorces PCR pour quatre espèces: *Harmonia axyridis, Coccinella septempunctata, Coleomegilla maculata* et *Propylea quatuordecimpunctata.* Nous avons également déterminé la durée de la période de détection de l'ADN des proies (DS_{50}) à la suite de leur ingestion pour chaque combinaison des espèces en interaction. Nous avons observé une grande variabilité des valeurs de DS_{50} selon les combinaisons prédateur-proie, soit de 5,2 h à 19,3 h. En conséquence, il est impossible de déterminer un patron commun de temps de digestion chez les coccinelles, qu'elles aient un rôle de proie ou de prédateur. Nous avons utilisé les valeurs de DS_{50} pour corriger les données provenant des coccinelles capturées au champ afin de démontrer l'importance de la compensation des temps de

détection, spécifiques à chaque combinaison prédateur-proie, dans l'interprétation des résultats.

2.2 Abstract

Intraguild predation (IGP) has been increasingly recognized as an important interaction in ecological systems over the past two decades and remarkable insights have been gained into its nature and prevalence. We have developed a technique using molecular gut-content analysis to compare the rate of IGP between closely-related species of coccinellid beetles (lady beetles or ladybirds) which had been previously known to prey upon one another. We first developed PCR primers for each of four lady beetle species: *Harmonia axyridis, Coccinella septempunctata, Coleomegilla maculata* and *Propylea quatuordecimpunctata.* We next determined the prey DNA detection success over time (DS_{50}) for each combination of interacting species following a meal. We found that DS_{50} values varied greatly between predator-prey combinations, ranging from 5.2 h to 19.3 h. As a result, general patterns of detection times based upon predator or prey species alone are not discernable. We used the DS_{50} values to correct field data to demonstrate the importance of compensation for detection times that are specific to particular predator-prey combinations.

2.3 Introduction

A predatory interaction between animals that compete for the same prey species is termed intraguild predation (IGP). IGP occurs widely in most ecological systems and is recognized to be important in structuring communities (Polis and Holt, 1992; Holt and Polis, 1997). An increasing number of studies have highlighted the complexity of intraguild interactions, and especially the impact of IGP on plant and animal communities as promoting or relaxing the biological control on herbivore pests (Rosenheim and Harmon, 2006; Vance-Chalcraft *et al.*, 2007b). IGP may also have important implications for conservation biology when an intraguild predator has a negative impact on threatened species (Müller and Brodeur, 2002; Suutari *et al.*, 2004).

Intraguild interactions between arthropods can be difficult to observe under field conditions because the organisms involved are often small and cryptic (Heimpel *et al.*, 1997; Rosenheim *et al.*, 1999). The occurrence and ecological consequences of IGP have traditionally been studied in Petri dishes (e.g. Lucas *et al.*, 1997; Burgio *et al.*, 2002; Labbé *et al.*, 2006), or field cage experiments (e.g. Rosenheim *et al.*, 1993; Hoogendoorn and Heimpel, 2004; Gardiner and Landis, 2007; Chacón *et al.*, 2008). Although the spatial scale and the complexity of the vegetation structure is greatly increased in field cages compared with Petri dish arenas, species interactions are still limited and dispersal is prevented in these studies. A recent meta-analysis by Janssen *et al.* (2007) revealed that increasing spatial complexity usually leads to a decrease in the strength of intraguild interactions by providing refuge for the intraguild prey (e.g. Hoogendoorn and Heimpel, 2004). Along similar lines, Briggs and Borer (2005) and Vance-Chalcraft *et al.* (2007) warned that conclusions resulting from short-term experiments examining IGP may be poor tests of ecological theory, which is based on long-term equilibrium models. Few studies have investigated IGP in complex ecosystems because traditional techniques are not well adapted to detecting and quantifying interactions that are usually considered to be relatively rare (Messing *et al.*, 2006).

Gut-contents analyses uncover predation events without interfering with the behavior of predators and prey and without disrupting ecosystem processes. In addition to the gut dissection method (Sunderland, 1988), which can only be applied to large chewing predators feeding on sclerotized prey, a range of biochemical and molecular techniques have been developed to assess the diet of predatory arthropods (reviewed by Symondson, 2002; Sheppard and Harwood, 2005; King *et al.*, 2008). The enzyme-linked immunosorbent assay (ELISA) method has been used for over two decades to identify arthropods and characterize predator-prey interactions (Greenstone, 1996). This technique can be used to process high numbers of samples and to distinguish between life-stages of a single prey species. However, the development of antibodies can be time-intensive and expensive. In recent years, gut-contents analysis using the polymerase chain reaction (PCR) has emerged as a powerful tool for identifying predator-prey interactions among arthropods in both laboratory and field studies (Symondson, 2002; Harwood and Obrycki, 2005;

Sheppard and Harwood, 2005; Gariepy *et al.*, 2007). This approach has recently been applied to the study of IGP between predator species (Harwood *et al.*, 2007b) and between predators and parasitoids (Chacón *et al.*, 2008; Traugott and Symondson, 2008) under field conditions.

The amplification of a primer pair designed for a specific fragment of prey DNA allows detection of small quantities of DNA in the gut of a predatory insect. However, digestive enzymes reduce prey detectability over time by cutting the target DNA into smaller fragments. A number of physiological and ecological factors modulate the digestion rate, thereby affecting the fate of target DNA fragments in the gut of predatory arthropods. Prey DNA amplification can be influenced by the characteristics of primer used (Hoy, 2003), fragment size (Hoogendoorn and Heimpel, 2001), temperature (Hosseini *et al.*, 2008; von Berg *et al.*, 2008b), age and size of the prey and predator, and feeding mode of the predator (Greenstone *et al.*, 2007; Hosseini *et al.*, 2008). The evaluation of prey detectability over time is critical in the ecological interpretation of gut-contents analysis data because variability in the post-feeding detection period within predator-prey associations can lead to over- or under-estimation of the actual occurrence of predation events. For example, comparing predation rates between two predator species with divergent digestion rates can result in a higher detection rate of prey in the "slower-digesting" species even if the other species has a higher predation rate. A number of studies utilizing PCR-based gut contents analyses have suggested that digestion times are longer for predators that ingest liquid rather than solid prey (Greenstone *et al.*, 2007; Hosseini *et al.*, 2008) (but see Chen *et al.*, 2000), although this may be due to greater consumption rates by sucking predators (Lundgren *et al.*, 2009). Working with two closely-related coccinellids, Hoogendorn and Heimpel (2002) found similar digestion rates for the same prey species. However, a recent study of carabid beetles has shown that closely-related predator species can have quite dissimilar digestion rates (von Berg *et al.*, 2008b), casting doubt upon the hypothesis that closely-related predators should have similar digestion rates. On the other hand, closely-related prey species do tend to produce similar digestion rates in single predator species (Agusti *et al.*, 2003; Sheppard *et al.*, 2004; Read *et al.*, 2006), at least in studies using PCR-based gut contents analysis.

The prey DNA detectability success (DS_{50}) is defined as the time after which half of the predators of a cohort that fed at the same time test positive for the presence of a species of prey, considering that the rate of prey decay is usually exponential (Greenstone *et al.*, 2007). The DS_{50} (or usually known as half-life) and maximum length of time for prey detectability have been measured in a number of studies including species from varied taxa (e.g. Chen *et al.*, 2000; Read *et al.*, 2006; Greenstone *et al.*, 2007; Harwood *et al.*, 2007b; McMillan *et al.*, 2007; Hosseini *et al.*, 2008; Lundgren *et al.*, 2009). Although several studies reported differences in prey detectability over time and stressed the importance of weighting this detectability (Chen *et al.*, 2000; Hosseini *et al.*, 2008; von Berg *et al.*, 2008b), such corrections are rarely made. To our knowledge, there is no demonstration of how correction of raw proportions of field-collected predators testing positive for prey DNA can change the interpretation of the ecological significance of predation events.

The aim of this study was to compare the DS_{50} of four closely-related species (same tribe) of potential intraguild predators in order to weight the significance of intraguild predation based upon PCR gut-contents analyses of predators collected under natural conditions. Specific primers for regions of the internal transcribed spacer of the ribosomial gene complex (ITS-1) or the mitochondrial gene cytochrome oxidase I (COI) were designed for four coccinellid species (Coleoptera: Coccinellidae): *Coccinella septempunctata* Linnaeus, *Propylea quatuordecimpunctata* Linnaeus, *Harmonia axyridis* (Pallas) and *Coleomegilla maculata lengi* Timberlake; the main arthropod predators of the soybean aphid, *Aphis glycines* Matsumara in soybean fields in Québec, Canada (Mignault *et al.*, 2006). We compared the DS_{50} for predation among all combinations of these four coccinellid species. We propose a simple correction including DS_{50} values to adjust for differences in prey detectability over time and applied this correction to field data on gut-contents analysis.

2.4 Materials and methods

2.4.1 Coccinellid rearing

Coccinellid colonies were established from adult beetles collected in soybean fields in Québec. Larvae and adults were reared in isolation in Petri dishes and fed on a mixed diet of soybean aphids and a commercial diet composed of protein, lipid and sugar ("Beneficial Insect Food"; Natural Insect Control Ltd., Ontario, Canada). Coccinellids were kept in growth chambers at 24 $\pm$1°C, 65-75% RH, and under a 16:8 photoperiod (light:dark).

2.4.2 DNA extraction and primer design

DNA was extracted from whole insects. Each coccinellid was ground in 1.5 ml microcentrifuge tubes using sterile plastic pestles, in 100 µl grinding buffer (Bender *et al.*, 1983). All other steps follow the protocol described in Hoogendoorn and Heimpel (2001). Gene sequences in ITS-1 or COI regions were found on Genbank for *C. septempunctata* (AJ272142)*, H. axyridis* (AJ272146) and *C. maculata* (AY615732). We sequenced the ITS-1 region for *P. quatuordecimpunctata* from specimens collected in soybean fields from seven different regions in Québec in 2004. The ITS-1 was amplified by PCR using primers BD1 (5′-GTC GTA ACA AGG TTT CCG TA-3′) and 4S (5′-TCT AGA TGC GTT CGA A(G/A)T GTC GAT G-3′) (Bowles and McManus, 1993). Voucher specimens (one per region) are housed in the Collection d'insectes du Québec, MAPAQ, Qc. This ITS-1 region was deposited in Genbank (FJ013050), representing a unique sequence that was identical for all specimens. All primers were designed within these sequences using the software Primer 3 (Rozen and Skaletsky, 2000). The primers were also tested for cross-reactivity with the other coccinellid species used in the experiment. The specificity of the primers was further tested against nine invertebrate species [*Chrysoperla* sp. (Neuroptera: Chrysopidae), *Euaresta bella* (Diptera: Tephritidae), *Aphis glycines* (Homoptera: Aphididae) *Nabis americoferus* (Hemiptera: Nabidae), *Lygus lineolaris* (Hemiptera: Miridae), *Euschistus servus* (Hemiptera: Pentatomidae), *Scaphytopius* sp. (Hemiptera: Cicadellidae), *Systena frontalis* (Coleoptera: Chrysomelidae), *Hippodamia convergens* (Coleoptera: Coccinellidae) and *Formica fusca* (Hymenoptera: Formicidae)] which could be found in

soybean fields. Primer sequences and fragment sizes detected for all coccinellid species are shown in Table 2.1. Finally, a dilution of 1:10 (intraguild prey: intraguild predator) DNA mix was used to simulate IG prey in the predator gut and assess the suitability of each primer pairs with heterospecific DNA (Figure 2.1).

2.4.3 PCR amplification

We used a conserved primer pair derived from a mitochondrial 12S rRNA sequence of *Drosophila yakuba* Burla (Diptera) to confirm the presence of DNA in each sample [12Sai and 12Sbi, in Noda *et al.* (1997)]. Subsequently, all PCR reactions were conducted using the primer pair representing the species eaten (intraguild prey). Amplifications (for universal primers and coccinellids primers) were performed in total volumes of 25 ml, composed of 20.25 µl of 1× buffer (0.25 mM of each dNTP and 1.5 mM of $MgCl_2$), 2.5 µl of primer mix (20 µM), 0.25 µl of *Taq* (i.e. 1.75 units) (Promega), and 2 µl DNA sample. Samples tested with universal primers were placed in the thermocycler with an initial step of 3 min at 90°C followed by 30s at 94°C, 45s at 50°C, 1 min at 72°C (the 3 last steps were repeated 30 times), and a final step of 7 min at 72°C. For the coccinellid primers, the thermocycling program consisted of an initial step of 30s at 94°C, followed by 30s at 94°C, 30s at 52°C, and 30s at 72°C. The three last steps were repeated 30 times and were followed by a step of 5 min at 72°C. Because *H. axyridis* primers were less specific, we used a hot start for this primer pair (i.e. first step 5 min at 94°C prior to the addition of *Taq*), with all the following steps being similar, except for the annealing temperature that was 55°C rather than 52°C. All PCR products (10 µL) were electrophoresed at 120V in 2% agarose gels for approximately 1 h and then stained in ethidium bromide solution for 20 min and then visualized using a UV light-transiluminator. Because samples are likely to contain minute amounts of prey DNA, negative samples were tested three times.

2.4.4 Post-feeding detection period of intraguild prey

To evaluate the period of time that prey remains could be detected within guts after feeding, we allowed coccinellids to feed on individuals of each of the other species. The bioassay consisted of a fourth-instar larva feeding on 5 eggs of one of the other coccinellid species.

Last larval instars and eggs were selected because these developmental stages are more likely to be engaged in, and suffer from IGP, respectively (Cottrell and Yeargan, 1998b; Lucas *et al.*, 1998). All coccinellid larvae were reared in isolation as described above. Prior to the feeding experiment, individuals that had moulted to the fourth instar were starved for 24 h to standardize their nutritional state and to increase their motivation to feed. Eggs of the intraguild prey were less than 5 days old and stored at 4°C, as DNA is detectable throughout coccinellid embryonic development (A.-E. Gagnon*, unpublished data*). The feeding arena consisted of a 9 cm diameter Petri dish containing a piece of filter paper and a piece of moistened cotton. Each individual larva was allowed to feed on the eggs for a maximum of 30 min; those that did not consume eggs were discarded. After feeding, the number of eggs consumed was recorded and the predators were transferred to a new Petri dish. They were then placed into a growth chamber at 24°C for time periods ranging from 0, 4, 8, 12 and 16 hours. We chose 16 hours as the maximum digestion period because a previous study by Hoogendoorn and Heimpel (2001) did not detect prey DNA fragments after 12 h in coccinellid guts. However, in two combinations, we had to extent the digestion time to 32 h to reach the period corresponding to a decrease in prey detection. Following the holding period, coccinellids were frozen and stored at -80°C until DNA extraction. We sampled 10 individuals for each predator-prey time treatment. However, *P. quatuordecimpunctata* did not eat *H. axyridis* eggs so this combination was not evaluated. Also, because the extraction of DNA from *P. quatuordecimpunctata* and *C. septempunctata* was not always successful (7.52% of negatives ± 2.18 [mean ± SD]) and some larvae refused to eat eggs, less than 10 individuals were tested for some combinations (see Table 2.2 for *n* values).

2.4.5 Statistical analysis

We used two complementary approaches to compare prey detectability over time for each predator-prey combination and to estimate the DS_{50} values. First, we used a logistic regression with the GENMOD procedure of SAS 9.1.3 (SAS Institute 1996) to compare the intercept (b0) and the slope (b1) for all predator-prey combinations (Hosmer and Lemeshow, 2000). In this model, an adjustment of the deviance was made for

overdispersion to reflect the heterogeneity among observations. The effects of time since feeding, predator and prey species and meal size (number of eggs eaten) were also analyzed with logistic regression. The second approach allows estimation of the DS_{50} parameter, -b0/b1. The variance attributed to this parameter was first estimated by the delta method (Bieler and Williams, 1993). DS_{50} parameters for each IGP combination (one IG predator species with the three IG prey species) were then compared using Cochran's chi-square statistic. A Bonferonni correction was used to reduce type I error. Following a significant effect of prey detectability over time, pairwise comparisons were made with Student's t-test.

2.4.6 Weighting prey detectability

We used a method to weight prey detectability in gut contents and to better interpret the extent of predation under natural conditions. The method aims to correct raw data of predation events by considering differences in duration of detectability of prey DNA among combinations of predator and prey species. As suggested by Chen *et al*. (2000), digesting values (DS_{50}) for each predator-prey combination were first weighted to obtain the $DS_{50}^{weighted}$ as follows: the shortest DS_{50} was assigned a value of 1.0 and all other DS_{50} values were obtained by placing this benchmark DS_{50} in the numerator and each other DS_{50} values in the denominator. The corrected predation value ($IGP_{weighted}$) is calculated by multiplying the proportion of field-collected predators found to contain prey remains by their specific $DS_{50}^{weighted}$. Although this correction does not provide a quantitative value of prey consumption, it affords an estimate of the relative impact a predator may have in comparison to other predator or prey species.

We used a sample of field-caught *H. axyridis* and *C. septempunctata* to examine the impact of weighting prey detectability on the importance of IGP for both predator species. Predators were sampled by sweep net in a soybean field in Hérouxville, QC (46°40'00'', 72°37'00'') on July 25th and August 15th 2005 and brought back to the laboratory on ice. We used 40 field-collected 4th-instar larvae of *H. axyridis* and 35 of *C. septempunctata* to estimate the incidence of IGP on the three other coccinellid species for which primers had

been developed. Specimens were washed in 70% ethanol to prevent contamination when predators were all placed in the same bag. Harwood (2008) found no evidence that sweep netting overestimated predation frequency in molecular gut content analysis versus hand collected specimens.

2.5 Results

2.5.1 Primer specificity

All primer pairs were highly specific, except for the primer pair for *C. maculata,* which also amplified *H. convergens* DNA. This primer was nevertheless retained for our study since *H. convergens* occurs at very low densities (< 1%) in Québec soybean fields (Mignault *et al.*, 2006). For all other coccinellid species, cross-reactivity tests showed that no DNA was amplified from species other than the species for which primer pairs were developed.

2.5.2 Post-feeding detection period of intraguild prey

The detectability of prey DNA decreased significantly with time after feeding with DS_{50} values ranging from 5.2 to 19.3 h (Tables 2.2, 2.3). Meal size (from 1 to 5 eggs) had an only marginally-significant influence on the probability of prey detection (improved detection with the increase of the meal size) (Table 2.3). The rate of DNA degradation was not significantly different among predator species ($p = 0.7294$), but significantly different between prey species ($p = 0.0094$) and according to specific predator-prey combinations ($p < 0.0001$; see below).

To illustrate the specificity of predator-prey combinations, we compared DS_{50} values of predator species consuming a given prey species (Figure 2.2) and of prey species being eaten by a given predator species (Figure 2.3). The DS_{50} of *H. axyridis* eggs was shorter within *C. septempunctata* than within *C. maculata* (d.f.= 1, χ^2= 5.2527, p= 0.0219; Figure 2.2a). However the DS_{50} of *P. quatuordecimpunctata* was significantly longer within *C. septempunctata* than within *C. maculata* (d.f.= 1, χ^2= 8.0753, p= 0.0176; Figure

2.2d). No differences in DS_{50} were detected for the two other prey species: *C. septempunctata* (d.f.= 2, χ^2= 1.9238, p= 0.3822; Figure 2.2b) and *C. maculata* (d.f.= 2, χ^2= 0.4532, p= 0.7972; Figure 2.2c).

Inconsistent patterns emerged when prey detectability half-lives are compared for a given predator species as well (Figure 2.3). For example, while *C. maculata* exhibited significantly higher DS_{50} when feeding on *H. axyridis* than on *P. quatuordecimpunctata* (d.f.= 2, χ^2= 10.7936, p= 0.0045; Figure 2.3c), no significant differences in DS_{50} were detected for the three other predator species: *H. axyridis* (d.f.= 2, χ^2= 2.9643, p= 0.2272; Figure 2.3a), *C. septempunctata* (d.f.= 2, χ^2= 3.7404, p= 0.1541; Figure 2.3b) and *P. quatuordecimpunctata* (d.f.= 1, χ^2= 2.3846, p= 0.1225; Figure 2.3d).

2.5.3 Weighting prey detectability of field-caught predators

The incidence of IGP (proportion of predator individuals positive for the DNA of other coccinellid species) ranged from 12% to 65% for *H. axyridis* and from 9% to 23% for *C. septempunctata* (Table 2.4). However, applying the DS_{50} correction provides a different estimate of the relative importance of guild interactions for each predator-prey association. For example, from the proportion of positive PCR amplifications, it appears that *H. axyridis* preys upon *C. maculata* almost twice as frequently as *H. axyridis* preys upon *P. quatuordecimpunctata* (IGP detected at 17.9% vs 11.7%, respectively). However, using the $IGP_{weighted}$ value, IGP was similar between the prey *P. quatuordecimpunctata* and *C. maculata* (score of 0.08 and 0.07, respectively) (Table 2.4). Another noteworthy comparison involves *H. axyridis* - *C. maculata* and *C. septempunctata* - *H. axyridis* that have dissimilar raw proportion of IGP (17.9% and 8.6%, respectively). In contrast, weighted IGP values suggest a similar proportion of intraguild prey with scores of 0.07 and 0.08, respectively (Table 2.4).

2.6 Discussion

2.6.1 Primer specificity

Detection of prey DNA in the gut of predators is most likely to be successful if the gene amplified occurs in multiple copies, and if sequences are relatively short (Zaidi *et al.*, 1999; Chen *et al.*, 2000). The internal transcribed spacer of the ribosomial gene complex (ITS-1) and the mitochondrial cytochrome oxidase I gene (COI) are both present in multiple copies in arthropod cells (Hoy, 2003). Furthermore, fragment sizes amplified by the four primer pairs were all short and of similar size (from 105 to 137 bp), allowing relatively long detection over time and appropriate comparison between species. Since the ITS-1 region possesses high genetic variability among coccinellid species (von der Schulenburg *et al.*, 2001), specific markers can be easily designed for closely related species. King *et al.* (2008) pointed out that high intra-individual and intraspecific variation can be found in ITS-1, making this region perhaps too variable to obtain consistent results. However, the primers we designed elicited high specificity to coccinellid species collected from seven geographically distant localities in the province of Québec, suggesting low genetic variability among populations, at least for this gene region

2.6.2 Prey DNA detection success analysis

The prey detectability half-lives for coccinellids ranged from 5.2 h 19.3 hours. Such relatively short DS_{50} values would facilitate the interpretation of predation rate data from field-caught predators, because long detection periods impair the determination of frequencies of predatory events, recent feeding events being not distinguishable from older ones, and may lead to overestimation of predation rates (Hagler and Naranjo, 1997; Sheppard and Harwood, 2005). On the other hand, longer detectability times have the advantage of revealing relatively rare predation events.

When studying predation of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) eggs by adults of *C. maculata* and *H. axyridis,* Hoogendoorn and Heimpel (2002) showed that the rate of decrease in prey detectability over time was similar for both coccinellid

species. However, studies in other systems suggest that prey DNA detection period for a given prey species can vary greatly among predator species. For example, Symondson and Liddell (1993) found that two closely related carabid beetles *Abax parallelepipedus* Piller and Mitterpacher and *Pterostichus madidus* Fabricus, show considerable differences in immunoreactivity after feeding on slug prey, with a detection period 2.5 times longer for the former species. Similar results were more recently found with PCR gut-content analysis for two other carabid species by von Berg *et al.* (2008b). As suggested by McMillan *et al.* (2007) who discussed prey DNA detection time in Coccinellidae, our results clearly indicate that prey detectability half-lives in molecular gut-content analysis are predator-prey specific rather than predator- or prey-specific. Broad patterns of detection times are not detectable even if both predator and prey are closely related (the same coccinellid tribe in our study), and targeted DNA fragments are from the same region and of similar length. This conclusion represents a serious drawback to the interpretation of molecular gut-content analysis (see below).

At least two inter-related ecological factors may have contributed to the temporal patterns of prey DNA detection we observed: their diet range and the toxicity of coccinellid eggs. For many herbivorous arthropods, polyphagous species have a larger diversity of digestive enzymes and a better capacity to excrete toxic compounds than oligophagous species (Krieger *et al.*, 1971; Schoonhoven *et al.*, 1998). These attributes can contribute to a more efficient (rapid) digestion of non-common food items for generalists. Such a pattern may also apply to generalist predators. For instance, our results showed that *C. maculata* and *H. axyridis*, which have a broad diet (Hodek and Honek, 1996) including various insects (aphids, mites, insect eggs) and plant material (pollen, fruit) (Roger *et al.*, 2000; Michaud, 2002), exhibit some of the lowest prey detection periods (more rapid decay) compared to the more specialized aphid feeder *P. quatuordecimpunctata* (Figure 2.3). However, this hypothesis does not hold true for all associations in our study, as a few polyphagous coccinellids showed high DS_{50} values. For example, the decay rate of *H. axyridis* eggs was very slow within the polyphagous predator *C. maculata*. In our feeding experiment, we used last larval instar coccinellid eating heterospecific eggs. But coccinellid eggs may possess alkaloids or other substances that can be toxic to other coccinellid species

(Hemptinne *et al.*, 2000; Ware *et al.*, 2008). The degree of toxicity is species-dependent as for example *H. axyridis* can survive well on a diet of *C. septempunctata* eggs while the opposite is not possible (Sato and Dixon, 2004). In our bioassay, *H. axyridis* had toxic/repellant eggs that apparently prevented predation from *P. quatuordecimpunctata* and likely slowed down digestion in *C. maculata* (DS_{50} = 18.9 h). Conversely, *C. septempunctata* fed on eggs of *H. axyridis*, and the decay rate was rapid (DS_{50} = 8.2 h). In this case, however, we cannot exclude that the amount of food intake was reduced because coccinellid larvae are known to regurgitate toxic food (Wiles and Jepson, 1993). Dark spots were sporadically observed on the filter paper in Petri dish after the feeding experiment and these could be the result of regurgitation. Furthermore, the biochemistry of specific primer/template combinations would likely influence prey DNA detectability in predator guts. In addition, the capacity of binding DNA target may be influenced by the physiology or morphology of the gut (e.g. the difficulty of grinding certain guts).

2.6.3 Weighting prey detectability

Although multispecies comparisons of predation rate using prey DNA molecular detection from field-caught predators have to be interpreted through models that consider detection time (Harwood *et al.*, 2007a), and although detection times are now commonly measured and compared for predator-prey combinations, weighting prey detectability has never to our knowledge, been attempted for field-caught invertebrate predators.

A primary application of such an approach is the determination of the relative importance of a given predator-prey combination in the ecosystem. Our results suggest that weighting prey detectability using DS_{50} values provides an improved estimate of IGP occurrence in field-caught coccinellids. Corrections implying interactions with high DS_{50} such as *H. axyridis* - *C. maculata* reduced their importance compared to other interacting species where the detection time was short, like *C. septempunctata* - *H. axyridis*. When considering only raw data, those two interactions had strongly different proportions of IGP detected. However, taking into account differences in digestion times, those interactions similar a same level of predation.

2.6.4 Limitations

Despite several advantages, a number of potential drawbacks have been associated with the molecular identification of prey in arthropod predator guts. DNA prey detectability success analyses are essential for estimating field predation rates but require extensive laboratory evaluation. This study illuminates the necessity to investigate each predator-prey combination, as decay rates are specific to particular predator-prey combinations. Furthermore, several other intrinsic factors may influence the prey DNA detection period. Metabolism may vary according to the developmental stage, size and sex of the predator, its level of satiety and activity, the presence of more than one prey type in the gut (chaser) (Weber *et al.*, 2009). Although most of these parameters can be accounted for in field-caught predators, such is not the case for prey. These observations bring out all the complexity of analyzing molecular data from the gut content of a predator.

Other disadvantages of the DNA technique include the inability to discriminate secondary predation (Sheppard *et al.*, 2005) and scavenging behavior (Foltan *et al.*, 2005; Juen and Traugott, 2005) although the four species of coccinellids tested in our study are not regarded as scavengers. Secondary predation occurs when a predator eats another predator containing the prey of interest in its gut. This indirect type of predation can potentially occur within coccinellid communities but remains difficult to quantify. An additional significant limitation of the technique is the incapability to detect cannibalism because conspecific DNA cannot be discriminated from predator DNA. Cannibalism is a very common phenomenon in Coccinellidae, mostly on eggs (Osawa, 2000; Gagné *et al.*, 2002) and can play an important role in population dynamics, especially in systems where IGP predators are abundant (Rudolf, 2007).

In conclusion, our results provide baseline information to study IGP among coccinellid species using molecular gut analyses. This study emphasizes the importance of considering prey DNA decay rate for each predator-prey species interaction, even for closely related species. We have proposed and tested a method of weighting prey

detectability in field-caught predators that take into consideration prey DNA decay rates. The results suggest that this approach is essential to correctly assess the relative importance of predator-prey interactions.

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2.9 Tables

Table 2.1 Coccinellid primer sequences (5'-3') and DNA amplified fragment sizes.

Species	Region	Forward primer sequence	Reverse primer sequence	Size (bp)
Coccinella septempunctata	ITS-1	CGA AAG ACG ATC CCT ACG AA	AAG TTC GCT CGT CCT GGT TA	105
Propylea quatuordecimpunctata	ITS-1	GAT ATA TCG GCG CGT TTC TC	ATC GCT TTC TCC ACC TCG TA	115
Harmonia axyridis	ITS-1	AAG AGG AGA CGC CGA CCA GA	AGG TAG CTT CAA TCG ATC GG	120
Coleomegilla maculata	COI	AGT GAA AAT GGG CAA CAA CA	GCC TTC TCC TTC CCT TCT TT	137

Table 2.2 Number of positive detected (presence of intraguild prey DNA within an intraguild predator) for each predator-prey combination at different times since feeding and the prey detection success over time for 50% of the predators (DS_{50})

IG Predator	IG Prey	Time since feeding (h)							DS_{50} ± SE
		0h	4h	8h	12h	16h	>16h	>24h	
Ha	C7	9 (10)[a]	6 (8)	2 (9)	1 (9)	3 (10)	-	-	7.2 ± 2.3
	Cmac	10 (10)	10 (10)	7 (10)	6 (10)	8 (10)	-	-	19.3 ± 7.0
	P14	10 (10)	7 (10)	5 (10)	5 (10)	2 (10)	-	-	10.0 ± 2.1
C7	Ha	19 (21)	5 (10)	10 (11)	1 (7)	0 (6)	-	-	8.2 ± 1.9
	Cmac	8 (8)	8 (8)	4 (6)	4 (6)	3 (6)	-	-	14.8 ± 3.5
	P14	6 (6)	6 (6)	4 (6)	2 (6)	4 (8)	-	-	13.2 ± 2.8
Cmac	Ha	10 (10)	8 (10)	3 (10)	7 (10)	8 (9)	5 (10) [24h]	1 (12) [32h]	18.9 ± 4.4
	C7	10 (10)	10 (11)	3 (10)	2 (12)	3 (10)	-	-	9.0 ± 1.7
	P14	9 (10)	6 (10)	2 (10)	1 (10)	0 (10)	-	-	5.2 ± 1.5
P14	C7	8 (8)	7 (7)	5 (10)	6 (10)	1 (7)	-	-	11.1 ± 1.8
	Cmac	8 (10)	6 (6)	6 (7)	3 (6)	5 (6)	4 (9) [20h]	1 (10) [28h]	17.4 ± 3.6

[a] Numbers in parenthesis represents the number of samples (*n*). Ha = *Harmonia axyridis*, C7 = *Coccinella septempunctata*, Cmac = *Coleomegilla maculata*, P14 = *Propylea quatuordecimpunctata*.

Table 2.3 Results of logistic regression on detection of coccinellid prey DNA over time within coccinellid predators

Sources	**d.f.**	χ^2	**P**
Predator	3	1.30	0.7294
Prey	3	11.47	0.0094
Time	8	155.40	<0.0001
Meal size	1	3.52	0.0606
Predator*Prey	4	27.57	<0.0001

Table 2.4 Calculation of the $IGP_{weighted}$ values with field-collected predators

Predator – Prey	**% IGP**	**DS_{50} (h)**	**$DS_{50}^{weighted}$**	**$IGP_{weighted}$[a]**
Ha – C7	65.4	7.2	1.00	0.65
Ha – Cmac	17.9	19.3	0.37	0.07
Ha – P14	11.7	10.0	0.72	0.08
C7 - Ha	8.6	8.2	0.88	0.08
C7 – Cmac	22.9	14.8	0.49	0.11
C7 – P14	17.1	13.2	0.55	0.09

[a]$IGP_{weighted}$ was obtained by multiplying the proportion of positives and $DS_{50}^{weighted}$

2.10 Figures

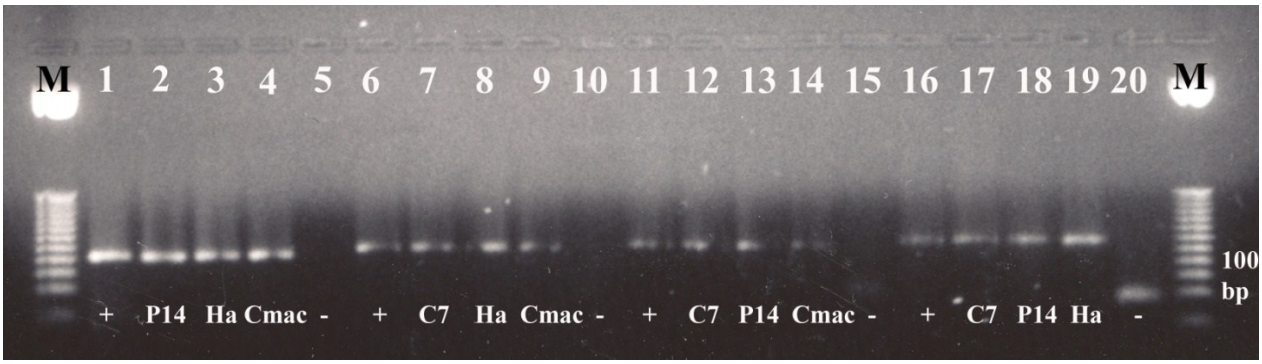

Figure 2.1. DNA amplification of mixed DNA [1:10 (prey: predator)] using all of the prey: predator combinations. Lanes 1-5 represent *Coccinella septempunctata* with positive control (+), the 3 IG prey and negative control (-); lanes 6-10 *Propylea quatuordecimpunctata* with +, the 3 IG prey and -; lanes 11-15 *Harmonia axyridis* with +, the 3 IG prey and -; lanes 16-20 *Coleomegilla maculata* with +, the 3 IG prey and -. IG prey are C7: *C. septempunctata,* P14: *P. quatuordecimpunctata,* Ha: *H. axyridis* and Cmac: *C. maculata. "*M" is the molecular-size marker (25 bp DNA step ladder, Promega).

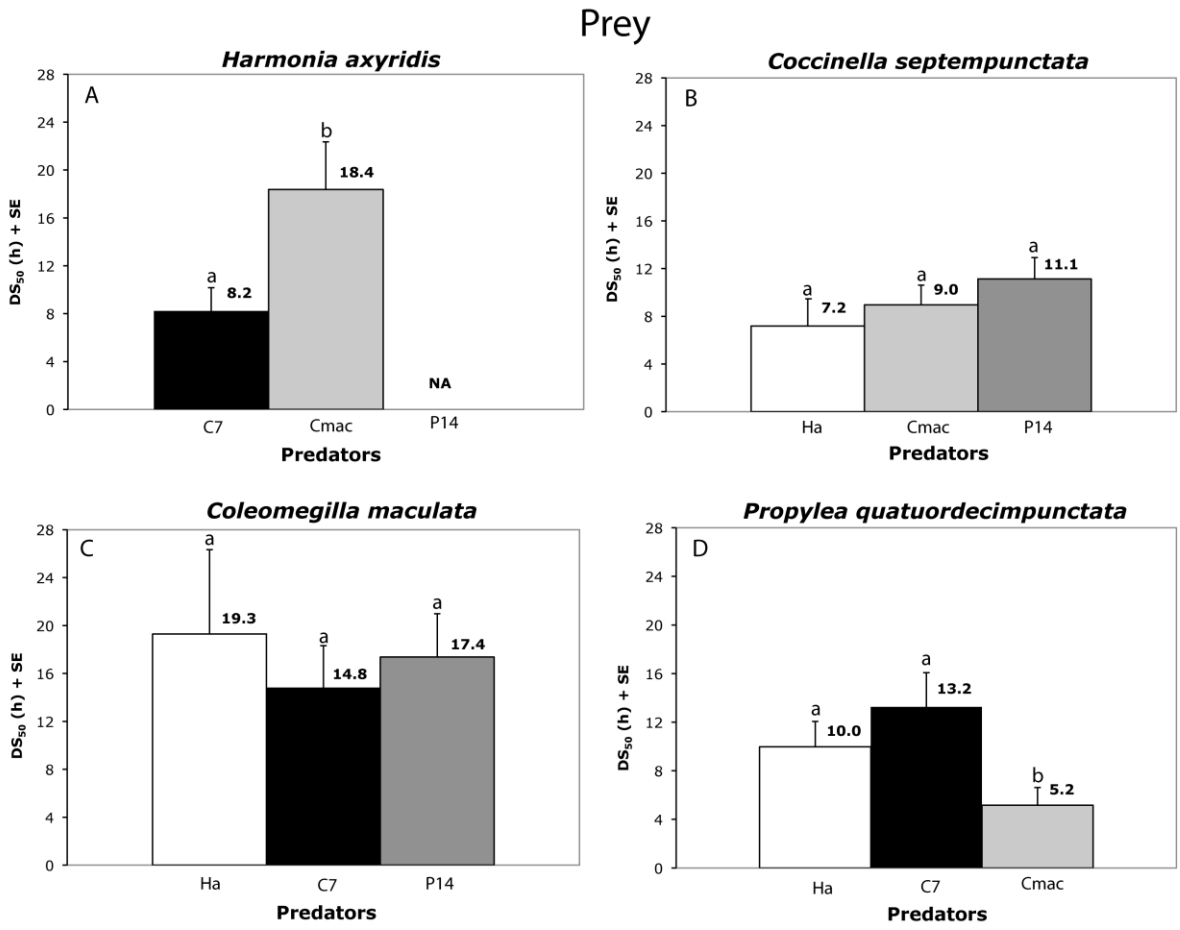

Figure 2.2. Effects of coccinellid prey species on DNA detectability success (DS_{50} ± SE; hours) following intraguild predation: a) *Harmonia axyridis* (Ha); b) *Coccinella septempunctata* (C7); c) *Coleomegilla maculata* (Cmac); and d) *Propylea quatuordecimpunctata* (P14). Detectability success values (DS_{50}) are shown above the histogram bars. Means followed by different letters are significantly different (Bonferonni corrected alpha). In a), the predator *P. quatuordecimpunctata* refused to eat *H. axyridis* eggs and was therefore removed from the analysis.

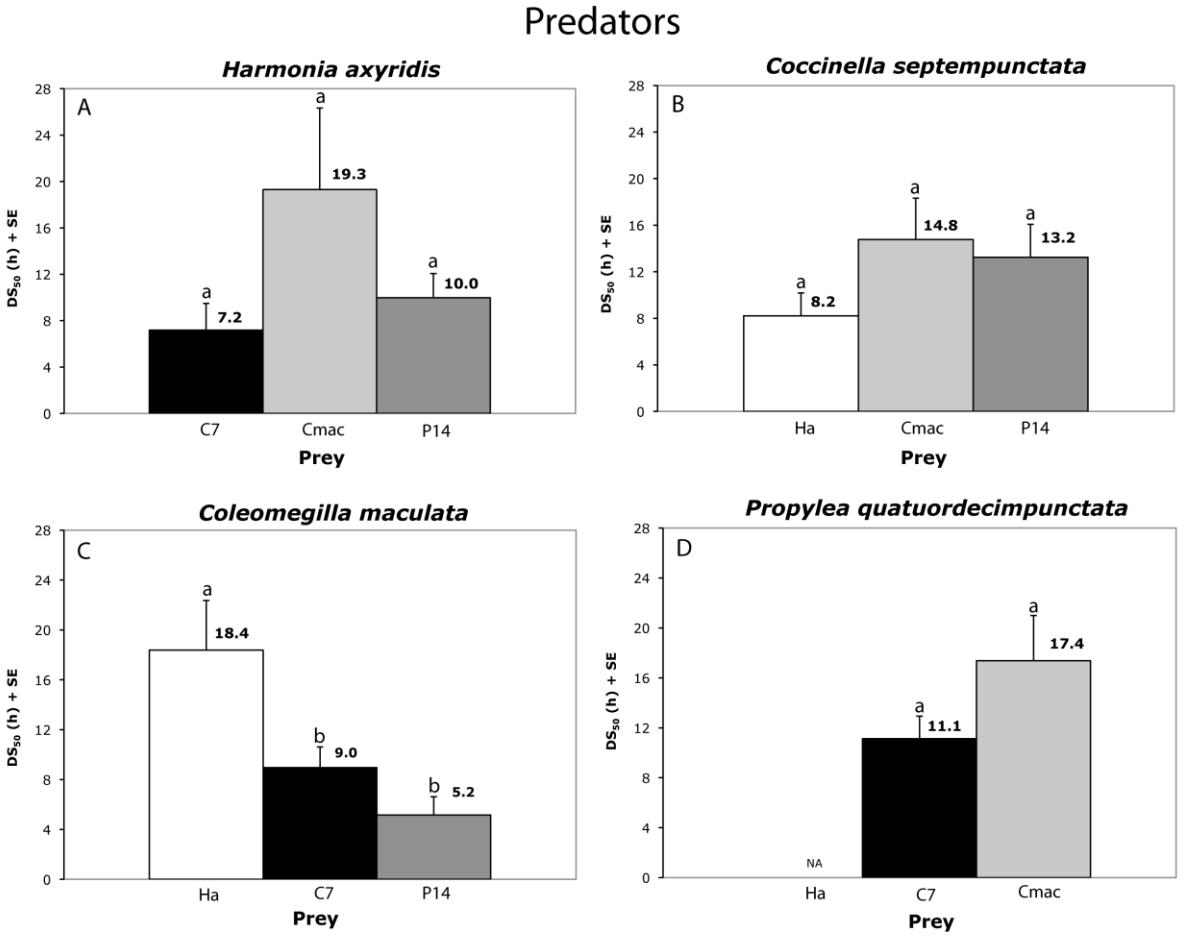

Figure 2.3. Effects of coccinellid predator species on DNA detectability success (DS_{50} ± SE; hours) following intraguild predation: a) *Harmonia axyridis* (Ha); b) *Coccinella septempunctata* (C7); c) *Coleomegilla maculata* (Cmac); and d) *Propylea quatuordecimpunctata* (P14). Detectability success values (DS_{50}) are shown above the histogram bars. Means followed by different letters are significantly different (Bonferonni corrected alpha). In d), the predator *P. quatuordecimpunctata* refused to eat *H. axyridis* eggs and was therefore removed from the analysis.

CHAPITRE III

The ubiquity of intraguild predation among coccinellids*

* Ce chapitre a été préparé pour une soumission à la revue scientifique ***Ecology Letters***. Les auteurs sont Annie-Ève Gagnon (Centre de Recherche en Horticulture, Université Laval), George Heimpel (University of Minnesota) et Jacques Brodeur (Institut de recherche en biologie végétale, Université de Montréal).

3.1 Résumé

La prédation intraguilde (IGP) survient lorsqu'un prédateur attaque un autre prédateur avec qui il est aussi en compétition pour la même proie. Malgré l'omniprésence apparente des interactions intraguildes, autant dans les écosystèmes naturels que manipulés, peu d'études ont quantifié les taux d'IGP dans différents taxa en conditions naturelles. Nous avons déterminé, en conditions naturelles et en utilisant des analyses moléculaires du contenu gastrique, la nature et l'incidence de l'IGP entre quatre espèces de coccinelles prédatrices dans les champs de soya. Nous avons démontré que l'IGP est une interaction extrêmement fréquente entre les coccinelles avec la détection de proies intraguildes chez 52,9% des 368 prédateurs récoltés au champ. Par ailleurs, 13,2% des individus échantillonnés contenaient deux voire même trois autres espèces de coccinelles dans leur tractus digestif. L'interaction était de type mutuel puisque les quatre espèces de coccinelles avaient la capacité de se nourrir l'une sur l'autre. À notre connaissance, cette étude représente la mise en évidence expérimentale la plus convaincante d'une fréquence élevée d'IGP entre des arthropodes prédateurs.

3.2 Abstract

Intraguild predation (IGP) occurs when one predator species attacks another predator species with which it competes for a shared prey species. Despite the apparent omnipresence of intraguild interactions in natural and managed ecosystems, very few studies have quantified rates of IGP in various taxa under field conditions. We used molecular analyses of gut contents to assess the nature and incidence of IGP among four species of coccinellid predators in soybean fields. Over half of the 368 predator individuals collected in soybean contained the DNA of other coccinellid species indicating that IGP was ubiquitous at our field site. Furthermore, 13.2% of the sampled individuals contained two and even three other coccinellid species in their gut. The interaction was mutual, as each of the four coccinellid species has the capacity to feed on the others. To our knowledge, this study represents the most convincing observational evidence of a high prevalence of IGP among predatory arthropods.

3.3 Introduction

Contemporary ecologists struggle with complexity. Communities involve thousands of species interacting in many diverse ways within the spatial and temporal variability of natural ecosystems (Paine 1969). In the late 1980's it became apparent that models based on functional trophic levels were not sufficiently universal to understand the dynamics and structure of communities (Rosenheim 1998). The necessity of integrating non-trophic and indirect relationships has prompted theoretical and empirical work aimed at examining the role of omnivores. One form of omnivory is intraguild predation (IGP), where one predator species attacks another predator species with which it also competes for a shared prey (Polis *et al.* 1989).

Following the pioneering field study of Polis and McCormick (1987) on species of desert scorpions that feed on each other, a fertile and rapidly growing literature on IGP has led to a reconsideration of several classical topics in ecology such as stability and diversity

of communities, trophic cascades in food webs, niche shift and species exclusion, as well as the effects of ecosystem productivity on species interactions. IGP also rapidly became relevant to aspects of applied ecology such as biological control, management of endangered species and the establishment of exotic invasive predators (Rosenheim *et al.* 1995; Muller & Brodeur 2002; Snyder *et al.* 2004). IGP is now considered to be ubiquitous in aquatic and terrestrial ecosystems, occurring in a great diversity of taxa from bacteria to mammals (Polis *et al.* 1989). According to an analysis conducted by Arim and Marquet (Arim & Marquet 2004) using 113 food webs, 58-87% of animal species are involved in IGP interactions.

Despite this apparent ubiquity of intraguild interactions in both natural and managed ecosystems, and despite their importance in structuring communities, very few studies have quantified rates of IGP in various taxa under field conditions. This is especially true for predatory arthropods, most likely because of the perceived difficulty of performing field observations of predation events (Messing *et al.* 2006). Intraguild interactions among arthropod species have traditionally been studied in Petri dishes (e.g. Lucas *et al.* 1998), or in field cage experiments (e.g. Rosenheim *et al.* 1993; Hoogendoorn & Heimpel 2004; Chacon *et al.* 2008). Although important for identifying potential functional trophic and guild links among species, these approaches are inadequate for predicting the full complexity of both direct and indirect interactions (Messing *et al.* 2006; Vance-Chalcraft *et al.* 2007). Consequently, results from experiments conducted in experimental arenas that have a limited number of interacting species and are conducted for short periods of time have led to skepticism about the actual occurrence and significance of IGP in nature (e.g. Hemptinne & Dixon 2005; Kindlmann & Houdkova 2006).

Some studies have examined IGP in more natural settings using different methodological techniques and are important in complementing the less natural enclosure-based experiments. First, a number of semi-quantitative food-web studies documenting the existence (presence/absence) of trophic linkages between omnivores have shown that predators also include predatory species in their diet (e.g. Polis & McCormick 1987). Second, purely observational field studies have quantified predator-predator interactions

(e.g. Rosenheim *et al.* 1999). Third, experimental studies have been conducted in which the full, natural community of predators and prey were retained, and there was little if any constraint imposed on predator foraging (e.g. Rosenheim *et al.* 2004). Finally, a range of biochemical and molecular techniques have been developed to analyse gut contents and assess the diet of predatory arthropods under field conditions (Sheppard & Harwood 2005).

In this study we assess the nature and incidence of IGP among four species of coccinellid predators (Coleoptera: Coccinellidae) in soybean fields under natural conditions. This system has several favourable attributes for the study of IGP. Coccinellids are generalist predators, voracious both during their larval and adult stages. In soybean fields of Québec, Canada, they can be abundant and play a role in aphid control (Rhainds *et al.* 2007). They show an aggregative response to prey density (Evans & Youssef 1992; Donaldson *et al.* 2007; Chacon & Heimpel 2010), thereby increasing encounter rates with conspecific or heterospecific coccinellids. Furthermore, a number of laboratory or exclusion cage experiments have shown that IGP is potentially a common interaction among coccinellids (e.g. Hironori & Katsuhiro 1997; Lucas *et al.* 1998) and have identified major ecological determinants of IGP such as relative size of the protagonists, mobility and aggressiveness, feeding specificity and aphid density (Lucas *et al.* 1998; Lucas & Brodeur 2001).

A second advantage for using coccinellids as model system is that we have developed molecular gut-content analyses to assess levels of IGP (Gagnon *et al.*, *submitted*). This approach uncovers predation events without interfering with the behavior of predators and prey and without disrupting ecosystem processes (Symondson 2002; Harwood & Obrycki 2005). Gut-contents analysis using the polymerase chain reaction (PCR) has recently been applied to the study of IGP between predator species (Harwood *et al.* 2007) and between predators and parasitoids (Chacon *et al.* 2008; Traugott & Symondson 2008).

3.4 Methods

3.4.1 The study system

We studied the community of coccinellids associated with the soybean aphid, *Aphis glycines* Matsumura (Homoptera: Aphididae). The four dominant species in soybean fields in the province of Québec are: *Coccinella septempunctata* Linnaeus, *Propylea quatuordecimpunctata* Linnaeus*, Harmonia axyridis* (Pallas) and *Coleomegilla maculata lengi* Timberlake, the only native species in this system (Mignault *et al.* 2006). These four coccinellid species are sympatric and observed throughout the season, with *H. axyridis* arriving later than the others. Their abundance in soybean is mostly correlated with aphid densities, as commonly observed in agroecosystems (Ives *et al.* 1993).

Our primary objective was to estimate IGP levels within coccinellid assemblages in soybean fields. In this paper we do not attempt to examine the multitude of ecological factors that can promote the occurrence of IGP (predator and prey densities, predator:prey ratio, predator stage structure, etc) across fields or sampling dates; these analyses will be presented elsewhere. However, to place the present study in context we provide here general information about aphid and coccinellid populations. *Aphis glycines* populations were relatively high with a seasonal mean of 266 and 371 aphids per plant in 2004 and 2005, respectively (A.E. Gagnon, *unpublished data*). The coccinellid community in 2004 was dominated by *H. axyridis* and *C. septempunctata* (representing 48 % and 41 %, respectively, of all four species) with a small proportion of *C. maculata* (5 %) and *P. quatuordecimpunctata* (6 %). In 2005, the proportions of each species were as followed: *H. axyridis* (59 %), *C. septempunctata* (18 %), *C. maculata* (14 %) and *P. quatuordecimpunctata* (9 %).

Coccinellids were sampled in soybean fields in 2004 and 2005 with sweep netting, put in an electric icebox at 4ºC, and brought to the laboratory. Specimens were then washed in 70% ethanol to prevent possible contamination stemming from the time that predators had been held together in the collecting bag (Harwood 2008). Samples were preserved in vials with 70% ethanol at 4°C until DNA extraction. Coccinellids were sampled in four

different fields, located at municipalities of Maskinongé (46°14’00’’, 73°01’00’’), Hérouxville (46°40’00’’, 72°37’00’’), Nicolet-Sud (46°13’00’’, 72°37’00’’) and St-Augustin-de-Desmaures (46°44’00’’, 71°28’00’’) in the province of Québec. A total of 188 and 180 coccinellids were sampled in 2004 and 2005, respectively (Figure 3.1 provides details per species). Insects were sampled from mid-July to mid-September (Appendix 1). We only used fourth larval instars in our analyses because they are more likely to be engaged in IGP than other stages (Hironori & Katsuhiro 1997).

3.4.2 DNA extraction and PCR cycles

DNA extraction and PCR protocols were modified from Hoogendoorn and Heimpel (Hoogendoorn & Heimpel 2001). DNA was extracted from whole coccinellid larvae. Each insect was ground in a 1.5 ml microcentrifuge tube using sterile plastic pestles (Ultident Scientific inc.) with 100 µl of grinding buffer (see Bender *et al.* 1983). PCR amplifications were done separately for each primer pair (*H. axyridis* [F-5'-AAG AGG AGA CGC CGA CCA GA-3' and R-5'-AGG TAG CTT CAA TCG ATC GG-3']; *C. septempunctata* [F-5'-CGA AAG ACG ATC CCT ACG AA-3' and R-5'-AAG TTC GCT CGT CCT GGT TA-3']; *C. maculata* [F-5'-AGT GAA AAT GGG CAA CAA CA-3' and R-5'-GCC TTC TCC TTC CCT TCT TT-3']; *P. quatuordecimpunctata* [F-5'-GAT ATA TCG GCG CGT TTC TC-3' and R-5'-ATC GCT TTC TCC ACC TCG TA-3']). Details for development of PCR markers are described in Gagnon *et al.* (*submitted*). All predators were screened against the primers of all three potential intraguild prey and against a universal primer (12Sai and 12Sbi (Noda *et al.* 1997)). The screening against the universal primer pairs was done to ensure that DNA could be successfully detected in all specimens. Amplifications were performed in total volumes of 25 µl, composed of 20.25 µl of 1× buffer (0.25 mM of each dNTP and 1.5 mM of $MgCl_2$), 2.5 µl of primer mix (20 µM), 0.25 µl of *Taq* (i.e. 1.75 units) (Promega), and 2 µl DNA sample. The thermocycling program consisted of an initial step of 30s at 94°C (for *H. axyridis*, we used a hot start, i.e. addition of the *Taq* after the first step), followed by 30s at 94°C, 30s at 52°C (*H. axyridis* = 55°C), and 30s at 72°C. The three last steps were repeated 30 times and were followed by a step of 5 min at 72°C. All PCR products (10 µL) were electrophoresed at 120V in 2% agarose gels for approximately

1 h and then stained in ethidium bromide solution for 20 min and then visualized using a UV light-transilluminator. DNA is detectable at very low concentrations (from 35.5 ng x 10^{-4} to 35.5 ng x 10^{-6} depending on species primers) under optimal conditions (without heterospecific DNA).

3.4.3 Weighting IGP

We used the proportion of individuals of a given coccinellid species containing the DNA of one, two or three different coccinellid species to express the species-level intensity of IGP. However, when comparing intensity of IGP between different coccinelid species, we used corrected data. Because the prey DNA detection success over time (DS_{50}, the time after which 50% of the predators of a cohort that fed at the same time test positive for the presence of a species of prey using the PCR assay) varied for each combination of interacting coccinellid species following a meal (ranging from 5.2 h to 19.3 h; Gagnon *et al. submitted*), we used DS_{50} values to correct field data. Such a correction confers more importance to a "rapid-digesting" species where probability of detecting an intraguild prey is lower than for a "slower-digesting" species. We did not attempt to estimate predation rate per predator because no strong relationship had been found between the number of prey eaten and the duration of DNA in gut-content of coccinellids (Gagnon *et al. submitted*)

3.5 Results

Three novel results emerge from our study. First, levels of IGP were extremely high with averages of 46.8% and 58.9% (non-weighted data) of all coccinellids containing DNA of other coccinellids in their gut in 2004 and 2005, respectively (Table 3.1). The intensity of IGP for each coccinellid-coccinellid interaction, expressed as the proportion of each species of IG prey detected in the gut of IG predators, following DS_{50} corrections, is shown in Figure 3.1.

Second, the results indicate that IGP is mutual with each of the four coccinellid species feeding on each of the other three species (Figure 3.1). However, although levels of IGP were high in both years, the relative proportion of intraguild prey species varied

between years. In 2004, *H. axyridis* was strongly represented as an intraguild prey species, whereas in 2005 *P. quatuordecimpunctata* and *C. septempunctata* were the dominant intraguild prey species.

Third, we report multiple prey detection (Table 3.1). When results from both years are combined, 11.8% of the intraguild predators contained in their gut the DNA of two other coccinellid species when they were captured in the field, and we detected the three intraguild prey species simultaneously in the guts of 1.4% of the sampled coccinellids. Consumption of two intraguild prey species was most common in *H. axyridis* (48.1% of all cases) and *C. maculata* (35.7%), whereas only *H. axyridis* was feeding on three intraguild prey species.

3.6 Discussion

Our results indicate that IGP is a very common interaction among coccinellid species in soybean fields. Levels of IGP were high, with 52.9% of all sampled individuals containing in their gut the DNA of one, two and even three other coccinellid species. The interaction is mutual, as each of the four coccinellid species has the capacity to feed on the other three species. To our knowledge, this study represents the most convincing observational evidence of the prevalence of IGP among predatory arthropods.

Our demonstration reflects the reality of the field situation. We used a sampling technique that entails no perturbation to the ecosystem or to the members of the community. Coccinellids were sampled *in situ*, without altering their behavior or distribution**,** thereby reducing potential artifacts that invariably arise through experimental manipulations conducted under laboratory conditions or within field cages. Molecular biology allows the detection of minute amount of prey material by PCR after DNA extraction. Molecular gut-contents analyses led to a demonstration of complex predation events between co-existing species and opens the opportunity to better understand the dynamics and structure of communities. However, molecular gut-content analyses have their limits as well (Sheppard & Harwood 2005). Quantification of prey DNA is not

possible with traditional PCR detection where presence or absence of target DNA can only be revealed, although quantitative PCR protocols have been developed for this application (Lundgren *et al.* 2009). We cannot determine how many heterospecific individuals had been consumed by predators that tested positive. Secondary predation, when a predator eats another predator containing the prey of interest in its gut, as well as scavenging behavior cannot be discriminated (Foltan *et al.* 2005; Sheppard *et al.* 2005). Furthermore, because conspecific DNA cannot be discriminated from predator DNA, PCR detection of cannibalism is not achievable. Thus, we still lack a basic understanding of the relative importance of IGP and cannibalism, a very common phenomenon in Coccinellidae (Majerus 1994), for population dynamics in soybean fields.

While IGP models of predator-predator interactions, as well as the effects of omnivory on extraguild prey suppression have recently received considerable attention from both empiricists and theoreticians (Holt & Polis 1997; Rosenheim 1998; Rosenheim & Harmon 2006; Holt & Huxel 2007; Vance-Chalcraft *et al.* 2007), conspicuously, very few studies have explicitly measured levels of IGP in arthropods under field conditions. To our knowledge only three other field studies using molecular techniques have directly quantified levels of IGP in arthropods. In the soybean agroecosystem, Harwood *et al.* (Harwood *et al.* 2009) examined predation between *H. axyridis* and the predatory bug *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) using molecular gut-content analysis. Less than 2.5% of *O. insidiosus* tested positive for the detection of *H. axyridis*. Chacon *et al.* (Chacon *et al.* 2008; Chacon & Heimpel 2010) detected aphid parasitoid DNA in seven predator species using PCR in a study examining IGP of released parasitoids of the soybean aphid. In those studies, percentages of predators testing positive for parasitoid DNA ranged from 4 to 33. Hautier *et al.* (Hautier *et al.* 2008) reported that 9 out of 28 *H. axyridis* collected in potato fields had fed on heterospecific species of coccinellids, based on alkaloid quantification by GC-MS. Although this technique is promising, identification of prey species is only possible at the genus level. More information about IGP levels measured under natural conditions is available for larger predators from different taxa (see Table 2 for selected examples), probably because predation events can more easily be detected through different sampling techniques. The first published study quantifying the

incidence of IGP in nature was conducted by Polis and McCormick (Polis & McCormick 1987) who observed relatively high proportions of intraguild prey in the diet of desert scorpions, from 8 to 21.9%, and up to 45% for the species *Paruroctonus mesaensis.* Feeding information was easily collected on scorpions because they digest their prey externally. Nevertheless, available data, both for arthropods and other taxa containing predators, are still too few to suggest patterns about the relative strength of IGP.

Several factors may contribute to the very high levels of IGP we quantified in coccinellids. First, coccinellids respond numerically to high aphid densities (Evans & Youssef 1992; Donaldson *et al.* 2007; Chacon & Heimpel 2010) a condition that may favour encounters between predators; although high prey abundance may also lead to predator satiation and thereby a reduction in intraguild interactions. Second, by eating a heterospecific they eliminate a competitor and thereby improve access to the aphid resource. Third, aphids are a relatively low quality prey resource (Snyder *et al.* 2000), and coccinellids can benefit by complementing their diet by feeding on other coccinellids. All these potential factors remain to be tested. Furthermore, we still have a poor understanding of ecological determinants that determine the strength and direction of intraguild interactions, and there is a need for more empirical studies that examine the effect of factors such as seasonality, vegetation-structured complexity, habitat productivity, extraguild prey density, as well as the behaviors and life histories of protagonists.

Over the past 20 years, several models and experimental studies have examined the nature and role of intraguild interactions in both terrestrial and aquatic communities. Intraguild predation is now considered to be ubiquitous in most species assemblages (Arim & Marquet 2004). However, previous studies conducted in natural or managed ecosystems have largely overlooked the prevalence of IGP among top predators. Our results on coccinellids emphasize the importance of quantifying IGP in a given community. This basic information is central for understanding the role of top predators in population dynamics and community structure, and from a more applied perspective, to predict their impact in programs devoted to the biological control of pest species or the management of native endangered or invasive exotic species.

3.7 Acknowledgements

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3.9 Tables

Table 3.1 Number (N) of specimens tested and levels of IGP (raw data) among four coccinellids species with molecular gut-content detection of one to three different intraguild prey species in a same predator, in 2004 and 2005.

	N	One intraguild prey		Two intraguild prey		Three intraguild prey		Total IGP	
		n	**%**	***n***	**%**	***n***	**%**	***n***	**%**
2004	188	72	38.30	14	7.45	2	1.06	88	46.81
2005	180	74	41.11	29	16.11	3	1.67	106	58.89

Table 3.2 Selected examples of IGP among different taxa.

IG predator	IG prey	Extraguild prey	% IGP	Method of detection	Region	Authors
White-tailed sea eagle (*Haliaeetus albicilla* L.)	Mink (*Mustela vison* Schreb.)	Fish and birds	<7% (for all mammal species)	Behavior observation	Finland	(Sulkava et al., 1997; Salo et al., 2008; nonlethal IGP)
Cougar, wolf	Coyote	Small mammals	43-67%	Radio-tracked animals	Alaska, Idaho South Africa, Tanzania	(in Palomares and Caro, 1999)
Lion, spotted hyena	African wild dog		13-50%			
Ref fox	American marten		4%		Ontario	
Scorpion *Paruroctonus mesaensis*	*P. luteolus* *H. arizonensis* *V. confuses*	Insects	8-22% (in some months higher than 40%)	External digestion (direct observation)		(Polis and McCormick, 1987)
Eagle own	Tawny owl	Mammals, birds, fish, invertebrates	0.6%	Pellets and prey remains found under nests and roost sites	Italy	(Sergio et al., 2007)
Dingo	Feral cat Red fox	NA	1.2-6.1%	Dissection of gut-content	Australia	(Glen and Dickman, 2005)
Many intertidal herbivores	Many intertidal herbivores	NA	0.37-10%	Dissection of intestinal content	Chile	(Camus et al., 2008)

3.10 Figures

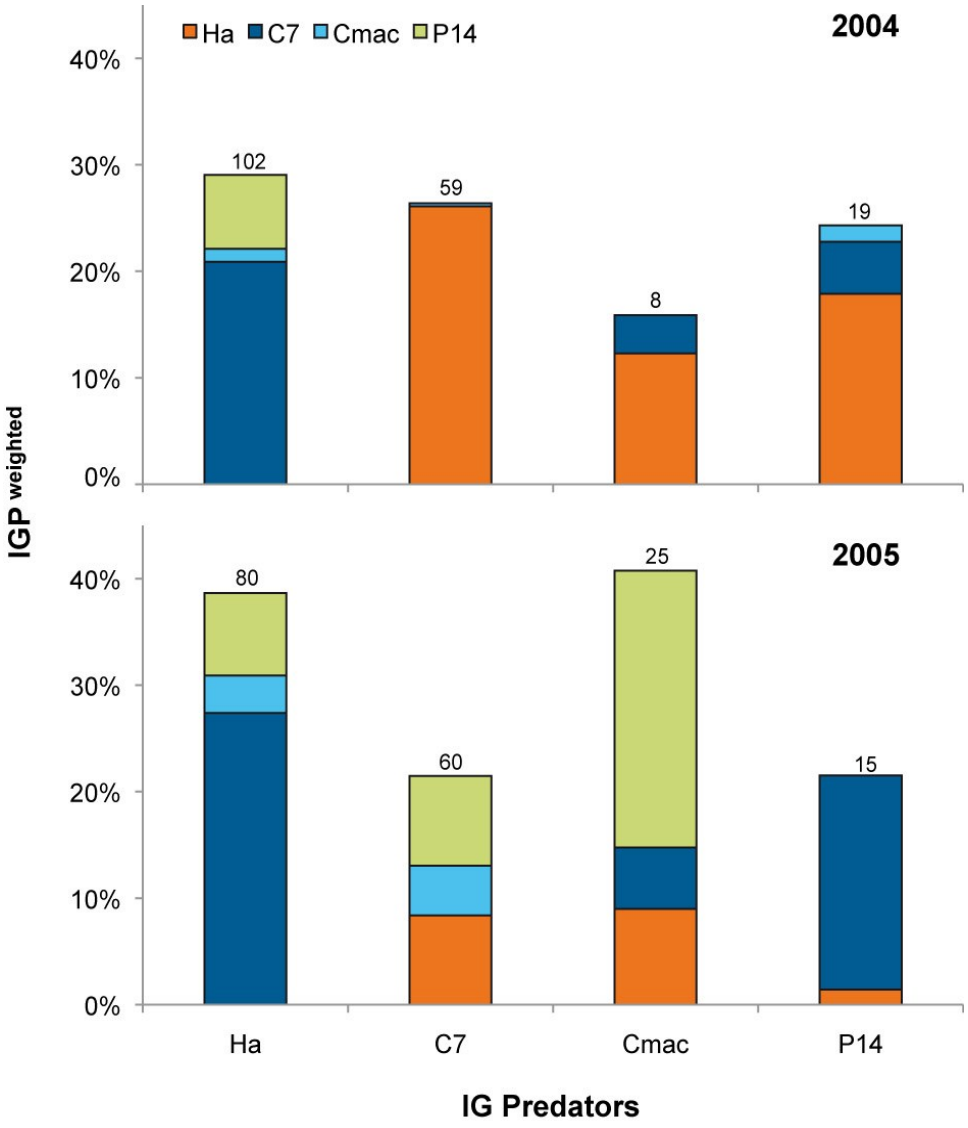

Figure 3.1. Levels of intraguild predation among four species of coccinellids measured by molecular gut content analysis in soybean fields in Québec, Canada, in 2004 and 2005. Results are expressed as the proportion of each species of IG prey detected in the gut of IG predators. DS_{50} values (see Methods section) were used to correct for differences in digestion times between predators and prey species. Ha = *Harmonia axyridis*, C7 = *Coccinella septempunctata*, Cmac = *Coleomegilla maculata*, P14 = *Propylea quatuordecimpunctata*. Numbers above histogram bars represent the number of coccinellid tested.

Appendix 1. Numbers of intraguild predation events for fourth instar larvae of four coccinellid species in four soybean fields in Québec, Canada, in 2004 and 2005. Ha= *Harmonia axyridis*, C7= *Coccinella septempunctata*, Cmac= *Coleomegilla maculata*, P14= *Propylea quatuordecimpunctata*.

Years	Field/Date	Number of specimens collected				
		Ha	C7	Cmac	P14	Total (all species combined)
2004	NICOLET					
	3-Aug - 16-Aug	5	11	-	1	17
	23-Aug - 6-Sept	14	3	1	5	23
	MASKINONGÉ					
	3-Aug - 16-Aug	7	3	3	3	16
	23-Aug - 6-Sept	14	-	3	3	20
	HÉROUXVILLE					
	27-July	5	5	-	-	10
	3-Aug - 16-Aug	13	10	-	2	25
	23-Aug - 6-Sept	14	12	-	1	27
	14-Sept	5	1	-	-	6
	ST-AUGUSTIN					
	3-Aug - 16-Aug	-	1	-	-	1
	23-Aug - 6-Sept	15	13	1	3	32
	14-Sept - 21-Sept	10	-	0	1	11
2005	NICOLET					
	2-Aug	-	-	15	4	19
	30-Aug	-	-	10	-	10
	MASKINONGÉ					
	26-July	-	-	-	3	3
	16-Aug	20	15	-	3	38
	HÉROUXVILLE					
	25-July	20	20	-	-	40
	15-Aug	20	15	-	-	35
	ST-AUGUSTIN					
	8-Aug	-	-	-	5	5
	15-Aug	20	10	-	-	30

CHAPITRE IV

Ecological factors promoting intraguild predation between ladybeetles*

* Ce chapitre a été préparé pour une soumission à la revue scientifique ***Oecologia*.** Les auteurs sont Annie-Ève Gagnon (Centre de Recherche en Horticulture, Université Laval) et Jacques Brodeur (Institut de recherche en biologie végétale, Université de Montréal).

4.1 Résumé

La prédation intraguilde (IGP) est une interaction commune au sein des ennemis naturels, structurant les communautés des systèmes terrestres et aquatiques. La multitude de facteurs écologiques, ainsi que leurs interactions pouvant promouvoir la présence d'IGP complexifie les approches expérimentales. Différentes études ont ciblé un ou deux facteurs spécifiques régulant l'IGP en laboratoire, mais peu d'études ont investigué tous les facteurs potentiels pouvant interagir entre eux en conditions naturelles. Nous avons examiné certains paramètres écologiques (densité des prédateurs et des proies, ratio prédateur:proie, stade de développement du prédateur et saisonnalité) promouvant l'IGP entre les coccinelles. Nous avons échantillonné un total de 611 *Harmonia axyridis* et 261 *Coccinella septempunctata* durant trois années dans les champs de soya, infestés du puceron *Aphis glycines*. À l'aide d'amorces PCR spécifiques aux espèces de proies intraguildes, l'IGP entre ces deux coccinelles a été détectée dans leur contenu gastrique, en plus de deux autres proies intraguildes: *Coleomegilla maculata* et *Propylea quatuordecimpunctata*. Bien que plusieurs facteurs écologiques liés à l'IGP se soient avérés significatifs, aucun patron général ne s'en dégage. Cette étude démontre donc que la présence d'IGP semble dépendre du contexte puisqu'une grande variabilité quant aux facteurs retenus dans les modèles

multivariés a été observée entre les espèces prédatrices et entre les années d'échantillonnage.

4.2 Abstract

Intraguild predation (IGP) is considered a ubiquitous interaction among natural enemies that structures the communities in terrestrial or aquatic systems. The multitude of ecological factors that can promote the occurrence of IGP makes experimental approaches difficult to address because possible interactions between factors can occur. Different studies have focused on one or two specific factors that may regulate IGP under laboratory conditions but few works have investigated all potential interacting factors under natural conditions. We examined a number of ecological parameters (predator and prey densities, predator:prey ratio, predator developmental stage and seasonality) that can promote IGP between ladybeetles. We sampled a total of 611 *Harmonia axyridis* and 261 *Coccinella septempunctata*, during three years in a soybean crops infested by the aphid *Aphis glycines*. Specific PCR primers of intraguild prey species were used to measured IGP between those two coccinellids species in their digestive tract, with two more intraguild prey: *Coleomegilla maculata* and *Propylea quatuordecimpunctata.* Even if multiple ecological factors were significantly associated with IGP, no general pattern can be drawn. This study shows that the occurrence of IGP is context-dependent, factors explaining IGP being variable among predatory species and years.

4.3 Introduction

Intraguild predation (IGP) occurs when a predator consumes a species that competes for the same resource (Polis and McCormick 1987; Polis et al. 1989). This interaction can promote trophic cascades with the diminution of one or both predator populations, resulting in an augmentation of the common prey and then a negative impact on plants (Polis and Holt 1992; Holt and Polis 1997). IGP can also displace species when the intraguild predator

exerts a strong pressure on the intraguild prey species (Dick and Dirk 2003). IGP is considered a ubiquitous interaction across different ecosystems and could be of high frequency under natural conditions in a specific system. For example, Arim and Marquet (2004) examined more than a thousand potential intraguild predators and intraguild prey in 113 food webs and revealed that the presence of IGP varied between 58.4% and 86.7%. Furthermore, the proportion of IGP detected in an assemblage of aphidophagous predators can be very high. Gagnon and collaborators (*Chapter* 3) observed IGP among four Coccinellidae at a frequency ranging between 15.9% and 40.8%.

Several ecological factors may determine the nature and frequency of IGP interactions, resulting from variation in the (i) climatic conditions, (ii) identity, age-structure and density of intraguild predators, (iii) distribution and density of the common prey, and (iv) host plant phenology and architecture (Brodeur and Rosenheim 2000). For example, many studies have been realized on the impact of the common prey density (Chacón and Heimpel 2010; Lucas et al. 1998; Lucas et al. 2009) or through the productivity of a system (Morin 1999; Diehl and Feissel 2001; Borer et al. 2003). In many cases, increasing the extraguild prey density will result in a decrease of IGP (Lucas et al. 1998). Because IGP is a phenomenon composed of predation and competition (Polis et al. 1989; Polis and Holt 1992), it is not surprising to find less IGP when the competition between protagonists is reduced. However, increasing the productivity of a system will not necessarily result ultimately in a decrease of IGP because both extraguild prey and predator populations can be increased (Chacón and Heimpel 2010). Predator densities can change through a gradient of productivity: efficient predator species to consume the common prey will be more abundant at low productivity while the less efficient species (and usually the intraguild predator) will be more important at high productivity (Morin 1999). However, many works have shown that coexistence of both intraguild predator and intraguild prey is possible when the productivity of the system is high (Mehner et al. 1996; Morin 1999; Amarasekare 2007; 2008).

Habitat structure can also influence IGP by reducing the interaction strength between the intraguild predator and the intraguild prey in complex-structured vegetation (Finke and Denno 2002). In a meta-analysis, Janssen et al. (2007) showed that IGP is considerably reduced when the spatial complexity is increased, principally by decreasing the encounter rate between protagonists and providing more refuges. Many other factors that modulate IGP have been identified such as the relative body weight of intraguild prey and intraguild predator (Woodward and Hildrew 2002; Félix and Soares 2004), the mobility of protagonists (Provost et al. 2006), the behavioral interference (Wissinger and McGrady 1993), the age-structure of the prey or predator (Wissinger 1992; Crumrine 2005), the chemical defenses of the prey (Hemptinne et al. 2000; Ware et al. 2008), and the identity of the common prey species (Lucas et al. 2009). It is difficult to generalize the impact a specific factor can have on IGP because all factors are potentially correlated and are variables with the peculiarity of each ecosystem (Rosenheim and Harmon 2006) and multiple coexistence mechanism between protagonist may occur at a same time (Amarasekare 2007). Also, it is unclear which factor or combination of factors is promoting IGP in nature because most of the experiments focused only on one or two factors in simplified arena. To our knowledge, no study has observed, under natural conditions, a complex of factors that can promote IGP (but see Chacon and Heimpel, 2010).

To determine the influence of multiple ecological factors on IGP between Coccinellidae, we used a molecular technique for the detection of intraguild prey in predator gut-content analysis. This methodology gave the opportunity to study IGP under natural conditions without disturbing (i) the spatial complexity and mobility of individuals, (ii) the species density and diversity, (iii) the natural temporal occurrence of species/stages. Previous work showed that this technique is effective in detecting IGP interactions among species under natural conditions (Gagnon et al. *Chapter 3*). Specific primers were designed for four coccinellid species (see Gagnon et al. *Chapter 2*): *Harmonia axyridis* (Pallas), *Coccinella septempunctata* L., *Coleomegilla maculata lengi* Timberlake and *Propylea quatuordecimpunctata* L. We used these primers to evaluate the incidence of IGP between coccinellids collected in soybean fields in Québec, Canada, during the summers 2004, 2005 and 2006. The goal of this study was to identify which ecological factors promote IGP in an

aphidophagous predator assemblage. Parameters that have been evaluated are: intraguild predator and intraguild prey densities, aphid density, ratio predator:prey, developmental stage and seasonality. We used logistic regression to establish relationships between the intensity of IGP and those ecological parameters.

4.4 Methods

4.4.1 Predator and aphid densities

To evaluate ladybeetle density, a weekly sampling of predators was conducted by sweep net for each field between late-June to early-September. Sampling consisted of 100 sweep netting through a zigzag transect to allow a random sampling. For each of 10 stations, separated by 100 footsteps, 10 strokes were done during walking. All insects (larvae and adults) were put in a plastic bag, frozen and then identified at the species level. In our analyses, only four coccinellid species were used because they represent the dominant predators in the soybean system (Mignault et al. 2006). Soybean aphid densities were estimated by counting directly all individuals on 50 plants per week and per field. The sampling consisted of the observation of five randomly selected plants at each station, through a zigzag transect, as described above. Results are expressed as total coccinellids per 100 sweeps, and mean aphids per plant (divided by 10) for each field during the three years.

4.4.2 Sampling

Coccinellid specimens for DNA extraction were collected in soybean fields in 2004 and 2005 with sweep netting, put in an electric icebox at 4°C, brought back to the laboratory and put in vials with 70% ethanol. Samples were preserved at 4°C until DNA extraction. In 2006, specimens were collected manually on soybean plants, and then placed in vials with ethanol. Harwood (2008) found no evidence that sweep netting overestimated predation frequency in molecular gut content analysis versus hand collected specimens of spiders in

alfalfa fields. From 2004 to 2006 samples were collected in five different fields (located on the same farm each years), in the province of Québec, Canada: Maskinongé (46°14'00'', 73°01'00''), Hérouxville (46°40'00'', 72°37'00''), Nicolet-Sud (46°13'00'', 72°37'00''), St-Augustin-de-Desmaures (46°44'00'', 71°28'00'') and Verchères (45°47'00'', 73°21'00''). In 2004, 326 specimens of different larval stages were collected [59 second (L2), 106 third (L3) and 161 fourth (L4)], 140 larvae of the last instar (L4) in 2005 and 406 individuals of different developmental stages (14 L2; 82 L3; 113 L4 and 197 adult) in 2006 (*H. axyridis* represents 76% of all specimens). Samples of coccinellids were collected during their period of appearance in soybean fields (see Figure 4.1-4.3)

4.4.3 Detection of IGP in field-caught predators

For a given sampling date, the number and developmental stages of specimens varied between species due to their different density in soybean fields (see Figures 4.1-4.3). Over the three years, a total of 611 *H. axyridis* and 261 *C. septempunctata* were used for PCR amplification. For each predator, presence of three potential intraguild prey was tested: *C. maculata, P. quatuordecimpunctata* and *H. axyridis* or *C. septempunctata* (depending which predator species was tested). For the two predatory coccinellids species, the rate of IGP was established as the proportion of sampled individuals containing the DNA of a minimum of one intraguild prey species divided by the total number of coccinellids from the same species caught per sampling date. In some instances, more than one intraguild prey specieswas detected in the gut of a predator (in 13.2 % of all intraguild predators species) (Gagnon et al. *Chapter 2*), but those predators with multiple intraguild prey were considered as unique intraguild predators.

DNA extraction of coccinellids was done with the protocol described by Hoogendoorn and Heimpel (2001). Details of the protocol used for PCR amplification and primers design for each coccinellid species are described in Gagnon et al. (*Chapter 2*). For each sample, four different PCR amplifications were done using four different primer pairs: one for the control (universal primer) and three for each of the heterospecific coccinellids.

Furthermore, IGP rates were corrected because of significant differences in digestion rates between coccinellid species (Gagnon et al., *Chapter 2*). Comparing IGP rates of two species having distinctive digestion rate can bias the result by detecting more intraguild prey in the gut-content of species having a slower metabolism. For this purpose, we used the DS_{50} value, which is the time where half of the specimens of a same cohort are testing positive for the presence of prey DNA. Full methodological description to correct field data can be found in Gagnon et al. (*Chapter 2*).

4.4.4 Relative prey consumption

To evaluate if predators engage in IGP at the same rate as they encounter a potential intraguild prey, examination of relative prey consumption were performed. The proportion of IGP detected in a predator for a particular prey species and the relative density of this prey species have been used as follows:

(% IGP on ladybeetle *x*) – (ladybeetle *x* density / density of all ladybeetle species)

A positive result indicates a higher consumption rate relative to prey abundance, and consequently a marked preponderance for this prey. Conversely, a negative result suggests avoidance/repulsion of this prey. A result near zero corresponds to a species that consumes intraguild prey randomly, along with the probability of encounter. This simple measure of relative prey consumption focused on encounter rate and prey density but does not take into account other parameters such as size or mobility of the species.

4.4.5 Statistical analyses

IGP rates were analysed in relation to different ecological variables: aphid density, coccinellid density (all species), predator:prey ratio (the ratio of coccinellid:aphid), developmental stage of the intraguild predator, sampling date. Because population dynamics are different between fields and years, results are analyzed separately for 2004, 2005 and 2006 and, fields were considered as bloc units. Logistic regressions were employed to build a model that retains variables that contributed to explain the best the

intensity of IGP under field conditions. A univariated analysis was first conducted for each parameter measured (described above) using a logistic regression for each year (LOGISTIC procedure, SAS et al. 1996). Significant parameters ($\alpha < 0.05$) were retained and, next, the selection of final variables was realized with a stepwise process. When more than one parameter were selected, possible interactions were tested. Evaluation of the goodness-of-fit of the model was then performed with the curve ROC (Hosmer and Lemeshow 2000). Furthermore, ANOVAs were used to compare relative prey consumption for each intraguild predator. Multiple comparisons between relative prey species consumption were addressed separately for each predator species with LSD (Least Significant Difference). SAS statistical software was used for all analyses (SAS Institute, 1996).

4.5 Results

4.5.1 Predator and prey densities

Coccinellid and soybean aphid densities during the growing seasons 2004, 2005 and 2006 are shown in Figures 4.1, 4.2 and 4.3, respectively. As commonly observed in aphidophagous systems, aphid populations start to increase during mid-summer, raises rapidly and then decrease during late summer. Coccinellid populations follow the aphid populations with a little delay. Soybean aphid densities varied greatly between fields and years (Figure 4.1 to 4.3). For example, higher aphid densities were observed in Maskinongé (mean of 311.9 and 525.1 aphids/plant in 2004 and 2005, respectively) compared to St-Augustin-de-Desmaures (mean of 126.6 and 88.1 aphids/plant in 2004 and 2005, respectively) (Figures 4.1 and 4.2). Mean aphid densities varied between years with, in increasing order, 109.2 ± 39.8, 265.6 ± 100.1 and 370.9 ± 131.5 aphids/plant in 2006, 2004 and 2005, respectively. Mean ladybeetle densities varied from 15.5 to 67.0 ladybeetles/100 sweep-net; means per year (Figures 4.1, 4.2 and 4.3). This high variability in both coccinellid and soybean aphid densities provides anopportunity to build our model with different responses.

4.5.2 Levels of IGP detected

The mean levels of IGP detected in *H. axyridis* and *C. septempunctata* throughout the season were variable within fields and years and are shown in Figures 4.1-4.3. We found considerably high levels of IGP for the two predatory coccinellids with mean rate ranging between 5.1-38.7% and 0.7-25.8% for *H. axyridis* and *C. septempunctata*, respectively. The level of IGP per field was variable with, for example, mean rate of IGP by *H. axyridis* in 2004 detected at Maskinongé of 8.8 ± 2.9% (mean ± SE) and Nicolet-Sud of 42.8 ± 6.7% ($F=26.00$, $df=1$, $p=0.0005$).

4.5.3 Models

Tables 4.1 and 4.2 present results of the models testing for ecological factors determining the occurrence of IGP for *H. axyridis* and *C. septempunctata*, respectively. For each species, results will be presented for each year and discussed in the next section to expose different patterns.

For the predator *H. axyridis*, different factors have been retained in the multivariate analyses within the three years of sampling (Table 4.1). In 2004, older larval stages were more involved in IGP interactions with 51.1% of IGP by L4, 27.8% by L3 and 9.4% by L2. In 2005, only L4 stages have been retained for our analysis and in 2006, the developmental stage was not a significant parameter. IGP in 2004 and 2006 was also less intensive late in the season with an average diminution of risk of IGP of 9.2% per week (according to the model) following the first detection. In 2005, time was not a significant parameter because sampling of coccinellids was done, in some fields, only on one day. However, in 2005, aphid density and the ratio coccinellid:aphid were both significant factors explaining the rate of IGP. When mean aphid density increased by one (mean aphid per plant), risk of IGP decreased by 0.8%. Risk of IGP was also reduced (-23.7%) when the coccinellid:aphid ratio increased by one (mean number of coccinellids per 100 sweep net/mean aphid per plant), all other parameters being fixed. No interaction was found between significant factors in all analyses. Robustness of the models, evaluated with the goodness-of-fit, was

excellent in 2004 and 2005 (ROC= 0.847 and 0.926, respectively), while in 2006, the explanatory variable was less robust with a ROC of 0.635. However, we sampled the highest number of coccinellids during this last year (406 specimens).

For the predator *C. septempunctata,* only two factors were retained in the models to explain IGP in 2004 and 2005, no factor being significant in 2006 (Table 4.2). In contrast to *H. axyridis*, seasonality had a positive impact on IGP, as more IGP was detected late in the season. In 2004, IGP increased by 3.6% per week. In 2005, increase of aphid density had a significant negative impact on IGP with a reduction of IGP of 0.6 %. The robustness of the models was inferior compared to models with *H. axyridis* with ROC of 0.625 and 0.689 in 2004 and 2005, respectively.

4.5.4 Relative prey consumption

A relative prey consumption index was used to examine a potential relationship between the proportion of IGP on an intraguild prey species and its relative abundance in the field (Figure 4.4). For *H. axyridis*, levels of IGP followed the relative prey abundance, except for the prey *C. septempunctata* in 2004 where less prey were eaten compared to its density (Figure 4.4A). In 2006, intraguild prey consumed by *H. axyridis* were slightly under-represented compared to the relative density of intraguild prey species in the field (Figure 4.4C). In other cases, *H. axyridis* did not show a relative prey consumption tendency. On the other hand, the prey *H. axyridis* was inversely represented in gut-content analyses of *C. septempunctata* compared to its relative density in the field for all three years. These results suggest that *C. septempunctata* avoid eating *H. axyridis* or that it escapes predation. Other intraguild prey species (*C. maculata* and *P. quatuordecimpunctata*) where not different from zero or slightly under-represented.

4.6 Discussion

We determined IGP with the amplification of prey DNA in gut-content of a total of 872 ladybeetles. This molecular method allowed us to collect individuals of *H. axyridis* and *C.*

septempunctata without disturbing their feeding habits. Molecular gut-content analyses have the advantages of studying interactions between species in a completely natural setting, without manipulation of species densities and phenology, habitat-structural arrangement and environmental conditions. The intensity of IGP is context-dependant with different ecological variables associated with each species, varying between the three years of sampling. Nevertheless, we have identified four ecological factors that contribute to explain the occurrence of IGP in this aphidophagous system: predator developmental stage, aphid density, coccinellid:aphid ratio, and seasonality.

4.6.1 Factors promoting IGP

4.6.1.1 Density-related effects

The intensity of predation by *H. axyridis* and *C. septempunctata* on other coccinellid species is inversely related to aphid density in some cases. It has been previously demonstrated under laboratory conditions that the increase of prey density reduced the intensity of IGP. For example, Obrycki et al. (1998) observed IGP between *C. septempunctata* and *C. maculata* when prey density was low (one aphid per day) while no IGP was recorded when more than 20 aphids were provided. Furthermore, Yasuda and Shinya (1997) show, in a field experiment, that high mortality occurred all along the development stages of *C septempunctata* and *H. axyridis* on hibiscus trees. This mortality was mainly attributed to cannibalism and IGP and their intensity increased when prey abundance was low. One of the reasons that ladybeetles consume heterospecifics in conditions of prey scarcity is probably the competition between species (Polis and Holt 1992). Furthermore, IGP can provide food, and sometimes even more nutritive compared to the common prey (Dixon 2000). In addition, low prey abundance promotes the movement of coccinellids, and this induced activity may lead to an increase in the intensity of IGP (Yasuda and Shinya 1997). A dilution effect can also occur when prey density become abundant, reducing thereby the frequency of IGP (Lucas and Brodeur 2001). However, Chacón and Heimpel (2010) show that in an open field experiment, a dilution effect for the parasitoid *Binodoxys communis* (Gahan) is overcome by short-term intraguild predator aggregation. Even if our study was also realized under natural conditions, our results are

contrasting with this recent study because for both coccinellid species, a decrease of IGP was recorded when prey density was high. This difference may be explained by a high mobility for coccinellid larvae that allow escape from predation while mummies of *B. communis* are completely immobilized, and represent a vulnerable insect.

4.6.1.2 Time-related effects

Moreover, our study shows that coccinellid density was not a significant factor retained in our analyses. It has also been observed by Chacón and Heimpel 2010that increasing aphidophagous predator density alone did not affect IGP. Increasing predator density can induce competitive intraguild interference, *i.e.* predators may change their behavior to avoid an intraguild predation event. Use of different niches has been revealed for *C. maculata* that exploit a lower strata of maize plant to avoid predation by *H. axyridis* (Hoogendoorn and Heimpel 2002). Other studies found an alteration of species behavior, where a mink reduced its distance cover to search food when the risk of IGP was high (Salo et al. 2008) and a weasel also reduced its activity levels when abundance of guild members increase (St-Pierre et al. 2006). This demonstrates that other mechanisms may alter the fitness of a species without detecting a 'direct' intraguild event, and this may have been the case in our study. We also identified that the increase of the coccinellid:aphid ratio has a negative effect on IGP exerted by *H. axyridis*. We could have attempted to see an increase of IGP in conditions where high competition between coccinellids is observed due to a high predator density and low aphid density. However, this counterintuitive result may be explained by the fact that the ratio coccinellid:aphid, like predator density alone, may increase interference between predators without necessary having direct IGP (see above).

We found that IGP exerted by *C. septempunctata* on other ladybird species increased throughout the soybean growing season. In contrast, predation by *H. axyridis* was high early in the season, when aphid populations begin to develop in soybean culture. Seasonality was one of the factors explaining IGP in more than one year and for the two species. However, it is not clear how time can directly have an impact on IGP as other

parameters are undoubtedly linked. One of the factors that we did not take into account is the plant-structured complexity that is dependant of time. As the plant grows, the complexity increases as more branches on the plant can be observed. This can be viewed as a high-structure habitat. A meta-analysis done by Janssen et al. (2007) showed that highly structured habitats provides protection from IGP to intraguild prey compared to habitats with little structure. However, when manipulating plant-structured complexity, we did not find a significant impact on the frequency of IGP among coccinellid species (Gagnon et al. *Chapter 5*). Many other factors may be linked to the variable 'time', such as the developmental stage of predators, where a sequence from eggs to adult cohorts can be observed (Yasuda and Shinya 1997). In our study, second larval stages of *H. axyridis* were less voracious at eating heterospecifics with a rate of IGP five times lower compared to the last larval stage. In concordance with our results, Yasuda and Shinya (1997) and Jansen and Hautier (2008) observed that *H. axyridis* faced with prey scarcity at the fourth larval stage and are more likely to engage in IGP. However, *H. axyridis* engage in IGP more frequently early in the season, when larval stages are prevalent. Maybe the increase of alternative prey late in the season, at high productivity, can promote coexistence between intraguild predators (Daugherty et al. 2007).

4.6.2 Ecological considerations

IGP can also be responsible for the displacement of native species (Evans 2004; Snyder et al. 2004; Pell et al. 2008). *Harmonia axyridis* is considered as a top predator in an aphidophagous assemblage, which can have negative impacts on other aphidophagous species (Koch 2003; Koch and Galvan 2008). This species had a greater ability than other guild members to engage in intraguild interactions. For example, higher attack and escape rate (Yasuda et al. 2001) and higher voracity (Lucas et al. 1997) can lead to the displacement of other vulnerable species. We found that intraguild prey preference for the predator *H. axyridis* was not different from the relative abundance of prey in the field, while *C. septempunctata* eat less intraguild prey compared to species density in the field. *Harmonia axyridis* consume *C. septempunctata* more often than vice versa (Yasuda and Shinya 1997; Yasuda et al. 2001; Sato and Dixon 2004). The behavior of this former one

can be more detrimental to the survival of heterospecifics because it can engage in, but also win often a confrontation. We also showed that rates of IGP by *H. axyridis* were more intense early in the season, when the control of the pest is critical to ensure biological control. Furthermore, because this species is now dominant in agricultural systems since its first detection in Canada in 1995 (Coderre et al. 1995), this could have an impact on the long-term biological control. Diminution of other coccinellid species due to competition and predation exerted by *H. axyridis* may delay the arrival of early-coccinellid species, such as *P. quatuordecimpunctata* (Honek et al. 2008) in infested cultures. Since 2002, we have seen a drastic diminution of this species in soybean fields, resulting probably from displacement by the dominant *H. axyridis*.

4.6.3 Conclusion

To summarize, ecological factors retained in our model varied considerably from year to year and between coccinellid species. This may reflect all the complexity in generalizing interactions among species that are strongly context-dependant (Chacón and Heimpel 2010). However, this study is one of the first demonstrating the impact of specific ecological parameters under completely natural conditions.

4.7 Acknowledgements

We are grateful to G. Daigle for statistical advices and to É. Lemaire, V. Janelle, J. Mainguy, M.-E. Tanguay and G. Boivin for assistance in the field. This work was funded by the Fonds Québécois de Recherche sur la Nature et les Technologies and the Canada Research Chair program.

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4.9 Tables

Table 4.1 Parameters explaining the occurrence of intraguild predation for the predator *Harmonia axyridis*.

Variables	ß	SE	DF	t	P > | t |	Means[d] ± SE
2004[a]						
Intercept	3.1347	1.0341	207	3.03	0.0562	-
2nd larval stage	-2.3071	0.5519	207	-4.18	< 0.0001	9.4 ± 8.2
3rd larval stage	-0.9961	0.3980	207	-2.50	0.0131	27.8 ± 17.7
4th larval stage	0.0000	.	.	.	.	51.1 ± 21.5
Date	-0.0953	0.0178	207	-5.35	< 0.0001	9.1 ± 1.8
2005[b]						
Intercept	36.1581	10.6108	77	3.41	0.0010	-
Aphid density	-0.0082	0.0000	77	< 0.0001	< 0.0001	0.1 ± 0.0
Ratio C:A	-0.2707	0.1737	77	-3.28	0.0015	23.7 ± 18.97
2006[c]						
Intercept	-0.6824	0.6065	315	-1.13	0.2613	-
Date	-0.0884	0.0334	315	-2.65	0.0085	9.2 ± 3.4

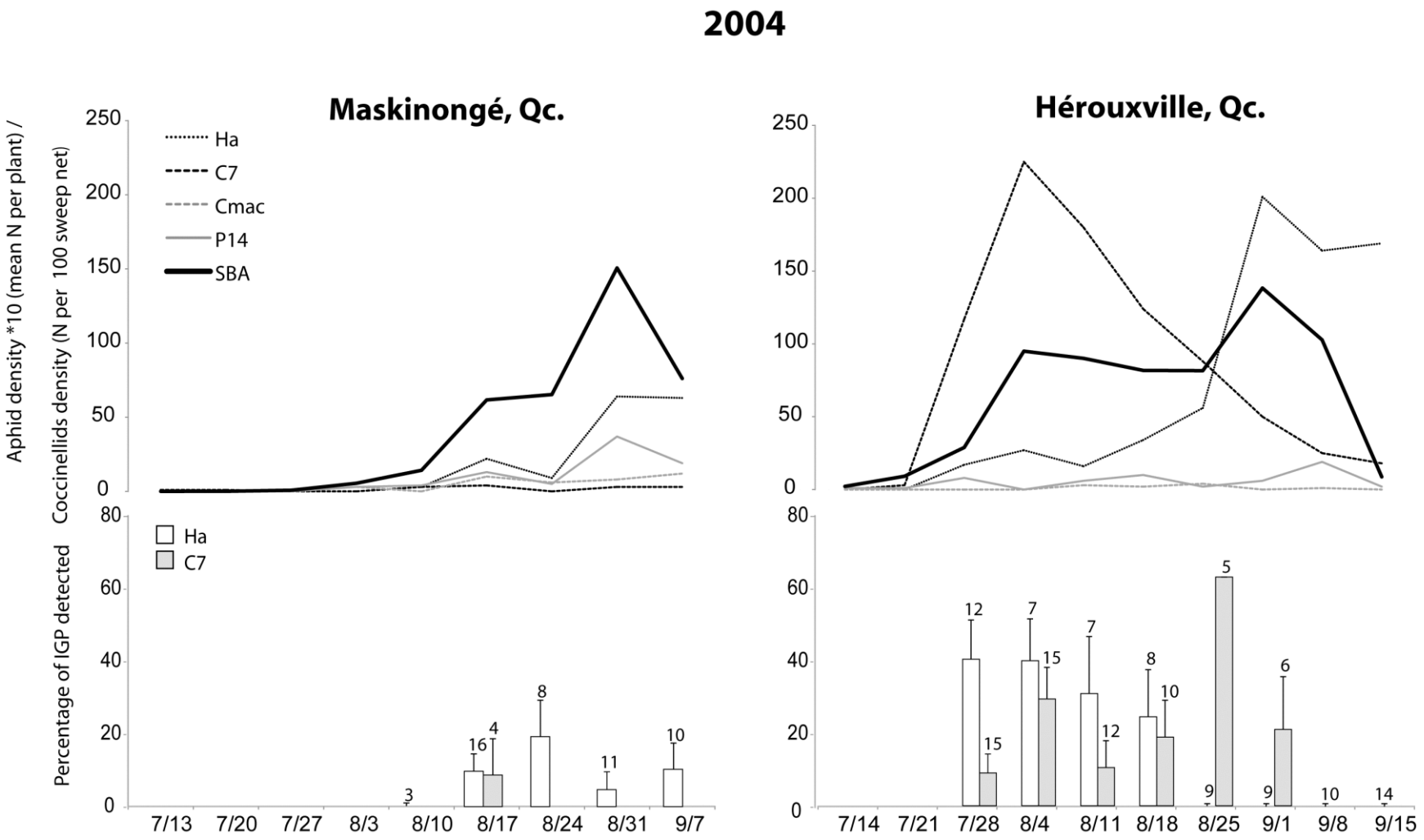
[a] Roc= 0.847
[b] Roc= 0.926
[c] Roc= 0.635
[d] Interpretation of response predicted (ß) for each variable retained. For qualitative variables: mean risk of IGP per class. For quantitative variables: mean increase or decrease of risk of IGP when the variable increase by one unit (100*(exp(ß)-1).

Table 4.2 Parameters explaining the occurrence of intraguild predation for the predator *Coccinella septempunctata.*

Variables	**ß**	**SE**	**DF**	**t**	**P > | t |**	**Means[c] ± SE**
2004[a]						
Intercept	-1.1603	0.3983	110	-2.91	0.0043	-
Date	0.0350	0.0155	110	2.26	0.0260	3.6 ± 1.6
2005[b]						
Intercept	1.2392	0.5380	58	2.30	0.0249	-
Aphid density	-0.0061	0.0021	58	-2.84	0.0061	0.6 ± 0.2
2006						
No variables retained						

[a] Roc= 0.625
[b] Roc= 0.689
[c] Interpretation of response predicted (ß) for each variable retained. For quantitative variables: mean increase or decrease of risk of IGP when the variable increase by one unit (100*(exp(ß)-1).

4.10 Figures


2004
Maskinongé, Qc.
Hérouxville, Qc.
Aphid density *10 (mean N per plant) / Coccinellids density (N per 100 sweep net)
Ha
C7
Cmac
P14
SBA
Percentage of IGP detected
7/13
7/20
7/27
8/3
8/10
8/17
8/24
8/31
9/7
7/14
7/21
7/28
8/4
8/11
8/18
8/25
9/1
9/8
9/15

2004 (continued)

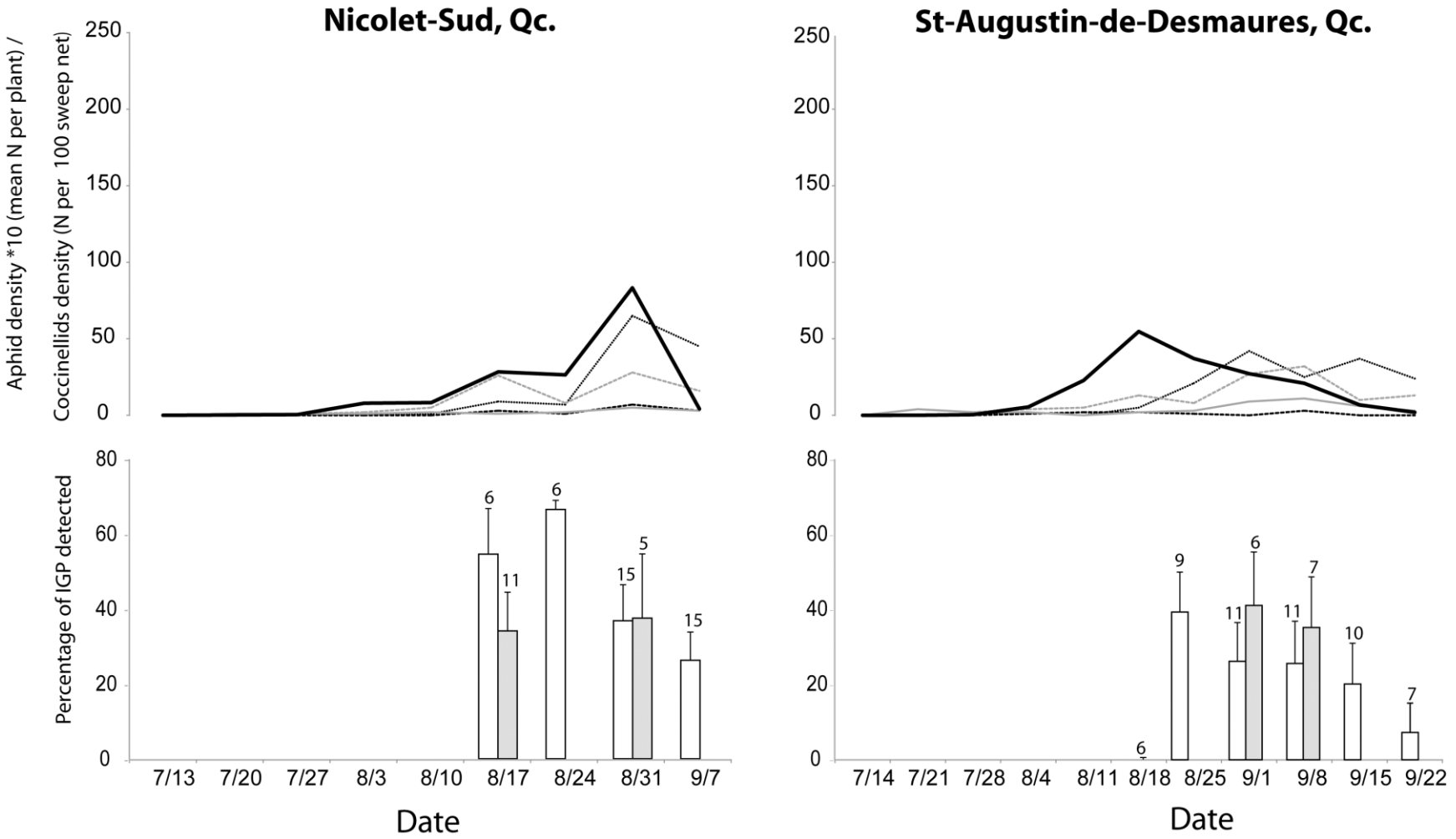
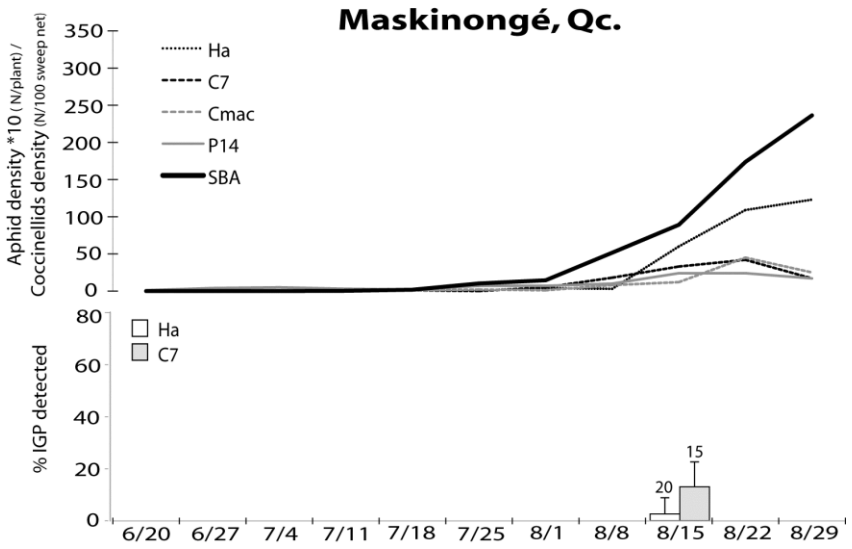
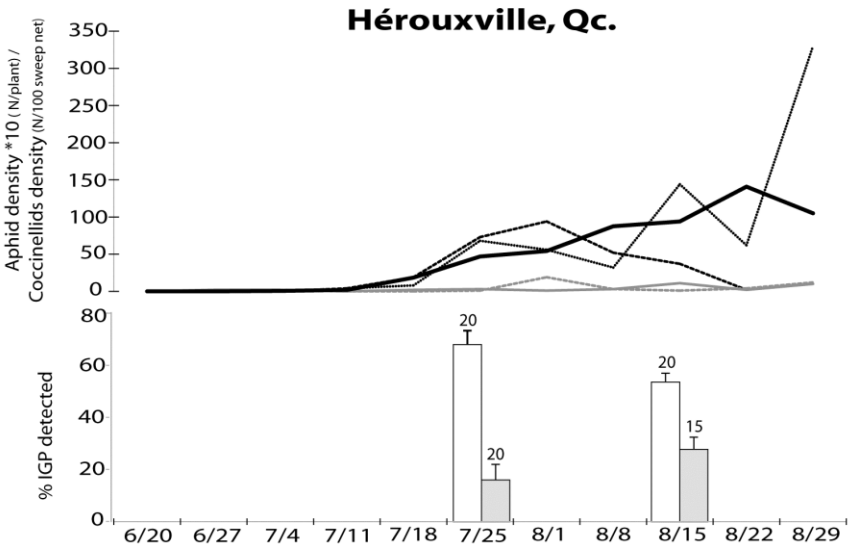


Figure 4.1. Coccinellids and soybean aphid densities in four soybean fields, during the summer 2004 (upper section). Ha= *Harmonia axyridis*; C7= *Coccinella septempunctata*; Cmac= *Coleomegilla maculata*; P14= *Propylea quatuordecimpunctata* and SBA= *Aphis glycines*. B) Proportion of IGP detected in *H. axyridis* and *C. septempunctata*, during the summer 2004 (lower section). One positive intraguild predator is represented by the presence of DNA of at least one intraguild prey species. Intraguild prey species tested for *H. axyridis* were: *C. septempunctata*, *C. maculata* and *P. quatuordecimpunctata*. Intraguild prey species tested for the predator *C. septempunctata* were: *H. axyridis*, *C. maculata* and *P. quatuordecimpunctata*. Proportion of IGP has been adjusted with digestion times (see in method section).

2005


Maskinongé, Qc.
Aphid density *10 (N/plant) / Coccinellids density (N/100 sweep net)
Ha
C7
Cmac
P14
SBA
% IGP detected
Ha
C7
20
15
6/20
6/27
7/4
7/11
7/18
7/25
8/1
8/8
8/15
8/22
8/29


Hérouxville, Qc.
Aphid density *10 (N/plant) / Coccinellids density (N/100 sweep net)
% IGP detected
20
20
20
15
6/20
6/27
7/4
7/11
7/18
7/25
8/1
8/8
8/15
8/22
8/29

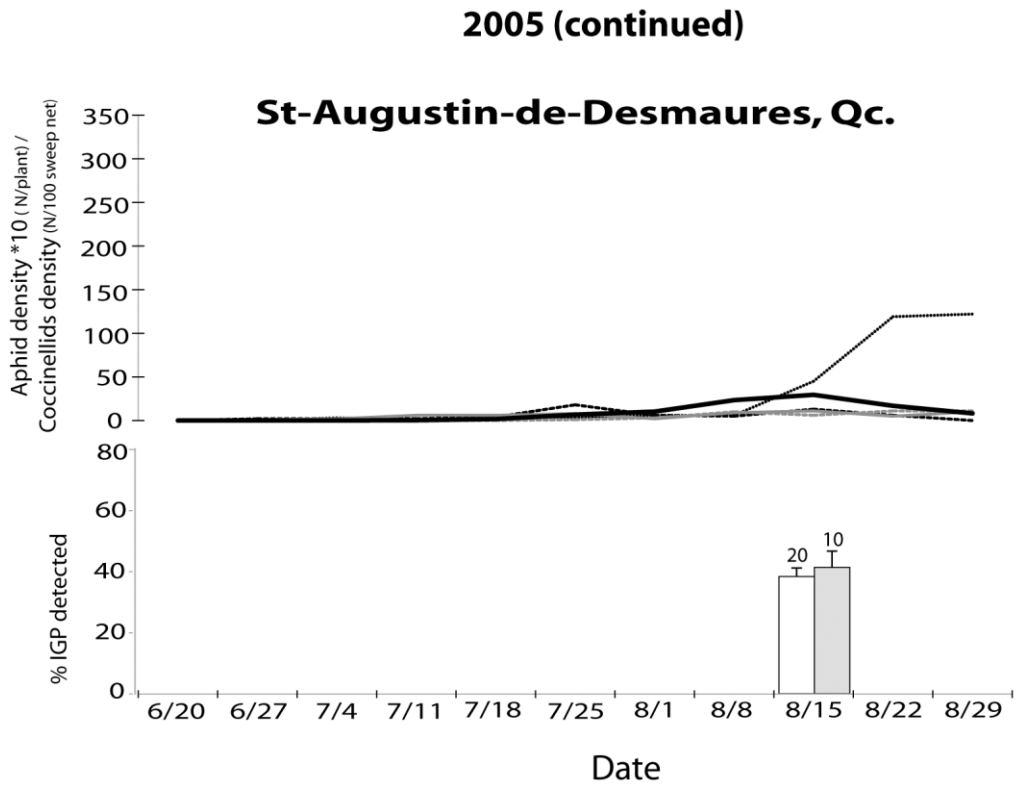


Figure 4.2. Coccinellids and soybean aphid densities in three soybean fields, during the summer 2005 (upper section). Ha= *Harmonia axyridis*; C7= *Coccinella septempunctata*; Cmac= *Coleomegilla maculata*; P14= *Propylea quatuordecimpunctata* and SBA= *Aphis glycines*. B) Proportion of IGP detected in *H. axyridis* and *C. septempunctata*, during the summer 2005 (lower section). One positive intraguild predator is represented by the presence of DNA of at least one intraguild prey species. Intraguild prey species tested for

were: *C. septempunctata*, *C. maculata* and *P. quatuordecimpunctata*. Intraguild prey species tested for the predator *C. septempunctata* were: *H. axyridis*, *C. maculata* and *P. quatuordecimpunctata*. Proportion of IGP has been adjusted with digestion times (see in method section).

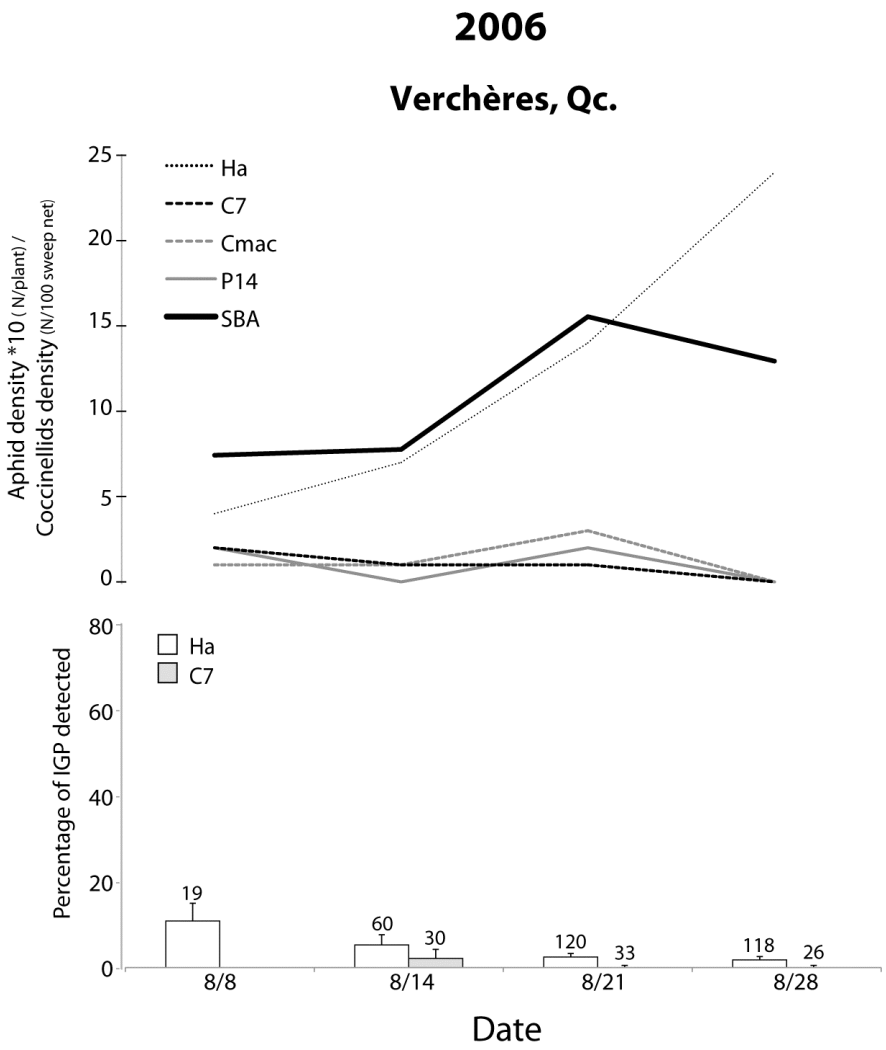
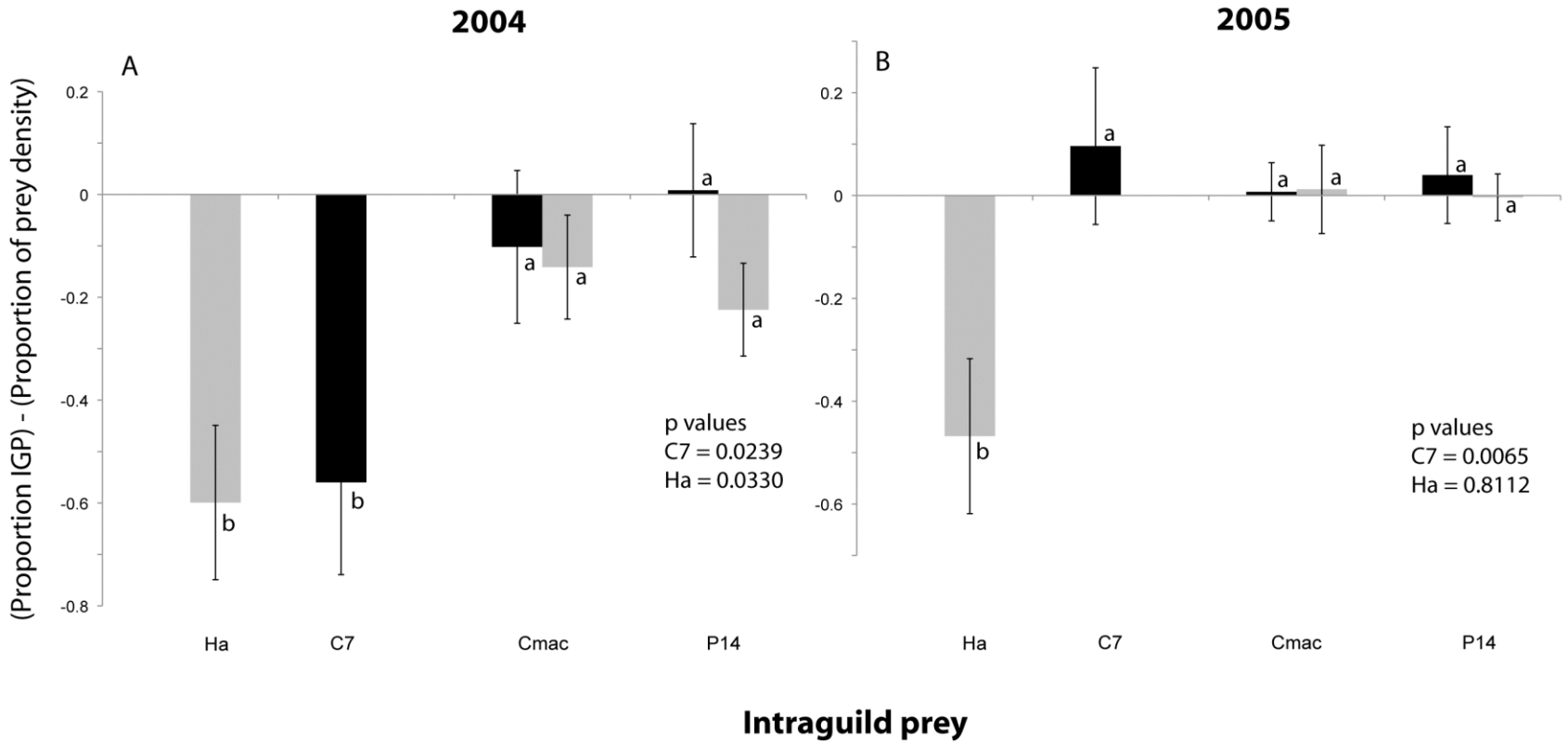

2006
Verchères, Qc.
Aphid density *10 (N/plant) /
Coccinellids density (N/100 sweep net)
25
20
15
10
5
0
Ha
C7
Cmac
P14
SBA
Percentage of IGP detected
80
60
40
20
0
Ha
C7
19
60
30
120
33
118
26
8/8
8/14
8/21
8/28
Date

Figure 4.3. Coccinellids and soybean aphid densities during the summer 2006 (upper section). Ha= *Harmonia axyridis*; C7= *Coccinella septempunctata*; Cmac= *Coleomegilla maculata*; P14= *Propylea quatuordecimpunctata* and SBA= *Aphis glycines*. Proportion of IGP detected in *H. axyridis* and *C. septempunctata*, during the summer 2006 (lower section). One positive intraguild predator is represented by the presence of DNA of at least one intraguild prey species. Intraguild prey species tested for *H. axyridis* were: *C. septempunctata*, *C. maculata* and *P. quatuordecimpunctata*. Intraguild prey species tested for the predator *C. septempunctata* were: *H. axyridis*, *C. maculata* and *P. quatuordecimpunctata*. Proportion of IGP has been adjusted with digestion times (see in method section).


2004
2005
A
B
(Proportion IGP) - (Proportion of prey density)
0.2
0
-0.2
-0.4
-0.6
-0.8
a
b
p values
C7 = 0.0239
Ha = 0.0330
p values
C7 = 0.0065
Ha = 0.8112
Ha
C7
Cmac
P14
Intraguild prey

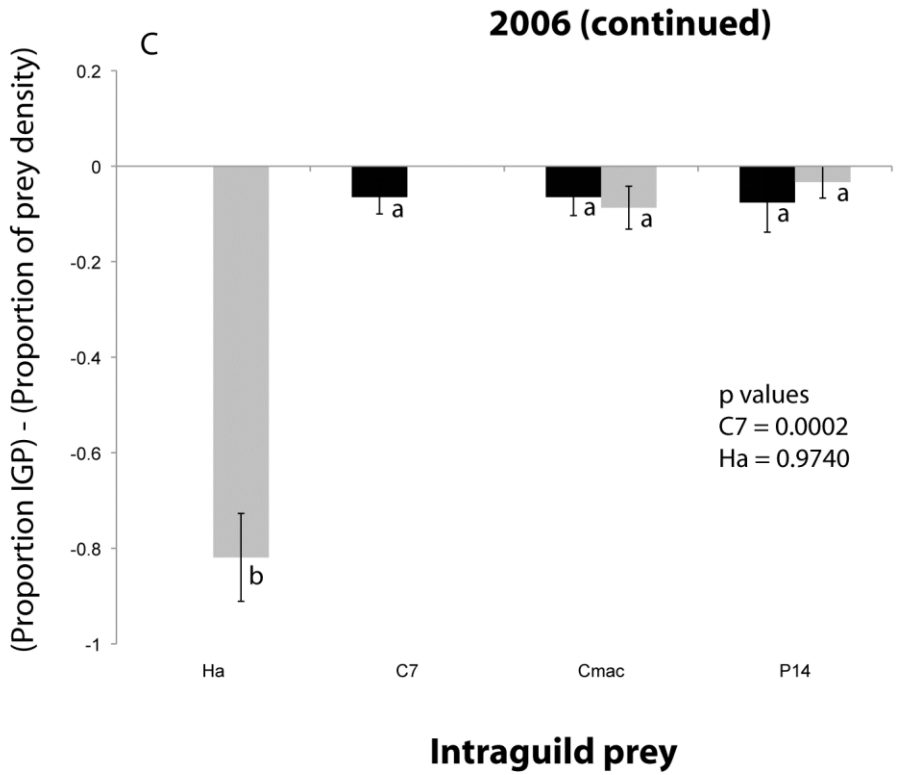


Figure 4.4. Intraguild prey preference (proportion of IGP on prey *x* – relative abundance of prey *x*) for *Harmonia axyridis* (black) and *Coccinella septempunctata* (gray): A) 2004; B) 2005; and C) 2006. Positive result shows a prey preference, a negative result means avoidance of this type of prey, while a result near zero (when error bar cross the zero line) show no preference. Results from a same predator have been analysis trough an ANOVA (p value presented on the graph) and multiple comparisons have been made among intraguild prey for the same predator species with LSD (least significant difference). Histograms with different letters are significantly different (α<0.05). Error bars indicate the standard deviation. Cannibalistic interactions have not been tested (NA).

CHAPITRE V

Impact of plant structural complexity and extraguild prey density on intraguild predation*

* Ce chapitre a été préparé pour une soumission à la revue scientifique ***Biological Control***. Les auteurs sont Annie-Ève Gagnon (Centre de Recherche en Horticulture, Université Laval) et Jacques Brodeur (Institut de recherche en biologie végétale, Université de Montréal).

5.1 Résumé

Plusieurs études de modélisation ou expérimentales ont démontré que la prédation intraguilde (IGP) pouvait engendrer un impact négatif sur la lutte biologique. Plusieurs facteurs écologiques et abiotiques déterminent la nature et la fréquence des interactions intraguildes, et ainsi la probabilité que l'IGP puisse affecter les populations d'herbivores. Notre étude a examiné l'effet de la complexité de la structure de la plante et de la densité des proies extraguildes, ainsi que leur interaction, sur la fréquence de l'IGP entre deux espèces de coccinelles (*Harmonia axyridis* et *Propylea quatuordecimpunctata*). La théorie prédit que les taux d'IGP devraient augmenter avec une diminution de ces deux facteurs: la densité des proies extraguildes et la complexité de la structure de la plante. Nous avons conduit une expérience factorielle au champ, en milieu ouvert, où des larves de coccinelles étaient introduites pour une durée de cinq jours. Nous avons manipulé deux niveaux de densité de pucerons, faibles (100 pucerons par parcelle) ou élevées (1000 pucerons par parcelle), ainsi que deux niveaux de complexité de la structure de plante en retirant la moitié des branches sur un plant de soya ou en laissant le plant intact. L'utilisation de

marqueurs moléculaires spécifiques à *P. quatuordecimpunctata* nous a permis de détecter sa présence dans le contenu gastrique de *H. axyridis*. L'analyse moléculaire du contenu gastrique de *H. axyridis* a révélé des taux d'IGP supérieurs (environ 20%) à faible densité de pucerons que lorsque les densités étaient élevées (< 6%). De plus, l'augmentation de la complexité de la structure de la plante a eu une influence négative sur la densité des pucerons, mais seulement dans les traitements à faible densité de proies extraguildes (réduction de 5% vs 29% à forte et faible complexité de la plante, respectivement). Par ailleurs, la structure de la plante n'a pas eu d'impact sur la fréquence de l'IGP (12% et 13% à faible et forte complexité, respectivement). En accord avec la littérature existante, cette étude démontre que l'IGP est amplifiée à faible densité de proies extraguildes. Par contre, aucun lien n'a été révélé entre l'IGP et la complexité de la structure de la plante, s'expliquant peut-être par la nature de la structure de l'habitat modifiée dans notre expérience.

5.2 Abstract

Several models and experimental studies conducted in confined environments have shown that intraguild predation (IGP) can interfere with biological control of arthropod pests. A number of ecological and abiotic factors determine the nature and frequency of intraguild interactions, and thus the potential of IGP to affect herbivore populations. This study examined the effect of plant structural complexity and extraguild prey density, and their interactions, on the occurrence of IGP between two species of ladybirds (*Harmonia axyridis* and *Propylea quatuordecimpunctata*). The theory predicts that IGP levels would increase with a decrease of both factors: extraguild prey density and plant structural complexity. We conducted a factorial experiment in an open soybean field into which coccinellid larvae were introduced in experimental plots for a period of five days. We tested two levels of aphid density, low (~100 aphids per plot) and high (~1000 aphids per plot), and two levels of plant structural complexity, low by removing half of the branches from the soybean plants and high by leaving plants intact. We used species-specific molecular markers to detect the presence of *P. quatuordecimpunctata* in the digestive tract

of *H. axyridis*. Molecular gut-content analysis of *H. axyridis* revealed that rates of IGP were higher (≈ 20%) at low aphid density than at high aphid density (< 6%). Increased plant structural complexity had a negative influence on aphid density, but only at low aphid density treatments (reduction of 5% vs. 29% at high and low plant complexity, respectively). However, plant structure did not impact the frequency of IGP (12% and 13% at low and high complexity, respectively). According to the existing literature, this study demonstrates that IGP is amplified at low extraguild prey density. However, no relationship was found between IGP and plant structural complexity, perhaps as a consequence of the nature of the habitat structure we modified in our experiment.

5.3 Introduction

Intraguild predation (IGP), an interaction between species that compete for the same resource, occurs widely in ecological systems and is recognized to be functionally important (Arim and Marquet 2004). Theoretical models and empirical evidence suggest that IGP can modulate population abundances and structure communities (Polis and Holt 1992; Holt and Polis 1997; Rosenheim 1998). Within agroecosystems, several empirical studies have shown that IG predators may either enhance (Cardinale, Harvey et al. 2003), have no effect (Colfer and Rosenheim 2001; Labbé, Cloutier et al. 2006), or disrupt biological control (Rosenheim, Wilhoit et al. 1993; Snyder and Ives 2001; Borer, Briggs et al. 2003; Chacón, Landis et al. 2008). The intensity and outcome of interactions between biological control agents may change over time, resulting from variation in the (i) environmental conditions, (ii) identity, age-structure and density of intraguild predators, (iii) distribution and density of the pest, and (iv) host plant phenology and architecture. The latter two factors are the object of our study.

The density of the extraguild prey (the common prey of intraguild predators), which usually reflects the productivity of an ecosystem, is considered to be one of the major determinant of the magnitude and stability of intraguild interactions (Morin 1999). Theoretical models have considered how resource productivity may modulate the densities

and persistence of both the intraguild predator and its intraguild prey. In biological control, these considerations are important since they determine the capacity of interacting predators to suppress pest populations, as well as the stability of trophic and guild interactions over time. With regards to productivity, the following predictions have been made. Conditions of intermediate productivity should favor the coexistence of interacting species. In contrast, intraguild predators and intraguild prey are likely to be excluded under conditions of low and high productivity, respectively (Holt and Polis 1997; Mylius, Klumpers et al. 2001; Borer, Briggs et al. 2003). However, a number of empirical studies showed that intraguild predators do not persist with intraguild prey in low productivity systems while, in contrast to theory, intraguild prey populations are maintained at higher levels of productivity (Morin 1999; Amarasekare 2000; Diehl and Feissel 2000; 2001). This discrepancy between theoretical predictions and empirical results about the consequences of IGP on population dynamics suggests that other factors or mechanisms are involved in the persistence/exclusion of protagonists. These include spatial and temporal refuges (Lucas and Brodeur 1999; Finke and Denno 2006; Amarasekare 2008; see below), antipredator defense of intraguild prey (Lucas, Coderre et al. 1997; Okuyama 2002), intraspecific interference (Amarasekare 2008), and natural enemies of the intraguild predator (McCann, Hastings et al. 1998). All these factors can influence the intensity of IGP by decreasing both competition and encounter rate between protagonists. Furthermore, this discrepancy may also arise from studies conducted in confined environments suggesting that IGP weakens with increasing extraguild prey density. Chacon and Heimpel (*in press*) showed that such results may be a consequence of experimental procedures that do not allow for predator aggregation.

Habitat structure - the physical arrangement of objects in space (McCoy and Bell 1991) - influences the diversity and number of refuges available, and thereby the encounter rate between interacting species (Janssen, Sabelis et al. 2007). Variations in plant structural complexity can be expressed at three different levels: i) plant size or surface area, ii) plant parts (seeds, flower, stem), and iii) connectivity of parts or plant form (architecture) (Andow and Prokrym 1990). Structurally complex vegetation can reduce predation on the intraguild prey and thus reduce negative effects of IGP on biological control (Finke and

Denno 2002). In agroecosystems, monocultures are non-diversified habitats. However, the plant structure changes throughout the growing season, and the increase of foliar abundance, blooming and growth of fruits are likely to provide additional refuges for arthropods. We might therefore expect changes in the intensity of IGP interactions over time.

Very few studies carried out in the field have examined ecological factors governing the amplitude, frequency and outcome of IGP. The occurrence of IGP has largely been assessed indirectly through estimation of animal abundance (Wise and Chen 1999; Meyhofer and Hindayana 2000; Finke and Denno 2002), an approach that does not reveal underlaying interactions. IGP is only one mechanism among several others that can contribute to variations of prey or predator abundances. A powerful sampling technique to examine mortality due to IGP under natural conditions is the use of PCR primers specific to prey species to detect target DNA in the gut-content of potential intraguild predator (Harwood, Desneux et al. 2007; Chacón, Landis et al. 2008; Harwood, Yoo et al. 2009; Gagnon et al. Chapter 2; Chacón and Heimpel, in press).

The soybean aphid, *Aphis glycines* Matsumara (Homoptera: Aphidiae), a species native to Asia, has been introduced to North America in 2000 (Ragsdale, Voegtlin et al. 2004). Several aphidophagous predators have responded to the invasion of soybean fields by *A. glycines*, coccinellids being the most abundant in north-eastern North America (Mignault, Roy et al. 2006). Several species frequently contribute to an effective control of *A. glycines* (Costamagna and Landis 2006; Costamagna, Landis et al. 2007; Rhainds, Roy et al. 2007). However, they are widely-foraging and opportunistic predators that often engage in IGP (Weber and Lundgren 2009), and we have observed very high levels of predation between four coccinellid species in soybeans (Gagnon et al. *unpublished data*). Among them, *Harmonia axyridis* (Pallas), an exotic species introduced in 1916 in North America (Gordon and Vandenberg 1991), is recognized as an important intraguild predator (Yasuda and Katsuhiro 1997; Sato and Dixon 2004; Snyder, Clevenger et al. 2004; Gardiner and Landis 2007; Hautier, Grégoire et al. 2008; Pell, Baverstock et al. 2008) because of its large size (4.9–8.2 mm (Koch 2003)) and high voracity (Michaud 2002).

Propylea quatuordecimpunctata is an accidently introduced species observed for the first time in Montréal, Québec, in 1968 (Day, Prokrym et al. 1994). In contrast to *H. axyridis*, *P. quatuordecimpunctata* is a relatively small beetle (3.5-5.2 mm (Gordon and Vandenberg 1991) that may suffer more frequently from IGP. The magnitude and direction of IGP are often determined by body size; smaller individuals being more vulnerable to large predators (Lucas, Coderre et al. 1998; Félix and Soares 2004). Furthermore, we observed a change over time in the composition of coccinellid species within soybean fields in Québec with a shift from *P. quatuordecimpunctata* being the most abundant species in 2002 to *H. axyridis* being the dominant one in recent years, perhaps because of IGP (A.-È. Gagnon, *unpublished data*).

The aim of this work was to evaluate the impact of plant structural complexity and density of an extraguild prey species *A. glycines*, as well as their interaction, on the incidence of IGP between two ladybirds, *H. axyridis* and *P. quatuordecimpunctata.* Based on the theory, we predicted that: i) a more complex plant structure benefits the intraguild prey by reducing the encounter rate with its intraguild predator; ii) IGP frequency is higher in low aphid density treatments. For this purpose, in an open field experiment, we manipulated aphid density and plant structure complexity and quantified the impact of these treatments on both predator and aphid abundances. Rates of IGP between *H. axyridis* and *P. quatuordecimpunctata* were revealed using molecular gut-content analyses.

5.4 Materials and methods

5.4.1 Study site and experimental design

The experiment was carried out in 2008 in a site located on the Laval University campus, Québec (Canada), characterized by a mosaic of small plots (<0.5 ha) with high crop diversity, and surrounded by an urban residential area. The soybean field was sown with the cultivar Lynx-RR (2550-2750 UTM) at a density of 16 seeds per linear meter within rows and a distance of 18 cm between rows. Applications of herbicide (Glyphosate)

followed local agricultural practices and insecticides were not used. Soybean plants were at the R3 to R5 stages during the experiment.

The effects of plant structural complexity and extraguild prey density on the incidence of IGP and soybean aphid population growth were tested using a 2 x 2 factorial design in nine completely randomized blocks. The four treatments were: (1) low plant structural complexity and low aphid density; (2) high plant structural complexity and low aphid density; (3) low plant structural complexity and high aphid density; and (4) high plant structural complexity and high aphid density. Each experimental plot consisted of a square of 1 m^2 (Figure 5.1). The four treatments were established in each block, with a distance of 10 m between blocks. Within a block, all treatments (plots) were isolated from each other and from the surrounding vegetation by removing one meter of soybean plants. Plots were randomly assigned to each treatment. Just before each repetition, all plants were carefully examined to manually remove predators and other arthropods. Because *A. glycines* has not yet colonized the field at the beginning of the experiment we inoculated aphids in experimental plots at two different densities (see below). The experiment lasted for five days and was repeated three times (Trials 1 to 3) in three different sites within the same field, for a total of three weeks (from August 4 to August 22).

5.4.2 Aphid prey density and soybean structural complexity

Two levels of aphid density were tested, low (~100 aphids per plot) and high (~1000 aphids per plot). Inoculations were done by stapling soybean leaves infested with *A. glycines* (all life stages combined) to leaves located in the middle of the plant. The aphids came from a *A. glycines* strain from Québec maintained in the laboratory since 2007 at 23°C; 16:8, L:D. Aphids were inoculated one day before the beginning of the experiment to favor their dispersal onto the plant. Aphid density was determined at the beginning (Day 1) and at the end (Day 5) of the experiment by recording the numbers of aphids on all leaves from five randomly selected plants per plots, for a total of 15 plants per treatment per trial.

Plant structural complexity (architecture) was manipulated by removing, or not, half of the branches from the soybean plant. The treatment of high complexity corresponds to the entire plant and the treatment of low complexity was the plant with half of the branches removed. In order to maintain similar total foliar surfaces between treatments, the numbers of plants per plots were corrected. A leaf-area meter was used (Li-3100, LI-COR Biosciences) to measure mean foliar surface of five plants in both the non-manipulated plot (high complexity treatment) and the manipulated plot (low complexity treatment). Because foliar surface of plants in the low complexity treatment was reduced by 48% following pruning (manipulated plants 395.97 ± 14.99 cm^2 vs. control plants 828.64 ± 118.67 cm^2 [means ± SE]), the number of plants was set to 10 and 20 in high and low plant structural complexity treatment, respectively. This procedure allows variation in plant architecture without modifying plant surface area.

5.4.3 Predator populations

All coccinellid larvae introduced into the experimental plots were laboratory-reared individuals (23°C; 16:8, L:D) taken from populations stemming from individuals that were originally collected from soybean fields surrounding the region of Montréal, Québec (Canada) in 2008. For each treatment, 8 *P. quatuordecimpunctata* 4^{th}-instar larvae and 8 *H. axyridis* 3^{th}-instar larvae were added in the plot. This protocol allows us to mimic the natural occurrence of larval stages of those two coccinellid species, as *P. quatuordecimpunctata* occurs earlier in soybean fields than *H. axyridis* (Gagnon, A.-E., *personal observation*). Four larvae of each species were introduced on middle leaves of plants and the remaining four on top leaves. Coccinellids density was assessed the first, third and fifth day following their introduction on plants. For *H. axyridis*, larvae were collected, put individually into a microcentrifuge tube filled with 95% ethanol, and brought back to the laboratory for molecular gut-content analyses (see below). These individuals were substituted with new 3^{th}- or 4^{th}-instar larvae after the first two sampling periods.

5.4.4 Molecular gut-content analysis

DNA for each *H. axyridis* larva collected in the experiment was extracted using the protocol described in Gagnon et al. (*Chapter 2*). PCR amplification was first confirmed using universal insect universal primer (Sai and Sbi on Mitochondrial 12S rRNA, in Noda et al. (1997)) to assess the quality of DNA extraction. Amplifications were then performed for the detection of *P. quatuordecimpunctata* (primers sequences on ITS-1: F-5'-GAT ATA TCG GCG CGT TTC TC-3' and R-5'-ATC GCT TTC TCC ACC TCG TA-3') in total volumes of 25 µl, composed of 2.5 µl of 10× buffer (1.5 mM of $MgCl_2$) (GenScript, Piscataway, United States), 0.5 µl of each dNTP (200 µM) (Applied Biosystems, Streetsville, Canada), 4 µl of primer mix (20 µM), 0.25 µl of *Taq* (i.e. 1.25 units) (GenScript), 15.75 µl of ddH_2O and 2 µl of DNA sample. Samples tested with the universal primers were placed in the thermocycler with an initial step of 3 min at 90°C followed by 30s at 94°C, 45s at 50°C, 1 min at 72°C (these 3 last steps were repeated 30 times), and a final step of 7 min at 72°C. For coccinellid primers, the thermocycling program consisted of an initial step of 3 min at 94°C, followed by 30s at 94°C, 45s at 52°C, and 30s at 72°C. The three last steps were repeated 30 times and were followed by a step of 3 min at 72°C. All PCR products (10 µL) were electrophoresed at 120V in 2% agarose gels for approximately 1 h and then stained in ethidium bromide solution for 20 min and finally visualized using a UV light-transiluminator. The detection times for *P. quatuordecimpunctata* within *H. axyridis* have been evaluated in a previous study with a DS_{50} of 10h (time where the prey DNA detectability was detected in 50% of predators tested) (Gagnon et al. *Chapter 2)*. *Propylea quatuordecimpunctata* was not sampled because 4th-instar larvae do not have the capacity to feed on larger *H. axyridis* 3th-instar larvae.

5.4.5 Immigration and emigration from field plots

Throughout this experiment, we studied interactions in the field without confining soybean plants in cages. We used physical barriers and repeated hand-removal of predators to maintain as much as possible the integrity of the experimental treatments. To prevent

migration and immigration of crawling and ground-living insects, a physical barrier 13 cm in height, made of black plastic lawn edging, was staked 10 cm into the soil around each plot. Tanglefoot®, a sticky material, was applied to the top of the barrier. Coccinellids and other insects glued on the barrier were collected at the end of the experiment and identified. To prevent naturally occurring predators to interfere with experimental treatments, migrant flying insects (mainly adult aphidophagous species) were all removed manually on day 1, 3 and 5 by carefully inspecting all soybean leaves, as well as the soil surface within each plot. The identity and number of immigrating predators were noted for each treatment. Predator hand-removals, however, cannot lead to a complete exclusion of immigrating insects (Rosenheim et al., 2004). Furthermore, because predators typically show a numerical response to aphid colonies (Frazer, 1988) we expect more immigrant predators to colonize high aphid density treatments. As a result, our data cannot be used to compare the impact of predation on aphid populations between treatments with low vs. high *A. glycines* densities (see Discussion).

5.4.6 Statistical analyses

The effects of extraguild prey density and plant structural complexity on aphid and predator abundances (expressed as per capita change in population size over the entire experiment: [initial insect count – final insect count] / initial insect count]) were analyzed with a factorial ANOVA using a randomized block design structure (Proc glm, SAS, 1996). *Harmonia axyridis* and *P. quatuordecimpunctata* abundances were first analyzed by comparing mean proportions of specimens recovered at the end of the experiment, and next, for each species, the effect of treatments was determined using ANOVA. The proportion of cumulative IGP detected in *H. axyridis* (number of larvae with *P. quatuordecimpunctata* DNA/sum of all larvae collected on Day 1, 3 and 5) for each treatment was analyzed with a factorial ANOVA (Proc glm, SAS 1996). Finally, differences in mean numbers of insects entering the plot between treatments were analyzed for the three sampling days (Day 1, 3 and 5) through a factorial ANOVA.

5.5 Results

5.5.1 Predator populations

Numbers of ladybird larvae recovered in experimental plots after five days were low and differed significantly between species (F=5.39, df=1, p=0.0234), with more *H. axyridis* (1.11 ± 0.30 individuals per plot; mean ± SE) than *P. quatuordecimpunctata* (0.36 ± 0.11 individuals per plot). Proportions of *P. quatuordecimpunctata* specimens recovered at the end of the experiment were similar among treatments (F=0.62, p=0.6096; Figure 5.3). However, more *H. axyridis* were recovered in high rather than low aphid density treatments (F=14.51, df=1, p=0.0013). On the other hand, plant structural complexity did not influence *H. axyridis* abundance (F= 0.72, df=1, p= 0.4084; Figure 5.3), and the interaction between aphid density and plant structural complexity was not significant (F=0.18, df=1, p=0.6772).

5.5.2 Intraguild predation

We extracted DNA from a total of 177 *H. axyridis* larvae sampled in the four treatments (35 in C-A-; 35 in C+A-; 45 in C-A+; and 62 in C+A+; Figure 5.4). Percentages of IGP were significantly higher in low (20.13% ± 1.81%; mean ± SE) rather than high (5.31% ± 1.28%) aphid density treatments (Table 5.1). Plant structural complexity did not significantly impact on the frequency of IGP, and the two factors did not interact (Table 5.1).

5.5.3 Immigration

A total of 153 insects were removed from the plots during the three trials, and all of them were aphidophagous predators. Predators that migrated in the plot were adults of *H. axyridis* (89.29% ± 2.70%), *P. quatuordecimpunctata* (9.96% ± 2.45%) and *Coccinella septempunctata* (0.74% ± 0.40%). Low aphid density treatments attracted fewer predators

than high aphid density treatments ($F=66.53$, $df=1$, $p<0.0001$; Figure 5.5), the difference being less apparent on Day 3 and 5.

5.6 Discussion

This study examines how herbivorous pest density and plant structural complexity influence intraguild interactions between coccinellids. Our results show that the aphid prey is the template that influences interactions between predators, IGP being higher when aphid density is low. Biological control of soybean aphid populations cannot therefore be fully evaluated without referring to aspects of the interaction between *H. axyridis* and *P. quatuordecimpunctata*.

5.6.1 Predator populations

We found more immigrant aphidophagous predators in high aphid treatments, suggesting a numerical response, but mean numbers of predators entering the plots per period of observation were relatively low (0.5 and 2.3 predators per plot, in low and high aphid density treatments, respectively) compared to the number of predators introduced at the onset of the experiment (N=16). However, we cannot exclude that immigrant predators may have had an impact on aphid populations. Our experimental approach is thus likely to have overestimated the impact of predation at high aphid densities. Previous reports by Donaldson et al. (2007) and Chacon and Heimpel (*in press*) showed that predator aggregation is a common phenomenon in the soybean aphid system. Furthermore, Costamagna and Landis (2007) observed that transient predation can be important for soybean aphid populations. Non-desired immigrant individuals are a drawback of the type of field experiments we conducted without cages. However, we were successful in establishing and maintaining our experimental treatments by removing flying predators entering the plots three times during the experiment. Furthermore, because these immigrant predators are adults, they are not likely to be engaged in IGP. Several aphidophagous taxa do not feed on prey during the adult stage (e.g. lacewing, syrphid fly, predatory midge) or

for those that actually behave as predators, like coccinellids, the adults do not have great capacity to feed on large larvae as those used in our tests (Félix and Soares 2004). We did not recover any predator on the glued barrier, indicating that emigration from the plots was negligible. However, for both coccinellid species, few larvae were recovered at the end of the experiment.

Throughout our experiments, *P. quatuordecimpunctata* larvae were less often recovered than *H. axyridis*. *Propylea quatuordecimpunctata* is a more vulnerable species to IGP because of its small size compared to other aphidophagous coccinellids (Félix and Soares 2004). A recent molecular gut-content analysis conducted by Gagnon et al. (*unpublished data*) showed that *P. quatuordecimpunctata* is frequently eaten by *H. axyridis*. In the present study, survival was higher for *H. axyridis* than for *P. quatuordecimpunctata*, more specifically in treatments of high aphid densities. However, similar numbers of *P. quatuordecimpunctata* larvae were recovered between treatments at the end of the experiment even though IGP was much higher in the two treatments with low density of aphids. Other ecological factors would therefore have reduced *P. quatuordecimpunctata* survival/presence. Emigration from experimental plots due to competitive exclusion exerted by *H. axyridis* is unlikely since no larvae were found on the glue barrier. However, it might be that coccinellid larvae were laying on the ground and went undetected even if we carefully examined the soil surface. Furthermore, mortality from cannibalism may have contributed to the disappearance of *P. quatuordecimpunctata* as coccinellids are well known to engage in cannibalistic interactions (Agarwala and Dixon 1991). Cannibalism evolved as a self-regulating mechanism in coccinellids, preventing exponential growth of populations when prey become scarce (Fox 1975; Polis 1981).

5.6.2 Intraguild predation

Molecular gut-content analysis was a useful approach to examine interactions between the intraguild predator *H. axyridis* and the intraguild prey *P. quatuordecimpunctata.* The proportion of IGP detected was higher in treatments with low aphid densities, as observed in other studies (e.g. Lucas, Coderre et al. 1998; Obrycki, Giles et al. 1998; Schellhorn and

Andow 1999; Kajita, Takano et al. 2000; Hindayana, Meyhofer et al. 2001; Burgio, Santi et al. 2002). However, *P. quatuordecimpunctata* did not benefit from an increase in plant structural complexity to avoid attacks from *H. axyridis*. This result contrasts with the conclusion of a meta-analysis by Janssen et al. (2007) indicating that intraguild prey are usually less subject to predation in more complex habitat. This may arise from the nature of the habitat complexity. On soybean plants, having more plant materials (stems and leaves) does not provide a new type of physical refuge to the intraguild prey. In contrast, Finke and Denno (2002), for example, showed that the incorporation of thatch on the ground surface increased survival of the intraguild prey, *Tytthus vagus*, by providing new refuges from predation by the wolf spider *Pardosa littoralis*. Furthermore, beneficial effects of naturally growing vegetation are not always due to the structural complexity of the plant, but may arise when alternative food resources are present (herbivores, pollen, nectar) (Zehnder, Gurr et al. 2007; Birkhofer, Wise et al. 2008), which was not the case in our experimental set-up.

5.6.3 Biological Control

Biological Control

Ladybirds are very important for biological control of the soybean aphid (Fox, Landis et al. 2004; Costamagna, Landis et al. 2007; Rhainds, Roy et al. 2007). They contribute to significantly reduce aphid populations, although levels of control vary from year to year (Costamagna, Landis et al. 2007). As mentioned above, because our cage-free approach precludes complete exclusion of immigrating predators from feeding on aphids in experimental plots, our data cannot be used to directly interpret aphid population dynamics. However, our results indicate that high levels of IGP interfere with *A. glycines* control in low aphid density treatments (Figure 5.2).

In aphid biological control, it has been shown that the window of action of predators early in the season, when pest populations start to build up, determines the success and long-term impact of biological control agents (Landis and Van der Werf 1997; Ragsdale,

McCornack et al. 2009). Within this context, we hypothesize that *H. axyridis* is likely to be detrimental to biological control because this species is more involved in intraguild interactions at the beginning of the soybean growing season (Gagnon *et al.*, *unpublished data*), and because IGP is more intense at low aphid density. These conditions can be combined in the field in Québec, when *A. glycines* infestations are developing and soybean plants aborted pods and seeds (R4-R5). However, several other ecological factors influence the incidence of IGP, as well as the consequences on biological control. Large-scale and long-term experiments would be needed to test this scenario.

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5.9 Tables

Table 5.1 Factorial ANOVA of the effects of plant structural complexity and extraguild prey density (*Aphis glycines*) on the incidence of IGP between two coccinellid species (*Harmonia axyridis* and *Propylea quatuordecimpunctata*).

	df	ss	*F*	p-value
Trial	2	0.8229	3.14	0.0459
Plant structure	1	0.0412	0.31	0.5756
Aphid density	1	0.8063	6.15	0.0141
Plant structure x Aphid density	1	0.0019	0.01	0.9040
Trial x Plant structure	2	0.0626	0.24	0.7878
Trial x Aphid density	2	0.1465	0.56	0.5730
Trial x Plant structure x Aphid density	2	0.0650	0.25	0.7808

5.10 Figures

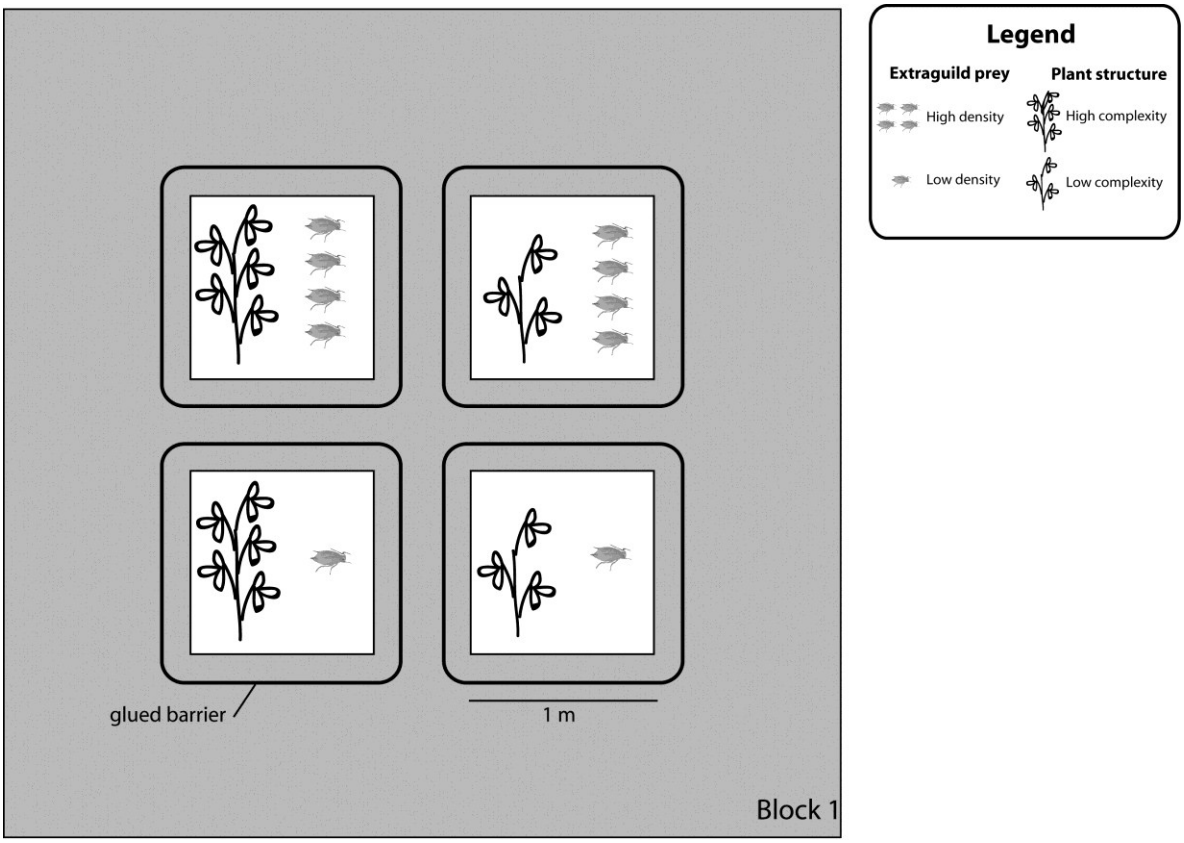
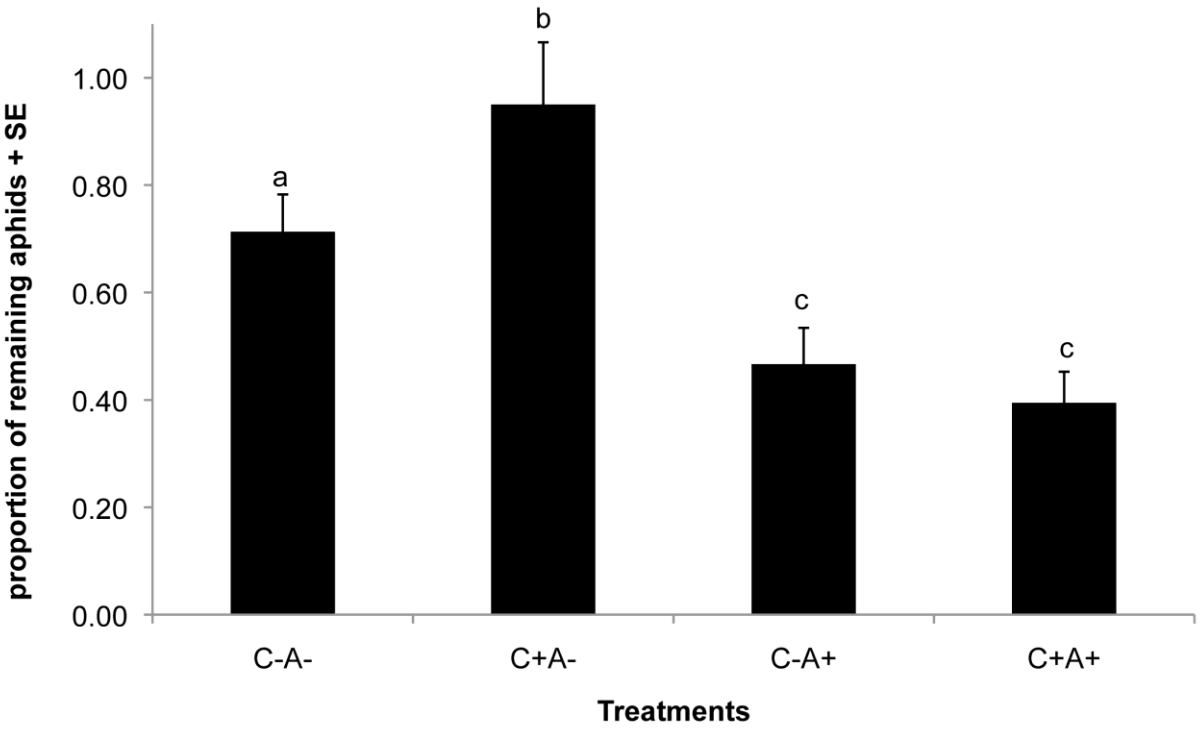


Figure 5.1. Schematic diagram of an experimental plot used to examine the effects of plant structure complexity and *Aphis glycines* (extraguild prey) density on the incidence of intraguild predation between *Harmonia axyridis* and *Propylea quatuordecimpunctata*. *White zone*, soybean plants; *gray zone*, no plant.

Figure 5.2. Proportions (mean±SE) of *Aphis glycines* remaining on five soybean plants at



the end of the experiment: Low plant structural complexity x Low aphid density (C-A-), High plant structural complexity x Low aphid density (C+A-), Low plant structural complexity x High aphid density (C-A+), High plant structural complexity x High aphid density (C+A+). Different letters above histogram bars represent significant differences ($p<0.05$) using LSD (least significant difference).

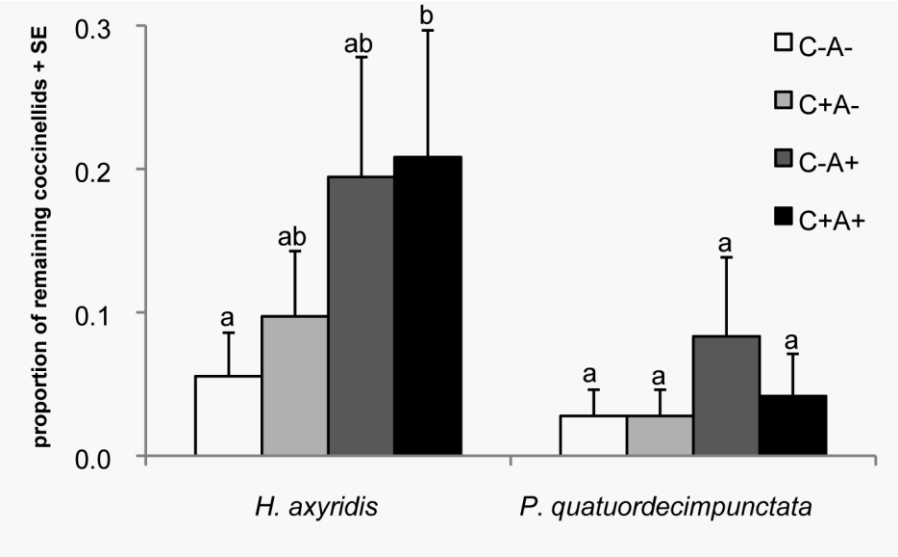


Figure 5.3. Proportion of coccinellids remaining in experimental plots at the end of the experiment. See legend of Figure 5.2 for treatment description. For each coccinellid species, different letters above histogram bars represent significant differences ($p<0.05$) using LSD (least significant difference).

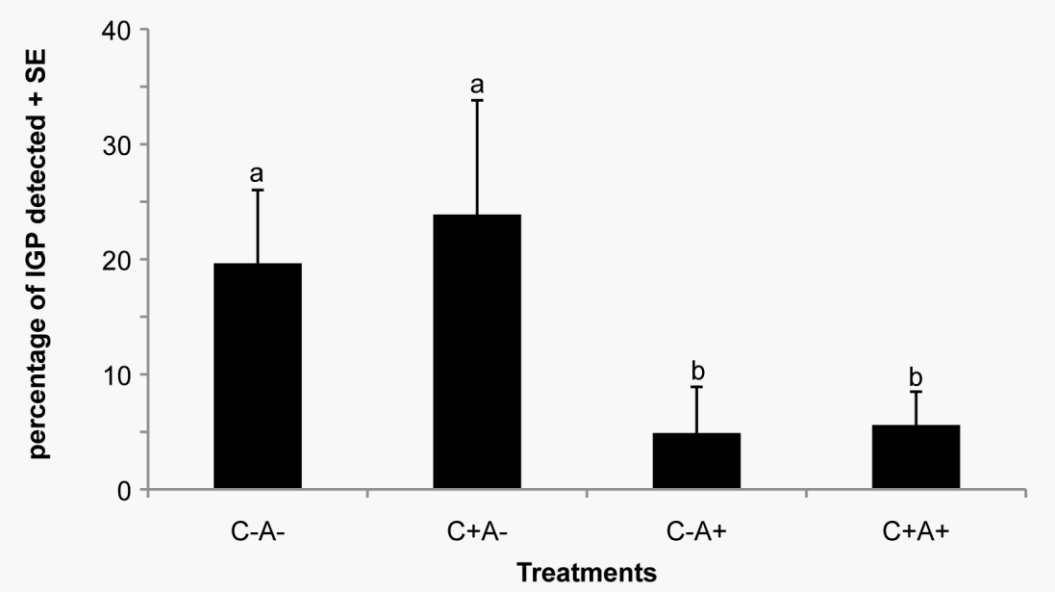


Figure 5.4. Percentages (mean ± SE) of *Propylea quatuordecimpunctata* found in the gut-content of *Harmonia axyridis*. See legend of Figure 5.2 for treatment description. Different letters above histogram bars represent significant differences ($p<0.05$) using LSD (least significant difference).

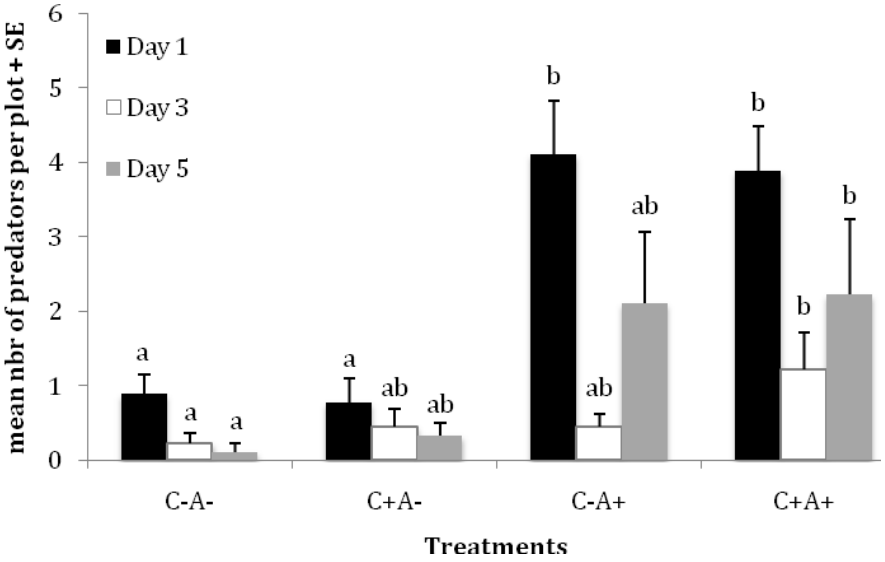


Figure 5.5. Mean number (± SE) of predators entering in experimental plots 1, 3 and 5 days following the beginning of the experiment. See legend of Figure 5.2 for treatment description. Different letters above histogram bars represent significant differences ($p<0.05$) using LSD (least significant difference).

CHAPITRE VI

Conclusion générale

6.1 Conclusion générale

Depuis les premières démonstrations expérimentales du phénomène à la fin des années 80, la prédation intraguilde (IGP) suscite beaucoup d'intérêt chez les écologistes et les utilisateurs de la lutte biologique (Polis et McCormick, 1987; Polis et Holt, 1992). L'IGP est un phénomène où un individu dévore un organisme appartenant à la même guilde, *i.e.* partageant les mêmes ressources alimentaires. Il a été démontré que l'IGP pouvait altérer le contrôle d'un ravageur puisque les populations de certains de leurs ennemis naturels diminuent suite à l'action de prédateurs intraguildes (Rosenheim *et al.*, 1993; 1995). Inversement, d'autres études ont démontré que dans certaines situations, l'IGP ne perturbe pas le contrôle du ravageur (Janssen *et al.*, 2006 et références citées). Ainsi, l'impact de l'IGP sur la lutte biologique dépend de plusieurs facteurs tels la nature et la composition des espèces ou la densité des prédateurs. Par ailleurs, puisque les organismes impliqués dans des événements d'IGP sont souvent petits, cryptiques ou nocturnes et que la fréquence de ces interactions peut parfois être très faible, les probabilités d'observer l'IGP en milieu naturel sont rares, rendant son étude et sa compréhension ardues. Les principales approches utilisées jusqu'à présent impliquaient le confinement des espèces protagonistes dans des microcosmes à l'intérieur desquels l'expérimentateur faisait varier certains paramètres de l'écosystème afin d'identifier et de quantifier une interaction. Ces méthodes sont adéquates pour répondre à des hypothèses impliquant des interactions simples et directes entre les individus. Par contre, bien qu'elles nous permettent très bien de décoder certaines relations potentielles au sein d'un réseau trophique, il est difficile, voir impossible d'extrapoler ces résultats au milieu naturel (Messing *et al.*, 2006). En effet, ces techniques minimisent les niveaux de complexité spatiale et structurelle de l'écosystème, de même que la diversité et l'abondance des espèces, en plus d'être limitées temporellement (Janssen *et al.*, 2007). Les approches réductionnistes utilisées tant au niveau des études empiriques que théoriques ont sans contredit contribué à la perception que l'IGP engendrait invariablement un impact négatif sur la lutte biologique. Plus récemment, l'utilisation d'outils moléculaires s'est répandue dans les études écologiques puisqu'elle offre de nombreux avantages (Hoogendoorn and Heimpel, 2001; Harwood et al., 2009). En particulier, ces outils

permettent de caractériser les habitudes alimentaires des prédateurs en n'altérant aucunement leur environnement.

Ce projet visait l'étude de l'IGP au sein de quatre espèces de coccinelles: *Harmonia axyridis*, *Coccinella septempunctata*, *Coleomegilla maculata* et *Propylea quatuordecimpunctata*. Elle a été réalisée dans des champs de soya où un nouveau ravageur, le puceron du soya (*Aphis glycines*), cause problème et où l'assemblage d'ennemis naturels se caractérise par la dominance de ces quatre Coccinellidae. Les principaux objectifs de cette étude étaient de : i) développer des outils moléculaires permettant de détecter et de quantifier *in situ* les interactions intraguildes au sein des communautés de coccinelles; ii) établir des relations entre l'IGP et certains paramètres écologiques; et iii) évaluer l'impact de l'IGP entre les coccinelles sur le contrôle des populations du puceron du soya.

Dans un premier temps, nous avons développé des amorces PCR (régions ITS-1 ou COI des gènes) pour chacune des espèces de coccinelles (Chapitre 2). Ces amorces ont été testées face à différentes espèces de prédateurs et ravageurs afin d'établir leur spécificité. Par la suite, afin d'être en mesure de comparer adéquatement les taux de prédation entre les espèces, nous avons établi un outil de correction pour tenir compte des variations dans les temps de digestion de chaque prédateur en interaction avec chacune des proies. Ainsi, la période de détection d'une proie dans le contenu gastrique d'une espèce de prédateur digérant plus rapidement sera moins longue puisque le processus de digestion diminue la viabilité de l'ADN. Toutes les combinaisons prédateurs-proies entre les quatre espèces de coccinelles ont été testées et nous avons observé des temps de digestion (DS_{50}) variant entre 5,3 h à 19,3 h, concordant ainsi avec d'autres études (McMillan *et al.*, 2007; von Berg *et al.*, 2008). Cette variabilité entre les espèces de prédateur pour une même proie, et pour un même prédateur face à différentes proies pourrait s'expliquer par : i) la présence de complexes d'enzymes digestives différents, *a priori* déterminés par le régime alimentaire du prédateur; et ii) la composition chimique des proies intraguildes (œufs). Ces résultats démontrent la nécessité de corriger les résultats de détection par amorces PCR afin d'éviter

de sous- ou surestimer le phénomène de prédation entre les espèces. Il est à noter que cette correction, qui selon nous est essentielle, demeure pratiquement absente dans la littérature et nécessite donc une plus grande considération. Toutefois, l'application systématique de ce système de correction ne s'avère pas toujours envisageable puisque la réalisation des tests en laboratoire est exigeante, d'autant plus si le système à l'étude comporte de nombreuses espèces pouvant potentiellement interagir. D'autre part, dans cette étude, seul un type de combinaison impliquant deux stades de développement (un individu de quatrième stade larvaire s'attaquant à des œufs) a été évalué. Puisque l'IGP entre les coccinelles ne se limite pas qu'à ce type de combinaison, il aurait été tout aussi intéressant d'observer la variabilité des temps de digestion avec différents stades de développement, et ce autant pour le prédateur intraguilde que pour la proie intraguilde.

Par la suite, nous avons déterminé la nature et la fréquence de l'IGP au champ en caractérisant le contenu gastrique de plus de 350 prédateurs récoltés sur deux années (Chapitre 3). Nous avons échantillonné les quatre espèces de coccinelles à l'étude, et ces dernières ont été testées afin de détecter chacune des trois proies intraguildes potentielles. L'utilisation des marqueurs moléculaires a permis d'établir des taux d'IGP en milieu naturel variant entre 15,9% et 40,8% chez les Coccinellidae, démontrant ainsi l'omniprésence de cette interaction dans les champs de soya du Québec. Ces taux d'IGP sont très élevés comparativement à d'autres études ayant utilisé une approche moléculaire de détection d'IGP. Par exemple, entre 0% et 2,5% des punaises *Orius insidiosus* récoltées dans des champs de soya contenaient la proie intraguilde *H. axyridis* (Harwood *et al.*, 2007; 2009). De plus, nos travaux ont révélé que l'IGP était de type mutuel, *i.e.* chacune des espèces de coccinelle pouvait à la fois être prédateur ou proie. Également, dans 13,2% des prédateurs échantillonnés, deux, voire trois espèces de proie intraguilde ont été détectées. Ces résultats confirment l'efficacité de la méthode moléculaire de détection de l'IGP en milieu naturel ainsi que l'omniprésence de cette interaction chez les coccinelles qui exploitent l'agroécosystème du soya. Alors que les études en milieu confiné s'attardent à établir les conséquences de l'IGP sur la dynamique des populations, seule l'approche moléculaire permet de quantifier de façon juste les niveaux d'IGP en milieu naturel.

Néanmoins, bien qu'il s'avère impossible de le déterminer avec des amorces PCR, il aurait été intéressant d'établir les taux de cannibalisme chez ces quatre espèces de coccinelles. Selon Dixon (2000), les populations de prédateurs faiblement soumises à l'IGP seraient au contraire très fortement contrôlées par le cannibalisme.

Par ailleurs, nous avons déterminé les principaux facteurs écologiques qui influencent la fréquence des interactions intraguildes chez deux espèces de coccinelles, *H. axyridis* et *C. septempunctata*, au cours de trois années d'échantillonnage (Chapitre 4). À l'aide d'une modélisation réalisée par régressions logistiques, plusieurs facteurs écologiques ont été considérés: les densités des proies extraguildes et intraguildes, le ratio prédateur:proie, le stade de développement du prédateur et la période d'échantillonnage. De ces facteurs, plusieurs se sont avérés significatifs et variaient selon l'espèce de prédateur et l'année d'échantillonnage. Par exemple, chez le prédateur *H. axyridis*, les stades de développement étaient fortement corrélés à l'IGP: plus le développement de l'insecte évolue, plus il est susceptible de s'engager dans une interaction intraguilde. De plus, l'augmentation de la densité de pucerons, l'augmentation du ratio prédateur:proie ainsi que l'avancement dans la saison de croissance sont autant de facteurs qui tendent à réduire la fréquence d'IGP exercée par *H. axyridis*. Chez *C. septempunctata*, seule l'augmentation de la densité de pucerons avait un effet réducteur sur la fréquence d'IGP, alors qu'au contraire, l'avancement dans la saison de croissance augmentait les probabilités d'IGP. Ainsi, l'impact de l'IGP exercée par *H. axyridis* serait probablement plus dommageable que celle exercée par *C. septempunctata* sur la lutte biologique du puceron puisque la première consomme plus de proies intraguildes tôt en saison, période où le contrôle des populations de pucerons est déterminant pour le succès à long terme. Bien qu'ils n'aient pas été significatifs dans tous les cas, la saisonnalité ainsi que la densité des proies extraguildes s'avèrent deux facteurs récurrents dans l'explication de l'occurrence de l'IGP au sein des coccinelles dans les champs de soya.

Nous avons également évalué l'impact de la densité des proies extraguildes (pucerons) et de la complexité de la structure de la plante sur l'IGP entre *H. axyridis* et *P. quatuordecimpunctata*. Pour ce faire, nous avons réalisé une expérience factorielle en champ, sans cages, où l'effet des deux facteurs était considéré simultanément (Chapitre 5). Le traitement à faible densité de pucerons a eu un impact négatif sur le contrôle biologique du puceron en augmentant l'IGP entre les prédateurs. En effet, l'IGP était beaucoup plus importante dans ces traitements (20% d'IGP) que lorsque la densité de pucerons était plus élevée (< 6% d'IGP). Cette interaction semble donc être modulée principalement par l'accessibilité des proies extraguildes. Pertinemment, Lucas et collaborateurs (1998) ont démontré en laboratoire que l'augmentation de la densité des proies extraguildes diminuait, de façon générale, la fréquence de l'IGP. Par ailleurs, la structure de la plante n'a pas eu d'impact direct sur la fréquence de l'IGP, mais semble avoir altérée la lutte biologique au puceron. À faible densité de pucerons, la structure complexe de la plante semble avoir avantagé les pucerons en leur procurant des refuges. Nos résultats divergent de certaines autres études (Finke et Denno, 2002; Janssen *et al.*, 2007) démontrant que l'augmentation de la complexité de la plante diminuait le taux d'IGP, augmentant d'autant l'efficacité de la lutte biologique. Le traitement utilisé au cours de notre étude pour modifier la structure de la plante hôte (altération de la structure foliaire du plant au lieu de l'ajout d'un couvre sol) pourrait contribuer à expliquer cette différence. Dans un contexte agricole, il serait intéressant d'observer l'impact d'une augmentation de la diversité végétale (par exemple, en utilisant des cultures intercalaires) sur l'intensité d'IGP entre les ennemis naturels. Bref, cette analyse démontre l'importance d'évaluer un système de façon holistique, *i.e.* en considérant systématiquement tous les facteurs pouvant potentiellement influencer la fréquence d'IGP puisque que ceux-ci n'agissent pas de façon individuelle. La généralisation de l'impact des facteurs modulant ce type d'interaction demeure encore à ce jour difficile à réaliser.

Cette thèse a permis de démontrer l'omniprésence de l'IGP dans les interactions entre les coccinelles. Notre vision des coccinelles dites aphidiphages, où les pucerons représenteraient leur diète de prédilection, s'élargit considérablement puisque ces

prédateurs incluent une abondance de proies intraguildes à leur diète. Il demeure encore délicat d'établir les conséquences de l'IGP sur la structure des populations, et plus particulièrement sur la lutte biologique. Néanmoins, nous savons que l'IGP peut avoir un effet néfaste sur la lutte biologique, notamment lorsque la densité de l'herbivore est faible. Nous avons mis en lumière la grande complexité quant à la compréhension des facteurs régissant l'intensité de l'IGP, leurs interactions rendant leur analyse laborieuse. Bien que chaque situation soit différente, nous pouvons tout de même affirmer que la densité des proies extraguildes représente un facteur d'une grande importance, probablement parce qu'il diminue la compétition entre les protagonistes.

6.2 Perspectives de recherche

Dans un avenir rapproché, il serait intéressant d'utiliser une approche en PCR quantitatif (qPCR) afin de quantifier les proies du contenu gastrique des prédateurs. Bien que nous ne puissions savoir le nombre de proies consommées (la quantité d'ADN pouvant varier selon la masse de la proie, le temps de digestion, la présence d'autres espèces de proies, etc.), nous pourrions déterminer la proportion relative d'une proie par rapport à d'autres espèces de proie. Il serait donc intéressant de développer des amorces moléculaires pour la proie extraguilde (le puceron du soya, en l'occurrence) et les autres proies alternatives. Ceci permettrait, entre autres, d'identifier si l'IGP survient lorsque la proportion de proies extraguildes est faible par rapport aux proies intraguildes. Dans le même ordre d'idées, il serait possible de considérer le séquençage complet du contenu gastrique d'un prédateur afin d'observer l'éventail des proies consommées. Cette méthode permettrait d'obtenir des résultats intéressants sur les proies n'ayant pas encore été considérées pour un prédateur.

Toutefois, les méthodes moléculaires présentent également des limites. Notons par exemple l'impossibilité de détecter le cannibalisme, tel que mentionné ci haut. De plus, les interactions indirectes (Lima, 1998; Werner et Peacor, 2003), reconnues pour leur rôle important dans la structure des communautés, demeurent difficiles à évaluer en milieu naturel (Messing et al., 2006). L'utilisation de marqueurs moléculaires du contenu

gastrique n'apporte qu'une partie des informations liées à l'interaction directe entre deux espèces. Tout ce qui a trait aux comportements anti-prédateur, à la compétition et au déplacement des espèces, ne peuvent être mesurés par des techniques moléculaires. L'observation directe des individus en milieu naturel demeure une méthode précise pour identifier un comportement animal. Pour terminer, l'application des outils moléculaires en écologie se répand de plus en plus, permettant ainsi l'étude complémentaire *in situ* de plusieurs interactions et comportements autrefois impossibles à évaluer avec un tel degré de résolution.

6.3 Références

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