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Les stratégies écologiques de la coccinelle myrmécophile *Coccinella magnifica* Redtenbacher.

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Introduction

Les interactions entre les fourmis et les coccinelles

Les observations qui vont être présentées ici concernent les interactions entre plusieurs niveaux d'une chaîne trophique. Il sera ici question de prédateurs (coccinelles et fourmis) et d'herbivores (pucerons). Le niveau de production primaire, à savoir les plantes hôtes des pucerons, sera étudié via l'influence des paramètres de l'habitat sur les insectes. La majorité des espèces de Coccinellidae sont des prédateurs de pucerons ou de cochenilles, or, les fourmis sont à la fois des prédateurs généralistes d'invertébrés et des mutualistes récoltant le miellat produit par divers hémiptères. Une coccinelle peut donc entrer en compétition avec des fourmis lorsqu'elles exploitent les mêmes ressources (Figure 1, à gauche). Quand les coccinelles attaquent des proies qui ne sont pas protégées par des fourmis, les différents prédateurs exercent une pression de compétition les uns envers les autres (Figure 1, à droite).

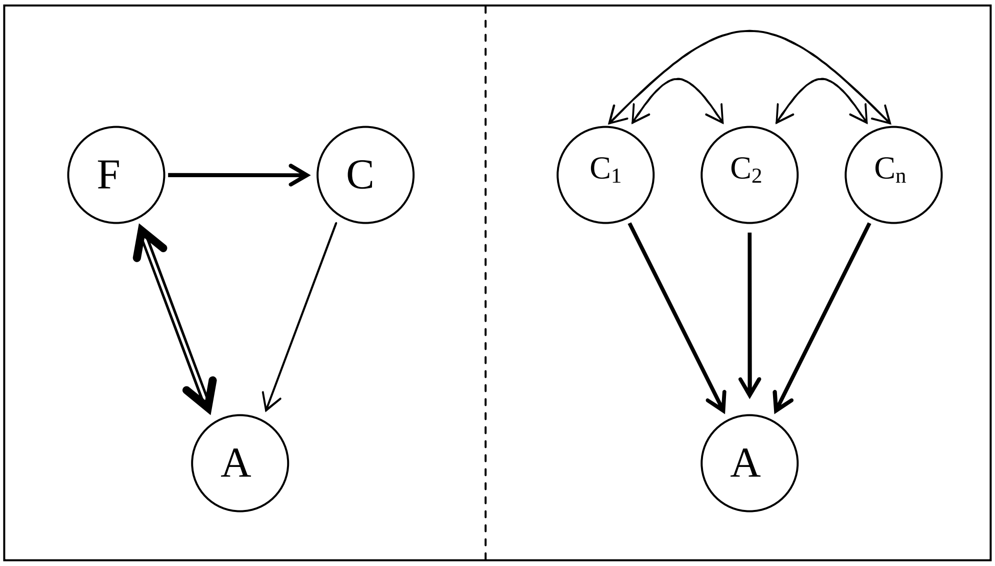

Figure 1 : Schéma des interactions de prédation (simples flèches) et de compétition (doubles flèches) entre des pucerons (A), des fourmis (F) et des coccinelles aphidiphages (C, C_1, C_2,…, C_n). A gauche : situation lorsque des fourmis entretiennent des interactions mutualistes avec des pucerons ; les coccinelles subissent la pression de prédation exercée par les fourmis. A droite : situation lorsque les fourmis sont absentes. Les prédateurs entrent en compétition pour une même ressource.

Le plus souvent, les coccinelles sont donc repoussées des colonies de pucerons mutualistes par les fourmis (Banks, 1962 ; Jiggins *et al.*, 1993). On observe par ailleurs une grande diversité d'espèces de coccinelles exploitant des ressources qui ne sont pas protégées par des fourmis. La compétition qui s'exerce entre ces coccinelles est un moteur évolutif important expliquant notamment les mécanismes de spécialisation pour des ressources qui sont a priori inaccessibles aux autres compétiteurs.

Pourtant, on rencontre, en Europe et, plus largement, dans presque tout le paléarctique, une espèce de coccinelle, *Coccinella magnifica* Redtenbacher, qui a pour particularité de vivre en interaction étroite avec des fourmis (e.a. Donisthorpe, 1920a et 1920b ; Majerus, 1989).

C. magnifica est une espèce de grande taille (long. 5,5 - 8 mm), morphologiquement proche de la très commune coccinelle à sept points, *Coccinella septempunctata* L. (5 – 8 mm). Elle vit exclusivement en association avec des fourmis. En Europe occidentale, les fourmis hôtes les plus fréquentes sont les espèces de fourmis rousses des bois, *Formica rufa* L. *sensu lato* (5 - 9 mm).

Les coccinelles myrmécophiles

C. magnifica n'est pas la seule coccinelle myrmécophile connue. Au moins 12 autres espèces ont une biologie étroitement associée avec des fourmis. Les stratégies écologiques de ces espèces sont pourtant assez variées, on y distingue les catégories suivantes :

a. **Myrmécophilie « compétitive »**. Les larves et les adultes se nourrissent des hémiptères (Pucerons, cochenilles, aleurodes, …) entretenus par les fourmis en étant plus ou moins bien acceptés, voire ignorés par les fourmis : *C. magnifica* ; *Platynaspis luteorubra* (Goeze) (Majerus, 1994 ; Völkl, 1995) ; au moins deux espèces du genre *Brachiacantha spp.* Dejean (Smith, 1886; Mann, 1911, Wheeler, 1911; Montgomery & Goodrich, 2002) ; *Anisolemnia tetrasticta* Fairmaire (Dejean, 2002) ; *Scymnus fenderi* Malkin (MacKay, 1983 ; Hölldobler & Wilson, 1990) ; *Scymnus formicarius* (Wasmann, 1894 ; identification suspecte ?!) ; *Hyperaspis reppensis* Herbst (Silvestri, 1903) ;

b. **Myrmécophilie suspectée.** Les adultes ont été observés dans des nids de fourmis mais leur régime alimentaire reste inconnu : *Thalassa saginata* Mulsant (Berti *et al.*, 1983 ; Corbara *et al.*, 1999 ; Orivel *et al.*, 2004) ; une ou plusieurs espèces (?) de la tribu Monocorynini (Kovár, 1996) ; *Hyperaspis acanthicola* n. sp. (Chapin, 1966) ; *Tetrabrachis (Litophilus) connatus* Panzer (Koch, 1989a et b, 1992) ;

c. **Myrmécophagie occasionnelle.** Les larves ont été observées en compagnie des fourmis, dont elle se nourrissent en complément d'autres proies plus classiques : *Ortalia pallens* Mulsant (Harris, 1921) ; *Scymnodes lividigaster* (Mulsant) (Pope & Lawrence, 1990).

Aperçu de la systématique des Coccinellidae

La famille des Coccinellidae est composée, en Europe, de 7 sous-familles, dont les Coccinellinae, Chilocorinae et Scymninae sont les plus diversifiées et représentent la majorité des espèces de notre faune. Les Coccinellidae appartiennent à l'infra-ordre des Cucujiformia, au même titre que les Chrysomelidae ou les Curculionidae. En tant que prédateurs, elles diffèrent de la majorité des autres espèces de la super-famille. Le comportement prédateur est une évolution qui est apparue à la base de l'arbre phylogénétique des coccinelles. Les taxons les plus primitifs (Sticholotidinae, Coccidulinae et Lithophilinae) sont d'ailleurs principalement prédateurs. D'autres stratégies non prédatrices sont apparues chez les Epilachninae, parmi lesquelles toutes les espèces sont phytophages, et chez plusieurs genres de Coccinellinae, qui sont mycophages (Figure 2 et Annexe). La grande majorité des espèces connues du grand public sont de grande taille et brillamment colorées, elles appartiennent à la sous-famille des Coccinellinae. Les espèces prédatrices de cette sous-famille consomment surtout des pucerons mais on y trouve aussi des prédateurs plus généralistes s'attaquant à des larves de chrysomèles ou à des psylles (Iablokoff-Khnzorian, 1982). Les espèces de taille moyenne, généralement noires à taches rouges, appartiennent aux Chilocorinae. Celles-ci sont prédatrices de cochenilles, de pucerons ou des deux. Parmi les Chilocorinae, on trouve la tribu des Platynaspidini, représentée chez nous par une seule espèce, *Platynaspis luteorubra* qui est, avec *C. magnifica*, la coccinelle myrmécophile la mieux connue (Majerus, 1994 ; Völkl, 1995).

Les espèces de la sous-famille des Scymninae sont beaucoup plus discrètes que les précédentes. Elles sont de petite taille, de couleur noire, parfois maculée de rouge-orange et elles sont toutes prédatrices de cochenilles. Les Coccidulinae et les Lithophilinae ne sont représentées que par 6 espèces en Europe et le régime alimentaire de certaines d'entre elles est mal connu. Les Sticholotidinae et Ortaliinae sont des taxons absents d'Europe occidentale mais bien représentés en Asie.

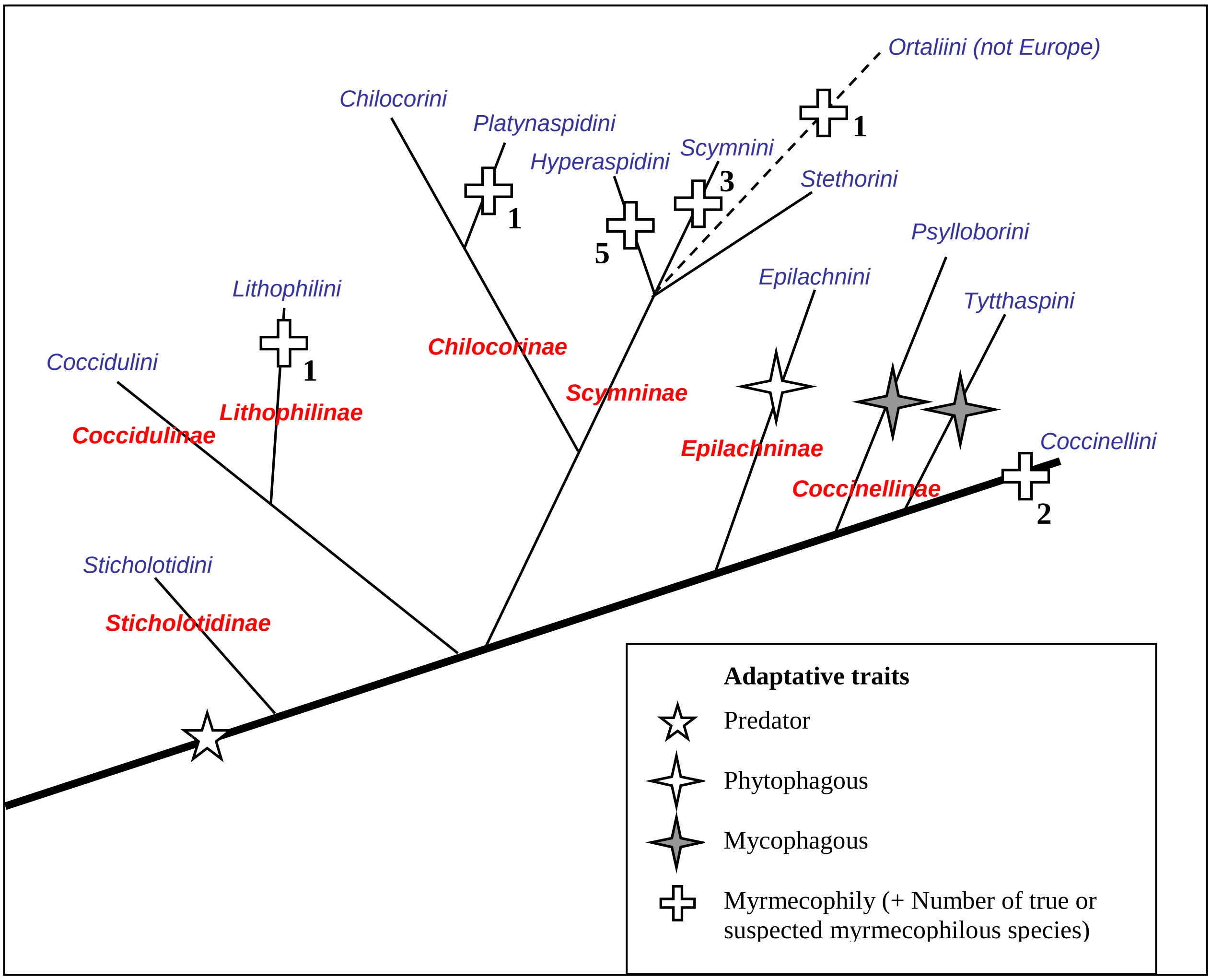

Figure 2. Arbre phylogénétique probable pour les tribus de Coccinellidae d'Europe (cf. Annexe). Adapté d'après Sasaji (1968, in Majerus 1994) et Gregory *et al.* (2003).

Les informations publiées au sujet de C. magnifica

Les premières observations de *C. magnifica* datent de la fin du 19ème et du début du 20ème siècle, avec la publication des premières descriptions de l'association avec les *Formica rufa s.l.* (Morris, 1888 ; Donisthorpe, 1910, 1920a et 1920b ; Pontin, 1959).

Dès les premières observations, les auteurs ont pu constater que *C. magnifica* se rencontrait uniquement en compagnie des fourmis rousses des bois. Mais les informations sur

C. magnifica et la nature de ses interactions avec les fourmis sont finalement restées très laconiques jusqu'aux publications de Majerus (1989 et 1994). Il a d'abord été proposé que la coccinelle cohabitait avec les fourmis dans leurs nids mais, rapidement, on a compris que les interactions avaient lieu au niveau des colonies de pucerons. Les adultes et les larves sont capables de se nourrir des pucerons pourtant protégés par les fourmis. Pourtant, la coccinelle n'est pas complètement acceptée par les fourmis : si on dépose un adulte de *C. magnifica* sur un nid de fourmis ou dans une colonie de pucerons, il sera attaqué par les fourmis mais moins systématiquement que s'il s'agissait de *C. septempunctata*. Sur les pistes olfactives des fourmis, les *C. magnifica* sont à peu près ignorées (Sloggett *et al.*, 1998 ; Sloggett & Majerus, 2003).

La figure 3 schématise les interactions qui interviennent dans l'habitat de *C. magnifica.* Les prédateurs de pucerons (C_1, C_2) sont attaqués par les fourmis et repoussés hors de la sphère d'influence de celles-ci (contour en pointillé). Le seul prédateur subsistant en présence des fourmis, grâce à une réduction de l'agressivité de celles-ci, est *C. magnifica*. La coccinelle peut dès lors s'attaquer aux pucerons à l'abri de la compétition des autres prédateurs de pucerons. Dans ces conditions, les fourmis entretiennent un véritable stock de nourriture disponible pour la reproduction de la coccinelle myrmécophile.

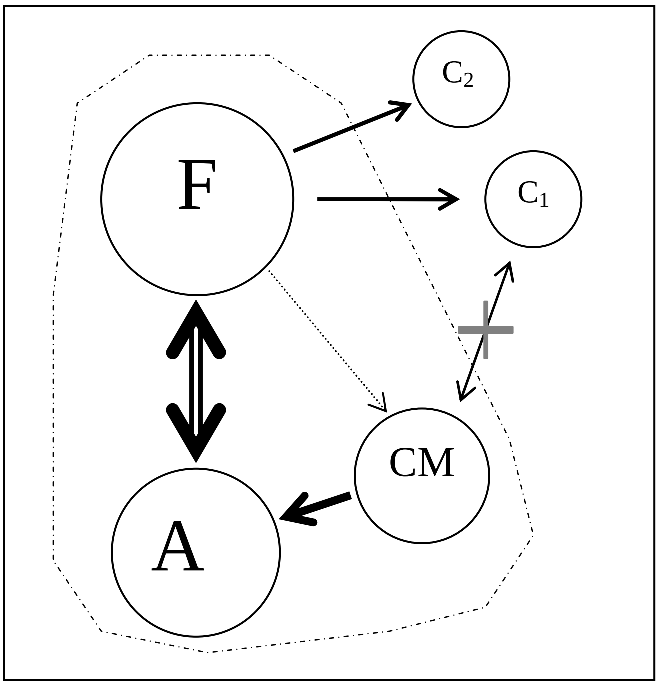

Figure 3. Schéma des interactions de *C. magnifica* (CM) avec des fourmis (F) et des pucerons mutualistes (A). Les autres coccinelles prédatrices (C_1, C_2) évitent l'aire dominée par les fourmis (contour discontinu) et n'entrent donc pas en compétition avec *C. magnifica*.

En Europe occidentale, *C. magnifica* est préférentiellement associée à *F. rufa s.s.* ou *F. polyctena* mais, dans le reste de son aire de répartition, elle est susceptible d'entrer en interaction avec d'autres espèces de fourmis. En Europe centrale, *C. magnifica* est également associée à *Formica cinerea* (P. Cerygnier, comm. pers.). Cette espèce occupe la place de fourmi dominante dans les habitats sableux, à l'instar de *F. rufa* en forêt (Czechowski & Marko, 2005). *C. magnifica* a aussi été observée en Sibérie, en association avec des fourmis indéterminées (Sloggett, 2002). D'une manière générale, *C. magnifica* a été observée en compagnie de fourmis d'assez grande taille et qui exploitaient des pucerons.

On s'accorde pour considérer *C. magnifica* comme distribuée dans tout le paléarctique sauf au Japon et dans l'Est de l'Asie (Iablokoff-Khnzorian, 1982). La majorité des observations précises proviennent d'Europe centrale (Haviar, 2004) et occidentale (Sloggett *et al.*, 2002).

Une bonne partie de la distribution probable reste donc à confirmer (Figure 4). Ceci sous-entend que nos connaissances des interactions myrmécophiles de *C. magnifica* sont partielles. La recherche de *C. magnifica* en Asie et au Moyen-Orient pourrait bien apporter des informations précieuses pour une description plus complète de l'espèce.

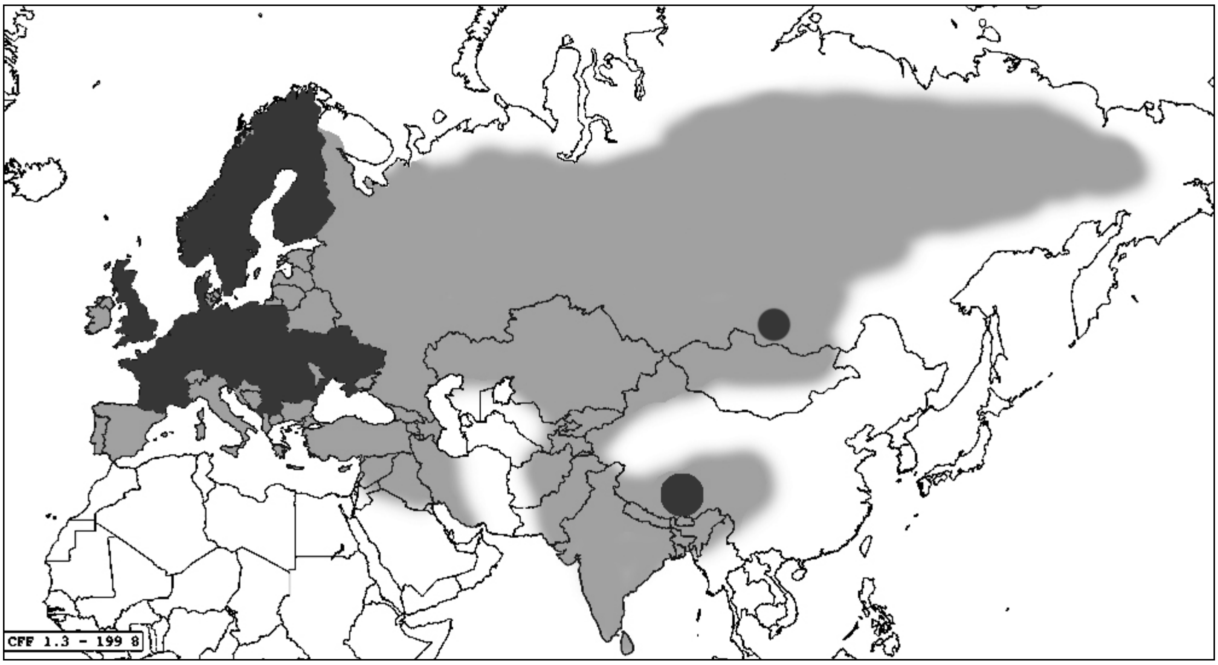

Figure 4. Aire de distribution de *C. magnifica*. En grisé : enveloppe approximative (Iablokoff-Khnzorian, 1982). En noir : pays pour lesquels il existe des données publiées. Les deux points correspondent, l'un, aux observations de Majerus près du Lac Baïkal (Sloggett *et al.*, 2002), et l'autre, au taxon *Coccinella lama*, décrit du Tibet, et apparenté à *C. magnifica*.

Les fourmis rousses des bois

Les fourmis rousses des bois forment un complexe d'espèces nommé *Formica rufa sensu lato*. Ce groupe est un des représentants majeurs du genre Formica, qui compte environ 150 espèces dans l'ensemble des régions Holarctiques (Hölldobler & Wilson, 1990). Ce groupe est aussi un des plus connus du grand public et des forestiers car les espèces qui le composent sont d'assez grande taille (5 - 9 mm) et elles construisent des nids de taille imposante, souvent interconnectés en super-colonies (Chérix, 1986). Leur impact positif sur les forêts est connu de longue date (Nef, 1963 ; Bradley, 1973 ; Rosengren, 1979 ; Skinner, 1980 ; Skinner & Whittaker, 1981 ; Hyo Kim & Murakami, 1983). Toutes ces études ont pour objectif de mesurer les effets positifs et négatifs des colonies sur l'état sanitaire de la forêt, au sens sylvicole. La conclusion unanime étant que les effets délétères, dus à l'exploitation et à la protection des colonies de pucerons, sont largement compensés par la prédation exercée sur les ravageurs herbivores tels que les larves de Tenthredinidae et les chenilles d'Hétérocères. Pour ces différentes raisons, des projets de protection et de « bouturage » des nids ont vu le jour dans plusieurs pays avec plus ou moins de succès (Nef, 1963 ; Chérix, 1986). C'est aussi pourquoi *F. rufa s.l.* est un insecte bénéficiant d'un statut légal de protection en Belgique (Décret du Gouvernement wallon relatif à la conservation des sites Nature 2000 ainsi que de la faune et de la flore – 6.XII.2001). Plus récemment, Mabelis (2007) a proposé que les *F. rufa s.l.* soient protégées à une plus grande échelle. L'argument pour le choix de ces fourmis étant qu'elles sont de bons indicateurs pour le suivi de l'évolution de la biodiversité puisque leurs colonies abritent un grand nombre d'espèces associées.

Le complexe *F. rufa s.l.* compte huit espèces en Eurasie. On peut les différencier sur base de critères morphologiques (principalement la pilosité) et de la pigmentation, ainsi que sur base de leurs préférences écologiques (Yarrow, 1955 ; Seifert, 1996). Cependant, les espèces *F. rufa s.s.* et *F. polyctena* ne sont pas toujours différenciables sur le terrain. Elles forment parfois des colonies mixtes et elles sont interfécondes. Ces deux taxons sont donc plutôt à considérer comme un complexe d'espèces incomplètement différenciées (El-Showk, 2005). En Belgique, il existe trois espèces du groupe 'rufa' : *F. rufa s.s., F. polyctena* et *F. pratensis*.

Interactions des fourmis rousses avec la faune environnante

Chaque nid de fourmis est le point de départ d'un réseau de pistes rejoignant les différentes sources de nourriture, principalement des colonies de pucerons disséminées dans la végétation. Les ouvrières qui suivent les pistes peuvent ainsi s'éloigner de plusieurs dizaines de mètres de la colonie pour trouver de la nourriture, mais de nombreuses ouvrières quittent les pistes et investiguent chaque recoin de l'environnement immédiat du nid. A part les insectes volants, toutes les espèces vivant à proximité d'une colonie de fourmis sont donc susceptibles de rencontrer et d'interagir avec celles-ci. Il existe deux types d'études de l'influence des fourmis sur la faune d'arthropodes environnante : les études concernant la faune du sol (Von Sörensen & Schmidt, 1987 ; Laasko, 1999 ; Laasko & Setälä, 2000 ; Hawes *et al.*, 2002 ; Lenoir *et al.*, 2003 ; Reznikova & Dorosheva, 2003) et celles concernant la végétation arborée et la canopée (Nef, 1963 ; Bradley, 1973 ; Rosengren, 1979 ; Skinner, 1980 ; Skinner & Whittaker, 1981 ; Hyo Kim & Murakami, 1983). Les auteurs observent généralement que la présence des fourmis influence significativement les macro-invertébrés du sol (e.a. Van Sörensen & Schmidt, 1987). Lorsque l'on s'intéresse aux organismes de petite taille, on constate que les fourmis n'influencent pas la composition de la faune, même avec de fortes densités de fourmis (Lenoir *et al.*, 2003). Les fourmis n'atteignent donc pas tous les organismes de la même manière. Elles ne sont pas aptes à capturer les proies les plus agiles (Brüning, 1991) et elles détectent difficilement les organismes très lents et vermiformes (Hassell & Southewood, 1978).

Le mutualisme

Le mutualisme est un phénomène connu de longue date et qui consiste en une association réciproquement bénéfique. L'origine du nom provient du bénéfice mutuel obtenu par deux groupes d'organismes qui interagissent. Ce terme s'applique aux interactions entre des fourmis et divers Hémiptères, il a notamment été défini par Way (1963) comme suit : « *Association between ants and other insects which is mutually beneficial without necessarily implying obligate dependance or interdependance* ». Il a été proposé de remplacer le terme mutualisme par « trophobiose », qui désigne une association symbiotique basée sur un échange de nourriture (Delabie, 2001). Ce dernier ne sous-entend pas qu'il y ait nécessairement d'avantage direct mutuel entre les organismes intervenant dans l'association, il inclut donc aussi les interactions qui seraient défavorables à l'un des intervenants. Par souci de cohérence avec la majorité des travaux publiés, nous utiliserons le terme de mutualisme, qui est plus généralement usité.

Les interactions entre des fourmis et des mutualistes sont répandues dans le monde entier. On connaît des fossiles où des fourmis sont associées à des pucerons puis à des cochenilles, respectivement depuis l’Oligocène (30 MA) et le Miocène (20 MA) (Hölldobler & Wilson, 1990 ; Johnson *et al.*, 2001). Depuis Carl von Linné et Charles Darwin, des descriptions sur les interactions entre divers hémiptères et des fourmis ont commencé à apparaître dans la littérature scientifique (Huxley, 1954). L’importance économique des pucerons sur l’agriculture a continuellement promu la description d’un nombre croissant d’associations mutualistes.

Caractéristiques des mutualistes

Les hémiptères suceurs de sève pompent leur nourriture en insérant leur stylet buccal au travers des tissus de la plante hôte pour atteindre le phloème. La sève qui y est pompée est très riche en eau et en sucres, les hémiptères doivent donc extraire de grandes quantités pour obtenir les différents éléments nutritifs nécessaires à leur développement et à leur reproduction. L’eau et les sucres excédentaires sont ainsi déféqués sous forme d’un liquide nommé le miellat. La production du miellat est un inconvénient pour les hémiptères car l’accumulation de cette matière sucrée au sein de la colonie favorise l’apparition de maladies et les odeurs qui sont émises risquent d’attirer des prédateurs. Pour l’état sanitaire de la colonie, il est donc impératif que les excréments puissent être évacués le plus loin possible de celle-ci. Chez les pucerons, une partie des espèces a acquis des adaptations morphologiques et comportementales permettant d’éjecter au loin les gouttes de miellat. Les autres espèces de pucerons interagissent avec des fourmis qui récoltent le miellat et les protègent de leurs ennemis naturels.

Objectif du travail

Malgré les publications traitant de *C. magnifica*, de nombreuses questions fondamentales restent en suspens. Dans les chapitres qui suivent , nous présentons les résultats de plusieurs approches complémentaires menées en laboratoire et sur le terrain, visant à répondre à ces questions. Les démarches scientifiques suivies dans chacun de ces chapitres sont résumées sous forme de questions et d’hypothèses sous-jacentes.

Chapitre 1. The populations of *C. magnifica* in Belgium. A rare but locally abundant myrmecophilous ladybird

Question : Quelle est l’abondance de *C. magnifica* en Belgique ? Comment les individus d’une population sont-ils répartis à l’échelle d’un site et quelles sont les ressources alimentaires utilisées ?

Hypothèse : *C. magnifica* est une espèce écologiquement spécialisée, dont la distribution géographique est restreinte aux sites dominés par les fourmis. Elle pourrait donc être assez rare en Belgique, mais localement abondante lorsque les fourmis hôtes sont présentes. Au sein de la sphère d’influence des fourmis, *C. magnifica* consomme une grande diversité de proies, à la manière d’une espèce généraliste.

Chapitre 2. Reaction of ants to, and feeding biology of a congeneric myrmecophilous and non-myrmecophilous ladybird

Question : Quelle est l’influence directe des fourmis sur le succès de prédation de *C. magnifica* lorsque celle-ci attaque une colonie de pucerons protégée par les fourmis ?

Hypothèse : *C. magnifica* est capable de se défendre des attaques des fourmis, lorsqu'elle pénètre dans une colonie de pucerons, grâce à ses caractéristiques morphologiques, comportementales et physiologiques, qui la différencient de *C. septempunctata*.

Chapitre 3. Ant trail : a highway for *Coccinella magnifica* Redtenbacher (Coleoptera : Coccinellidae).

Question : *C. magnifica* possède t-elle des aptitudes à utiliser les signaux chimiques émis par les fourmis avec lesquelles elles sont associées ?

Hypothèse : les fourmis utilisent des réseaux de pistes phéromonales reliant le nid aux colonies de pucerons. Si *C. magnifica* peut suivre les pistes des fourmis, alors elle pourrait réduire considérablement son effort de recherche des proies.

Chapitre 4. A comparison of the reproductive traits of a generalist and a specialist *Coccinella* species.

Question : L'investissement énergétique des adultes envers leur progéniture est-il identique chez *C. magnifica* et *C. septempunctata* ? La pression exercée par les fourmis sur *C. magnifica* est-elle mesurable au niveau des sites de ponte, de la taille et du nombre d'œufs produits quotidiennement ?

Hypothèse : si *C. magnifica* est écologiquement spécialisée, alors elle devrait trouver des sites de ponte propices moins fréquemment qu'une espèce généraliste. Dans ce cas, on peut s'attendre à ce que *C. magnifica* produise des œufs plus gros qu'une espèce généraliste de taille comparable. Les larves de *C. magnifica* pourraient aussi avoir des pattes de taille réduite si elles peuvent exploiter des ressources plus stables dans l'espace et le temps, grâce à l'action des fourmis.

Chapitre 5 Is variability of adult size an indicator of specialization in predatory insects ?

Question : Où se situe *C. magnifica* sur le continuum spécialiste-généraliste, au-delà du simple constat que sa distribution géographique est fort localisée ? Peut-on estimer le degré de spécialisation alimentaire des coccinelles au moyen des paramètres de la taille du corps ?

Hypothèse : On pose l'hypothèse que le degré de variabilité de la taille du corps reflète la diversité des choix alimentaires des espèces. *C. magnifica* exploite un large spectre de ressources au sein des aires influencées par les fourmis. Elle y bénéficie de ressources plus abondantes et stables mais conserve un régime alimentaire généraliste.

Deux questions seront également abordées dans la discussion

Question : Quel est le mode de vie de *C. magnifica* en dehors de l'aire de répartition des *F. rufa s.l.* ?

Hypothèse : *C. magnifica* trouve un optimum avec les espèces de *F. rufa s.l.* bien qu'elle soit probablement capable de tirer profit d'autres espèces de fourmis de grande taille et qui exploitent massivement des colonies de pucerons.

Question : Quels sont les points communs et les différences entre *C. magnifica* et les autres coccinelles myrmécophiles ?

Hypothèse : Les mécanismes ayant conduit à l'apparition de la myrmécophilie chez *C. magnifica* ne sont pas les mêmes que ceux impliqués chez *Platynaspis luteorubra, Brachiacantha spp.* ou *Thalassa saginata*. Autrement dit, on peut distinguer différentes formes de myrmécophilie chez les coccinelles, en réponse à des pressions évolutives et des prédispositions morphologiques distinctes.

Résumé des résultats

En Belgique, *C. magnifica* a toujours été trouvée dans des sites caractérisés par la présence de colonies de *F. rufa s.l.* Dans le site étudié, plusieurs espèces de pucerons sont exploités par les fourmis durant tout l'été, principalement *Aphis sarothamni* (Franss.). Outre cette espèce, on observe d'autres proies consommées par des larves et des adultes de *C. magnifica*, dans les différentes strates de la végétation. Il s'agit principalement de pucerons ainsi que d'une espèce de psylle, tous visités par des fourmis. Plus occasionnellement, des proies non-mutualistes composent aussi le régime alimentaire de *C. magnifica*.

La caractérisation des sites de ponte montre que *C. magnifica* diffère des autres espèces de coccinelles par son choix des sites de ponte. Elle pond à plusieurs dizaines de centimètres des ressources les plus proches et le plus souvent sur des parties mortes de la plante hôte. On attribue cette singularité à la pression exercée par les fourmis, qui détruiraient les œufs s'ils étaient situés près des colonies de pucerons et ce, malgré l'existence probable, sur les œufs et les larves, de médiateurs chimiques réduisant l'agressivité des fourmis. D'autre part, *C. magnifica* produit des œufs plus gros que ceux de *C. septempunctata*, et donc des larves de premier stade possédant plus de réserves alimentaires. Avant d'atteindre les proies disponibles, les larves de *C. magnifica* ont ainsi la capacité de parcourir plus de chemin que les larves des autres espèces de coccinelles. Le coût énergétique qu'implique la production de gros œufs est compensé par une fécondité réduite, en accord avec la théorie sur l'allocation des ressources énergétiques aux ovaires. L'hypothèse selon laquelle l'investissement énergétique accru pour les larves de *C. magnifica* réduirait leur temps de développement et permettrait la capture de proies de grande taille n'est pas étayée par nos résultats. La différence de poids entre *C. magnifica* et *C. septempunctata* est rattrapée dès le deuxième stade larvaire et s'inverse au cours du reste du développement, car cette dernière espèce a un taux de croissance supérieur. *C. magnifica* ne raccourcit donc pas son temps de développement en pondant de gros œufs. D'autre part, on n'observe pas de tendance chez *C. magnifica* à une spécialisation pour des proies de grande taille. Les pattes des larves de premier stade, surtout les antérieures, sont plus courtes que celles de *C. septempunctata*, ce qui va à l'encontre d'une tendance à la spécialisation pour des proies de grande taille ou difficiles à manipuler.

Le « désavantage » apparent concernant le choix des sites de ponte chez *C. magnifica* est probablement contrebalancé par la capacité de *C. magnifica* à suivre les pistes olfactives des fourmis et donc à trouver facilement des ressources. Nous avons démontré que les adultes ont la capacité de détecter et de suivre des pistes chimiques de *F. polyctena*, dès lors, on peut supposer que ce soit aussi le cas pour les différents stades larvaires. Sur le terrain, on a fréquemment observé des adultes se déplaçant avec les fourmis sur plusieurs (dizaines de) mètres, le long de pistes menant du nid des fourmis aux colonies de pucerons. L'utilisation des indices chimiques des fourmis doit, très probablement, être un net avantage pour *C. magnifica* lors de la recherche des ressources nutritives.

La description des interactions comportementales entre *C. magnifica* et les fourmis a été abordée au moyen de deux dispositifs en laboratoire : des tests en arène n'offrant pas de refuge ni de possibilité de fuite (conditions artificielles) et des tests sur des arbres en pots colonisés par des pucerons (conditions semi-naturelles). Dans les conditions artificielles, on constate que les œufs et les larves de *C. magnifica* bénéficient d'une réduction de l'agressivité, comparativement à *C. septempunctata*. Dans cette situation, on ne peut pas attribuer les différences observées au comportement des coccinelles, dès lors, on suspecte l'intervention de médiateurs chimiques. Dans les conditions semi-naturelles, les adultes des deux espèces ont des comportements et des succès de prédation très différents. En présence de fourmis, *C. magnifica* consomme plus de pucerons et ce, avec un meilleur succès de capture que *C. septempunctata*. L'efficacité accrue de *C. magnifica* est associée à sa capacité à contourner les attaques des fourmis, alors que *C. septempunctata* fuit dès qu'elle est attaquée. Toutefois, *C. magnifica* capture plus de proies en l'absence des fourmis que lorsqu'elles sont présentes. Cette réduction du taux de consommation est attribué au dérangement de la coccinelle causé par les fourmis durant la prédation. D'autre part, les adultes de *C. magnifica* évitent les attaques des fourmis grâce à leur comportement et à leur morphologie (forme plus ronde, sans arrêtes saillantes du tégument).

La spécificité alimentaire de *C. magnifica* est faible puisqu'elle se développe très bien en consommant des pucerons myrmécophiles ou non myrmécophiles. Elle est pourtant moins apte que *C. septempunctata* à métaboliser les alcaloïdes toxiques du puceron *Aphis jacobaeae*. *C. magnifica* n'est donc pas associée à des proies vivant dans des habitats précis ou sur des plantes hôtes spécifiques. La spécialisation de *C. magnifica* concerne plutôt la restriction géographique déterminée par les populations de la fourmi hôte. *C. magnifica* est donc spécialisée pour des fourmis et non pour des pucerons !

C. magnifica a presque la même taille que *C. septempunctata*, mais la variabilité de la taille de *C. magnifica* est supérieure. La variabilité de la taille observée au sein des douze espèces correspond en partie à la diversité du régime alimentaire, on suggère donc que ce coefficient exprime la spécialisation alimentaire. La variabilité de la taille met en évidence la différence entre les stratégiques myrmécophiles de *C. magnifica* et *P. luteorubra*, cette dernière étant à la fois nettement plus oligolectique et de taille moins variable que toutes les espèces mesurées. En théorie, la variabilité intraspécifique de la taille des adultes doit être relative à la stabilité des ressources en qualité et en quantité. Les deux espèces myrmécophiles tirent probablement profit de l'effet des fourmis sur les pucerons et bénéficient ainsi d'une grande quantité de proies, stables dans le temps. Une première piste pour l'estimation de la spécialisation a été ouverte via l'utilisation du coefficient de variation de la taille. La relation observée est certainement entachée de l'une ou l'autre erreur due à l'ensemble des paramètres non mesurés tels que le polymorphisme génétique ou la variabilité des conditions environnementales. L'avantage de la méthode choisie est qu'elle permet d'utiliser une grande quantité d'individus disponibles dans les musées.

Discussion générale

La myrmécophilie est un phénomène observé à l'échelle mondiale, chez presque tous les ordres d'arthropodes (Hölldobler & Wilson, 1990). On estime qu'il existe dans le monde environ 100000 espèces d'insectes associées de près ou de loin aux fourmis (Schönrogge *et al.*, 2002). La pression évolutive conduisant à une association étroite avec des fourmis est donc un phénomène commun et répandu. Il n'y a rien d'étonnant à ce qu'il concerne aussi les coccinelles qui, lorsqu'elles recherchent des colonies de pucerons, doivent nécessairement entrer fréquemment en contact avec les fourmis (cf. Figure 1). Sachant que les colonies de pucerons protégées par les fourmis constituent une source de nourriture potentiellement très favorable à la reproduction des coccinelles, une espèce acceptée par les fourmis serait nettement avantagée. Dès lors, on pourrait s'attendre à ce que de nombreuses espèces aient évolué vers un comportement myrmécophile. La réalité est très différente, puisque les cas avérés d'espèces de coccinelles associées à des fourmis sont très rares. En aucun cas, des mécanismes d'acceptation de l'insecte myrmécophile par les fourmis n'ont pu être démontrés. En revanche, les mécanismes défensifs communs à toutes les espèces de coccinelles sont efficaces pour se défendre des oiseaux et des fourmis (Pasteels *et al.* 1973 ; Holloway *et al.*, 1991). Une coccinelle adulte a des élytres solides et glissants qui peuvent être utilisés comme bouclier contre les fourmis. Si nécessaire, la coccinelle peut se défendre en émettant une saignée réflexe collante et toxique. Des cires floconneuses sont sécrétées au niveau de la face dorsale des larves de certaines espèces de Scymninae. Ces cires constituent un obstacle physique temporairement résistant aux attaques des fourmis (Völkl&Volhand, 1996). Cette adaptation permet surtout aux larves de se dissimuler parmi leurs proies pour éviter l'ensemble de leurs ennemis naturels, dont les fourmis. Donc, lorsqu'une coccinelle à la recherche de nourriture atteint une colonie de pucerons protégés par des fourmis, elle est capable de s'enfuir sans dommage, après avoir éventuellement consommé quelques proies. Par contre, la reproduction y est impossible car les œufs et les larves sont plus vulnérables. On peut vraisemblablement déduire que la particularité d'une espèce myrmécophile est de pouvoir éviter l'agressivité des fourmis durant les stades d'œuf et de larve.

Caractéristiques des coccinelles myrmécophiles

Chez *C. magnifica*, le choix des sites de ponte assure une faible exposition des œufs aux fourmis et on suspecte aussi l'intervention de médiateurs chimiques réduisant l'intensité du comportement agressif des fourmis. Les larves sont identiques à celles de *C. septempunctata* mais parviennent pourtant à se nourrir des pucerons protégés, il est donc fort probable que *C. magnifica* possède une signature olfactive induisant une tolérance par les fourmis. Les observations menées sur le terrain révèlent que les larves sont très mobiles et quittent régulièrement la colonie de pucerons (et donc aussi les fourmis), emmenant parfois une proie pour la dévorer à l'écart. Ces déplacements permettent probablement de réduire le temps durant lequel la larve est exposée aux fourmis. Chez *P. luteorubra*, la morphologie des larves semble être particulièrement favorable au mode de vie myrmécophile. Celles-ci sont plates et lentes, avec des pattes courtes cachées sous le corps. La marge latérale du corps est munie d'une couronne de soies robustes assurant la protection de la face ventrale. Cette morphologie est rare quoique pas unique chez les coccinelles, mais elle diffère de la morphologie larvaire des autres Chilocorinae. Les œufs sont pondus isolés et cachés sur la plante-hôte. Le principal trait adaptatif des adultes des deux coccinelles myrmécophiles est donc le comportement consistant à affronter les fourmis plutôt que de les fuir pour atteindre les colonies de pucerons.

L’apparition de la myrmécophilie chez les Coccinellidae

Les étapes évolutives nécessaires à la transition de l’ancêtre commun du genre *Coccinella* vers *C. magnifica* se traduisent par des adaptations ténues. Le seul « trade-off » que nous ayons mesuré est la tendance à pondre moins d’œufs mais de plus grande taille, comparativement au « modèle *C. septempunctata* ». Hormis cela, *C. magnifica* répond aux mêmes lois que *C. septempunctata* et les autres espèces de coccinelles. Les larves de *P. luteorubra*, quoique différentes des autres Chilocorinae, sont très similaires à celles des *Phymatosternus sp.* ou de certains *Scymnus sp.* (Scymninae ; réf - photo). La morphologie des larves de *P. luteorubra* n’est donc pas propre à la cohabitation avec des fourmis mais semble bien être un avantage. Sur l’arbre phylogénétique des Coccinellidae, les différentes espèces myrmécophiles avérées (Majerus *et al.*, 2007) se trouvent dans des sous-genres différents et éloignés les uns des autres (Fig 2). Il est donc fort probable que la myrmécophilie soit apparue séparément dans ces différents groupes. Ceci pourrait expliquer pourquoi les stratégies de chacune des espèces myrmécophiles sont si différentes.

Pourquoi n’y a t-il pas plus d’espèces de coccinelles myrmécophiles ?

On estime qu’il existe dans le monde entre 5000 et 20000 espèces d’insectes myrmécophiles obligatoires (Schönrogge *et al.*, 2002), mais seules quelques dizaines ont évolué vers cette stratégie pour se nourrir des mutualistes protégés par les fourmis. La stratégie myrmécophile existe chez les Chrysopidae, les Syrphidae ou les hyménoptères parasitoïdes, qui se nourrissent de pucerons (Tauber & Tauber, 1987 ; Albuquerque *et al.*, 1997 ; Schönrogge *et al.*, 2002 ; Hübner, 2000). Dans chacune de ces familles, très peu d’espèces sont étroitement associées à des fourmis. Chez les Syrphidae, plusieurs espèces (e.a. les *microdon spp.*) sont myrmécophiles mais en tant que prédatrices du couvain des fourmis (Donisthorpe, 1927 ; Schönrogge *et al.*, 2002). Certaines espèces des genres *Paragus, Chrysotoxum, Pipizella, Anthogramma* et *Platycheirus* sont connues pour s’attaquer aux pucerons hypogés élevés par des fourmis (Rotheray *et al.*, 1996 ; Dziock, 2005). Si l’on s’intéresse plus particulièrement aux coléoptères, on dénombre en Scandinavie 73 espèces myrmécophiles dont 46 (63%) sont associées à *F. rufa s.s.* (Päivinen *et al.*, 2003). Il s’agit principalement de coléoptères vivant dans ou à proximité du nid et aucun n’est prédateur de pucerons. *C. magnifica* est un coléoptère myrmécophile parmi beaucoup d’autres mais c’est un des rares à s’attaquer aux pucerons utilisés par ses hôtes. La rareté du phénomène pourrait en partie s’expliquer par la complexité des interactions entre le myrmécophile, les fourmis, les mutualistes et les plantes hôtes. Plus un système est complexe et/ou rigide, plus il est fragile par sa sensibilité aux variations des paramètres environnementaux (Hoeksema & Bruna, 2000). La myrmécophilie chez les prédateurs de pucerons apparaît donc très marginale par rapport à la diversité des formes et des stratégies écologiques de cette guilde. *P. luteorubra* et *C. magnifica* font exception par leurs comportements et leurs morphologies spécifiques. Cette forte spécialisation leur donne accès à des ressources délaissées par leurs compétiteurs.

Myrmécophilie obligatoire ?

Hormis quelques adultes erratiques, on ne rencontre jamais *C. magnifica* en dehors de la zone d’influence des fourmis. Pourtant, le régime alimentaire de *C. magnifica* est comparable à celui des espèces généralistes, elle pourrait donc théoriquement se reproduire à la fois dans les habitats où les fourmis sont présentes ou absentes. En présence des fourmis, *C. magnifica* bénéficie de l’abondance et de la pérennité des colonies de pucerons ainsi que de l’exclusion des compétiteurs. En l’absence de fourmis, ces deux avantages disparaissent. On peut émettre l’hypothèse que *C. magnifica* soit absente des habitats non dominés par les fourmis à cause de la pression de compétition exercée par les autres prédateurs de pucerons. Une explication envisagée serait que *C. magnifica* bénéficie d'un espace libre d'ennemis (Enemy free space theory : Jeffries & Lawton, 1984), grâce à l'effet des fourmis sur la faune environnante. Cette hypothèse a pourtant été en partie invalidée suite aux expériences menées avec l'hyménoptère parasitoïde *Dinocampus coccinellae*. Cette espèce est un des principaux ennemis de *C. septempunctata*, sa prévalence dans les populations naturelles atteint 10 à 20 %. Par contre, lorsque *D. coccinellae* attaque *C. magnifica*, sa larve ne se développe pas dans le corps de la coccinelle et l'on n'observe jamais l'émergence d'adulte ni de mortalité accrue des coccinelles infectées (Sloggett *et al.*, 2004). *C. magnifica* est intrinsèquement protégée de cet ennemi naturel, son avantage à cohabiter avec les fourmis ne peut donc pas s'expliquer par la réduction du parasitisme par *D. coccinellae*. Le bénéfice pour *C. magnifica* serait plutôt de trouver un espace libre de compétiteurs !

Les principaux « ennemis potentiels » de *C. magnifica* seraient donc les fourmis avec lesquelles elle est associée ! On en déduit que les adaptions de *C. magnifica* lui permettent de minimiser à fois la pression de prédation provenant des fourmis et la pression de compétition intraguilde. Ce qui correspond à un avantage sélectif important pour *C. magnifica*, mais uniquement au sein de la sphère d'activité des fourmis.

C. magnifica est une espèce spécialisée pour les habitats influencés par les fourmis, à l'instar d'autres coccinelles qui sont spécialisées pour les roselières ou les landes à callune. Elle est donc inféodée à une caractéristique de l'habitat (la présence de colonies de *F. rufa s.l.*) plutôt qu'à la présence de proies ou de plantes hôtes.

Pourquoi les Formica spp. ?

En Europe de l'Ouest, on trouve majoritairement *C. magnifica* en compagnie des espèces du complexe *F. rufa/polyctena* et plus ponctuellement avec *F. fusca, F. rufibarbis, F. pratensis, F. sanguinea,…* En Europe centrale, elle peut être aussi associée à *Formica cinerea* Mayr (P. Cerygnier, comm. pers.) et en Scandinavie à *Formica lugubris* (Sloggett *et al.*, 2002). Toutes ces fourmis sont d'assez grande taille (4,5 - 9 mm) et ont une influence majeure sur la faune environnante (e.a. Skinner, 1980). Elles entretiennent des interactions mutualistes avec de nombreuses espèces d'hémiptères, principalement avec des pucerons. *C. magnifica* n'est jamais observée avec des fourmis de taille moyenne telles que *Lasius niger* (3 – 5 mm) qui exploite aussi de nombreux hémiptères et avec qui la coccinelle *P. luteorubra* est associée. Il existerait une correspondance entre la taille des coccinelles myrmécophiles et celle de la fourmi associée, à l'instar de la correspondance observée entre la taille des coccinelles et celle de leurs proies (Dixon & Hemptinne, 2001). *C. magnifica* pourrait difficilement se protéger d'attaques de petites fourmis comme elle le fait avec les *Formica* en utilisant ses élytres comme un bouclier. De petites espèces pourraient attaquer la face ventrale de la coccinelle et ainsi accéder aux pattes et aux sutures intersegmentaires qui en sont les points sensibles.

C. magnifica n'est donc pas spécialisée pour une espèce de fourmi en particulier mais bien pour une ou des espèces de grande taille, localement dominantes et entretenant des colonies d'homoptères. Au sein de son aire de distribution eurasienne (Figure 4), *C. magnifica* rencontre ces conditions au contact des différentes espèces de *F. rufa s.l.* Dans une partie de l'aire de distribution de *C. magnifica*, les *F. rufa s.l.* sont absentes ou remplacées par d'autres espèces. Alors on trouve *C. magnifica* avec d'autres espèces de fourmis. Ce caractère opportuniste de *C. magnifica* expliquerait pourquoi elle n'est pas associée de manière stricte avec une espèce de fourmi, à la différence de *P. luteorubra*.

Les informations écologiques sur l'association de *C. magnifica* avec d'autres espèces que les *Formica spp.* sont rares, de même pour les situations dans lesquelles les coccinelles sont observées sans fourmis associées. Dans l'état actuel de nos connaissances, il serait logique de considérer que l'association avec des *Formica spp.* (et tout particulièrement avec *F. rufa s.l*). est une situation optimale. Les autres situations seraient marginales et ne se rencontreraient qu'en périphérie de l'aire de distribution de la coccinelle.

Perspectives

Au terme des investigations qui viennent d'être présentées, nous avons identifié deux pistes de recherches qui devraient être investiguées en priorité. La première option est plutôt une approche de laboratoire et concerne la nature des communications chimiques entre *C. magnifica* et les fourmis. La seconde option nécessiterait de dédier des efforts importants à des prospections de terrain afin d'élargir les connaissances de l'espèce à l'ensemble de son aire de répartition.

Ecologie chimique : les signaux de communication

Nous avons constaté qu'il existe probablement des signaux chimiques sur les œufs et les larves de *C. magnifica* réduisant l'agressivité des fourmis. Lorsqu'un adulte est attaqué, les fourmis atteignent rarement la face ventrale ou les membres de la coccinelle. Des signaux chimiques pourraient être responsables d'une réduction locale de l'agressivité des fourmis. A cet égard, nous avons remarqué que les adultes de *C. magnifica* effectuent de longues séances de toilettage entre deux interactions avec des fourmis. Durant le toilettage, des molécules produites par les glandes labiales pourraient être enduites sur les membres et les sternites. A partir d'extraits des molécules de surface des différents stades de *C. magnifica*, on testera la réponse comportementale des fourmis afin d'identifier d'éventuelles molécules actives. Corollairement, il faudrait identifier les signaux des fourmis détectés par *C. magnifica*, et que celle-ci utilise probablement lorsqu'elle suit les pistes olfactives de son hôte. Pour ce faire, il serait nécessaire de tester sur *C. magnifica* (et d'autres espèces) des extraits des glandes des fourmis produisant des signaux olfactifs et en identifier la composition.

Biogéographie évolutive : liens de parenté entre les populations de C. magnifica

On pourrait comparer la proximité génétique (marqueur à déterminer !) entre les individus provenant de populations réparties dans l'aire de distribution de *C. magnifica*. A partir de cette information, on pourrait déterminer des centres de dispersion et formuler des hypothèses sur l'historique biogéographique de l'espèce. Cette approche a déjà été utilisée chez les fourmis du groupe *F. rufa* et il serait intéressant de rechercher des similitudes entre la structure des populations des deux espèces. Les *F. rufa s.l.* sont associées aux zones de forêts tempérées caducifoliées et de conifères. Au moment des épisodes glaciaires du Pléistocène, les ancêtres des fourmis rousses ont été repoussées, avec la limite des forêts, vers le Sud de l'Europe dans des zones refuges péri-méditerranéenne et baltique ainsi qu'autour des Mers d'Azov et d'Aral. Au cours des 10 à 15 derniers milliers d'années, les espèces de F. rufa s.l. se sont stabilisées, principalement dans les aires boréo-alpines mais aussi dans les plaines boisées. Les différences entre plusieurs sous-populations de *F. pratensis* correspondent aux refuges occupés durant la dernière époque glaciaire (El-Showk 2005). La transition des habitats depuis la fin de la dernière glaciation est un facteur majeur de l'évolution de notre faune et flore. Si on peut en observer aujourd'hui les conséquences sur les fourmis, il est probable que la coccinelle associée ait subi des influences comparables.

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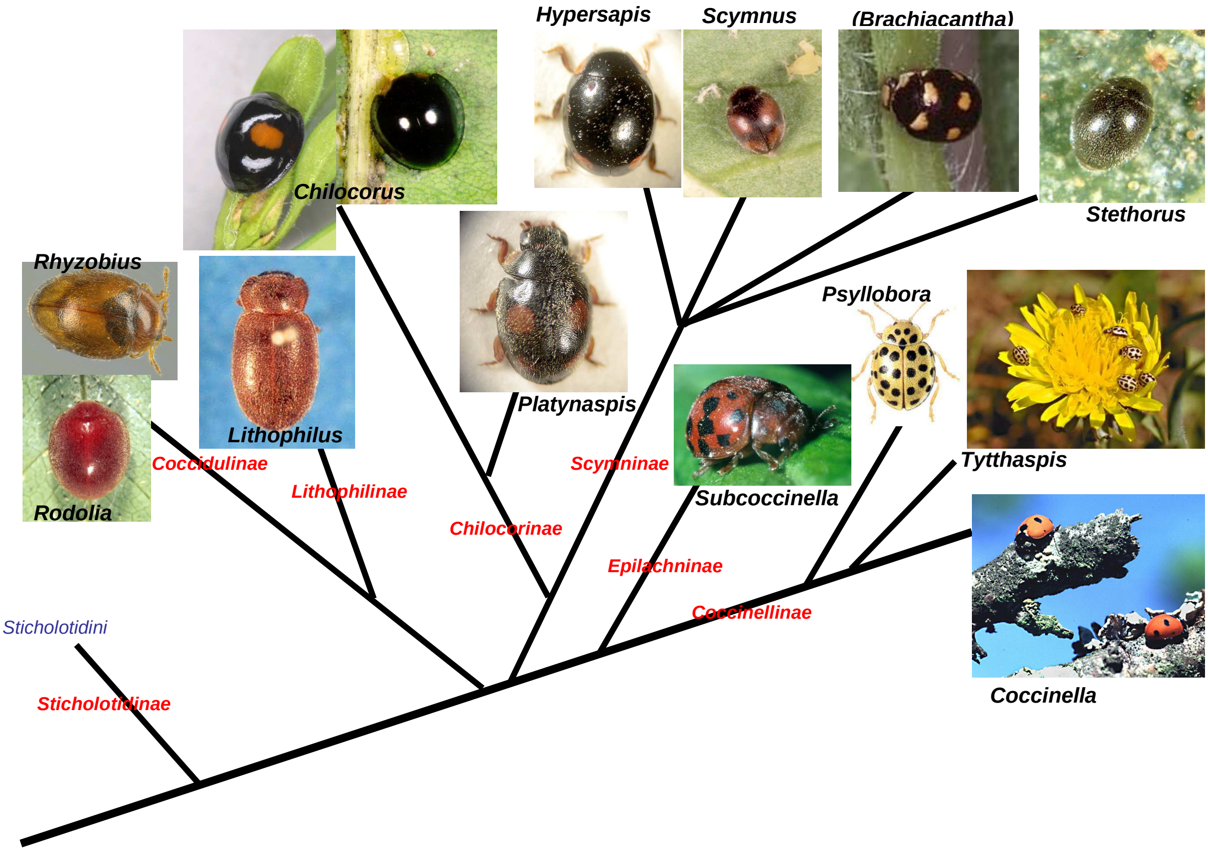
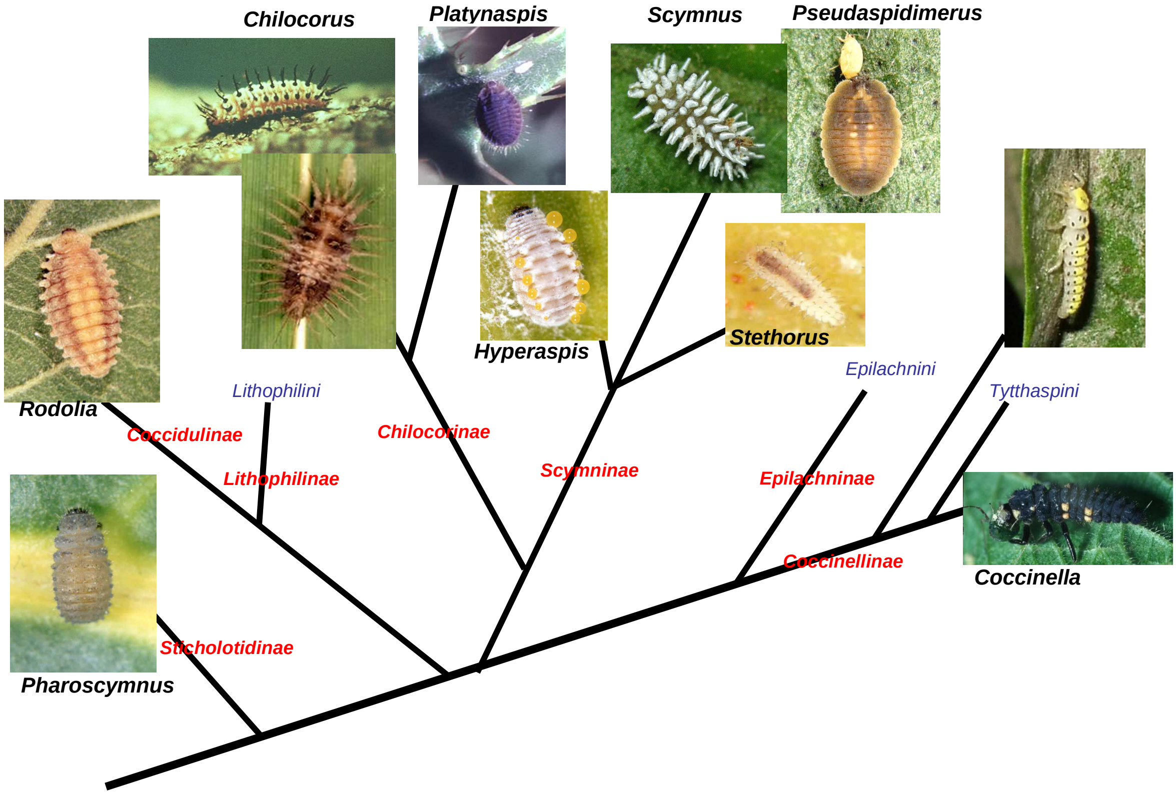
Annexe

Cladogrammes des Coccinellidae d’Europe accompagnés de photos de genres illustrant les types morphologiques chez les adultes et chez les larves. La branche du genre Brachiacantha (Amérique du Nord) a été ajoutée à titre de comparaison (adultes), ainsi que Pseudaspidimerus qui appartient à la tribu des Scymnini (larves). Adapté d’après Sasaji (1968, in Majerus 1994) et Gregory *et al.* (2003).





ARTICLE I : The populations of *C. magnifica* in Belgium. A rare but locally abundant myrmecophilous ladybird

Jean-François Godeau and Jean-Louis Hemptinne

Introduction

The distribution of insects is usually badly known even if some families, such as ladybirds, benefit from more attention from naturalists in the field. This can be due to a lack of taxonomical knowledge but, most often, the occurrence of species is probably underestimated because they are cryptic, confounded with another species or they live in localized habitats. Even if the European ladybirds are relatively well described and the list of the ladybirds of Belgium established some time ago, some species remain badly known., This is the case of *Coccinella magnifica* Redtenbacher (Branquart & Maes, 2003). It is very similar in size, shape and colour to the very common seven spot ladybird *Coccinella septempunctata* L., which is believed to be its closest relative (Iablokoff-Khnzorian, 1982). The two species can only be differentiated on the basis of rather inconspicuous morphological traits such as the colouration of two metepimeres or the curvature of their elytra and of the pronotum front angles (Iablokoff-Khnzorian, 1982; Baugnée & Branquart, 2000). Although similar the two species do not exploit the same prey and do not live in the same habitats. In western Europe, *C. septempunctata* is the most common species, living in a range of habitats and showing nevertheless a preference for aphids of herbaceous plants. *C. magnifica* is much rarer and is only found in the close vicinity of ants (Iablokoff-Khnzorian, 1982; Majerus, 1994; Hodek & Honek, 1996). The lack of field observations of *C. magnifica* is then probably due to its actual rarity combined with its likeness with *C. septempunctata*.

Compared to *C. septempunctata*, which was already described in 1756 by Linneaus, *C. magnifica* was recognized as a species much later as it was described in 1837 and 1843 (Iablokoff-Khnzorian, 1982). The first field observations of this rare ladybird date back to the late 19th century when Morris (1888) described its association with ants and considered it as a myrmecophilous species. These earlier observations were later reinforced by Donisthorpe (1910, 1920a and b). This species is mainly found in association with *Formica rufa s.l.* (the Red Wood Ant group of species) and preys on ant-attended aphids as well on some species of insects encountered in ant territories. For a long time, it was admitted that *C. magnifica* was not attacked by ants and was accepted by the latter (Donisthorpe 1920a and b, Berti & Bourlard, 1983; Majerus, 1989). However, recent studies challenged this old point of view (Sloggett *et al.*, 1998; Sloggett *et al.*, 2003; Godeau et al. in prep). Therefore, *C. magnifica* is probably competing with ants for the exploitation of a common resource, the aphids. As *C. magnifica* successfully coexists with ants, it should be able to forage efficiently regardless to ants' activity.

Adult ladybirds are morphologically well resistant to ants bites thanks to their hardened cuticle but the soft-bodied eggs and larvae can be killed by ants (Sloggett *et al.*, 1998; Sloggett *et al.*, 2003; Godeau et al. in prep). It has been proposed that larvae and eggs of *C. magnifica* are protected from ants aggressiveness by defensive chemicals or by behavioural traits (Sloggett & Majerus, 2000; Godeau *et al.*, in prep). As *C. magnifica* is the only ladybird able to feed on ant-attended mutualists, it would therefore benefit from the absence of intraguild predation.

So far, the distribution of *C. magnifica* remains almost unknown in Belgium. In Great-Britain, the data collected by the British Ladybird Survey confirmed that it belongs to the most rare species among the ladybird fauna and that it was always found in association with *Formica rufa* L. and *F. polyctena* Förster (Majerus 1989, 1994). A similar survey began in 1998 in Belgium and it increased considerably the amount of field observations of ladybirds.

We report here the new data of *C. magnifica* and the results of our field investigations. We collected field information about *C. magnifica* to have better understanding of its myrmecophilous behavioural characteristics and to improve knowledge about its populations.

Material & Method

Distribution map

The distribution of *C. magnifica* in Belgium was updated by our field survey, recent observations made by members of the workgroup 'Coccinula' as well as data from museum collections (Institut Royal des Sciences Naturelles de Belgique and Faculté universitaire des Sciences Agronomiques de Gembloux).

During the Summers 1997 to 1999, fourteen sites where *F. rufa s.l.* had been previously observed were visited and the occurrence of *C. magnifica* recorded (Table 1). Each site was searched using a combination of techniques such as sweeping net, beating tray and visual inspection. We sampled adults and larvae in herbs, on bushes, on small trees and on lower branches of tall trees, but we could not search in the canopy, above 3m. Two large populations were discovered in Chanly and Grande Hoursinne, where the communities of ladybird species in ant-foraged area were compared with those in areas not visited by ants. In Grande Hoursinne, we performed 3 sampling sessions in ant-foraged areas and 3 other in ant-absent areas, each session lasted 1 hour. In Chanly, 39 sampling sessions in ant territories (see below) were compared with 3 sampling sessions in ant-absent areas.

Table 1 : The fourteen sites where *F. rufa s.l.* had been previously observed and where the occurrence of *C. magnifica* was recorded.

	Altitude (m)	Natural districts	Forest type	Habitat	Sandy soil	Presence of *Coccinella magnifica*	Presence of *F. rufa s.l.*
Burg-Hampstede (NL: Zélande)	15	Maritime	Mixte	Clear forest	I	I	I
Bois de Lauzelles (Louvain-la-Neuve)	100	Mosan	Mixte	Clear forest with heath	I	I	I
Plombières	200	Mosan	Feuillus	Fallow area	O	I	I
Wiesmes (Beauraing)	210	Mosan	Feuillus	Fallow area	O	I	I
Chanly (Wellin)	250	Mosan-Ardennais	Mixte	Fallow area	O	I	I
Roche-à-Frêne (Erezée)	250	Mosan-Ardennais	Mixte	Clear forest	O	I	I
Camp Milit. Lagland (Arlon)	380	Lorrain	Mixte	Heath	I	I	I
Grande Hoursinne (Erezée)	420	Ardennais	Conifères	Fallow area	O	I	I
Baudour (Saint-Ghislain)	70	Mosan	Mixte	Fallow area	I	O	I
Masnuy-Saint-Jean (Jurbise)	70	Mosan	Mixte	Forest	I	O	I
Treignes (Viroinval)	260	Ardennais	Conifères	Forest	O	O	I
Treignes (Viroinval)	290	Ardennais	Feuillus	Fallow area	O	O	I
Oignies-en-Thiérache (Viroinval)	325	Ardennais	Conifères	Forest	O	O	I
Luchy (Bertrix)	450	Ardennais	Conifères	Fallow area	O	O	I

The population of *C. magnifica* in Chanly (Wellin ; N 50°04'30'' – E 5°09'30'')

During the Summers 2000 and 2001 and the Spring 2002, the position of the nests of *Formica rufa s.l.* were reported on a map (Fig. 4). There it was found that *C. magnifica* preyed upon the following species of aphids :

Aphis sarothamni Franssen on *Cytisus scoparius* (L.) Link;
Aphis pomi de Geer and *Cacopsylla sp*. on *Crataegus monogyna* Jacq.;
An unidentified aphid on *Prunus spinosa* L.;
Aphis urticata Gmelin and *Microlophium carnosum* (Buckton) on *Urtica dioica* L.;
Symydobius oblongus (Von Heyden) on *Betula pendula* Roth;
Myzocallis castanicola Baker and *Lachnus pallipes* (Hartig) on *Quercus robur* L.

Every ten days, the populations of these aphids were monitored from March to November and their demography described by an index as follows: 0 = no colony; 1 = colony increasing or decreasing in size; 2 = maximal number of individuals in the colony. The index obtained for each species is summed by host plant to give a semi-quantitative index of phenology.

In 2000 and 2001, there were 39 sampling sessions of 4 hours in the ant-foraged area (Fig. 6). During each session the site was search using a combination of techniques such as sweeping net, beating sheet and visual inspection. The *C. magnifica* encountered were sexed. The sex-ratio of the samples are pooled and compared to an even proportion with a Chi-square test.

The distances of *C. magnifica* batches of eggs found during one day of intensive search on broom (*Cytisus scoparius*) and oak (*Quercus robur*) to the nearest aphid colony were measured. The distribution of frequencies of these distances were compared with a random proportion with G-test for goodness of fit (Sokal & Rohlf, 1995).
Similarly, the distances of egg clusters of *Adalia bipunctata* (L.) laid on a bush of *Euonymus europaeus* L. to the nearest colony of *Aphis fabae* Scopoli in an ant-absent site were measured in April 2003. The log-transformed distances of eggs to the prey recorded for the two ladybird species were compared with a Student T-test.

Results

From 1859 to 1996, there were only 17 records of the presence of *C. magnifica* in the two entomological collections searched. Compared to this low occurrence, *C. magnifica* was observed 182 times from 1997 to 2007 at various altitudes from the seaside to the “Ardennes” (Fig. 1.). Nevertheless, most ladybirds were seen in the Campine district, which is characterised by sandy soil.

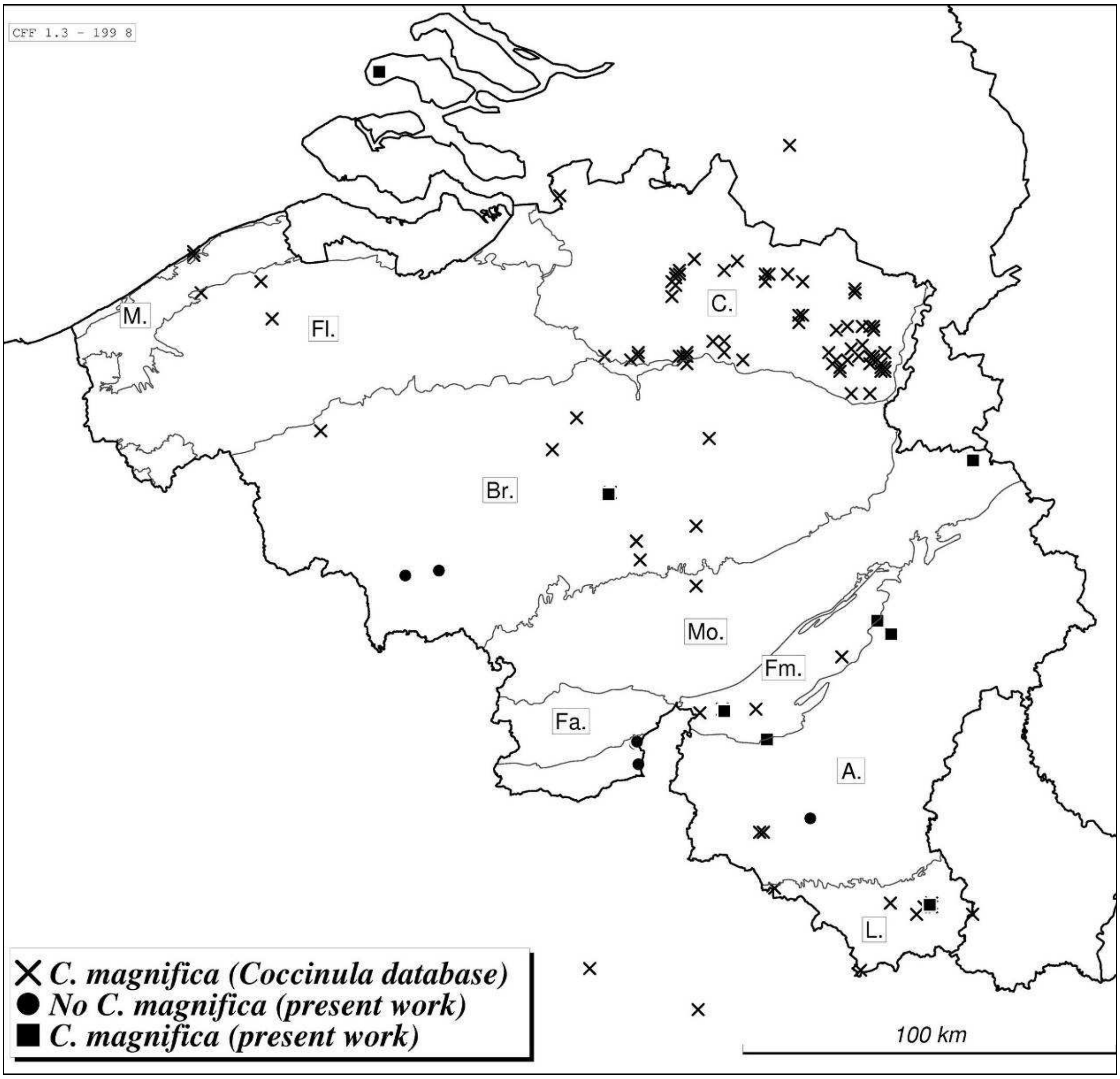


Fig. 1 : The distribution of *C. magnifica* in Belgium. Legend of the natural districts: Maritime (M.); Flandrien (Fl.); Brabançon (Br.); Campine (C.); Mosan (Mo.); Fagne (Fa.); Famenne (Fm.); Ardennes (A.); Lorraine (L.). Note: 2 dots are overlaying for the "No *C. magnifica*" distribution.

Fourteen locations where *C. magnifica* had been previously encountered by naturalists were again visited in the course of this study. The ladybirds were present in 8 out of these 14 places. Five of these sites were situated at a forest edge, a fallow area or clear cut parcel, the 3 other were clear forest with a low density of trees. There, *C. magnifica* coexists mainly with *F. polyctena*, *F. rufa* and less often with *F. partensis*. On one occasion, it was found with *Formica fusca* L. (Obs. T. Adriaens, 'Coccinula' database).

The ladybird communities in ant-foraged and ant-absent areas show strong differences in species composition (Fig. 2 and 3). *C. magnifica*, which is always the dominant species in the presence of ants becomes extremely rare in the absence of ants. In such situations, the dominant species is *C. septempunctata*.

Fig. 2 : The proportions of *C. magnifica*, *C. septempunctata* and the other Coccinellids species sampled in Chanly, in the areas where ants were present or absent.

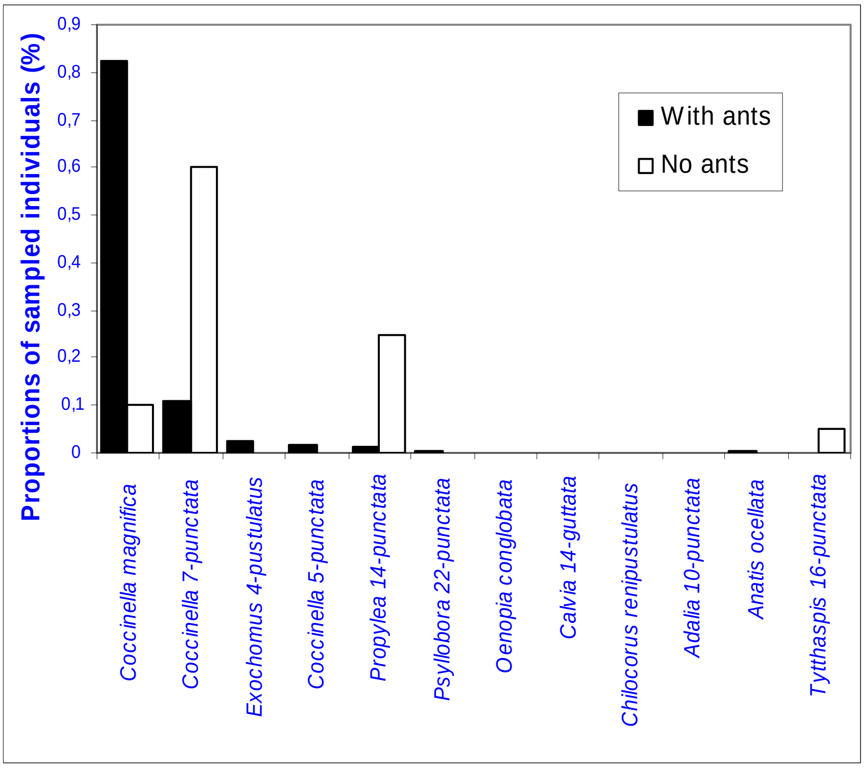
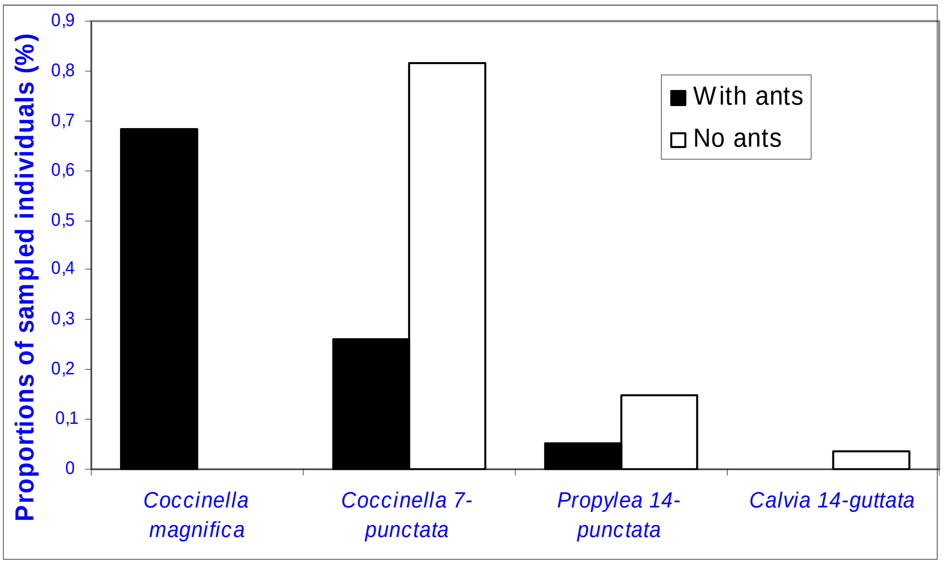


Fig. 3 : The proportions of *C. magnifica*, *C. septempunctata* and the other Coccinellids species sampled in Grande Hoursinne, in the areas where ants were present or absent.



Ants, aphids and ladybirds in Chanly

The ant populations were scattered in a mixed forest, especially along the edges and in fallow areas (Fig. 4). In these areas aphid colonies on nettles, various bushes and trees were the most crowded from early June to late August (Fig. 5). Most of the ladybirds were found on *C. scoparius* and *U. dioica; C. magnifica* was the dominant species during the 7 month, especially from May to August (Fig. 6).

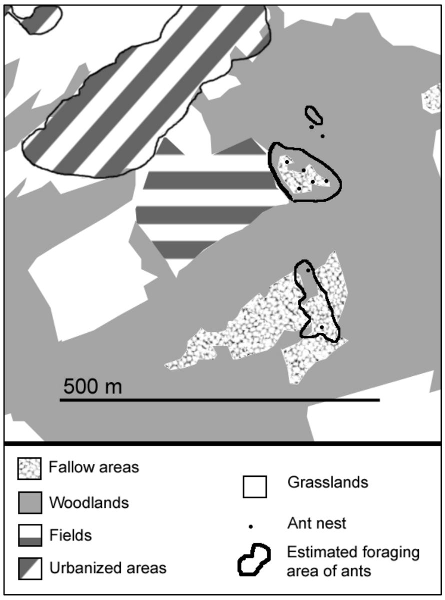


Fig. 4 : The study site in Chanly with the localization of the ants nests and the estimated ant-foraged areas.

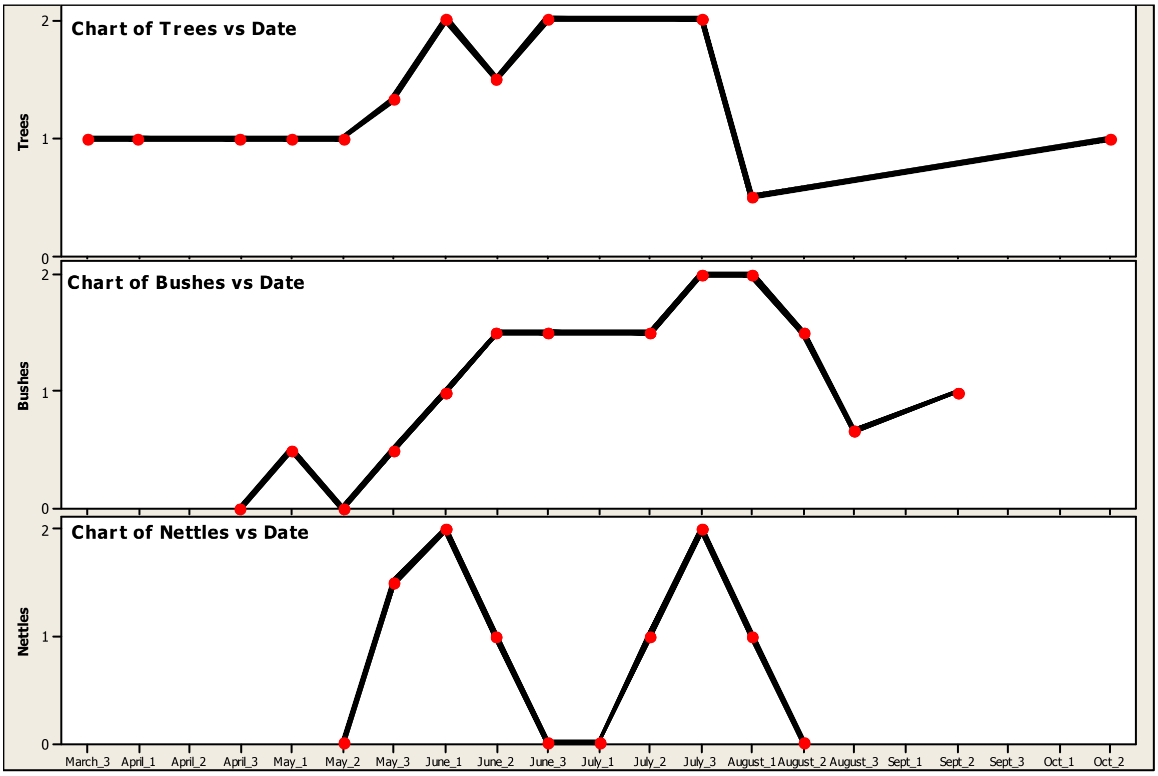


Fig. 5 : Charts of the evolution of the monitored aphid colonies (see text for the description of the index of phenology).

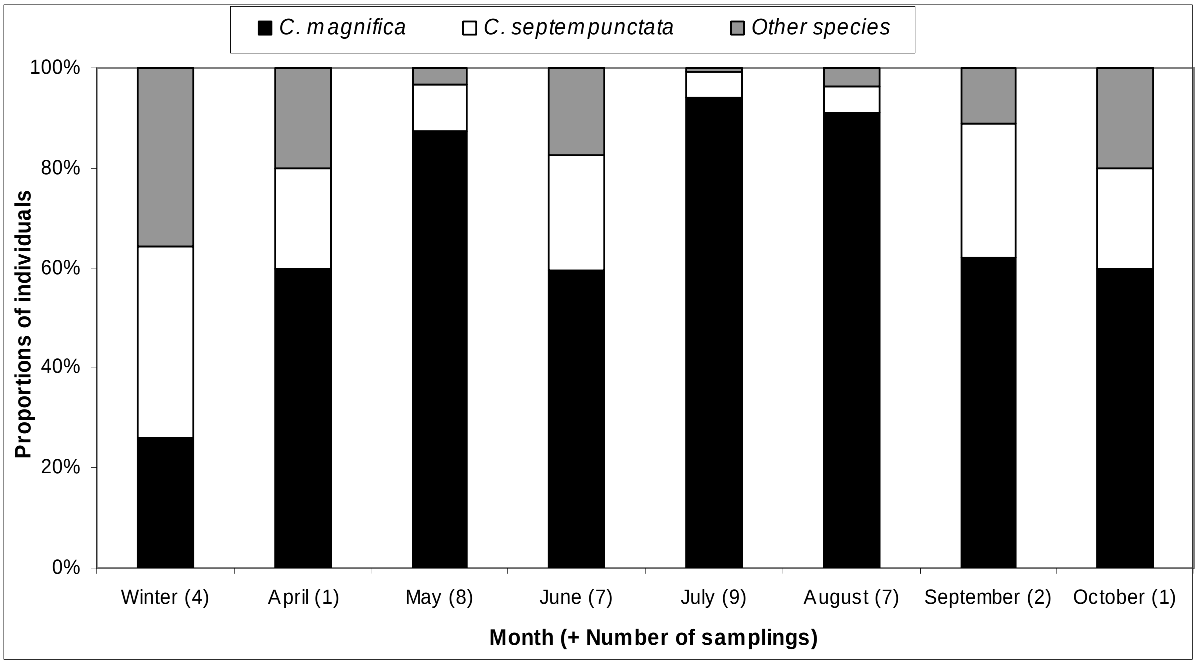


Fig. 6 : The proportions of *C. magnifica*, *C. septempunctata* and the other Coccinellids species sampled in Chanly + sampling effort between brackets.

The global sex-ratio of all the samples was nearly even with 174: females for 159 males (Chi-Sq = 0.677; d. f. = 1; P = 0.41).

On infested brooms and oaks, the egg clusters of *C. magnifica* were on average located at 8.2 ± 2.4 m from the nearest ant nest. The mean distance from the nearest aphid colony was of 45.5 ± 61.4 cm, ranging from 2 to 150 cm. These clusters were mainly situated on dry parts of the plants, such as dead branches, dried broom pods or lichens, which represent together 71.4% of all the observations (Table 2). The non-myrmecophilous ladybird *A. bipunctata* laid its cluster at an average distance of 11 ± 6.1 cm from the nearest aphid colony. *C. magnifica* egg clusters are significantly further from aphid colonies than those of *A. bipunctata* (T = 4.87; d. f. = 51; P ~ 0).

Table 2: The egg-laying sites of *C. magnifica* found within a natural population (Chanly, Belgium) which mainly foraged on broom (*Cytisus scoparius*) and oak (*Quercus robur*). The egg-laying sites are not randomly chosen (Gadj = 13.29; DF = 4; P-value < 0.01).

Location	Number of egg clusters (%)
Dead branch	18 (39.1%)
Leaf	10 (21.7%)
Pod	9 (19.6%)
Lichen	6 (13%)
Bark	3 (6.5%)

Discussion

C. magnifica is less rare in Belgium than previously thought Due to the activity of the amateur entomologists of the Coccinula working group, new populations of *C. magnifica* were found in many different natural districts of the country. However, the majority of the new records were made in the Campine. This is probably the result of an increased sampling effort in that Region but also to the high densities of *F. rufa s.l.* (Dekoninck *et al.*, 2003). The Campine offers lot of thermophilous habitats due to its sandy soil, where clear pine forest are often planted. Such conditions appears favourable to *C. magnifica.* The populations of *C. magnifica* have always been found in association with ants, most often with *Formica rufa s.l.* On the contrary, there were no *C. magnifica* in Baudour and Treignes despite the existence of ant populations in clear forests and fallow clear cuttings. From these observations, it appears that its habitats is therefore situated in woodlands and along edges.
We observed mutualistic interactions between aphids and sometime psyllids, ants and *C. magnifica* in every vegetation layers. This indicates that the ladybird is a generalist forager. We could not investigate the occurrence of *C. magnifica* in the tree canopy but we often saw adults climbing tree trunks following ant trails leading to the canopy where they probably found food resources.

At Chanly, our study site was largely colonized by brooms infested by ant attended colonies of *A. sarothamni*. This food resource was present from early June to September and reached its highest density in August. At that time, there were 1,000 to 3,000 individuals per plant.
Mating adults, eggs, larvae and freshly emerged adults were observed on brooms. Therefore, the aphid *A. sarothamni* is suitable food resource even if it is known to contain toxic alkaloids (Witte et al., 1990; Pasteels, 2007). This resource was not the only one suitable for the reproduction of *C. magnifica.* Larvae were also observed on nettles and various grasses such as *Holcus lanatus* L., *Dactylis glomerata* L. and *Molinia caerulea* (L.) Moench. All together in Chanly, the adults were observed feeding on about 10 other aphid species living on: *Acer pseudoplatanus, B. pendula, C. monogyna, Heracleum sphondylium, Picea abies, Pinus sylvestris, Populus canescens, P. spinosa* and *Q. robur*. These new records are in agreement with the statement that *C. magnifica* is a generalist predator geographically restricted to areas dominated by *F. rufa s.l.* ants (Sloggett & Majerus, 2000).

Compared to an non-myrmecophilous ladybird beetle, *C. magnifica* lays its eggs further from aphids colonies. One can hypothesized that this is an adaptation to avoid the destruction of egg batches due to the ant aggressiveness. A cost is probably entailed to such an adaptation but it is probably counterbalanced by the abundance and the stability of the food resources for which *C. magnifica* does suffer from the competition of other specialized aphidophagous predators.

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ARTICLE II: Reaction of ants to, and feeding biology of a congeneric myrmecophilous and non-myrmecophilous ladybird

Godeau, J.-F., Hemptinne, J.-L. and Verhaeghe, J.-C.

Introduction

Some species of ants occasionally feed on aphids but more usually they tend them and collect the honeydew they produce (e.g. Nixon, 1951; Way, 1963; Skinner, 1980; Morales, 2000). The majority of Coccinellid species feed on either aphids or coccids during their larval development and as adults and show various degrees of prey specificity (e.g. Iablokoff-Khnzorian, 1982; Hodek & Honek, 1996; Majerus, 1994). Therefore, ants and ladybirds sometimes compete for the same resources. Studies on the interaction between aphids, ants and ladybirds are rare (review in Majerus et al., 2007). Among the 6000 species in the Family Coccinellidae (Slipinsky, 2007), only 7 are considered to be true myrmecophiles and 4 others are thought to live in association with ants. Each of these ladybird species has developed a specific type of association with ants, which allows them to exploit the same resource as the ant (Majerus et al., 2007). Both the adults and larvae of myrmecophilous ladybirds feed on aphids, which normally benefit from the protection of ants (Völkl, 1995; Majerus, 1989).

In Western Europe, there are two myrmecophilous ladybirds. *Platynaspis luteorubra* Goeze, which measures 3.03 mm, is associated with small ants, mainly *Lasius niger* L., most often on thistle (*Cirsium arvense* (L.) Scop.) or tansy (*Tanacetum vulgare* L.) and is morphologically well differentiated from its close relatives (Völkl, 1995). The second species, *Coccinella magnifica* Redtenbacher, is much bigger (6.71 mm) and is only found in the immediate vicinity of *Formica rufa* L. and *F. polyctena* Förster nests in Great-Britain and Belgium. It is very similar in appearance to *Coccinella septempunctata* L., which is a generalist predator found in a wide range of habitats but not associated with ants. These two species of *Coccinella* constitute therefore a good model for investigating the mechanisms by which *C. magnifica* has adapted to living in close association with ants (Majerus, 1989; Majerus et al., 2007).

Unlike *C. septempunctata*, *C. magnifica* is only found foraging in the territories of *F. polyctena* colonies (Majerus, 1994, Sloggett & Majerus, 2000a). This constraint on their distribution is probably counterbalanced by *C. magnifica* out-competing other ladybirds in ant-foraged habitats. Whether this conforms to the Enemy Free Space hypothesis (Jeffries & Lawton, 1984) has not been tested experimentally (Sloggett et al., 2004). Another benefit might be a reduced risk of intraguild predation (Bristow, 1983; Breton & Addicott, 1992; Bishop & Bristow, 2003) because *C. magnifica* is the only ladybird present in these ant-protected areas (Majerus, 1989; Sloggett et Majerus, 2000b, Godeau *et al.*, in prep). However, this also remains to be tested.

Colonies of aphids tended by ants are generally larger, have a higher growth rate and last longer because winged forms appear and disperse later (e.g. El-Ziady & Kennedy, 1956; Banks, 1962; Way, 1963; Kleinjan & Mittler, 1975; Addicott, 1979; Bristow, 1984; Völkl, 1992; Flatt & Weisser, 2000; Sloggett & Majerus 2000b). It is suggested that the positive impact of ants on aphid colonies is the driving force in the evolution of myrmecophily in ladybirds (Sloggett & Majerus 2000a). Nevertheless, even in these rich food patches, *C. magnifica* still has to cope with or avoid inciting attack by the ants protecting these resources.

Species of ants belonging to the genus *Lasius* and *Formica* are the most common ants attending aphids in Europe (Hölldobler & Wilson, 1990). They do not sting but can bite or spray poisonous or acidic chemicals (Hölldobler & Wilson, 1990). Ants use their mandibles to kill eggs and larvae, which are easily seized and bitten (Bradley, 1973; Sloggett & Majerus, 2003). Adults, however, can easily escape ant attack by ducking down or dropping off a plant (Bradley, 1973; Sloggett *et al.*, 1998). Adult ladybirds are thought to be able to withstand attacks by ants thanks to their helmet shape and smooth dorsal surface. When molested, ladybirds may also reflex bleed. The substances contained in this exudate are repulsive or distasteful to ants (Happ & Eisner, 1961; Pasteels *et al.*, 1973, Holloway *et al.*, 1991).

Adults and larvae of *C. magnifica* are less frequently attacked by *Formica* workers than are those of *C. septempunctata* (Sloggett et al., 1998; Sloggett & Majerus, 2003) and unlike this species *C. magnifica* is more likely to remain in the vicinity of ant-tended colonies than to fly away (Sloggett & Majerus, 1998). This suggests that myrmecophilous species have evolved chemical and/or behavioural adaptations for living with ants. For example, chemicals on the body surface or in the droplets of haemolymph produced by *C. magnifica* when it reflex bleeds might reduce the aggressiveness of ants toward this species of ladybird. Despite the indications that *C. magnifica* is adaptated to living in aphid colonies tended by ants, the mechanism(s) by which they achieve this are unknown.

The objective of this study was to determine how *C. magnifica,* unlike *C. septempunctata,* is able to successfully coexist with ants. The foraging behaviour of these ladybirds in food patches in the presence of ants was studied in the laboratory, in particular (a) the intensity of aggression shown by ants to the eggs, larvae and adults of both species and (b) the impact of ants on the foraging efficiency of these ladybirds. The suitability of some non-myrmecophilous, facultative and obligate myrmecophilous aphid species as prey for *C. magnifica* and *C. septempunctata* was also investigated.

Material and methods

The ant colony

A *Formica polyctena* nest was collected from the field in Belgium (Mons) and maintained for five years in the laboratory (20 ± 1°C, LD 16:8) in a large plastic container (70*50*50 cm). This fully-functional nest contained several hundred workers and queens and produced brood every year. Wooden bridges linked the nest to foraging trays where the workers had access to dead cockroaches and to several glass tubes plugged with cotton wool soaked in a sugary solution. Fresh cockroaches and supplies of sugar were provided every week. Other bridges connected the nest to garbage and experiment trays. A large strip of Fluon® was painted around the rim of the nest container and the various trays to prevent ants from escaping.

Ladybird culture

Adults of *C. magnifica* and *C. septempunctata* were collected in the field and used to set up two laboratory cultures. They consisted of adults kept at 20 ± 1°C and under LD conditions of 16:8h, in 5-liter plastic boxes, which contained a piece of corrugated filter paper on which the females laid eggs. Three time a week the ladybirds were fed an excess of pea aphids, *Acyrthosiphon pisum* Harris reared on broad beans, *Vicia faba* L. Two stems of broad bean were added to each box to improve the survival of the aphids. Eggs were taken from the stock culture and incubated in 175-cm^3 plastic boxes kept under the same conditions as the stock culture. After hatching the larvae were fed three times a week with an excess of pea aphids until pupation.

Experiment 1 : Response of ants to ladybird eggs and larvae

Strips of corrugated filter paper with batches of *C. septempunctata* eggs were taken from the stock culture. The filter paper was carefully cut around the egg batches with fine scissors. Then, a batch of eggs was stuck to the bottom of an ant-foraging tray with a piece of adhesive tape. The ants were then given access to this tray and the number of times they palpated the eggs with their antennae and bit the eggs was counted over a period of 2 minutes. The number of antennal contacts with eggs was recorded in order to estimate the level of ant activity. This was repeated 10 times. The same experiment was then repeated 10 times using *C. magnifica* eggs.

A first instar larva of *C. septempunctata* was taken from the stock culture, put on a piece of filter paper and gently deposited in the experimental tray. Then, the ants were given access to the tray. The number of time the ants bit the larvae was recorded over a period of 5 minutes. This was repeated 10 times. The same experiment was then performed using 15 second - third and 15 fourth instar larvae. Larvae of *C. magnifica* were then similarly treated. The number of times the ants palpated and bit the eggs and larvae of the two species of ladybirds were compared using non-parametric Mann-Whitney tests.

Experiment 2 : Response of ants to ladybird adults

An adult of *C. septempunctata* was taken from the stock culture and placed on an ant foraging trail on an experimental tray. Care was taken not to stress the ladybird and so avoid reflex bleeding. Four categories of interaction were observed, ranging from low to high aggressiveness :

Level 0 (very low aggressiveness) = ants approach, walk, self groom, interact with another worker and ignore the ladybird ; level 1 = ants palpate the ladybird with their antennae or their labial mouthparts ; level 2 = ants try to bite the ladybird and simultaneously often straddle the ladybird ; level 3 (high aggressiveness) = ants bite the ladybird, bend their abdomen and spray acid or pull the beetle after biting. The interactions between the ants and the ladybird were observed for 10 minutes and the number of each type of interaction counted. This was repeated 10 times with *C. septempunctata* and the results pooled. Ten adult *C. magnifica* were then similarly treated. The frequencies of the four types of interactions recorded for both species of ladybird were compared using a G-test (Sokal & Rohlf, 1995).

Experiment 3: Predation efficiency of ladybird adults in the presence and absence of ants

Two saplings of *B. pendula*, measuring about 1.5 m, were dug up from a plot of land colonized by birches in autumn 2001 and planted individually in 30 cm diameter pots filled with standard compost. These saplings were kept outside during winter. The next spring they were placed in the laboratory; wooden bridges connected the pots to the nest of *F. polyctena*. In April 2002, 10 twigs of birch infested with small colonies (< 50 individuals) of *S. oblongus* were collected from the field and transferred to the laboratory. Aphids readily moved from the twigs to branches of the potted birches and formed new colonies. More than ten aphid colonies were present on each sapling in July 2002 when they were used for the experiments on predation efficiency.

An adult of *C. septempunctata* was taken from the stock culture and kept for 24 h without food in a Petri dish. At the end of this period it was gently transferred to the base of a birch branch on which ants were foraging. The number of aphids eaten by the ladybird, the predation success (= aphid eaten / aphid attacked) and the number of aphids leaving the colonies attacked by ladybirds were recorded over a period of 10 minutes. At the same time it was also noted whether the ladybird ducked down, reflex bled or fled when they encountered ants. This was repeated 17 times and a new aphid colony used for each replicate. During the

10 first replicates of this experiment, the aggressive behaviour of the ants was also recorded using the four categories defined in experiment 2. Seventeen other adult *C. septempunctata* were similarly treated, except that ants were excluded by a ring of glue round, which was placed round the base of the branch 24 h prior to the experiment.

The behaviour of 20 adults of *C. magnifica* were similarly recorded on birch saplings with and without ants.

The number of aphids eaten, capture efficiency and the number of aphids dislodged by the ladybirds were analyzed using two way ANOVA. Prior to these analyses, the data were respectively log, arcsin and square-root transformed to normalize the data and equate the variances. The number of times the ladybirds ducked down, reflex bled or flew away when encountered by ants were log transformed prior to being analyzed using a two way ANOVA followed by post-hoc Tukey tests (Sokal & Rohlf, 1995).

Experiment 4 : Survival of ladybird larvae fed on different species of aphids

The ability of the larvae of *C. magnifica* and *C. septempunctata* to survive and develop when fed ant-attended and non-attended aphids was investigated in the laboratory in the absence of ants. Two non-myrmecophilous aphids were used: *Metopolophium dirhodum* (Walker) collected in the field from *Arrhenaterum elatius* (L.) Beauv. ex. J. et C. Presl. and *A. pisum* reared on *Vicia faba* ; one toxic and facultatively ant-attended aphid (*Aphis jacobaeae* Schrank) collected in the field from *Senecio inaequidens* DC. where it was attended by *Lasius niger* L. (Witte et al., 1990; Pasteels, 2007) and an aphid that is always attended by ants (*Symydobius oblongus* (Von Heyden)) was also used. The later species was reared on potted *Betula pendula* L. to which ants from the laboratory nest had access (see below). Immediately after hatching, 20 first instar larvae of *C. magnifica* were fed *M. dirhodum* ad libitum until they pupated. The number of larvae surviving was recorded after each moult. Ten first instar larvae were similarly reared on *A. pisum*, 12 on *S. oblongus* and 15 on *A. jacobaeae*. This experiment was repeated with 10, 5 and 9 first instar larvae of *C. septempunctata* respectively fed *A. pisum*, *S. oblongus* and *A. jacobaeae*. The survival of the larvae of both species was compared using a Kolmogorov-Smirnov two-sample test (Sokal & Rohlf, 1995).

Results

Experiment 1: Response of ants to ladybird eggs and larvae

C. magnifica eggs were bitten significantly less often than those of *C. septempunctata*, even though the ants displayed the same level of activity in the foraging trays during the experiments on both these ladybird species (Table 1).

Similarly, *C. magnifica* larvae were bitten significantly less often than those of *C. septempunctata* (Table 1).

Table 1. The mean number of times *F. polyctena* workers palpated with their antennae (upper row) and bit the immature stages (other rows) of *C. septempunctata* and *C. magnifica*. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Stage	N	*C. magnifica*	*C. septempunctata*	Mann-Whitney (W)	P-value
Egg cluster (No. of palpations)	*10*	*29.5*	*28.7*	*100*	*0.7337 N. S.*
Egg cluster	10	2.5	11.6	154.5	0.0002 **
Instar 1	10	1.3	4.4	136	0.0184 *
Instar 2 and 3	15	4.2	12.8	326.5	0.0001 ***
Instar 4	15	4.5	8.3	290.5	0.0166 *

Experiment 2: Response of ants to ladybird adults

The ants responded to the adults of both species of *Coccinella* similarly. The most aggressive types of interaction were rather rare and *C. septempunctata* was not subjected to more attacks than *C. magnifica* (G = 2.6; DF = 3; N.S.; Fig. 1).

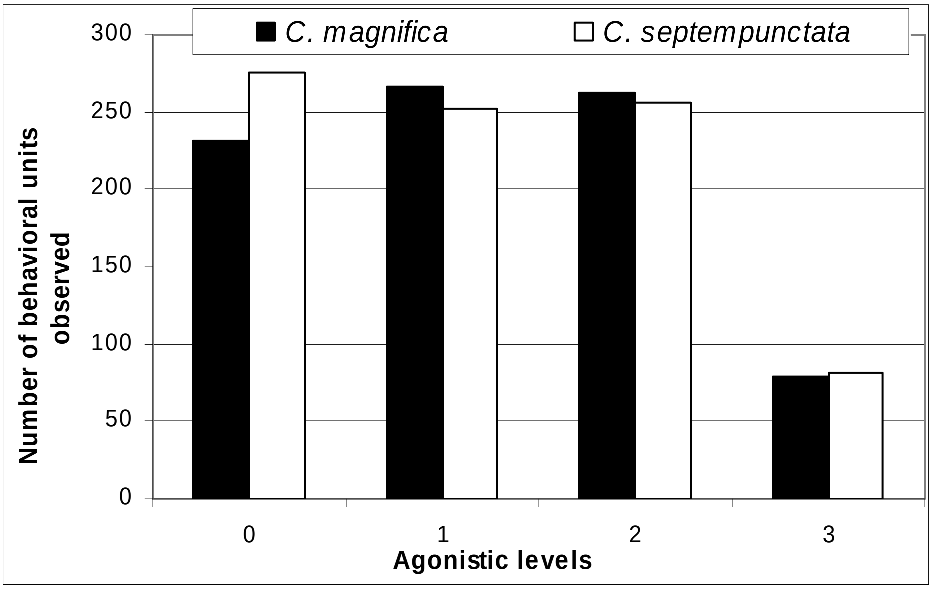

Fig. 1. The incidence of aggressive behaviour of increasing aggressiveness displayed by *F. polyctena* workers towards *C. magnifica* or *C. septempunctata* adults.

Experiment 3: Predation efficiency of ladybird adults in the presence and absence of ants

Ladybird predation

In terms of the number of aphids eaten, predation success and number of aphids escaping from ladybirds, there were no influence of the presence of ants on the differences between the two species of ladybird (ANOVA interaction of ant presence*ladybird species: number of aphids eaten : $F_{[1,70]} = 0.04$; P = 0.848; predation success : $F_{[1,70]} = 3.10$; P = 0.083; number of aphids escaping : $F_{[1,70]} = 4.07$; P = 0.051)

For both species of ladybirds, the presence of the ants resulted in a significant decrease in the number of aphids eaten and of aphids leaving colonies attacked by ladybirds compared to when ants were absent (number of aphids eaten : $F_{[1,70]} = 9.97$; P = 0.002; number of aphids leaving the colony : $F_{[1,70]} = 8.48$; P = 0.006). *C. magnifica* always ate more aphids and had a higher predation success than *C. septempunctata* (Fig. 2). The difference in the performance of *C. magnifica* and *C. septempunctata* increased and became more significant when ants were present (number of aphids eaten: Tukey comparison : T = -2.725; P = 0.0396; predation success : T = -4.49; P = 0.0002).

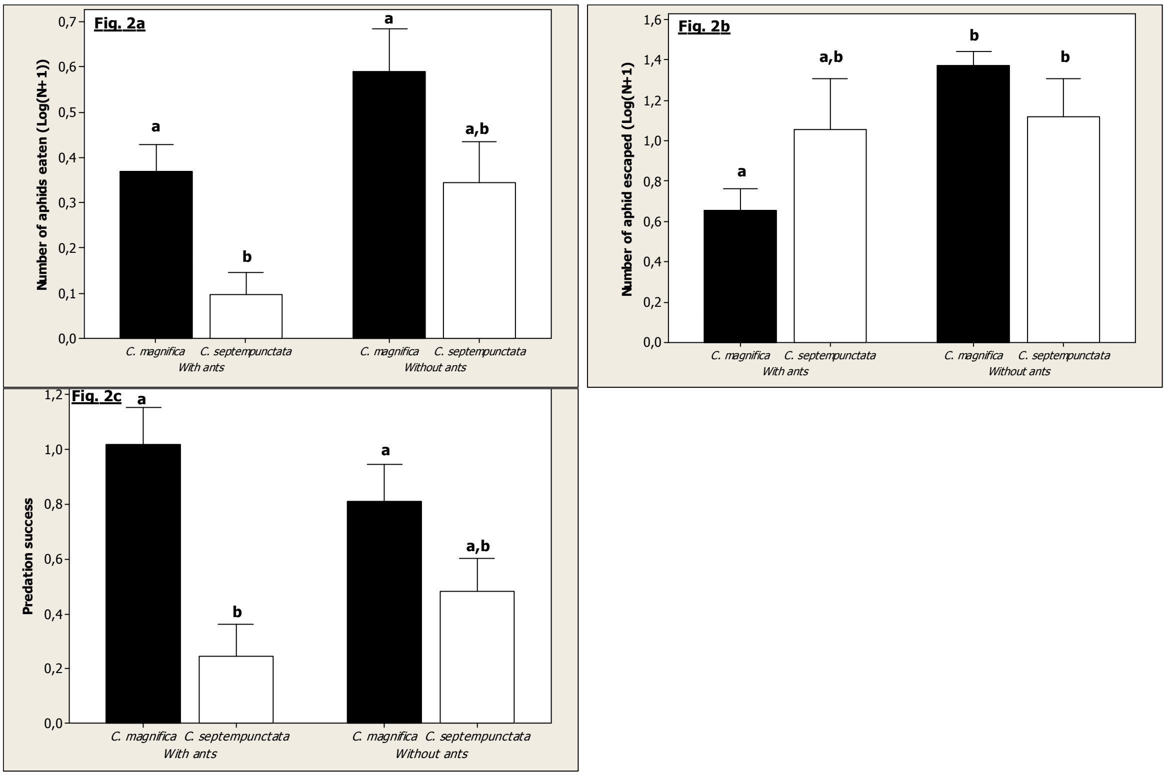

Fig. 2 a, b and c. The mean number(+ 1 SE) of aphids eaten (a) and escaped(b), and the mean value of the predation success (c). Bars with the same letter are not statistically different at P = 0.05 (Tukey post-hoc comparisons).

Ladybird behaviour

The two ladybirds differed significantly in their reactions to ant attack (interaction : $F_{[2,99]}$ = 15.72; P = 0.000; Fig. 3). The most common reaction of *C. septempunctata* was to move away from ants, which they did significantly more often than *C. magnifica* (T = -5.47; P ~ 0; Fig. 3). When attacked by ants *C. magnifica* tended to stay in or next to an aphid colony and duck down more often than *C. septempunctata* (T = 2.32; P = 0.19; Fig. 3). Reflex bleeding occurred less frequently than either ducking down (T = -3.04; P = 0.0084) or moving away (T = -5.83; P ~ 0) and to a similar extent in both ladybirds (T = -0.323; P = 0.99; Fig. 3). As a consequence *C. magnifica* on average spent significantly more time in an aphid patch than *C. septempunctata* (496 versus 114.5 seconds; W = 60; P = 0.0008).

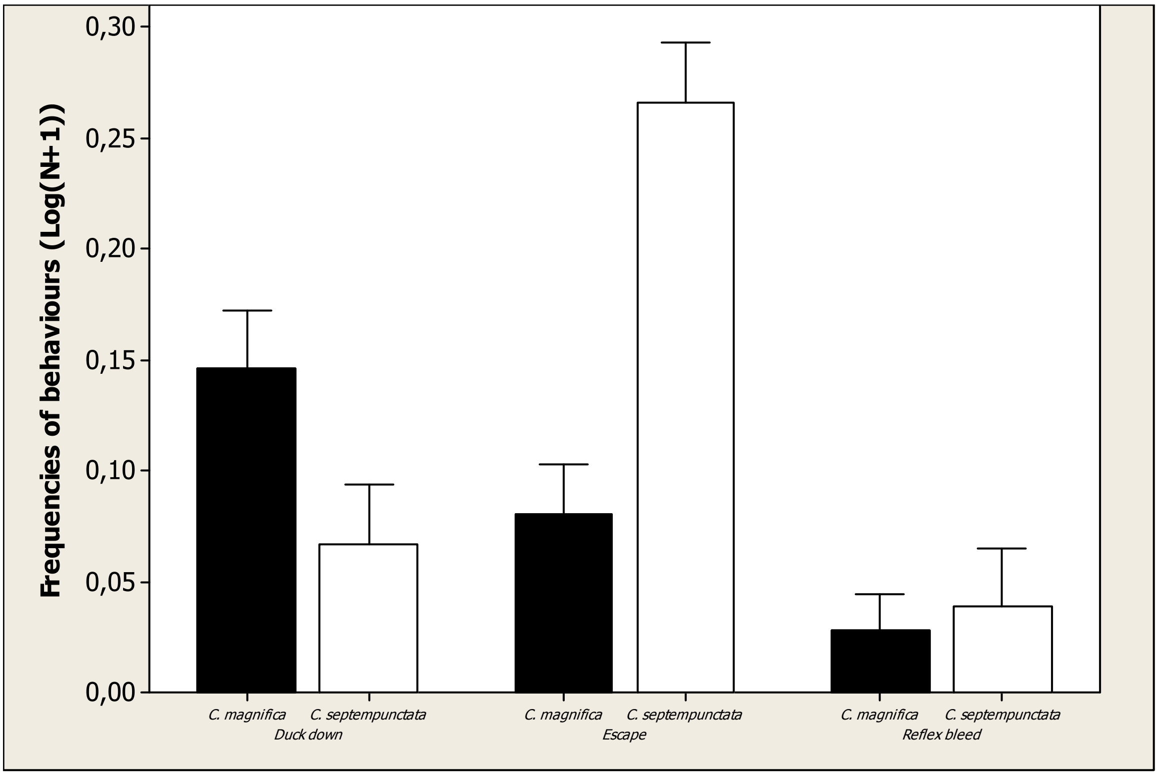

Fig 3. Ladybird behaviour when attacked by ants defending *Symydobius oblongus* colonies on a potted *Betula pendula*.

Ant behaviour

The response of ants to both ladybirds was similar. They showed a low level of aggression ('Contact' and 'Biting'), which rarely involved biting (Table 2).

Table 2. The incidence of different levels of aggression exhibited by ants encountering ladybirds in or close to an aphid colony.

Level of aggression	*C. magnifica*	*C. septempunctata*[1]
Contact (Level 1)	**24,74**	**42,11**
Biting (Level 2)	**48,97**	**39,64**
Bite elytra (Level 3)	**9,90**	**6,79**
Bite leg (Level 3)	**2,51**	**0,00**
Abdomen bending (Level 3)	**13,88**	**11,46**

[1]: The number of replicates was 9 for *C. septempunctata* because on one occasion no ants were encountered.

Experiment 4: Survival of ladybird larvae fed different species of aphids

The larvae of both species of *Coccinella* developed and survived equally well on a diet of *A. pisum*, *M. dirhodum* and *S. oblongus*. *A. jacobaeae* was a less suitable prey for *C. septempunctata* and a highly toxic prey for *C. magnifica*. That is, both species of ladybird survive equally well when fed on *A. pisum* and the myrmecophilous *S. oblongus*. *C. septempunctata* survives poorly but surprisingly better than *C. magnifica* when fed on the myrmecophilous *A. jacobaeae* in the absence of ants (Table 3).

Table 3. The survival of the different larval instars of *C. magnifica* and *C. septempunctata* fed different species of aphid.

Aphid species	*Metopolophium dirhodum*	*Acyrthosiphon pisum*		*Symydobius oblongus*		*Aphis jacobaeae*	
Ladybird species	*C. magnifica*	*C. septempunctata*	*C. magnifica*	*C. septempunctata*	*C. magnifica*	*C. septempunctata*	*C. magnifica*
Instar 1	100%	100%	100%	100%	100%	100%	100%
Instar 2	95%	100%	100%	100%	100%	100%	100%
Instar 3	90%	100%	100%	100%	100%	100%	33,3%
Instar 4	90%	100%	100%	100%	100%	88,9%	0%
N=	20	10	10	5	12	9	15

Discussion

The results of the experiments in which ladybird eggs, larvae and adults and ants were presented to ants suggest that two different but complementary mechanisms account for the relative immunity of *C. magnifica* from *F. polyctena* attack. This ladybird may be protected chemically and/or derive some protection from its behaviour.

The eggs and larvae of ladybirds are easily killed by ants biting them. Therefore, it would be adaptative if these instars of *C. magnifica* were protected by cuticular chemicals that reduced ant aggression. When placed on trails followed by ants in the laboratory, eggs and larvae of *C. magnifica* were less frequently attacked than those of *C. septempunctata*. The later were often destroyed by ants. That is, workers of *F. polyctena* are able to distinguish between the immature instars of these two ladybirds. Cuticular hydrocarbons are a good candidate as they are known to play a role in the communication between ladybirds (Hemptinne *et al.*, 1996 and 1998). Moreover, nestmate recognition in ants is mediated by cuticular hydrocarbons and fatty acids (Dettner and Liepert, 1994; Lenoir *et al.*, 2001) and the hydrocarbon profiles of myrmecophilous guests are similar to those of their host ants (Vander Meer and Wojcik, 1982; Akino, 2002; Orivel *et al.*, 2004).

The results indicate that *C. magnifica* larvae survived equally well to the fourth instar and adult stage (in prep.) when fed non-myrmecophilous (*M. dirhodum* and *A. pisum*) and an obligatory myrmecophilous aphid (*S. oblongus*). Thus *C. magnifica* is not a strongly specialized in terms of prey specificity, which is consistent with its large set of preferred prey (Sloggett *et al.*, 2002). Our results indicate that *C. magnifica* survived less well than *C. septempunctata* when fed *A. jacobaeae*. The latter species survives and sequesters pyrrolizidine alkaloids when it feeds on this toxic aphid (Witte *et al.*, 1990), whereas none of the larvae of *C. magnifica* survived to the fourth instar. Thus the *C. magnifica* we used appear poorly adapted to feed on some myrmecophilous aphids. This is not in accord with the results of our field study in which *C. magnifica* is recorded as mainly exploiting the myrmecophilous *Aphis sarothamni* (Franss.), an aphid that contains quinolizidine alkaloids (in prep). This natural population probably expressed a phenotypic adaptation allowing the metabolism of toxic chemicals as is the case with *A. bipunctata* eating *Aphis fabae* Scopoli (Rana *et al.*, 2002).

On the other hand, adults of both *C. magnifica* and *C. septempunctata* are attacked when dropped on ant nests (Donisthorpe, 1920a and 1920b), on foraging trails and on ant attended aphid colonies in the field (Sloggett et al., 1998; Sloggett & Majerus, 2003). In our experiments in the laboratory, *C. magnifica* provokes weaker aggression than *C. septempunctata*. These results also suggest that ant workers can distinguish between the two species of ladybird by tactile contact. The experiment using potted birches indicates another aspect of the problem. *C. septempunctata* has a natural tendency to leave aphid patches when it is attacked by ants whereas *C. magnifica* tended to assume a defensive position and to stay longer in ant attended aphid colonies. The adults frequently ducked down and were seen to move their elytra to shield themselves and fend off ant attacks. *C. magnifica* can even continue eating an aphid when it crouches closely against the substrate or when ants try to bite it (Pers. obs.; Sloggett et al., 1998). Sometimes *C. magnifica* grasps an aphid and retreats some distance from the colony to escape ant attacks (Pers. obs.; Sloggett & Majerus 2003). This behaviour is not only known for *C. magnifica* but is also proposed as the mechanism by which *C. septempunctata* is able to reduce aphid disturbance when foraging on *Centaurea sp.* (Stadler, 1991). Therefore, even though adult chemical defence probably plays a role in the protection of *C. magnifica* from ant aggression, its behaviour also allows this ladybird to forage in the territories of *F. rufa* and *F. polyctena*.

It is necessary to know more than it can coexist with ants to understand how *C. magnifica* populations develop successfully in ant-dominated areas. It remains to be shown that this ladybird forages optimally. The predation efficiency of *C. magnifica* and *C. septempunctata* foraging on potted birches in the absence of ants differed with the former species eating more aphids than the later. This advantage of *C. magnifica* is greater when ants have access to the birches. *C. magnifica* also disturbed the aphids less in ant-attended colonies. As ladybirds often experience difficulties in eating aphids in the presence of ants it is not surprising that *C. septempunctata* and other ladybird species are rarely found with *C. magnifica* in the field.
The results of the laboratory experiments reported here indicate that ants have a negative effect on ladybird foraging success, as is recorded for the hyperparasitoid *Alloxysta brevis* (Thomson) (Hübner, 2000). The number of aphids eaten by both ladybirds decreased when ants were present. Probably, the ants disturbed these ladybirds and therefore increased their prey handling time. *C. magnifica* stayed in ant attended aphid colonies, whereas *C. septempunctata* left when ants were present. This difference in behaviour enables *C. magnifica* to exploit a rich source of food, which is not available to many other aphidophagous insects.

The degree of protection of mutualistic Hemiptera by ants is highly variable in space and time, depending on the densities of the colonies (Breton & Addicott, 1992; Bishop & Bristow, 2003) or honeydew composition (Völkl *et al.*, 1999; Fisher *et al.*, 2002; Woodring *et al.*, 2004). Depending on the ecological parameters, an ant-attended species can benefit from both a high and low level of protection by ants. Similarly, two species of aphid can compete for ant protection in the same habitat yet be unequally protected because of their species specific characteristics. Mutualistic interactions therefore are very variable, which could explain the diversity of ant-associated mutualists' predators.
The two myrmecophilous ladybirds, *C. magnifica* and *P. luteorubra,* strongly differ in prey specificity, with the former a generalist (like *C. septempunctata*) but restricted to particular habitats, and the latter is oligophagous feeding on aphids infesting common plants like

C. arvense or *T. vulgare*. Myrmecophily is likely to have evolved independently several times in the Coccinellidae and as a result is likely to take different forms. Comparisons with other myrmecophilous Hemiptera-eaters are needed for a better understanding of these complex interactions.

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ARTICLE III : Ant trail : a highway for *Coccinella. magnifica* Redtenbacher (Coleoptera: Coccinellidae)

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Abstract : Red Wood Ant species chemically mark .trails connecting their nest to aphid colonies. The trail pheromone are produced in the ant's hindgut. The large trails are maintained thanks to visual clue and because new pheromone marks are regularly laid down. As a consequence, Red Wood Ant's workers create a network of trails equivalent to hundreds of meters around every nest mound. *Coccinella magnifica* is an aphidophagous ladybird species living only in the vicinity of Red Wood ant's nests. It preys upon aphid colonies regardless of ant's presence. We show here that, in the laboratory, *C. magnifica* can follow a recruitment trail laid down by *Formica polyctena* under artificial conditions with its hindgut's pheromone. This is confirmed by field observations of ladybirds walking along natural Red Wood Ant's trails. As *C. magnifica* appears to be a specialist, this behaviour is adaptive to efficiently locate prey.

INTRODUCTION

In most temperate forests, lives a well-known ant species : the red wood ant (*Formica rufa sensu lato*) which is a taxonomical complex including some ecological species separated by their habitat preferences (YARROW 1955; SEIFERT 1996). It lives in large colonies whose nests are built as a hillock of dead plant fragments. This mound can reach 1.5 to 2 meters high and shelter hundreds of thousands ant individuals (HÖLLDOBLER & WILSON 1990). The other striking aspect of that ant are their wide foraging trails, linking the nest to food resources scattered in the surrounding (SKINNER 1980). Trails are marked by workers which uses chemical pheromones probably produced in the hindgut and in other glands (GABBA & PAVAN 1970; PARRY & MORGAN 1979; ATTYGALE & MORGAN 1984). Moreover, visual cues have been demonstrated to play an important role in red wood ants orientation.

Ants are largely known as invertebrate predators but they also gather honeydew and occasionally prey upon aphids dwelling in trees and shrubs (e.g. WAY 1963; SKINNER 1980).

What are the relationships between ants, aphids and aphid predators?

Since more than half a century, it is largely admitted that ants increase attended aphid populations by cleaning the honeydew droplets glued on the colony and by protecting them against intruders, especially ladybirds, lacewings, bugs and syrphids (e.g. BANKS 1962; WAY 1963; HÖLLDOBLER & WILSON 1990).

Ladybirds found in red wood ant’s habitat are mainly woodland-specialised species whose adults and larvae feed on tree aphids. In comparison with open areas, aphid resources are more dispersed vertically in the forest vegetation stages. All these hotspots of attended aphids are scattered in a three-dimension maze of branches, which create a more complex situation for food location than a two-dimensions system in herbaceous vegetation. Some ladybirds species encountered in woodland are: *Myzia oblongoguttata* L. in deciduous ones, or *Myrrha octodecimguttata* L. in pine forests, or *Coccinella magnifica* Redtenbacher in mixed forests, which is morphologically very similar to *Coccinella septempunctata* L. but scarcer because of its localised distribution (MAJERUS 1994). Some of these species are occasionally observed on ants trails, being brought back to the nest. Field observations revealed that *M. oblongoguttata* and *Anatis ocellata* L. are sometimes observed as prey, carried by *F. rufa*

L. on foraging trails (SLOGGETT et al., 1999). In that cases, ladybirds have been killed by ants or found already dead. *C. magnifica* is the only species usually observed alive on trails, walking among ant workers (SLOGGETT et al., 1999; pers. obs.). This species is known to live around *F. rufa*'s colonies (DONISTHORPE 1920a and b; MAJERUS 1989), it is suspected to benefit from ant-attended colonies, by preying upon it, in spite of the ant aggressive behaviour.

C. magnifica has evolved as a specialised predator, adapted to avoid ants aggressiveness. Though, we ignore whether this apparent immunity is due to the use of appeasement chemicals produced by the ladybird or only to behavioural adaptations. Since it is clearly established that adults and larvae of *C. magnifica* eat attended aphids, there is no information attesting that it also follows ants trails to enhance its food location efficiency. If it was the case, this adaptation, combined with an apparent immunity toward ants, could represent a strong selective advantage.

We want to test the fact that *C. magnifica* is found along *F. rufa*'s trails not as a prey but as an intruder.

We report here the results of two experiments using a *Formica polyctena* Förester colony kept in laboratory and two specie of ladybirds, *C. magnifica* and *C. septempunctata* .

MATERIAL AND METHODS

Tests with a naturally-laid trail

In the first experiment, we used a naturally-laid ant trail which is obtained as follows. A nest is connected by a bridge to an area with a sugar solution. When the recruitment of workers was important, a cardboard sheet, on which a grid was traced with a pencil, was place on the bridge (shaded squares on Figure 1). Only a row of squares was accessible to the ants, the others were covered with two smaller cardboard sheets. Workers walked for two hours on this row when travelling back and forth between the nest and the sugary solution. Then, the cardboard sheet was uncovered and deposited in an arena where the tests were performed with *C. magnifica* adults.

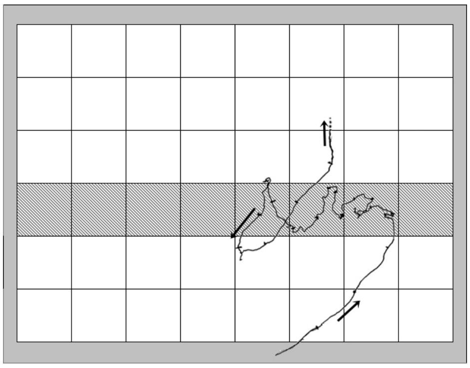

Fig. 1. Cardboard sheet on which a row of squares (shaded) have been marked by trail pheromones laid by foraging ants. An sample of a path walked by a ladybird is figured.

Marked and non-marked squares on which the ladybird walked were counted during two minutes. The comparison between ladybird's path and the rows of marked square was obtained by using a similarity index SI (1) (Verhaeghe 1982), indicating if there is a preference for marked squares :

$$\text{S.I.} = \frac{Nc^2}{Na \times Nb} \qquad (1)$$

where Nc = number of marked squares crossed by the ladybird, Na = total number of marked squares and Nb = total number of squares crossed by the ladybird.

Occupation time of each square was also measured as an estimation of walk speed.

Each adult was firstly tested with a marked cardboard and then presented with an unmarked cardboard. There were 9 replicates with the ant trails and 8 with a clean cardboard.

Tests with an artificial circular trail

In the second experiment, we aimed at confirming our first results and obtaining more precise information about the nature of the observed ant-trail. We made an artificial circular trail, by dissecting ants, extracting the gland which produces the trail pheromones. Ant workers were collected on the foraging trails from the laboratory nest and dissected one by one under water, with ophthalmologic dissection tools. When one hindgut was isolated, it was transferred to 75 ml water and crushed. The results of five dissections were gathered and deposited on a strong filter paper with a Stadler® metallic pen. This method, described by PASTEELS and VERHAEGHE (1974), has already been used in numerous trail-following experiments with ants and other invertebrates (CAMMAERTS et al. 1990; LENOIR et al. 1992; QUINET & PASTEELS 1995).

The 12-cm diameter circular trail on a filter paper was put in a circular arena of 25-cm in diameter and left for 10 minutes before the beginning of the tests. Two identical sequences of tests have been performed with the same trail: (1) four ants observed during two minutes; (2) four ladybirds (*C. magnifica* or *C. septempunctata*) during four minutes; (3) four ladybirds of the second species (*C. septempunctata* or *C. magnifica*) during four minutes; (4) four ants observed again during two minutes to check out whether the trail is still active). The first sequence took place 10 min after the trail has been laid down and the second 70 min later.

The artificial trail was compared to a control trail obtained with pure water. There were 5 replicates with the trail and 4 with the control.

All these observations were filmed and analysed afterward. For each sequence, the detection rate was measured and the “following score” was noted. The detection rate is the number of times an animal crossed the trail, stopped and eventually followed it. The “following score” is the number of 10° arcs consecutively followed by an insect. This score was log-transformed. Both the detection rates and the “following scores” are presented by their mean. The statistical difference between detection rates is obtained by comparing the proportion of defectives (Detection of trail or not) during all tests. The result is a binomial distribution on which we calculated a value for z (NIST/SEMATECH 2003), representing the significance level for the difference between the two proportions (test *vs* control). The differences between following scores were tested with a Median test.

RESULTS

Tests with a naturally-laid trail

As the trail is deposited by ants, we assume that the right pheromones are present on the cardboard sheet. In comparison with the control, *C. magnifica* walked more often ($\text{S.I.}_{\text{Test}}$= 0.4219 ; $\text{S.I.}_{\text{Control}}$= 0.0417; Mann-Whithney U = 109.0; P<0.01, Fig. 2) and slower (Median occupation time: marked squares: 2.313 sec; covered squares: 1.259 sec; Mann-Whithney U = 126.0, P< 0.001, Fig. 3) on trail-marked squares. These results are consistent with an olfactory detection and orientation of *C. magnifica* thanks to *F. polyctena* trail pheromones.

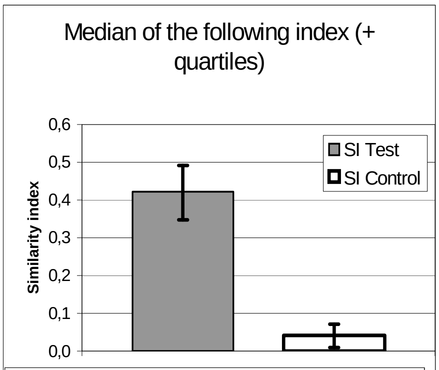

Fig. 2. Following index performed by C. magnifica *on a marked sheet (in grey) and on control (in white)*

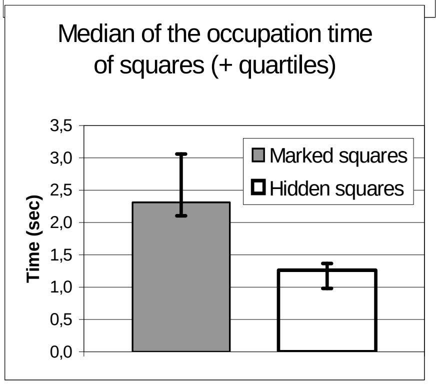

Fig. 3. Occupation time of squares, used as an estimation of walk speed on marked (in grey) and non-marked (in white) squares.

Tests with an artificial circular trail

Over the course of the experiments the quality of the trails deteriorated so that the detection rate of ant F5 was not different in the presence or absence of the pheromone (Table 1). *C. magnifica* show a strong tendency to respond positively to the pheromone and not to the control. On the contrary, the detection rate of *C. septempunctata* is not modified by ant trails. For all the insects tested, there was no difference between the “following scores” measured with and without pheromone.

Table 1. The detection rates and “following scores” of F. polyctena (F2, F3, F4 and F5), C. magnifica (CM_1 and CM_2) *and* C. septempunctata (C7_1 and C7_2) measured in two sequential experiments

Sequence		*Detection rate (%)*		
		Test (N=)	*Control (n=)*	*Z =*
Nr. 1	F2	60 (225)	45.99 (137)	4.28 (***)
	CM_1	45.78 (166)	36.14 (166)	2.58 (*)
	C7_1	56.25 (64)	51.19 (84)	0.81 (N.S.)
	F3	62.11 (190)	28.26 (92)	2.74 (**)
Nr. 2	F4	57.22 (180)	48.62 (181)	2.31 (*)
	CM_2	41.38 (145)	27.52 (109)	3.74 (***)
	C7_2	35.58 (104)	37.93 (58)	-0.61 (N.S.)
	F5	50.48 (198)	51.48 (154)	-0.61 (N.S.)

DISCUSSION

In the laboratory, C. magnifica, is able to follow a natural foraging trail delineated by pheromone spots.

Artificial trails made up of hindgut extracts are less efficient than natural trails. They however trigger detection and trail-following by ants and C. magnifica.

Our preliminary results might be confirmed by testing an extract of 10-hindguts instead of 5, expecting a response twice bigger with ants and *C. magnifica*.

Knowledge about pheromones produced by Dufour, poison and mandibular glands are quiet scant in the literature, so that we do not know their respective role in the formation of *F. rufa* foraging trails. Additional tests must be performed to check whether one or more of these glands could be used to enhance trail-following efficiency.

The fact that *C. magnifica* is able to follow ants trails is adaptive because these ladybirds are able to discover and exploit large aphid colonies attended by ants.

This study leads to another question : does the trail pheromone intervenes in mate encountering, selection of oviposition sites and egg laying ?

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ARTICLE IV: A comparison of the reproductive traits of a generalist and a specialist *Coccinella* species.

Jean-François Godeau and Jean-Louis Hemptinne

Introduction

The environmental conditions experienced by an organism are likely to influence its life history parameters, such as the achieved adult size or the reproductive rate. It is therefore challenging to investigate the influence of the habitat on the life histories trade-offs of comparable species. The ecological characteristics of an habitat, such as the period of time for which an organism has access to its preferred resources, leads to specific pressures which will influence the allocation of energy to various metabolic functions. In an habitat with a relative high cost of reproduction, a species can achieve similar fitness either by combining a high reproduction with a low growth or a low reproduction with a high growth. When the cost of reproduction is low, the resources are first allocated to growth and then to provide a short but intense bout of reproduction, usually followed by death (Begon et al., 1996). But an organism's life history is also constrained by the phylogenetical position it occupies. Hence, it can obscures our understanding of the habitat-characteristics trade-offs.

In predatory ladybirds, the two species *C. septempunctata* and *C. magnifica* constitute an interesting model. They belong to the same genus, they are sympatric, of the same size and look extremely similar. They are therefore believed to be closely related but they have contrasting habitat preferences (Sloggett & Majerus, 2000a). *C. septempunctata* is one of the commonest ladybird in temperate Palaearctic regions. It is a generalist predator present in most types of habitats especially those dominated by an herbaceous vegetation and it feeds on more than 30 species of prey (Iablokoff-Khnzorian, 1982, Hodek & Honek, 1996). Conversely, *C. magnifica* is one of the rarest species in the same regions. It is highly specialized to only live with the red wood ants (*Formica rufa s.l.* group) where it feeds on the aphids attended by those ants (Donisthorpe, 1920a and 1920b; Majerus, 1989; Sloggett & Majerus, 2000b).

The colonies of aphids tended by ants are generally larger, have a higher growth rate and last longer because winged forms appear and disperse later (e.g. El-Ziady & Kennedy, 1956; Way, 1963; Kleinjan & Mittler, 1975; Bristow, 1984; Flatt & Weisser, 2000). As *C. magnifica* can coexist with ants, it is considered to benefit from highly valuable aphid colonies, without the competition of other predators which avoid ants (Sloggett & Majerus 2000b). Nevertheless, *C. magnifica* is not totally ignored by the ants. The workers of red wood ants are very aggressive (Mabelis, 1984). When an adult of *C. magnifica* preys upon attended aphids, it is often attacked and is forced to escape (Sloggett et al., 1998; Godeau et al. in prep).

In the areas without the *F. rufa s.l.* species, the colonies of aphids last for shorter period of time. Therefore, *C. septempunctata* and the other competing aphid predators should develop fast enough to achieve their development before the depletion of the preys in the patch. Moreover, *C. septempunctata* is a big species which should probably arrive quite late in a developing aphid colony so that the aphid supplies are enough for its offspring.

Therefore, we suspect the two Coccinella species to show contrasted life history parameters. The females of all predatory ladybirds invest the same proportion of their biomass in gonads. Thus, there is a trade-off among the species between producing a lot of small eggs or less but bigger ones (Stewart et al., 1991). It is adaptative for specialized species to invest more energy in each egg because the probability of finding a suitable habitat is less than for the numerous habitats of generalists. Since *C. magnifica* is restricted to ant-influenced areas, we predict that it would lay eggs of bigger size than the generalist *C. septempunctata*. The latter would benefit to spread its eggs in many different aphid colonies as the risks will be shared between numerous places.

The aim of this paper is to test the prediction that the specialist species lays fewer but larger eggs than the generalist one. The actual effect of the eggs size on the growth rate should be also detected between the two Coccinella.

Material and methods

Ladybird culture

Adults of *C. magnifica* and *C. septempunctata* were collected in the field to set up two laboratory cultures. These consisted of a maximum of 40 adults reared at 20 ± 1°C, LD 16:8, in 5-liter plastic boxes, which contained a piece of corrugated filter paper on which the females laid eggs. Three time a week the ladybirds were fed an excess of pea aphids, *Acyrtosiphon pisum* Harris reared on broad beans, *Vicia faba* L. Two stems of broad bean were added to each box to improve the survival of the aphids. At the same time the piece of corrugated filter papers were changed and the eggs were taken from the stock culture and incubated in 175-cm^3 plastic boxes under the same conditions as the stock culture. After hatching the larvae were fed three times a week with an excess of pea aphids until pupation.

The Weight of the eggs and the first instar larvae

Seven batches of eggs of *C. magnifica* were taken from the ladybird culture. The pieces of filter paper on which they had been laid were moistened with distilled water. After a while the glue fixing those eggs dissolved and the eggs were gently separated using a fine paintbrush. The eggs were left to dry for one hour for drying before being weighted a Mettler microbalance to the nearest 0.01 mg. Six batches of *C. septempunctata* were similarly treated. The larvae from two egg clusters from the *C. magnifica* culture were weighed right upon emergence as were the larvae from on egg cluster of *C. septempunctata*. The mean weights were log-transformed before being compared with a Student T test.

The Reproductive rate

Twelve females of *C. magnifica* were taken from the ladybird culture and isolated into a 9-cm Petri dish containing a piece of corrugated filter paper. They were fed daily an excess of *A. pisum*. They were left to acclimatize for 24 h and then the number of eggs laid were counted daily for 10 days. Seven females of *C. septempunctata* were similarly treated. The females were subsequently weighted alive to the nearest 0.1 mg on a digital microbalance at the end of this 10 days period. The ladybirds were then frozen and dissected to count the number of their ovarioles. The reproductive rate (Rr) is obtained by multiplying the number of eggs laid per day by the average egg weight. The reproductive rate is plotted against the adult weight and we compared the two species with tests for equality of slopes and equality of intercepts between two regression lines (Sokal & Rohlf, 1995).

The Survival of the first instar larvae

Four batches of eggs of *C. magnifica* were taken from the ladybird culture and checked several times per day to spot the moment of emergence of the first instar larvae. As soon as the larvae began to walk away from their egg cluster, 66 individuals were taken up with a fine paintbrush. Each larva was immediately weighted on a Mettler microbalance (see before) and subsequently isolated in an Eppendorf tube with a piece of damp filter paper. Once a day, the tube were inspected to check for larval survival. The same experiment was repeated with 32 larvae of *C. septempunctata* born from 2 batches of eggs of the ladybird stock culture. Each species was analysed by linear regression of the time of survival against the initial weight. The reproductive rate is plotted against the adult weight and we compared the two species with tests for equality of slopes and equality of intercepts between two regression lines (Sokal & Rohlf, 1995).

The size of the legs of the first instar larvae

Five first instar larvae of *C. magnifica* that had recently dispersed from their batch of eggs were taken from the ladybird culture and were killed in ethanol. Their legs were clipped with a pair of fine scissors and placed in a balsam mounting medium between a glass slide and a cover glass. These preparations were used to measure the sizes of the tibia and the femurs from the front, mid and hind legs under a binocular fitted with a reticule with an accuracy of 25 µm . This was repeated with 5 *C. septempunctata* larvae. The lengths of the femurs and tibias of the two species were compared with a non-parametric Mann-Whitney test because of the non normality of the data.

The growth rate of the larvae of the two species of Coccinella on different species of aphids

The performances of the larvae of *C. magnifica* and *C. septempunctata* when fed different species of aphids was investigated in the laboratory. Two non-myrmecophilous aphids were used : *Metopolophium dirhodum* (Walker) collected in the field on *Arrhenaterum elatius* (L.) Beauv. ex. J. et C. Presl. and *Acyrthosiphon pisum* reared on *Vicia faba* ; one toxic and facultative ant-attended aphid (*Aphis jacobaeae* Schrank) collected in the field on *Senecio inaequidens* DC. and attended by *Lasius niger* L. (Witte et al., 1990; Pasteels, 2007) and one aphid always attended by ants (*Symydobius oblongus* (Von Heyden)) collected on potted *Betula pendula* L. tended by *F. polyctena* from a nest maintained in the laboratory (see Godeau et al. in prep for additional details). Immediately after hatching, 20 first instar larvae of *C. magnifica* were fed ad libidum *M. dirhodum* until pupation. The larvae were weighted after moulting and the duration of each instar recorded. Ten first instar larvae were similarly reared with *A. pisum*, 12 with *S. oblongus* and 20 with *A. jacobea*. This experiment was repeated with 10 and 9 first instar larvae of *C. septempunctata* respectively fed *A. pisum* and *A. jacobea*. The growth rate is calculated as the weight increase per hour for the first, second and third instars. The growth rates of the three stages are compared separately with an ANOVA (+ post hoc Fisher comparison). The duration of development from the egg to the adult is given for the two Coccinella species and compared with a Mann-Whitney test.

Results

The Weight of the eggs and the first instar larvae

The eggs of *C. magnifica* are significantly heavier than those of *C. septempunctata* ($E_{C.\ magnifica} = 0.25 \pm 0.025$ mg (N = 154) and $E_{C.\ septempunctata} = 0.20 \pm 0.034$ mg (N = 139);T = 14.15; d.f. = 247; P = 0). Not surprisingly, the first instar larvae of *C. magnifica* are heavier upon emergence than those of *C. septempunctata* (*C. magnifica* : 0.227 ± 0.0199 mg; *C. septempunctata* : 0.178 ± 0.0279 mg; T = 7.14; d.f. = 29; P = 0).

The Reproductive rate

C. magnifica lays significantly less eggs per day than *C. septempunctata* (Table 1; T = 3.88; d.f. = 95; P = 0). *C. magnifica* has less ovarioles than *C. septempunctata* (Table 1; W = 45; P = 0.016). The mean ovarioles number is nearly two times the amount of eggs laid per day, which suggests that eggs are produced alternatively by one ovary each day. The number of ovarioles is weakly correlated with the daily egg production (r = 0.35; N.S.) and with the adult weight (r = 0.27; N.S.). The slopes and the intercepts of the relationships of the reproductive rate (Rr) on adult weights are not significantly different (Fig.1; slope: $F_{[1,15]} = 0.0534$; P > 0.05; intercepts: $F_{[1,16]} = 1.224$; P > 0.05). *C. magnifica* has less ovarioles than

C. septempunctata (Table 1; W = 45; P = 0.016). The mean ovarioles number is nearly two times the amount of eggs laid per day, which agrees with the fact that eggs are produced alternatively by one of its two ovaries each day. Though unexpectedly, the amount of ovarioles is weakly correlated with the daily egg production (r = 0.35; N.S.) nor with the adult weight (r = 0.27; N.S.).

Table 1 : The number of eggs laid per day, the egg mass laid per day, the adult weight, the ratio of Rr on W_A and the number of ovarioles of Coccinella females. The means are compared with a Mann-Whitney test for all parameters but number of eggs which are compared with a Student T test.

	Eggs per day		Egg mass per day (Rr)		Adult weight (W_A)		Log(Rr)/Log(W_A)		Ovarioles	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C. magnifica	29,2	16,87	7,3	1,35	48,5	7,63	0,51	0,049	68,6	5,63
C. septempunctata	44,1	29,61	8,9	2,32	57,4	11,81	0,53	0,067	88,8	5,20
Difference between species	T = 3.88; d.f. = 95; P = 0		W = 98; P = 0.069		W = 76; P = 0.1877		W = 78; P = 0.2617		W = 45; P = 0.016	

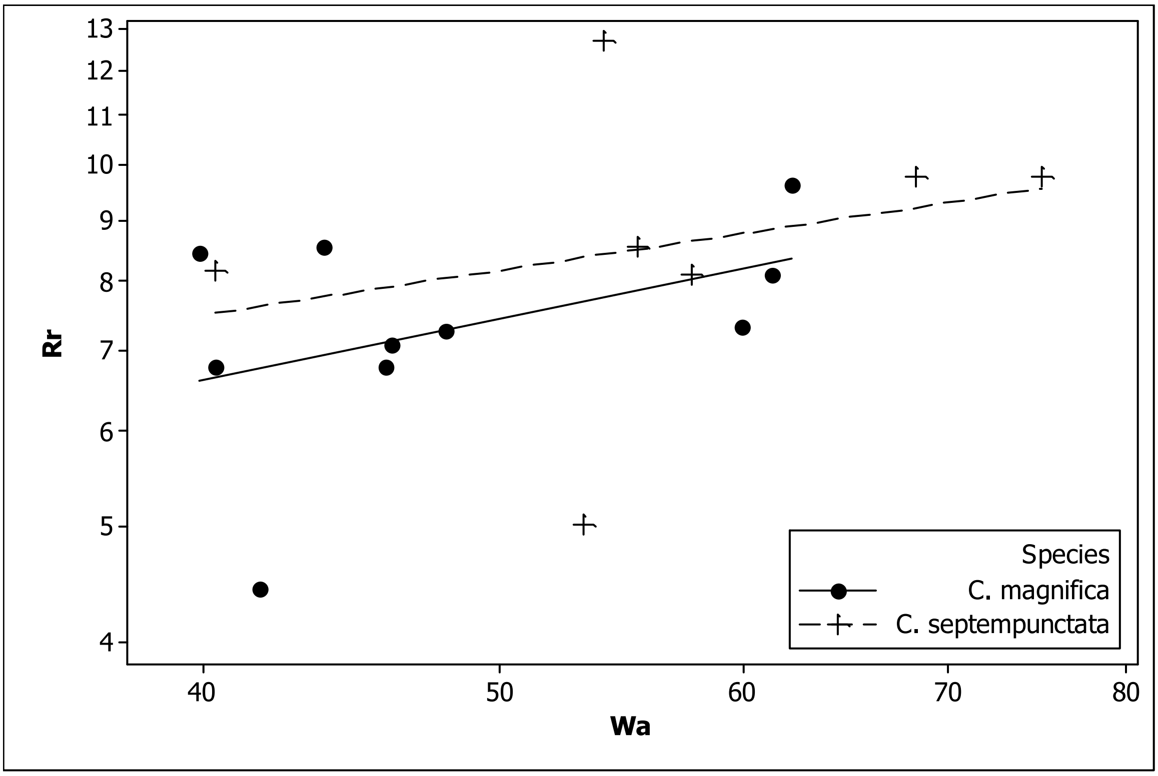

Fig. 1 : The reproductive rate Rr (Egg mass laid per day) plotted against the weight of the female (W_A), on Log-transformed scales.

The survival of the first instar larvae

The correlations between the duration of survival and the initial weight are significant for both species (Fig. 2; $r_{CM} = 0.74$; $r_{C7} = 0.6$) and the slopes and the intercepts are not different from each other (Slopes: $F_{[1,94]} = 0.158$; $P > 0.05$; intercepts: $F_{[1,95]} = 1.658$; $P > 0.05$). Thus a larva survives in direct proportion to its reserves of energy. We can infer a mean survival time from the mean weight of first instar larvae measured before. At 20°C, a *C. magnifica* larva would survive 89.3 hours and *C. septempunctata* 75.3 hours before catching its first prey (T = 11.16 ; df = 28 ; P ~ 0).

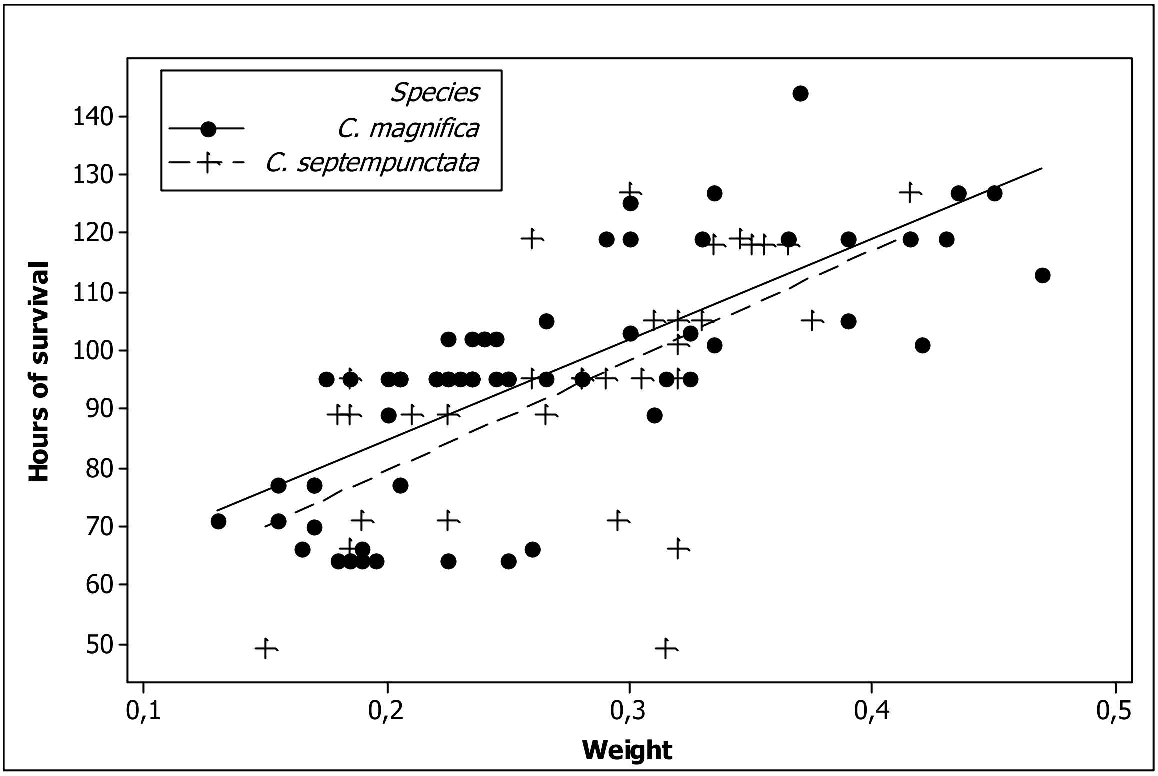

Fig.2 : The relationship between the duration of survival of starved first instar larva and their initial weight.

The length of the legs of the first instar larvae

The legs of *C. magnifica* are significantly shorter than those of *C. septempunctata* (Table 2). The length of the front, mid and hind legs of *C. magnifica* are respectively 84.4%, 91.8% and 94.6% shorter than those of *C. septempunctata*.

Table 2 : The size of the legs of the two Coccinella species.

		Femur			Tibia			Test between species (W=)	
Species	Leg	Mean	SD	N	Mean	SD	N	Femur	Tibia
C. septempunctata	Front	0.34	0.016	13	0.55	0.023	29	67 ***	193 ***
	Mid	0.32	0.015	14	0.48	0.022	24	126.5 *	150.5 ***
	Hind	0.31	0.013	13	0.48	0.022	17	122.5 N.S.	381.5 ***
C. magnifica	Front	0.31	0.011	10	0.46	0.024	19		
	Mid	0.30	0.013	12	0.43	0.030	15		
	Hind	0.30	0.013	11	0.44	0.023	25		

The Growth rate on different preys

The growth rates of the two ladybirds are different according to the prey species, for each larval instars (Table 3). The toxic aphid *A. jacobaeae* is a resource of lower quality than *A. pisum* for *C. septempunctata* and is lethal for *C. magnifica* since no larva reached the fourth instar (Godeau *et al.*, in prep). The growth rate of *C. septempunctata* fed *A. pisum* was higher than those of *C. magnifica*, except for first instar. *C. magnifica* developed well when fed *A. pisum, S. oblongus* or *M. dirhodum*. The highest growth rate was achieved during third instar with *M. dirhodum*.

Table 3 : The growth rate (mg/h) of the two Coccinella species fed different species of aphids during the first, the second and the third larval instars and the total duration of the development of the two Coccinella species fed *A. pisum*.

	C. septempunctata		*C. magnifica*				
	A. pisum	*A. jacobaeae*	*A. pisum*	*A. jacobaeae*	*S. oblongus*	*M. dirhodum*	F
First instar	$0.005^{a,b}$	0.005^{a}	0.011^{c}	0.004^{a}	0.004^{a}	0.007^{b}	$F_{[5,.68]} = 19.67$ ***
Second instar	0.022^{d}	$0.013^{a,b}$	$0.019^{c,d}$	0.009^{a}	0.014^{b}	$0.015^{b,c}$	$F_{[5,.51]} = 6.86$ ***
Third instar	0.059^{d}	0.026^{a}	$0.033^{a,b}$	-	$0.044^{b,c}$	0.047^{c}	$F_{[4,.39]} = 9.86$ ***
Development time (first to fourth instar)	554.7	-	585.75	-	-	-	W = 41; P = 0.134 N.S.

Discussion

Our measures confirm that the reproductive rate of both species is proportional to their body weight. We also confirm the predicted difference between the two Coccinella species in the size of their eggs: those of *C. magnifica* are 25% larger than those of *C. septempunctata*. The trade-off for fewer but larger eggs in *C. magnifica* may be attributed to its specialist myrmecophilous strategy but it does not explain which evolutionary pressures led to this trend.

The relatively large eggs of *C. magnifica* give birth to larvae which have more reserves of energy to overcome starvation than *C. septempunctata*. We have shown that *C. magnifica* laid its eggs at some distance from the aphid colonies, farther than other ladybirds do (Godeau & Hemptinne, in prep). Hence, we suggest that the higher survival of *C. magnifica* would allow its larvae to search longer for their first prey. Moreover, *C. magnifica* larvae have shorter legs than those *C. septempunctata*, thus the former cannot walk faster than the latter. The size of *C. magnifica* larvae is therefore likely to allow them for covering bigger distance to find a suitable patch of preys than the other ladybird species. As a larva of *C. magnifica* reaches an attended colony of aphids, it does probably not need to walk away to find other colonies because ant prolong the persistence of the aphid colonies. This can explain why its legs are relatively small (Dixon, 2000).

C. magnifica is sometimes observed to prey upon unattended aphids (Sloggett et al., 2004; Godeau & Hemptinne, in prep) and we found that its larvae can develop on different attended or unattended aphids, but not on toxic ones. *C. magnifica* looks therefore to be specialized for ants but not for a specific prey species.

When fed the same prey (*A. pisum*), the second and third larval instars of *C. magnifica* have a lower growth rate than *C. septempunctata* larvae. Even with starting with bigger eggs, *C. magnifica* achieves a smaller adult size during about the same period of growth. These results suggest that, conversely to the other ladybirds, the selection for big eggs is not likely to be explained by a reduction of the development time in *C. magnifica*.

We conclude that the females of *C. magnifica* invest more energy in each egg than *C. septempunctata* because of the presence of ants. But this cost is counterbalanced by the abundant and longer-persisting supplies of aphids provided by these ants.

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ARTICLE V : Is variability of adult size an indicator of specialization in predatory insects ?

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Introduction

Assessing the degree of specialization of a species may be of practical interest, for example, for conservation (Thomas & Morris, 1994). The strategies to implement in order to protect a species confined to a narrow range of habitats or food sources are probably different from those that will protect a species able to exploit a large set of resources. However, assessing correctly the diet breath of a species may be a daunting task and may require time-consuming field works. In addition, data sets assembled from field observations are often biased towards the records of food eaten by insects encountered in man made habitats where they aggregate in rich patches. For example, the preferences of the ladybird beetle *Coccinella septempunctata* L., which is a dominant species in herbaceous crops are rather well known. On the other hand, it is still a sort of a mystery for species dwelling in trees, like *Calvia decemguttata* (L.). It is tempting to bypass these difficulties and try to get some insight into diet breadth by laboratory observations. This has been done for some predators. The suitability of different aphid species have been investigated by comparing their development or by measuring their fecundity (Blackman, 1965; Albuquerque *et al.*, 1997; Obrycki *et al.*, 1997; Kalushkow, 1998; Rana *et al.*, 2002; Kalushkov & Hodek, 2005). These investigations, though bringing good information on the food requirements, remain marginal to assess the actual degree of specialisation of predators confronted with the huge diversity of prey available in the field.

On the other hand, Museum collections represent a source of data coming from the field. They are made of numerous specimens that have been caught over a large period of time and coming from diverse territories. This source of data is usually not rich in ecological information but their advantage is that all the specimens can be easily handled and measured. However, after a quick glimpse at insect collections one immediately gets the impression that some species are much more variable in size than others.

This questions about the processes that promote the intraspecific difference of size among a population. Size is dependent on the species genetical make-up but knowledge about the amplitude of size variation in species under constant environmental conditions are still rare (Grill *et al.*, 1997; Dixon, 2000). In contrast, the influence of the environment is better understood. Temperature has a large influence on size since it influences larval growth rate. (Butler, 1982; Katsarou *et al.*, 2005). The quantity and quality of resources also influence larval growth rate and adult size (Blackman, 1965; Ohgushi, 1987; Dixon & Guo, 1993; Hauge *et al.*, 1998; Fujiyama & Katakura, 2001). When a last-instar larva reaches a certain critical size, starvation can lead to premature adult molting via a cascade of hormonal interactions (Davidowitz *et al.*, 2003). These premature adults are smaller than adults resulting from an undisturbed larval growth.

Is a relationship between size variation and specialization likely? In situations of food scarcity, specialists are left with few options because the probability of finding a alternative suitable prey is very small. The possibility that the rarity of the normal prey would favour a gradual acceptance of an alternative prey is theoretically possible. However, this appears unlikely in predator-prey systems where the abundance of the prey fluctuates over a shorter time scale than that needed by predators to adapt to a novel prey (Rana *et al.*, 2002). The alternative prey is likely to become rare when the predators are still in the middle of their shift in prey preference. In such a context, it is more reasonable to assume that females will compete for rare suitable oviposition sites and that they will become less choosy (Begon *et al.*, 1996 ; Fréchette *et al.*, 2004). Therefore, their larvae will scramble for dwindling food sources and most if not all of them will die from cannibalism. The few individuals that survive will nevertheless have benefited from a good food regime constituted of a mixture of their normal prey and conspecifics so that they are expected to be of a size not too different from the optimum. On the other hand, generalists facing an absence or a scarcity of their preferred prey will add a new prey item to their food range providing that the average searching time of their normal prey becomes very large compared to the handling time (Begon *et al.*, 1996). According to the models developed for phytophagous insects (Courtney *et al.*, 1989; Nylin & Janz, 1999), they will start to explore less optimal foods which are known to reduce growth rate and lead to individuals of a smaller size (Ohgushi, 1987; Fujiyama & Katakura, 2001). When they will again have access to a high quality food, their performances and size will increase again.

The aim of this paper is to use predatory ladybird beetles feeding on aphids to test the idea that size variability recorded in Museum collections is an indicator of specialization. They constitute a good biological model because they always attracted the attention of amateur entomologists and scientists because of their colour variation, their shiny appearance or their practical impact in crop protection (Majerus, 1989; Majerus & Kearns, 1989; Hemptinne *et al.*, 2005). As a consequence of such an interest, they are quite abundant in collections and information on their preferred prey, their abundance, the plants on which they are usually caught are usually available in the literature. Some species like *C. septempunctata* are well known as generalist. They feed on more than 30 species of prey (Hodek & Honek, 1996) and are recorded in a wide range of habitats. Conversely, other species are highly specialized such as *Myrrha octodecimguttata* (L.) or *Anatis ocellata* (L.). They are strictly dependent on pine trees in which they feed on one or two species of aphids (Iablokoff-Khnzorian, 1982; Majerus, 1994). Most species lie somewhere between these above two extremes. Fortunately, Majerus (1994) gives information on the habitats and prey of a large sample of European species and provide some clues about the likely degree of specialisation of these species.

Beside specialization for food or habitat, some ladybird beetles are specialist because they lives in association with ants. There are two such myrmecophiles in Western Europe. *C. magnifica* lives in habitats dominated by *Formica rufa* s.l. where it feeds on attended aphids. This species looks similar to *C. septempunctata* but has a narrower geographic range (Donisthorpe, 1920a and 1920b; Majerus, 1989; Sloggett & Majerus, 2000). *Platynaspis luteorubra* (Goeze) is most commonly located on *Cirsium arvense* (L.) Scopoli where it feeds on *Aphis fabae cirsiiacanthoides* Scopoli tended by the ant *Lasius niger* (L.). Myrmecophily affects the abundance of resources by prolonging the life span of aphid colonies (Nixon, 1951; Way, 1963; Kleinjan & Mittler, 1975; Skinner, 1980; Flatt & Weisser, 2000). Therefore, myrmecophilous predators benefit from more stable resources than non-myrmecophilous ones (Stadler & Dixon, 1999; Sloggett & Majerus, 2000). Considering that ant-associated specialists are expected to exploit resources either of a better quality or more stable than generalists (Hoeksema & Bruna, 2000), they should be less variable in size.

This available ecological information on ladybirds beetles will be correlated with measurements taken in a Museum collection in order to seek for a relationship between size variability and specialization.

Material and methods

Sampling procedure of measured individuals

Ladybird beetles of different species were sampled in the collection “Faune belge” of The Natural Science Museum of Belgium in order to study their size variability. The specimens in the collection had been caught by various naturalists in Belgium from 1870 on. Fourty-two specimens of each species were randomly selected and measured following the first 42 digits of the number Pi, starting in the upper left corner of the collection boxes and following each lines of specimens from the left to the right. If a species was represented by too few insects to apply this procedure, all the specimens were measured.

Size was measured in four different ways (Fig. 1):

- the maximum length of the thorax and the abdomen measured dorsally ;
- the maximum width of the abdomen measured dorsally ;
- the maximum width of the pronotum measured dorsally ;
- the maximum height of the body from the metasternite to the highest point of the elytra.

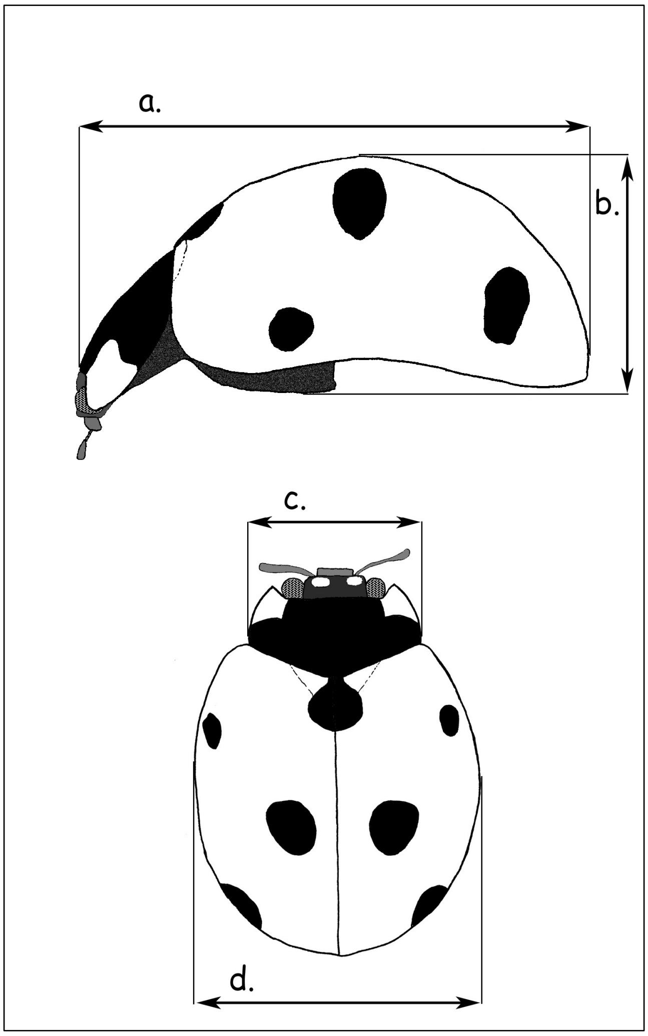

Fig. 1. Localization of the measurements done on the ladybirds from a Museum collection (see text for details) : a. Total length ; b. body height ; c. Pronotum width ; d. Abdomen width.

Measurements were carried out under a binocular fitted with a reticule, whose accuracy varies between 40 and 90 µm according to the magnification of the binocular. If these four measurements could not be done on a particular insect for some or another reasons, they were taken on the next specimen to the right. Therefore, the dataset does not have missing values. To reduce bias due to space autocorrelation the number of specimens coming from the same site was limited to two. If a third ladybird from the same site was again selected, it was substituted by the closest ladybird from another geographic location to its right.

Under-represented species

The Museum collections had very few specimens for two species. There were only 40 individuals for *P. luteorubra* that were all measured. There were only 18 *C. magnifica*. In order to complete the Museum sample, 12 males and 12 females were randomly selected among 89 individuals collected in the field (Chanly, Belgium) and measured.

The selection of a good size estimator

In order to find a good size estimator, a Principal Component Factor Analysis of the Correlation Matrix was used on the log-transformed measurements. The coefficient of correlation of Pearson of this axis with each of the log-transformed measurements was used to evaluate their aptitude at describing body size.

Statistical analyzes

Sex ratio

We could not distinguish the sex of the individuals of the Museum collection. As ladybird females are generally larger than males (Dixon, 2000), we determined the sex ratio and the mean pronotum width for each sex for a sample of *C. magnifica* and *C. septempunctata* caught in the field. The sex ratio have been compared between the two species with a Chi-square test and, for each species, we compared the sex ratio to an even proportion of males and females with a Fisher exact test. We tested the differences of sizes between the sexes with a Student t-test. These observed sex ratio and distributions of frequencies of size were then used to assigned a sex to the specimens measured in the Museum collection.

Normality of the data

An Anderson-Darling test was performed to check for the normality of the distributions of sizes of each species separately.

Interspecific differences of size

The values of the size estimator for each species were log-transformed and then submitted to a Bartlett's test to check for the homogeneity of the variances of the samples. Then, these samples were compared by an ANOVA followed by a post-hoc comparison (Tukey 95% individual confidence intervals).

Determination of the variability of the data

The coefficient of variation expressed in percentages was calculated to characterize size variability of each species. An estimate of the standard deviation of the coefficient of variation (Scv) was then calculated to test for the significance of the difference between two variances (Sokal & Rohlf, 1995):

$$Scv = CV\%/\sqrt{(2n)} \text{ (for } CV\% < 15)$$

Correlations between the coefficients of variation and ecological factors

We searched the literature and the database of the Coccinula Working Group for parameters that could explain a significant part of the variability in species size (Table 1). For each species of ladybird, the degree of association between the number of prey types (Iablokoff-Khnzorian, 1982), the abundance (Branquart & Maes, 2003), the preferred habitats (Majerus & Kearns, 1989), the number of host-plant taxa (Majerus, 1994; Data Coccinula, 2007 unpublished) and the coefficient of variation was investigated by a multiple regression. The coefficient of correlation of Spearman between parameters picked out by this first analysis and the coefficients of variation was then calculated.

Table 1. Summary of the ecological parameters associated to ladybirds beetles

Food div._GB : Number of alimentary types (aphids, coccids, adelgid, mildew, plants, mites, honeydew, pollen, nectar). Principal (bold) and secondary alimentary types have been pooled. * : British food is not known for this species.

Abund_B : Abundance Index 1→ 6: (1):Very Rare; (2): Rare; (3): Localized; (4): Widespread; (5): Common; (6): Very Common.

Habitats_GB : Index for Generalist-Specialist continuum: (1) host plant restricted specialist ; (2) conifer specialist; (3) broad-leaved deciduous woodland specialist; (4) arboreal generalist; (5) herbaceous generalist; (6) generalists with environmental constraints; (7) Generalists, breeding in a wide range of herbaceous and arboreal habitats.

Host plants_GB : number of host plant taxa.

Host plants_B : number of host plant genus.

Species	**Food div._GB**	**Abund_B**	**Habitats_GB**	**Host plants_GB**	**Host plants_B**
Anatis ocellata (L.)	5 (**aphids**, coccids, adelgid, honeydew, pollen)	4	1	9	39
Chilocorus renipustulatus (Scriba)	5 (**coccids**, aphids, adelgid, mites, mildew)	4	3	13	36
Coccinella hieroglyphica L.	2 (**aphids**, Chrysomelid*)	2	1	5	23
Coccinella magnifica Redtenbacher	5 (**aphids**, coccids, adelgid, honeydew, nectar)	2	6	18	21
Coccinella quinquepunctata L.	7 (**aphids**, coccids, adelgid, honeydew, pollen, nectar, mildew)	4	6	4	98
Coccinella septempunctata L.	8 (**aphids**, coccids, adelgid, Mites, honeydew, pollen, nectar, mildew)	6	7	21	255
Coccinella undecimpunctata L.	8 (**aphids**, coccids, adelgid, Mites, honeydew, pollen, nectar, mildew)	3	5	20	70
Exochomus quadripustulatus (L.)	7 (**coccids**, aphids, adelgid, mites, honeydew, pollen, nectar, mildew)	5	2	17	77
Myrrha octodecimguttata (L.)	3 (**aphids**, adelgid, pollen)	3	1	1	11
Platynaspis luteorubra (Goeze)	1 (**aphids**)	2	6	2	10
Subcoccinella vigintiquatuorpunctata (L.)	2 (**plants** (*Caryophyllaceae),* nectar)	3	5	17	27
Tyttaspis sedecimpunctata (L.)	3 (**mildew**, pollen, nectar)	4	5	12	69

Results

The selection of a good size estimator

The first axis of the Principal Component Analysis summarizes 97% of total variance (Var(Fact1) = 3.88) of the four measurements (Coefficients of correlation of the first factor to : Log(total length) = -0.989 ; Log(abdomen width) = -0.989; Log(pronotum width) = -0.985; Log(body height) = -0.977). Each of them is strongly correlated with the PCA axis and could therefore be used as a size estimator. Though, the pronotum width appears as a reliable estimator of the size because it is a measurement taken from one of the hardest part of the body and because it was already used as a size estimator for other ladybirds species (Koizumi *et al.*, 1999; Fujiyama & Katakura, 2001; Obrycki *et al.*, 2001).

Normality of distributions

All species are normally distributed except *A. ocellata* and *C. quinquepunctata* which show a slight departure from normality (table 2).

Table 2. The mean, standard deviation, coefficient of variation in percent (CV %), estimate of the standard deviation of CV %, values of the Anderson-Darling test (A-D test) and the statistical values associated to this test of the size estimator of twelve ladybird species. Means followed by the same letter are not statistically different, P > 0.05 (Tukey post-hoc comparison).

Species	**N**	**Mean**	**SD**	**CV%**	**S_{cv}**	**A-D Test**	**P**
T. sedecimpunctata	42	1.67 a	0.11	6.40	0.698	0.574	0.128*
P. luteorubra	40	1.75 b	0.08	4.75	0.531	0.613	0.103*
S. vigintiquatuorpunctata	42	1.99 c	0.11	5.54	0.604	0.617	0.101*
C. renipustulatus	42	2.115 d	0.14	6.41	0.699	0.55	0.147*
C. undecimpunctata	42	2.12 d	0.17	7.78	0.849	0.355	0.444*
C. hieroglyphica	42	2.14 d,e	0.13	6.12	0.667	0.702	0.062*
E. quadripustulatus	42	2.19 e	0.15	6.82	0.744	0.347	0.464*
M. octodecimguttata	42	2.27 f	0.11	5.04	0.55	0.648	0.084*
C. quinquepunctata	42	2.37 g	0.12	5.15	0.562	0.765	0.043
C. magnifica	42	3.34 h	0.2	6.10	0.666	0.389	0.37*
C. septempunctata	42	3.37 h	0.26	7.57	0.826	0.369	0.368*
A. ocellata	42	4.11 i	0.22	5.30	0.578	0.985	0.012

Sex ratio

The two field samples show a slightly female-biased sex ratio for both *C. septempunctata* and *C. magnifica* (table 3). However, these two ratios are not different from an even proportion of females and males and are not different from each other (Chi-Sq = 0.0043; DF = 1; P = 0.948). The pronotum width of females and males are not significantly different, neither for *C. magnifica* nor *C. septempunctata* (Table 2). The distributions of frequencies of the sizes in relation to the sex established for *C. septempunctata* and *C. magnifica* were used to estimate the sex ratio of the Museum sample. Therefore, the bias due to the unknown distribution of sexes among the Museum samples is considered to be low and equivalent for every species.

Table 3. The sex-ratio and the mean pronotum width (mm) of a field sample of *C. septempunctata* and of *C. magnifica*.

		Female	Male	Test between sexes
C. septempunctata	N =	26	16	Fisher test: P = 0.379
	Pronotum width	3.4 ± 0.16	3.32 ± 0.22	T = 1.29; d.f. = 40; P = 0.204
C. magnifica	N =	53	36	Fisher test: P = 0.228
	Pronotum width	3.28 ± 0.2	3.2 ± 0.19	T = 1.89; d.f. = 87; P = 0.062

Interspecific size differences and variability

There are significant differences in size between all the species t ($F_{[1,11]} = 820.46$; d. f. = 11 ; P ~ 0), but for *C. magnifica* and *C. septempunctata* which are identical (post-hoc comparison: t = 0.7; P = 0.99).

The coefficient of variation of the more variable species is 3% higher than that of the least variable. However, there are significant differences of variability between the species (Bartlett test = 23.81 ; P = 0.014; figure 2 and table 3).

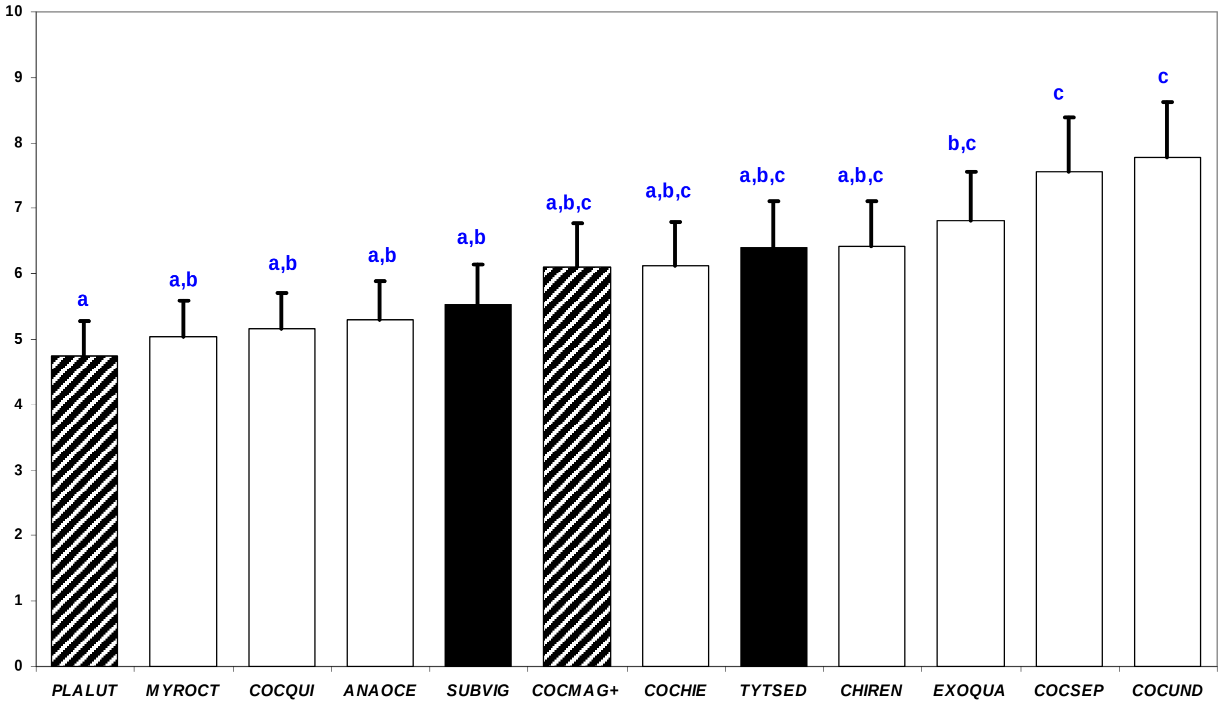

Fig. 2. The coefficients of variation (+1 estimated SD) of what for twelve species of ladybird beetles. Bar legend: open = predators; filled = myrmecophilous predators; solid = non-predators. Bars with the same letters are not statistically different, P > 0.05. Species: TYTSED = *T. sedecimpunctata*; PLALUT = *P. luteorubra*; SUBVIG = *S. vigintiquatuorpunctata*; CHIREN = *C. renipustulatus*; COCUND = *C. undecimpunctata*; COCHIE = *C. hieroglyphica*; EXOQUA = *E. quadripustulatus*; MYROCT = *M. octodecimguttata*; COCQUI = *C. quinquepunctata*; COCMAG+ = *C. magnifica*; COCSEP = *C. septempunctata*; ANAOCE = *A. ocellata*.

Multiple F comparisons on the estimated standard deviation of the coefficient of variation (fig. 2) emphasize 3 groups of species. Firstly, there is a pair of highly variable species : *C. undecimpunctata* (coefficient of variation = 7.78 ± 0.85) and *C. septempunctata*; (coefficient of variation = 7.57 ± 0.83). Secondly, the myrmecophilous *P. luteorubra* is among the least variable species (coefficient of variation = 4.75 ± 0.53). Between these two extremes lies a third group of species.

Despite the similarity in morphology and size of *C. septempunctata* and *C. magnifica* they are quite different in terms of variability. Their coefficient of variation differ by about 1.5 %, that is half of the amplitude of the variability of the entire dataset. However, they are not significantly different ($F_{[41,41]} = 1.54$; $P > 0.05$). A sample of 61 individuals instead of 42 would have been required to statistically separate these two species since $F_{0.05\,[60,60]} = 1.53$.

The two non-predators species (*S. vigintiqutuorpunctata* and *T. sedecimpunctata*) were not separated from predators; their coefficient of variation were close to the general mean of the data set.

Correlation between size variability and ecological parameters

There is no significant relationship between the abundance, the habitat preference, the number of prey types and the variability of size. The number of host plants explain a significant part of the variance in the multiple regression between the ecological parameters and size variability (table 4). It is strongly correlated to the coefficient of variation (Table 5 and figure 3). Although the maximum number of food categories was not an influent factor in the multiple regression, it is significantly correlated to the coefficient of variation of the size ($r_{Spearman} = 0.64$; $P = 0.025$).

Table 4. The results of the multiple regression of the coefficients of variation of the size of ladybirds to the ecological parameters.

Predictor	Coef	SE Coef	T	P
Constant	4.6777	0.6447	7.26	0.000
Abund_B	-0.0047	0.2062	-0.02	0.982
Host plant_GB	**0.0929**	**0.0343**	**2.71**	**0.03**
Habitats_GB	-0.0535	0.0928	-0.58	0.582
Food div._GB	0.1194	0.1153	1.04	0.335
R-Squared = 72.7%				

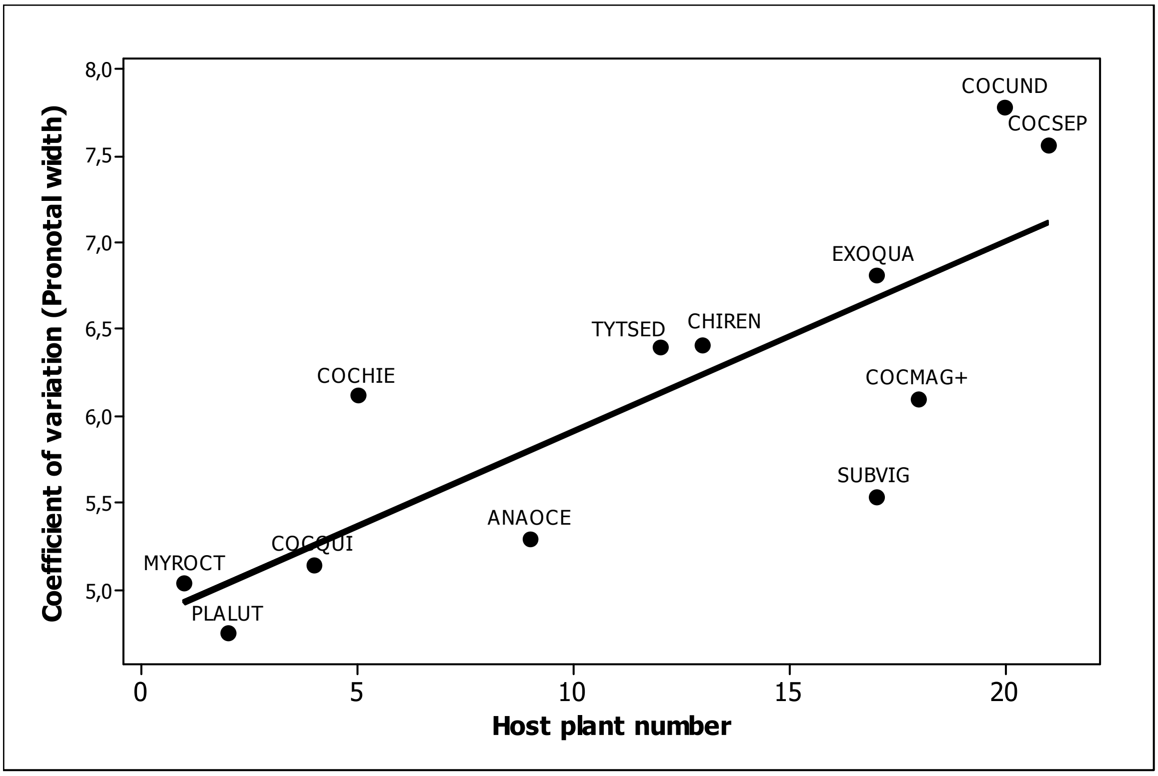

Fig. 3. The relationship between the number of host-plants on which twelve species of ladybird beetles have been observed and the coefficient of variation of the pronotal width of these species. TYTSED = *T. sedecimpunctata*; PLALUT = *P. luteorubra*; SUBVIG = *S. vigintiquatuorpunctata*; CHIREN = *C. renipustulatus*; COCUND = *C. undecimpunctata*; COCHIE = *C. hieroglyphica*; EXOQUA = *E. quadripustulatus*; MYROCT = *M. octodecimguttata*; COCQUI = *C. quinquepunctata*; COCMAG+ = *C. magnifica*; COCSEP = *C. septempunctata*; ANAOCE = *A. ocellata*.

The number of host plant genus taken from field observations in Belgium give a significant correlation with the coefficient of variation (Table 5). That is, generalist ladybird beetles appear more variable in size than more specialized species.

Table 5. The coefficients of correlation of Spearman of the coefficient of variation of the size of the ladybirds to the host plant number.

	Coefficient of correlation
Host plant (Great-Britain)	0.816 (P = 0.001)
Host plant (Belgium)	0.615 (P = 0.033)

Discussion

The degree of specialization of a species is usually estimated on the basis of the food resources it uses or the habitat preferences it displays. The two parameters can be assessed in the field and through experiments but it will not be easy to convert this information into a specialization index? We investigated if size variability is somehow connected with ecological parameters taken from the literature.

We suggest that size variability could be a parameter to take into account when one tries to measure the degree of specialization. We show that a substantial part of specific size variation is related to food specialization. This is in agreement with the hypothesis that generalists species have to prey upon low quality resource when food become rare, so that the variability of size is higher than for specialists. Our results point out that the two most size-variable species (*C. septempunctata* and *C. undecimpunctata*) are considered as generalist and that the two conifer specialists (*M. octodecimguttata* and *A. ocellata*) belong to the less variable group within our dataset. The two extremities of the gradient seem to fit the rule that specialists are less variable in size than generalists, but there is no correlation between the coefficient of variation of size and the index of specialization. Tough the index we used have been proposed from the results of survey in Great-Britain, we can expect to earn different results with an index adapted to the Belgian context. An improved index of habitat specialization, updated and corresponding to the geographic area, would be necessary for further investigations.

Furthermore, the importance of food in shaping individual size distribution among species is reinforced by the published results of experimental manipulation on diet of *Harmonia axyridis* Pallas (Grill *et al.*, 1997). Two groups of ladybird larvae were grown and fed *ad libitum* in laboratory, one group was fed optimally with aphids in excess (*Acyrtosiphon pisum* Harris), the other was provided with a sub-optimal artificial food made of meat. The mean size of adults obtained from the two treatments were different. Optimally-fed larvae gave birth to greater but less variable adults (coefficient of variation “Optimal food” = 5.02), whereas sub-optimally fed larvae produced smaller adult whose coefficient of variation was higher (coefficient of variation “Sub-optimal food” = 6.97). In this experiment, the only factor explaining differences between the two groups was food quality. As expected, food quality influenced mean size (Blackman, 1965; Ohgushi, 1987; Albuquerque *et al.*, 1997) but, additionally, individuals grown in optimal and artificially stable conditions reached a low size variability coefficient, comparable to the value we obtained for the highly specialized *P. luteorubra*.

The position of the two myrmecophilous species along the gradient of size variability questions about the processes we are intending to point out. Both species are considered as specialists because of their obligatory association with ants, but they are nevertheless different in terms of their diet diversity. *P. luteorubra* is occasionally seen on various host-plant but is mainly described as a predator of *Aphis fabae cirsiiacanthoides* living on *Cirsium arvense* or *Metopeurum fuscoviride* Stroyan living on *Tanacetum vulgare,* attended by ants of genus *Lasius* (Völkl, 1995). Conversely, *C. magnifica* has been reported on a lot of host plants in Belgium and Great-Britain, eating tenth of aphid species (Sloggett *et al.*, 2002; pers. obs.), psyllidae (pers. obs.) and chrysomelid larvae (Iablokoff-Khnzorian, 1982). The two species are highly specialized for living in contact with ants, although they show contrasting food preferences. As we knew that colonies of aphids are more crowded and live longer when attended by ants, we expected that predators that evolved the ability to tap these resources would have benefited from a more stable environment. As a consequence, they should exhibit a less variable size. Our results are in agreement with this hypothesis. One myrmecophilous ladybirds is located at the lower end of the gradient and the second is in the middle, far from the generalist morphologically similar *C. septempunctata*. The two myrmecophilous ladybirds have such differences in their food preferences that we propose that *C. magnifica* is more variable than *P. luteorubra* because of its generalist diet. The shift toward a lower coefficient of variation for both species can be attributed to the increased stability of their ant-attended prey populations. It was already proposed by other authors that myrmecophilous specialist predators are positively selected thanks to resource abundance since they can overcome ant aggression (Albuquerque *et al.*, 1997; Sloggett & Majerus, 2000).

To investigate further this postulate, other similar studies should be done with sex differentiation of the individual measured and with determined conditions of space and time scales. Additional results on other areas and with larger samples should be necessary to reinforce the hypothesis that a specialization index can be derived from intraspecific size variability. We also emphasize here the importance of naturalists observations and subsequent Museum collections for the useful information which then becomes available. It would also be interesting to see if other taxonomic groups will support the same conclusions.

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