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MECHANISMS OF PREY SELECTION IN THE LADYBEETLE *COLEOMEGILLA MACULATA LENGI* TIMB. (COLEOPTERA: COCCINELLIDAE)

BY

CAROLINE ROGER

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Ph.D.)

Department of Natural Resource Sciences
Macdonald campus of McGill University
Montréal, Canada

January 1999

0-612-50249-X

SHORT TITLE

Prey selection in the generalist predator *Coleomegilla maculata*

Caroline Roger

À Gabriel

Pour son amour, sa patience et sa compréhension

ABSTRACT

Ph.D. **Entomology**

Mechanisms of prey selection in the ladybeetle *Coleomegilla maculata lengi* Timb. (Coleoptera: Coccinellidae)

Foraging generalist predators are frequently confronted with a diversity of prey types that differ in profitability. Optimal foraging models predict that predators should select and exploit the most profitable prey types and reject unprofitable ones. The goal of this research was to evaluate the mechanisms underlying prey selection, prey exploitation and predation efficacy in the generalist predator *Coleomegilla maculata lengi* Timb. (Col., Coccinellidae).

The influence of prey species, prey size and predator age on predation efficacy and prey consumption by *C. maculata* was determined using non-choice laboratory tests. The three lepidopterous species occurring in cruciferous crops, namely, *Artogeia rapae* (L.), *Plutella xylostella* (L.) and *Trichoplusia ni* (Hübner), were used as prey. Results showed that, within a given prey instar, *C. maculata* preyed more on *P. xylostella* than on the other two species and that predation efficacy increased with increasing predator age. These experiments also revealed that coccinellid larvae had a higher prey weight consumption rate on intermediate-sized prey compared to smaller or larger prey even though they killed a higher number of small prey. It is suggested that this higher efficacy on prey of intermediate size could be a consequence of higher costs associated with the consumption of prey at both ends of the size spectrum.

To evaluate behavioral parameters of prey selection in *C. maculata* larvae, an image analysis system, initially developed for parasitoids searching for hosts, was modified. This system was programmed to locate the eggs used as prey and to detect and follow the movements of the *C. maculata* larval head. This user-friendly system can detect and follow the movements with less than 0.1% of error.

Using this tracking system, the discriminatory capacity and the prey selection behavior of *C. maculata* larvae were evaluated in choice situations in which prey differed in quality (unparasitized, parasitized, young or old *T. ni* eggs). Coccinellid larvae did not exhibit any preference between parasitized and unparasitized eggs of the same physiological age. However, when physiological age differed, coccinellid larvae always preferred the younger eggs despite parasitism. Longer handling time and detrimental effects on immature developmental time and survival was always observed on the less preferred egg type.

This study also revealed that coccinellid larvae can learn to gradually reject the less suitable parasitized eggs, thereby improving their prey selection behavior. It was also demonstrated that these learned behaviors could be forgotten and that previous experiences on other prey types could influence the initial preference of coccinellid larvae. This study clearly indicates that the generalist predator *C. maculata* can select and exploit prey according to their profitability by using discrimination and learning abilities.

RÉSUMÉ

Ph.D. **Entomologie**

Mécanismes impliqués dans la sélection de proies chez la coccinelle *Coleomegilla maculata lengi* Timb. (Coleoptera: Coccinellidae)

Les prédateurs généralistes en recherche de nourriture sont fréquemment confrontés à une diversité de proies de profitabilité différente. Les modèles de quête optimale prédisent qu'un prédateur devrait exploiter les proies les plus profitables et rejeter les moins profitables. Le but de cette recherche était d'évaluer les mécanismes sous-jacents à la sélection et l'exploitation des proies chez le prédateur généraliste *Coleomegilla maculata lengi* Timb. (Col., Coccinellidae).

La prédation et l'exploitation de proies par *C. maculata* a été évaluée en laboratoire à l'aide de tests sans possibilité de choix en fonction de l'âge du prédateur, de la taille et de l'espèce de la proie. Les proies utilisées ont été les trois espèces de lépidoptères retrouvées en culture de crucifères soit, *Artogeia rapae* (L.), *Plutella xylostella* (L.) et *Trichoplusia ni* (Hübner). Les résultats ont démontré, qu'à l'intérieur d'un même stade larvaire, *C. maculata* avait un taux de prédation plus élevé sur *P. xylostella* comparativement aux deux autres espèces et que l'efficacité de prédation augmentait avec l'âge du prédateur. Ces expériences ont également démontré que, même si les larves de coccinelles ont tué un plus grand nombre de petites proies, la quantité de biomasse de proies consommée en 24h était plus élevé pour les proies de taille intermédiaire. Il est suggéré que ce niveau de consommation plus élevé des proies de taille intermédiaire pourrait être la conséquence de coûts d'exploitation plus élevés pour les proies se retrouvant aux deux extrémités du spectre de taille.

Un système d'analyse d'images, initialement développé pour quantifier les comportements de recherche d'hôtes chez des parasitoïdes, a été modifié afin d'évaluer les paramètres comportementaux reliés à la sélection de proies chez les larves C. *maculata*. Ce système a été programmé pour localiser les oeufs utilisés comme proies et pour détecter et suivre les déplacements de la tête de la larve de coccinelle. Ce système peut détecter et suivre les mouvements avec une erreur de moins de 0.1%.

À l'aide de ce système d'analyse d'images, la capacité discriminatoire et le comportement de sélection des proies des larves *C. maculata* ont été évalués dans des situations de choix entre des proies de qualité différente (oeufs de *T. ni* parasités, non parasités, jeunes ou âgés). Les larves de coccinelles n'ont pas affiché de préférence entre des oeufs parasités et des oeufs non parasités de même âge physiologique. Toutefois, malgré le parasitisme, les larves de coccinelles ont toujours préférés des jeunes oeufs lorsque l'âge physiologique était différent. Des temps de manipulations plus longs et des effets négatifs sur le temps de développement des immatures et sur leur survie ont également été observés sur le type d'oeufs le moins préféré.

Cette étude a également révélé que les larves de coccinelles apprennent à rejeter graduellement les oeufs parasités alors que les oeufs non parasités sont pratiquement toujours acceptés. L'apprentissage permet donc aux larves de coccinelles d'améliorer leur comportement de sélection de proies. Il a également été démontré que cet apprentissage peut être oubliée et que l'expérience acquise antérieurement sur d'autres types de proies peut influencer la préférence initiale chez les larves de coccinelles. Cette étude démontre clairement que le prédateur généraliste *C. maculata* peut sélectionner et exploiter des proies selon leur profitabilité respective en utilisant des habiletés de discrimination et d'apprentissage.

ACNOWLEDGEMENTS

I wish to express my sincere gratitude to Dr. Guy Boivin and Dr. Daniel Coderre, supervisor and co-supervisor of my Ph. D., for their support, advice, encouragement and availability.

I gratefully acknowledge the valuable assistance and commentaries on my work from Dr. Éric Wajnberg and Dr. Clément Vigneault.

I also thank Dr. William Vickery and Dr. Bernard Roitberg for their constructive comments on specific manuscripts. Thanks are also extended to Sophie Demougeot, Nathalie Dauphinais and Josée Delorme for their technical assistance.

I would especially like to thank my very good friends, Dominique and Silvia for their unconditional support and for being there in good as well as in bad times. I also thank my colleagues in both labs for their friendship during all these years: François, Éric, Claude, Isabelle, Stéphan, Martin, David, Daniel, Danielle, Marie-Josée, Hélène, Sophie, Marjo and Sandra.

I am grateful to Marie Kubecki for her smiles, generosity, patience and help on all aspects of McGill administration and policies.

The author gratefully acknowledges the personal scholarship awarded by the Fonds pour la Formation de chercheurs et l'aide à la Recherche (FCAR).

My deepest gratitude goes to my parents who supported and encouraged me throughout my Ph. D. I especially thank my son Gabriel, who brings light and purpose to my life, for his understanding, encouragement, patience and love.

PREFACE

This thesis consists of a collection of four original manuscripts which have been either published or destined for publication in refereed journals. Because the manuscript-based structure for this thesis was chosen by the candidate, the following directions outlined in the "Guidelines for Thesis Preparation" published by the Faculty of Graduate Studies and Research of McGill University must apply:

> *Candidates have the option of including, as part of the thesis, the text of one or more papers submitted or to be submitted for publication, or the clearly-duplicated text of one or more published papers. These texts must be bound as an integral part of the thesis.*
>
> *If this option is chosen,* ***connecting texts that provide logical bridges between the different papers are mandatory****. The thesis must be written in such a way that it is more than a mere collection of manuscripts; in other words, results of a series of papers must be integrated.*
>
> *The thesis must still conform to all other requirements of the "Guidelines for Thesis Preparation".* ***The thesis must include****: A table of contents, an abstract in English and French, an introduction which clearly states the rationale and objectives of the study, a comprehensive review of the literature, a final conclusion and summary, and a thorough bibliography or reference list.*
>
> *Additional material must be provided where appropriate (e.g. in appendices) and in sufficient detail to allow a clear and precise judgment to be made of the importance and originality of the research reported in the thesis.*
>
> *In the case of manuscripts co-authored by the candidate and others,* ***the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent****. Supervisors must attest to the accuracy of such statements at the doctoral oral defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of all the authors of the co-authored papers.*

The thesis includes one published manuscript, two submitted manuscripts and one manuscript to be submitted, all are co-authored.

THIRD CHAPTER

Roger, C., D. Coderre & G. Boivin. 1998. Differential prey exploitation by the generalist predator *Coleomegilla maculata lengi* according to prey age and species. Submitted to Entomologia Experimentalis & Applicata.

FOURTH CHAPTER

Vigneault, C., C. Roger, K.P.C. Hui & G. Boivin. 1998. An image analysis system developed for evaluation of *Coleomegilla maculata* larvae's behavior. Canadian Agricultural Engineering 40: 55-60.

FIFTH CHAPTER

Roger, C., D. Coderre, C. Vigneault & G. Boivin. 1998. Discrimination of parasitized prey by the generalist predator *Coleomegilla maculata lengi* (Coleoptera: Coccinellidae): mechanisms and implication on intraguild predation. Submitted to Oecologia.

SIXTH CHAPTER

Roger, C., D. Coderre, E. Wajnberg & G. Boivin. 1998. Learning affects prey selection in a generalist coccinellid predator. To be submitted to Behavioral Ecology and Sociobiology.

CONTRIBUTION OF CO-AUTHORS TO MANUSCRIPTS

Under the guidance of her supervisors, the candidate was in charge of experimental design, laboratory set-up, experimental execution and management, data collection and statistical analyses as well as preparation of manuscripts for publication. The modifications brought to the automated system that was initially developed for minute insects were performed by Dr. Clément Vigneault. This system was modified at the request of the candidate to replace manual observations required for the study of prey discrimination by coccinellid larvae. The candidate defined the biological parameters and performed the tests to verify the exactitude of the results. She assisted Dr. Vigneault in providing valuable comments and guidelines during the modification process of the automated system. The candidate also made constructive suggestions and comments during the writing of the manuscript and revised the latter.

Dr. Guy Boivin contributed in general guidance through research supervision, administration and technical assistance and provided extensive comments on all manuscripts.

Dr. Daniel Coderre also contributed in general guidance through research supervision, contributed to technical help and provided encouragement and constructive comments on all manuscripts.

Dr. Clément Vigneault was responsible for the development of the automated system elaborated for the study of *Coleomegilla maculata* larvae's behavior and for comments and revision of the manuscript included in the fourth chapter. He also revised and provided comments on the manuscript of chapter 5.

Dr. Éric Wajnberg provided valuable assistance in statistical analyses reported in chapter 6 and read, revised and provided comments on the manuscript.

Ka Po Catherine Hui actively contributed to the preparation for publication of the manuscript on the automated system. This paper is included in chapter 5.

CLAIMS OF CONTRIBUTION TO KNOWLEDGE

Major contributions of this research are summarized in this section.

1. First mention of *Coloemegilla maculata* as a predator of the eggs and larvae of the three lepidopterous species occurring in cruciferous crops.

2. First study on the predation efficacy of a predator attacking the three lepidopterous pests.

3. First mention of the importance of prey species and prey size on the prey exploitation rate in Coccinellidae.

4. Development of a Protection Index (PI) that considers the differential predation caused by *C. maculata* and the relative importance of each species in terms of plant injury.

5. Confirmation of prey discrimination abilities in a generalist coccinellid predator.

6. First study to determine the importance of prey profitability in predaceous coccinellids and its influence of prey selection.

7. One of the very few studies demonstrating the presence of learning in insect predators and the first in Coccinellidae that does not involve a conditioning process.

8. An automated system initially developed for minute insects has been modified and used for the first time to quantify the behavioral components involved in prey selection by coccinellid larvae.

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CHAPTER 1

GENERAL INTRODUCTION

Foraging entomophagous insects are confronted to a diversity of environmental situations in which they may adopt different behavioral strategies. By their actions, they can influence the size, the structure and the population dynamics of their hosts or prey and of other predators/parasitoids present in the same guild and of the overall community (Jervis & Kidd 1996).

Most of an insect life is dedicated to actions related to the acquisition of food and to reproduction and, crucial decisions must be taken concerning these two activities. A foraging individual must decide where to seek for potential prey or hosts, what type of prey or hosts to accept and, when to move to a new habitat (Barnard 1983). The outcome of these decisions can greatly influence the survival and fitness of predators and parasitoids. In trying to understand what determines the decisional process of foraging insects, ecologists have increasingly turned to optimal foraging theory (Charnov 1976; Stephens & Krebs 1986).

Optimal foraging theory asks how natural selection may have shaped the insect behavior and assumes that individuals exploiting their resources most efficiently will be favoured (van Alphen & Vet 1986). Hence, this theory predicts that a forager should maximize its encounter rate with the most suitable and profitable prey or hosts and, when prey are abundant, avoid individuals of lesser quality (Stephens & Krebs 1986).

In the past two decades, parasitoids have been a preferential model for the study of foraging behavior because of the direct link between foraging efficiency and fitness (van Alphen & Vet 1986; Godfray 1994). On the other hand, the behavioral mechanisms underlying prey selection in in insect predators, such as prey discimination and learning in relation to prey quality, have been less studied. Although the link between prey selection and fitness is more difficult to quantify, predators face the same constraints as parasitoids and have also to optimize their survival and fitness.

Generalist predators search for prey in diverse habitats and encounter a broad range of insects of which only a small fraction are normally accepted for food. According to the optimal foraging theory, prey should be ranked by prey energetic value per unit of handling time, and should be added to the diet of a predator in decreasing order of profitability (Stephens & Krebs 1986). Many factors can influence the benefits and costs of searching, capturing and consuming a prey and thereby their overall profitability. For example, the value of a prey may be altered by physiological modifications provoked by parasitism (Al Rouechdi & Voegelé 1981) and by ageing process (Strand 1986; Vinson 1994). The costs of capturing a prey may be affected by factors that include predator-prey size ratio (Griffiths 1982; Sabelis 1992), prey density (Hassell *et al.* 1977) and aspects of prey vulnerability related to prey size (Thompson 1978; Pastorok 1981) and prey species (Dixon 1958; Hajek & Dahlsten 1987). Predators attributes that may affect their capture success rate, include prey detection capability (Malcolm 1992), mobility and rapidity of predator response following prey contact (Pastorok 1981) as well as their ability to respond to the presence of potential competitors (Polis *et al.* 1989).

As observed in many parasitoid species (van Lenteren 1981; van Baaren *et al.* 1994), the presence of discrimination ability (i.e. to distinguish parasitized from unparasitized prey) in predators could allow a predator to identify and eliminate potential competitors, especially under conditions of local resource competition (Polis *et al.* 1989). However, if parasitized prey are less profitable than healthy ones, it would be adaptive for a searching predator to use prey discrimination abilities to evaluate prey profitability and reject parasitized prey.

Many entomophagous insects are known to change and improve their foraging behavior with experience (Papaj & Lewis 1993). As suggested by van Alphen & Vet (1986), these learned behaviors may be adaptive, especially for habitat generalist insects that are frequently confronted to a diversity of prey types. However, the adaptive value of prey discrimination and learning by generalist predators have received little attention (Mackauer *et al.* 1990; Prokopy & Lewis 1993).

The study of the mechanisms underlying prey selection should also be a prerequisite for the utilization of generalist predators in biological control programs (van Alphen & Jervis 1996). Factors, such as the lack of consistency in the predation behaviors of natural enemies, have been invoked to explain why some species failed to control pests successfully (Ehler & Hall 1982; Stiling 1993). Therefore, a good understanding of the behavioral ecology related to prey selection and learning would give useful clues on how to optimize the utilization of natural enemies in managing pest populations.

In Québec, the use of the oophagous parasitoid *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) for the control of lepidopterous pests in cruciferous crops is under investigation (Boivin & Fournier 1993). Field survey in cruciferous crops located in southwestern Quebec have shown that many indigeneous natural enemies of lepidopteran eggs are also present in these cultures (Roger *et al.* 1995). This monitoring revealed that predaceous coccinellids were present on plants from the beginning of spring through the end of summer and that they were the most important predators. Among coccinellids, *Coleomegilla maculata lengi* Timb. (Coleoptera: Coccinellidae) was the most abundant species (Roger *et al.* 1995). Because *Trichogramma* has the potential to be used as a biological agent in inundative releases, competition for resources and interference are likely to occur with the indigenous populations of *C. maculata.* This generalist coccinellid could possibly attack the parasitized eggs containing immature parasitoids as reported in other generalist predators (Jones 1987; Brower & Press 1988; Ruberson & Kring 1991; Hoelmer *et al.* 1994).

Both potential competitors for lepidopteran eggs, the predator *C. maculata* and the parasitoid *Trichogramma*, were selected for this study. The three lepidopterous prey pests occurring in cruciferous crops were namely, the imported cabbageworm, *Artogeia* (=

Pieris) *rapae* (L.), the diamondback moth, *Plutella xylostella* (L.) and the cabbage looper, *Trichoplusia ni* (Hübner). The use of these three sympatric prey species has, from a functional perspective, several favorable attributes for the study of prey selection. First, they are attacked in most of their immature stages by *C. maculata* (Roger *et al.* 1995) and exhibit differences in size, morphology, mobility and defense responses. These differences may influence the profitability of these lepidopterous prey for foraging generalist predators such as *C. maculata* and consequently affect predation efficiency. Furthermore, parasitism by *T. evanescens* may induce physiological modifications that can alter prey suitability and change the energy and time payoffs for the predator *C. maculata*.

The main objectives of this thesis are to: (1) Determine the influence of prey species, prey size and predator age on prey exploitation by the polyphagous predator *C. maculata*. (2) Evaluate the discriminatory capacity of *C. maculata* larvae when presented cabbage looper eggs, both unparasitized and parasitized by *T. evanescens*. The second objective of this section was to study the influence of prey quality on prey selection and patch exploitation. (3) Determine if the behavior leading to prey selection involves a learning process. (4) Modify and use a method based on machine vision for the study of some behavioral parameters of prey selection in coccinellid larvae. All experiments were performed in a laboratory environment and most results obtained are discussed in relation to optimal foraging theory.

CHAPTER 2

LITERATURE REVIEW

Theoretical concepts that will be debated in manuscripts included in the next chapters will be presented in this section. Afterwards, behavioral aspects of prey location and prey selection in Coccinellidae as well as the published literature concerning optimal foraging, learning and behavioral data processing in predatory coccinellids will be discussed. Finally, the description of each insect species used in this research as well as the cruciferous crops in which they live, will be presented.

1. OPTIMAL FORAGING THEORY

All behavioral components take time and energy and these resources cannot be allocated to all behaviors simultaneously (Cuthill & Houston 1997). Therefore, insects must assess the costs and benefits related to foraging and reach the best trade-off possible (Crawley & Krebs 1992). Economic models of behavior (Charnov 1976; Cook & Hubbard 1977; Hassell & Southwood 1978) are powerful tools that can help to understand the nature of these trade-offs and subsequently determine the impact of the behavioral decisions of individuals on their fitness.

When a predator forages for food, it has to make decisions about where to search, which kind of prey to eat and so on. Optimal foraging theory assumes; (1) that the fitness associated with an animal's foraging behavior has been maximized by natural selection and, (2) that foragers can make decisions so as to maximize their rate of food intake (energy) (Stephens & Krebs 1986).

From these assumptions, theoretical models have been developed to account for two fundamental problems that a predator must face; which prey item to consume (Optimal diet selection) and when to leave a patch of prey (Patch time allocation) (Crawley & Krebs 1992).

1.1 Optimal diet selection

Predators are expected to choose food items so as to maximize their rate of food intake during a foraging bout (Schoener 1969). They should therefore be sensitive to various costs and benefits associated to different food types. Benefits are usually considered as being the energy value of a prey individual as well as its nutrient quality. The costs of taking prey items are in relation with the time and energy spent in searching and handling the food item and the risks associated with that action (Barnard 1983). Three main predictions emerge from this model: 1) predators should prefer more profitable prey, 2) should feed more selectively when profitable prey are abundant and, 3) when profitable prey are abundant, they should ignore unprofitable prey, regardless of how abundant they are. Profitability is defined as the ratio of the benefits and the time required to find and to handle the prey (Krebs & Davies 1997).

1.1.1 *Prey selection according to prey size and prey species*

Capture success, handling time and energetic value of prey are factors that are involved in prey size selection. When given a choice of different prey sizes, many predators prefer intermediate prey sizes that gives them the highest rate of energy return (Elner & Hughes 1978; Pastorok 1981). The number of prey offered being equal, handling time of large prey is often very long and therefore reduces their overall profitabilty. On the other hand, handling time of small prey is shorter but their energy content are lower compared to large prey.

The longer handling time of larger prey is frequently due to the difficulty of capture (Dixon 1958; Hajek & Dahlsten 1987; Chow & Mackauer 1991) attributed to their better defense responses or escape abilities (Evans & Schmidt 1990). Prey species can also exhibit variable defense strategies and consequently show different vulnerability to predation. A vast array of behaviors and morphological features has evolved in herbivorous insects to counteract the attacks of predators and parasitoids (Evans & Schmidt 1990; Gross 1993). These behaviors will be discussed in relation to the insects used in our biological model in subsequent sections of this literature review.

1.1.2 *Prey selection according to predator size*

Predator attributes such as its ability to search, capture and subdue prey items may also determine the lower and upper limits of prey size exploitation (Malcolm 1992). Generally, foraging efficiency is positively correlated to predator age (size) (Sabelis 1992). Older predators are usually better in detecting prey, have higher mobility and are more rapid following prey contact (Griffith 1982; Malcolm 1992). For example, young coccinellid predators experience more difficulty in detecting and exploiting prey than older instars or adults (Dixon 1959; Wratten 1976; Hajek & Dahlsten 1987). As suggested by Temple (1987), reduced efficacy in prey capture due to predator age or efficient prey defense responses may influence prey selection and drive a predator to include substandard prey (e.g. smaller prey) in its diet in a disproportionate way.

1.2 Patch time allocation

Because of resource requirements and patterns of social interaction, prey are often clumped in time and/or in space (Barnard 1983). When prey are clumped, we can say that they are patchily distributed. A foraging predator may encounter a range of food patches that may differ in quality. Therefore, if a predator is an efficient forager, it should select and exploit the most suitable patches (Crawley & Krebs 1992). However, differences in quality may not be readily apparent when the patches are first encountered. A predator may need to sample many patches before it exploits what it considers the most suitable and profitable ones.

Many models have been proposed over the years to explain patch time allocation (Nelson & Roitberg 1995). Early models used simple fixed rules such as "time expectation" (Gibb 1962), "number expectation" (Krebs 1973) and giving up time (GUT) (Hassell & May 1974) that were not allowing insects to change their time allocation according to their perception of patch quality. Giving up time was defined by Krebs *et al.* (1974) as the interval between the last prey capture and emigration.

These different methods can all be adaptive depending on the host distribution pattern (van Alphen & Vet 1986). For example, it has been shown that when the variance in patch densities is small, both the fixed time rule and the number expectation rule are adaptive (Iwasa *et al.* 1981). However, in cases where insects search for patches of prey or hosts that rapidly change in quality, it may be more profitable to use flexible decision rules (Nelson & Roitberg 1995). McNair (1982) proposed a flexible GUT and suggested that, in patches perceived has good, an insect should be more persistent and use larger GUTs. This was corroborated in studies performed with parasitoids (van Alphen & Galis 1983) and with predators (Nakamuta 1986) that showed variable GUTs according to fluctuations in patch quality.

In parasitoids, it has been suggested that the presence of parasitized hosts in a patch can affect patch time allocation (Bakker *et al.* 1985). While exploiting a patch, the ability to discriminate between parasitized and unparasitized hosts (van Baaren *et al.* 1994) allows the parasitoid to perceive the rate of encounter with unparasitized hosts and consequently provide the parasitoids with information about the profitability of the patch (van Alphen & Vet 1986). If the rate of encounter with parasitized prey is high, it may be more profitable to leave and search for another patch (van Alphen 1993). Several publications have addressed this question and it was found that female parasitoids searched longer on patches containing only unparasitized eggs than on patches containing parasitized eggs (van Alphen & Vet 1986; van Lenteren 1991). However, van Alphen & Galis (1983) found no evidence of a detrimental affect of the presence of parasitized hosts on patch time allocation by parasitoid females. It has been demonstrated that many factors such as previous experience, physiological state, presence of chemical cues and encounter with competitors can affect patch time allocation in foraging insects (van Alphen 1993; Nakamuta 1986).

In coccinellid predators, the effect of decreasing prey density on patch time allocation has been studied (Podoler & Henen 1986). It was shown *Chilocorus bipustulatus* L. and *C. kuwanae* Sylvestri responded to a decrease in prey density with a decrease in patch residence time (PRT). However, the presence of discrimination ability and the influence of intrinsic quality of prey on the tendency to leave a patch have not been studied.

There are a number of other factors which may affect patch residence time in foraging insects. These factors include encounters with competitors, travel time between patches and previous experience (learning) (Waage 1979; van Alphen & Vet 1986).

2. LEARNING

Learning has been broadly defined as a change in behavior as a consequence of experience (Kimble 1961; van Alphen & Vet 1986). However, many authors underlined the importance of memory and forgetting in the learning process (Thorpe 1963; Tully 1984). Because of the numerous ambiguous definitions proposed over the years (see Papaj 1993), Papaj & Prokopy (1989) suggested three criteria by which to define learning: an individual's behaviour should (1) change in a repeatable way as a consequence of experience, (2) change gradually with continued experience and, (3) wane in the absence of continued experience of the same type, or as a consequence of a novel experience or trauma.

2.1 Learning in insects

Pre-imaginal and adult learning appears to be widespread among phytophagous (Papaj & Propoky 1989), parasitoids (Turlings *et al.* 1993) and social insects (Menzel *et al.* 1993) (see Papaj & Lewis 1993 for a review). Recent publications have demonstrated the importance of learning in increasing the overall foraging efficiency of insects. For example, butterflies learn to handle a flower more effectively when the latter is associated with a high level of nectar extraction (Lewis 1993). Parasitoids learn to reject less suitable hosts more effectively after a few encounters (van Baaren & Boivin 1998). Similarly, locusts reject unpalatable plants more rapidly with experience (Blaney *et al.* 1985).

2.2 Associative learning

Learning may occur at any foraging level, from habitat location to host or prey acceptation, and insects are confronted to a diversity of stimuli at all these levels (Papaj & Lewis 1993). Usually, naive insects respond to a hierarchy of physical and/or chemical cues that guide them to potential habitats, prey or hosts (Vet *et al.* 1990). With experience, other cues can be encountered and, therefore, be involved in the decisional process of prey or host selection. If these cues are adequate, it should be adaptive for a foraging insect to include these novel cues and learn to associate them to the appropriate prey or host (Papaj & Prokopy 1989; Turlings *et al.* 1993). For example, a number of parasitoids associate colour, size, shape and odour with the presence of hosts (Lewis & Tumlinson 1988; Vet & Groenewold 1990; Schmidt *et al.* 1993). In a model proposed by Vet *et al.* (1990), these stimuli (fixed or learned) are ranked according to the preference of each individual. It has been suggested that previous experience may alter stimuli ranking and consequently affect habitat and host selection. It has also been shown that the physiological state and the previous oviposition experience could influence the learning response (Takasu & Lewis 1993) and preference (Bjorksten & Hoffmann 1995) of parasitoids.

2.3 Learning in predators

However, the adaptive value of learning in prey selection by generalist predators has received little attention. Most research report improvement in searching behavior (Punzo & Garman 1989; Ettifouri & Ferran 1993), prey preference (Houck 1986) and prey recognition in adults (Pasteels & Gregoire 1984; Blois & Cloarec 1985; Henaut *et al.* 1997) as a result of conditioning process. Many species of predators can also develop aversion to certain types of noxious food (Berenbaum & Miliczky 1984; Montllor *et al.* 1984; Giroux 1996). They can learn to associate the taste of a food to a subsequent nausea or other negative internal effects and, after a few encounters, systematically reject this food type. Learned behaviors in predatory coccinellids will be further discussed in the section on Coccinellidae.

2.4 Generalists vs specialists

It has been suggested that the ability to learn cues may be more adaptive for generalist insects because they attack a variety of prey species whose relative abundance and spatial distribution change over time (van Alphen & Vet 1986). With experience, they may gather information about current hosts or prey distribution and learn to concentrate their efforts on habitats or patches containing the most profitable prey (Lewis *et al.* 1990). However, these learned behaviors could also be advantageous for more specialist insects. Hence, many studies have shown that learning is not correlated with the degree of specialisation in phytophagous species (see review by Papaj & Prokopy 1989). In fact, both generalists and specialists have learning abilities but appear to apply them differently in similar foraging situations (Poolman Simons *et al.* 1992).

2.5 Learning and biological control

Intraspecific variation in prey location and attack abilities of entomophagous insects has often been considered as being a major source of inconsistent results in biological control (Lewis *et al.* 1990; Papaj & Vet 1990). It has been argued that a better understanding of the learning abilities of natural enemies is essential in enhancing these biological control programs (Vet 1987; Propoky & Lewis 1993). For example, in large insect colonies maintained in laboratory, the insect response to target prey is often altered by simplified rearing conditions. With adequate knowledge, it would be possible to give biological control agents an appropriate level of experience by using specific stimuli before they are released in the field (Lewis *et al.* 1990).

3. PREDACEOUS COCCINELLIDAE

The beneficial status of ladybeetles has a rich history that has long been recognized by the general public and by entomologists involved in the development of biological control programs (Majerus 1994; Hodek & Honek 1996). The family to which these insects belong, the Coccinellidae, are extremely diverse in their habits. They have been recorded from a wide range of habitats feeding on many different prey types and both monophagous and polyphagous species are known (Mills 1981; Hodek & Honek 1996). The vast majority of species are beneficial insects because of their predaceous nature, but a few are injurious, being mycophagous or phytophagous (Hagen 1962). Only the entomophagous coccinellids will be discussed in this work.

Because of their wide array of potential habitats and prey types, searching and selecting prey are crucial components of a ladybeetle behavior. Three successive phases can be distinguished in the prey searching and prey selection behaviors in predatory coccinellids: (1) prey habitat location, (2) prey location and, (3) prey selection (Hodek 1993). This review, however, mainly considers behavioral responses of coccinellids in relation to prey location and prey selection which are the principal aspects discussed in the following manuscripts.

3.1 Prey location

All larval instars and adult Coccinellidae are mobile and actively search for their food (Hodek & Honek 1996). Adult coccinellids are known to have higher efficiency for prey detection than larvae (Lambin *et al.* 1996). Adults can locate their prey in response to visual cues such as color (Meredia *et al.* 1992) and shape (Lambin *et al.* 1996), but only from a very short distance (Stubbs 1980; Heidari & Copland 1992; Hattingh & Samways 1995), whereas prey location in larvae occurs only upon physical contact (Dixon 1959; Storch 1976). Coccinellid larvae are known to possess gustatory sensillae located on the upper surface of the last segment of the maxillary palps (Barbier *et al.* 1989) that could be used to identify a potential prey (Storch 1976) and to evaluate prey quality. However, little is known about the olfactory cues perceived by coccinellids when searching for prey (Ferran & Dixon 1993). It has been shown that adult coccinellids were capable of responding to aphid odors (Evans & Dixon 1986) and to honeydew before actual contact occurred (Obata 1986).

Usually, an arrestment behavior is observed after an individual has consumed a prey (Dixon 1959; Nakamuta 1982; Ettifouri & Ferran 1993) or after it has detected honeydew (Carter & Dixon 1984; Heidari & Copland 1993). An arrestment behavior can also be observed after a coccinellid larva has successfully captured a prey, but failed to feed on it. According to Carter & Dixon (1984), these cues indicate the a patch of prey has been found.

The arrestant stimulus provokes a change in the searching pattern of coccinellids. It switches from one of rapid movement at random to one of more intensive search as reflected by a decrease in speed (orthokinesis) and more frequent turns (klinokinesis) (Dixon 1959; Podoler & Henen 1986) consequently increasing the probability of locating a further prey individual. This searching pattern has an obvious adaptive value for predators such as coccinellids seeking for aggregated prey (Hassell & Southwood 1978). This behavior, called intensive foraging, reverts to extensive foraging after a period of unsuccessful searching (Carter & Dixon 1984).

3.2 Prey selection

3.2.1 *Influence of prey capture efficiency*

Because of their poor visual perception, prey defense reactions or escape response may play an important role in the capture success of coccinellid predators. Usually, as mentioned above, coccinellid larvae are known to recognize prey only when palpal contact is made (Dixon 1959; Storch 1976) or when a prey is contacted with the tips of the foretarsi (Wratten 1973). Therefore, prey that can avoid physical contact may escape capture more successfully than prey using passive defense strategies. Hajek & Dahlsten (1987) demonstrated in a study comparing the interactions between *Adalia bipunctata* (L.) and three aphid species exhibiting different defensive responses, that the more mobile aphid species was more successful at actively escaping from coccinellid larvae by simply walking away from the predator. It has also been shown that young aphid instars (Dixon 1958; Wratten 1973; Hajek & Dahlsten 1987; Losey & Denno 1998) or caterpillars (Brodeur *et al.* 1996) are usually less efficient at avoiding capture compared to older prey. They dropped less frequently from the feeding site which increased their mortality risks (Chau & Mackauer 1997; Losey & Denno 1998) and walked away from the predator less frequently (Hajek & Dahlsten 1987). Young coccinellid larvae are less mobile compared to late instars larvae and adults (Wratten 1973; Hajek & Dahlsten 1987), have a handling time that is inversely proportional to their size (Dixon & Stewart 1991) and experience more difficulty in killing and exploiting prey (Dixon 1959). All these factors can influence the net energetic value (profitability) and the constraints associated to the capture of a prey item and might explain the differences in prey selection and exploitation rate often observed (Dixon 1959; Pastorok 1981). Hence, the outcome of predator foraging abilities and prey defense response may determine the upper and lower prey size limits that a predator can exploit (Pastorok 1981) and therefore define its optimal range of prey sizes.

3.2.2 *Influence of prey suitability*

Prey selection can be modulated by other factors involved in prey profitability such as prey suitability. Even if predaceous coccinellids frequently have a wide range of accepted food (Mills 1981; Hodek & Honek 1996), all food items may not be equally suitable. Lower suitability of prey may be caused by the lack of some essential nutrients (Obrycki & Orr 1990), the presence of toxic components (Dixon 1958; Blackman 1967a; Gruppe & Roemer 1988) or their lower palatability (Dixon 1958; Nishida & Fukami 1989). Hence, when a predator has captured a prey, its taste and nutritive value become decisive (Hodek 1993)

A resource can be considered as a suitable food if it enables larval development with low mortality and high oviposition rate for an important proportion of females (Hodek 1962). For example, when *C. maculata* adults were fed with a diet exclusively composed of pollen, they had a doubled pre-oviposition period and their fecundity was reduced by 50% compared to adults fed with aphids (Hodek *et al.* 1978). Therefore, this resource is not highly suitable for the development of coccinellid individuals. Hence, an adequate selection of suitable prey types may ensure higher profitability and fitness.

Food selection by immature coccinellids depends to a large extent on the adult preference for specific habitats (Hodek & Honek 1996). Compared to adults, coccinellid larvae are less selective in choice situations (Blackman 1967b, Hodek & Honek 1996). However, larvae are known to reject prey if the latter is distasteful or poisonous (Hodek & Honek 1996). On the first encounter with a distasteful aphid, *A. decempunctata* rejected the prey after piercing the body wall but on subsequent attacks the body was not pierced and was rejected on contact.

Even if they are less selective in their food choice compared to adults, coccinellid larvae also require specific nutrients for their growth and development and actively search for prey (Hodek & Honek 1996). However, foraging coccinellid larvae may be extremely vulnerable to predation by other aphidophagous predators (e.g. *C. maculata)* (Lucas *et al.* 1997; 1998) which may increase the risk of mortality for these larvae seeking for food.

3.3 Optimal foraging and Coccinellidae

Many questions were raised in the literature concerning the ability of predatory coccinellids to forage optimally (Ferran & Dixon 1993; Dixon *et al.* 1997) and only a few publications address aspects of optimal foraging (Carter & Dixon 1984; Nakamuta 1986; Podoler & Henen 1986).

Optimal foraging theory assumes that the time allotted to foraging by a predator will result in a maximal rate of successful encounters with prey (Hassell & Southwood 1978; Stephens & Krebs 1986). Predaceous coccinellids are a good model for the study of this theory, mainly because their searching strategy is developed for patchily distributed prey (Dixon 1959; Carter & Dixon 1984; Ferran *et al.* 1994). Searching time in coccinellids is spent either traveling between patches of prey or is spent intensively searching for prey within a patch (Ferran & Dixon 1993). This intensive searching pattern can easily be quantified in a way that corresponds to the giving up time (GUT) described by Krebs (1978) and Charnov (1976).

3.3.1 *Patch time allocation*

It was shown that foraging coccinellid larvae and adults respond to prey size and density according to the predictions mentioned in the optimal foraging theory. Giving up time (GUT), which is defined by the author as the searching time in a prey patch, increases with increasing prey size (Nakamuta 1986). In the following papers of this thesis, we used the GUT defined by Krebs *et al.* (1974) that was described earlier (P 10). Predatory coccinellids also allocate an increasing proportion of their time to patches containing high densities of prey (Podoler & Henen 1986), indicating that they can use flexible rules in their foraging behavior. However, the physiological state of coccinellids can modulate their response (Carter & Dixon 1982; Nakamuta 1987). It has been demonstrated that hunger provokes the adoption of intensive search even before physical contact with a prey occurred and that the duration of intensive search increased after a prey encounter. Furthermore, starved coccinellid larvae were found to be less selective in their choice of prey (Ferran & Dixon 1993).

3.3.2 *Prey quality and presence of conspecific predators*

Ovipositing coccinellids also respond to the quality of prey colonies and to the presence of conspecific predators (Hemptinne *et al.* 1992; 1993). Females refrained from laying eggs in aphid colonies already exploited by adult coccinellids and in those that will shortly decline in abundance and, showed a clear preference for egg laying on young leaves supporting young aphids. Kindlmann & Dixon (1993) proposed a model for quantifying the female ovipositing behavior in order to understand how they maximize their offspring production and, to eventually predict the number of offspring produced. However, this model has not been tested.

3.4 Learning in Coccinellidae

Virtually nothing is known about learning processes and its potential role in foraging by predaceous Coccinellidae. Prey preference (Houck 1986) and searching behavior (Ettifouri & Ferran 1993) of predatory coccinellids can be influenced by conditioning on a specific prey type. *Stethorus punctum* (LeConte) was found to have a weak preference for the tetranychid mite, *Tetranychus urticae* Koch, following preconditioning to this species (Houck 1986). The foraging behavior of coccinellid varied according to the food they had previously eaten. When fed the same food, *Harmonia axyridis* (Pallas) larvae changed from an extensive to an intensive search mode (Ettifouri & Ferran 1993). But when fed with a new food type, larvae either continued the extensive search mode or adopted a less sinuous path than in the typical intensive search pattern. Therefore, the conditioning process improved the larvae searching efficiency for the target prey.

3.5 Recording behavioral data

Observation of behavioral sequences and their duration in different foraging situations may help to understand the mechanisms underlying prey or host location and selection in parasitoids or predators (Jervis & Kidd 1996). In several studies, different tracking systems were used to quantify specific behavioral components involved in prey searching behavior by coccinellids.

Previously, the foraging behavior of coccinellids was quantified by manually recording their position in space at successive points in time, and then, by calculating the recorded parameters (Ferran & Dixon 1993). Generally, this tedious procedure was used in most studies describing the searching behavior of predatory coccinellids (Carter & Dixon 1982; Podoler & Henen 1986; Frazer & McGregor 1994). However, collecting behavioral data manually is time-consuming and increases the occurrence of human errors.

Commercial softwares such as The Observer (Noldus Information Technology) are now available for recording and analysing behavioral components. Data are recorded with a special keyboard, and stored in the memory of a computer (Jervis & Kidd 1996). The results can be subsequently statistically analysed. However, this technique still requires human interventions.

Other techniques have recently been elaborated to automatically record and analyze the behavioral components of parasitoids (Allemand *et al.* 1994) and predators (Ettifouri & Ferran 1993). In the laboratory, these components are filmed and recorded on a video tape and specific software are used to compute the search parameters (Ferran & Dixon 1993). These techniques can be very helpful in measuring modifications in the searching behavior of insects, such as the distance traveled over short distances, speed of walking, path tortuosity (Waage 1978), time spent searching in specific areas and so on.

4. BIOLOGY OF INSECTS USED

4.1 *Coleomegilla maculata*

4.1.1 *Biology*

As for all other coccinellid species, *C. maculata* is holometabolous, i.e. it has a complete metamorphosis, and passes through four larval instars, prepupa, pupa and adult. The relative duration of instars is influenced by environmental factors such as temperature (Obrycki & Tauber 1978; Wright & Laing 1978) and food (Smith 1965). When *C. maculata* larvae are reared on the aphid *Myzus persicae* (Sulzer), the first instar takes an average of 4.6, the second 3.0, the third 3.5 and the fourth 5.9 days at a temperature of 21°C (Wright & Laing 1978). Smith (1965) determined the effect of two aphid species on the duration of the development time of *C. maculata* immatures and found that the duration of instars was longer when they were fed with *Acyrthosiphon pisum* Harris compared to *Rhopalosiphum maidis* (Fitch).

In Canada, only a few generations are observed and longevity of *C. maculata* adults is approximately of one year (Gordon 1985). In fall, adults leave the crops and aggregate at the base of large willow trees (Benton & Crump 1979) or in woodland edges (Roach & Thomas 1991) to overwinter. Large aggregations provide a degree of heat conservation that enhance winter survival. Mating has also been observed before the dispersal from hibernation sites (Solbreck 1974). In early spring, adults migrate to adjacent fields in search of food such as pollen (Benton & Crump 1981).

4.1.2 *Distribution*

C. maculata is a neartic species (Hodek & Honek 1996) widely distributed east of the Rockies (Gordon 1985) and is often one of the most abundant coccinellid species found in herbaceous crops (Hodek & Honek 1996). It is commonly found on corn (Wright & Laing 1980, Coderre & Tourneur 1988), alfalfa (Hodek 1973), potato (Groden *et al.* 1990), oat (Shade *et al.* 1970) and cotton (Cosper *et al.* 1983). It has also been observed in peach (Putman 1957) and apple (Goonewardene *et al.* 1989) orchards.

4.1.3 *Food*

Predaceous ladybeetles have a wide range of accepted food, and *C. maculata* is known to be one of the most polyphagous coccinellid known (Hodek & Honek 1996). It can survive on a variety of food sources. Apart from feeding on aphids (Mack & Smilowitz 1980; Coderre *et al.* 1987), they prey on eggs and young instars of Colorado potato beetle (Groden *et al.* 1990; Giroux *et al.* 1995) and lepidopteran species (Pimentel 1961b; Andow 1990; Coll & Bottrell 1991) as well as on mites (Putman 1957), whiteflies (Labrecque 1994) and chrysomelid species (Shade *et al.* 1970; Weber & Holman 1976).

C. maculata may also complete its development on the pollen of several plants (Smith 1960; Hodek *et al.* 1978).

C. maculata can also be reared on dry food (Smith 1965) and artificial diet (Atallah & Newsom 1966). In nature, it can rely on alternate prey during periods of low density of the target prey (Hodek 1993). These are important advantages for the use of this species as a biological control agent (Murdoch *et al.* 1985).

Coccinellids are also known to eat eggs, larvae and pupae of their own species (Agarwala & Dixon 1992). Pienkowski (1965) has shown that young first-instar *C. maculata* larvae fed readily on eggs of their own egg mass. Sibling cannibalism is considered to have an adaptive value in that it improves the chances of survival for coccinellid immatures (Agarwala & Dixon 1992), lengthens their life span (Pienkowski 1965) and helps to maintain populations when prey are scarce (Banks 1956; Mills 1982).

4.1.4 *C. maculata and the lepidopterous species found in crucifers*

As part of a program to develop a biological control management system for lepidopterous pests of crucifers in Québec (Boivin & Fournier 1993; Godin 1997), a field survey of the natural populations of predacious coccinellids in cruciferous crops in southwestern Québec was conducted. This monitoring revealed that predaceous coccinellids were present on plants from mid-June through the beginning of October and that the second seasonal peak of the coccinellids was synchronized with the highest seasonal peak of the lepidopterous pests (Roger *et al.* 1995). Furthermore, predaceous coccinellids were the most important predators in those fields, *C. maculata* (59%) being the most abundant species followed by the sevenspotted *Coccinella septempunctata* L. (30%) and the fourteenspotted *Propylea quatuordecimpunctata* L. (11%) ladybeetles. In a study conducted in New York state, Pimentel (1961b) noted that *C. maculata* contributed to the control of the caterpillars. However, the predation efficacy of the coccinellid was not determined. Even if *C.*

maculata preys on many lepidopteran species (Conrad 1959; Warren & Taddic 1967; Coll & Bottrell 1991) and is found in cruciferous crops in North America (Pimentel 1961b; Roger *et al.* 1995), little is known on its prey range and possible impact on the lepidopteran species occurring on crucifers.

4.2 ***Trichogramma*** **spp**

Trichogramma spp. are minute endoparasitoids of insect eggs that mainly attack lepidopteran species (Olkowski & Zhang 1990). Until now, 532 species of the family Trichogrammatidae (Hymenoptera; Chalcidoidea) have been described (Godfray 1994). The genus *Trichogramma* comprises more than 100 species that occur on a large number of crops in diverse types of agroecosystems (Hassan 1994) and many of them are used worldwide in biological control programs (Smith 1996). It is estimated that over 32 million hectares are treated annually with *Trichogramma* spp. in more than 30 countries (Hassan 1994).

The study of *Trichogramma* behavior has proven to be extremely important for their utilization as biological agents (Hassan 1994). Many of these studies have focused on the processes involved in host acceptance and host suitability (Waage & Greathead 1986; Godfray 1994).

Among, *Trichogramma* spp., *Trichogramma evanescens* (Westwood) is one of the most studied species because of its enormous potential for the control of lepidopteran pests in many countries of the world (Hassan 1981; Felk *et al.* 1990). In North America, *T. evanescens* is an important factor of mortality for the imported cabbageworm (*A. rapae*) eggs and was proven to be an efficient biological agent against this species in cruciferous crops (Parker *et al.* 1971; Oatman & Platner 1972). However, results obtained for the control of the cabbage looper (*T. ni*) were variable (Parker & Pinnell 1972), and generally, most *Trichogramma* spp studied contribute little to the natural control of the diamondback moth (*P. xylostella*) and require frequent mass releases (Talekar & Shelton 1993).

All species of *Trichogramma* do not have the same host range and important behavioral differences exist between species and between local populations (van Dijken *et al.* 1986; Pak *et al.* 1990). These differences may, at least, partially account for the variable results frequently obtained in the field (Parker & Pinnell 1972; Losey & Calvin 1990).

4.2.1 *Host suitability*

When a suitable host egg is encountered, the female *Trichogramma* examines the egg by antennal drumming, drills into it with her ovipositor and lays one or more eggs within the host egg, depending on its size. Other factors such as host age (Godin 1997; van Huis *et al.* 1991), host species (van Dijken *et al.* 1986), structure of host egg chorion (Pak *et al.* 1990) and parasitism by other females (Nelson & Roitberg 1993) can also influence host suitability and selection by parasitoid females.

4.2.2 *Influence of host age on host suitability*

Although eggs are a finite stationary resource, they are usually rapidly changing and short lived (Vinson 1994). As embryogenesis proceeds, the resource contained inside the egg is converted into more complex structures (Pak 1986). These egg resource modifications may change the acceptability of the egg for female parasitoids and reduce their suitability for developing parasitoid larvae (Ruberson *et al.* 1987; Ruberson & Kring 1993). Many studies concerning host suitability for oophagous parasitoids indicate that ovipositing parasitoid females usually select younger eggs and that this selection corresponds to higher suitability for immature parasitoid development (Juliano 1982; Hintz & Andow 1990; Ruberson & Kring 1993). These authors observed a longer developmental time and a decrease in survivorship as well as a production of smaller individuals in older eggs, indicating an influence of host age on parasitoid fitness. In many cases, the time needed for penetrating the egg increased with host age (van Huis *et al.* 1991; Ruberson & Kring 1993), resulting in a decrease in egg profitability, and consequently, in fewer parasitized hosts.

Female egg parasitoids appear capable of assessing host age by detecting alterations in host size, shape and texture or by measuring curvature and surface area of the latter (Strand & Vinson 1983).

4.2.3 *Parasitism-induced modifications of the host egg*

During the acceptance behavior, *Trichogramma* females oviposit and inject a venom responsible for the cessation of host development and necrosis. After the oviposition phase, the female deposits an external chemical mark by wiping her ovipositor across the host after oviposition (Strand 1986). As a parasitoid larva develops within the host egg, it exploits the resources and secretes a surrounding membrane (Al Rouechdi & Voegelé 1981). These physiological changes harden the egg chorion and change the egg coloration that becomes gradually black. At the end of parasitoid larval development, most of the host resources are used up while air spaces form inside the egg (Al Rouechdi & Voegelé 1981).

4.2.4 *Intraguild Predation (IGP) on parasitized eggs*

Parasitism can influence host suitability and consequently host selection for other foraging parasitoid female (Godfray 1994) as well as prey selection for predators (Rosenheim *et al.* 1995). The attack of a parasitized host by a predator that consumes both the host and the developing immature parasitoid, is considered as unidirectional intraguild predation (IGP) (Rosenheim *et al.* 1995). IGP is defined as the killing and eating of species that use a common resource and thus are potential competitors (Polis *et al.* 1989). IGP frequently occurs under conditions of frequent local resource competition (Rosenheim *et al.* 1995).

Vinson (1975) was the first to suggest that parasitoid-induced host alterations could reduce predation (IGP) of parasitized hosts. This was observed mainly for predators attacking sessile prey like eggs or whiteflies (Al Rouechdi & Voegelé 1981; Hoelmer *et al.* 1994). In all cases where discrimination against parasitized prey occurred (see Rosenheim *et al.* 1995 for review), recently parasitized and unparasitized prey were consumed in the same proportion, but prey containing late larval and pupal stages of parasitoids were avoided. For example, Kindlmann & Ruzicka (1992) demonstrated that that syrphid larvae (*Metasyrphus corollae* (Fabr.)) consume recently parasitized aphids but reject parasitized aphids that have become partially or completely mummified. Similar behaviors were observed with other predators such as the two coccinellid species; vedalia beetle *Rodolia cardinalis* (Mulsant) attacking parasitized and unparasitized cottony-cushion scales (Quezada & DeBach 1973) and *Delphastus pusillus* (LeConte) attacking unparasitized whitefly prey and whiteflies parasitized by aphelinid wasps (Hoelmer *et al.* 1994). As suggested by many authors (Al Rouechdi & Voegelé 1981; Hoelmer *et al.* 1994), immature parasitoids may need to reach advanced developmental stages before they significantly change the physiological caracteritics of their hosts. Predators could then detect host modifications such as changes in the texture, size and shape of the prey, and select prey accordingly. In a study that involved *C. maculata* attacking parasitized aphids, the coccinellid removed the parasitoid pupae without attempting to feed on it (Wheeler *et al.* 1968). However, in some studies (generally involving aphid prey) the parasitized prey are readily consumed by the predators even after they have reached an advanced stage of parasitism (Frazer & Gilbert 1976; Wheeler 1977; Brodeur & McNeil 1992).

In most studies where discrimination have been observed, the mechanisms underlying parasitized prey discrimination in generalist predators such as *C. maculata*, and their possible influence on IGP, have not been studied.

4.3 The lepidopterous prey complex

4.3.1 *Problematic*

Crucifer production in Québec (cabbage, broccoli, cauliflower and Brussels sprouts) covers an area of 4120 ha, for an approximate value of 32 million dollars (1994) (Statistique Canada 1996). These crops are attacked throughout Northeastern America by caterpillars of three species, namely, the imported cabbageworm, *Artogeia rapae* (L.) (Pieridae), which is the most abundant species followed by the diamondback moth, *Plutella xylostella* (L.) (Plutellidae) and the cabbage looper, *Trichoplusia ni* (Hübner) (Noctuidae) (Harcourt 1962; Godin & Boivin 1998). Even though insecticides are routinely used in cruciferous crops, the annual estimated loss due to lepidopterous pest species in the United States is of 34 million dollars (Schwartz & Klassen 1981). *A. rapae* is the eight most important agricultural pest in Québec, while *P. xylostella* ranks fourteen and the sporadic pest *T.ni* causes important damage only when outbreaks occur (Chagnon *et al.* 1990).

4.3.2 *Seasonal occurrence*

In North America, populations of this lepidopterous species complex are present throughout the growing season and reaches its peak in August. However, populations of these multivoltine species differ markedly from location to location and between years (Harcourt 1957; 1962; Biever *et al.* 1992). In southeastern Canada, *P. xylostella* has three to six generations annually while both *A. rapae* and *T. ni* have three generations (Harcourt 1963; Godin & Boivin 1998). In New York, *A. rapae* and *P. xylostella* are detected earlier than *T. ni* which is always the most abundant species at harvest (Andaloro *et al.* 1982). In southwestern Québec, *P. xylostella* is more abundant early in the season whereas *A. rapae* is more abundant at the end of the summer (Godin & Boivin 1998). In this last study, only small populations of *T. ni* were observed and most *T. ni* larvae were collected in August and September.

4.3.3 *Plant damage and methods of control*

These lepidopterous pests are mainly responsible for damage to late crop (Harcourt 1962) and all three species can cause serious economic damage by feeding on the foliage and on the marketable portion of the plant or by contaminating these parts with frass or larvae (Harcourt *et al.* 1955). In Québec, control of lepidopterous pests necessitates five to six insecticide treatments annually, for a total of more than 4000 kg of active ingredients (Chagnon *et al.* 1990). However, over the years, these insects have become resistant to several synthetic insecticides (Talekar & Shelton 1993). *P. xylostella* and *T. ni* developed resistance to DDT in the 1950's (Harcourt 1957, Talekar & Shelton 1993) and, in recent years, to the bacterial insecticide *Bacillus thuringiensis* (Shelton & Wyman 1992). Because methods of controlling their populations through the application of pesticides have proven detrimental over the long term (Edelson *et al.* 1993), efforts have shifted to the development of alternative control methods more environmentally friendly.

Biological control, which involves the use of predators, parasitoids or pathogens of target pest species, has proven encouraging in limiting the outbreak of lepidopteran populations (Jaques & Laing 1978; Chagnon *et al.* 1990; Edelson *et al.* 1993). In Québec, the use of *T. evanescens* (Boivin & Fournier 1993) and *C. maculata* (Roger *et al.* 1995) for the control of the lepidopterous pests is being investigated. However, effective implementation of such programs requires a thorough understanding of the ecology of the lepidopterous pests.

4.3.4 *Development stages and larval defense behaviors*

The three lepidopterous species are of different sizes, have different morphologies and development time and exhibit different defense responses or escape abilities. These differences can influence their level of vulnerability to natural enemies and the extent to which they will be predated or parasitized (Jervis & Kidd 1996).

A. rapae

The females are active during the day and lay their eggs singly on the outer leaves of the host plant (Harcourt 1962). The eggs are yellow, have the shape of a bullet and are 1.0 x 0.4 mm in size (Pak *et al.* 1986). They stand erect and are attached to the leaf at their basal ends (Harcourt 1963). At a temperature of 22°C in laboratory conditions, the period of incubation for the eggs is four to five days (Richards 1940).

Five larval instars are observed in *A. rapae* and the average total larval period is 15 days during July and August in Ontario (Harcourt 1963). The last instar can reach a body length of 30 mm. The caterpillar is pale green with a velvety appearance due to a profusion of hairs that form a white bloom over the body (Harcourt 1963). The larvae feed from the lower surface of outer leaves until the end of the third instar. Afterwards, they move to the central part of the plant for the final two instars where they make most of the damage (Harcourt 1963).

In contrast to other *Pieris* butterflies, the caterpillars of this species do not usually use active avoidance mechanisms against potential natural enemies such as the parasitoid *Apanteles glomeratus* L. and are therefore extremely vulnerable to predation (Ohsaki & Sato 1990).

P. xylostella

The eggs are small, oval and flattened, having a scale-like appearance (Harcourt 1961). The average length and width are 0.4 and 0.3 mm respectively (Harcourt 1963). They are laid singly or in small groups, usually on the upper side of the leaf and preferentially in concavities (Telekar & Shelton 1993). In field cages, the average incubation period is of 5.6 days (Harcourt 1957).

P. xylostella has four larval instars and can reach a length of 1 cm. The caterpillars are smaller in size compared to the immatures of the other two species. The average duration of the developmental stages in the field are of 4.0, 4.0, 5.0, and 5.6 days for the first through fourth instars, respectively. Soon after emergence, the larva crawls to the lower surface of the leaf and mines the leaf tissues to feed on the mesophyll whereas older larvae feed freely from the upper surface (Harcourt 1957; Telekar & Shelton 1993).

The mines offer protection to the young and more vulnerable first-instar larvae. When disturbed, older larvae adopt more active and aggressive behavioral responses (Harcourt 1957). They wriggle backwards very rapidly, or drop from the leaf on a fine silken thread where they remain suspended.

T. ni

The eggs are white, roundly shaped (0.6 mm diameter x 0.4 mm height) and vertically striped (Sutherland 1966). In the field, they are usually laid to a vertical or lower surface of vegetation (Shorey *et al.* 1962). Even if the female does not deposit the eggs in masses, they may frequently be placed in small groups containing 2 to 11 eggs (Harcourt 1963). At a constant temperature of 24°C in the laboratory, McEwen & Hervey (1960) reported that approximately 3 days were required for hatching whereas at 23°C, an average of 4.8 days were needed to hatch with a percentage of viability of 75% (Shorey *et al.* 1962).

There are usually five larval instars (McEwen & Hervey 1960) but this number may vary depending on the conditions under which the larvae were maintained (Shorey *et al.* 1962). At a temperature of 27°C in laboratory conditions, the total larval development period lasted 35.9 days (Toba *et al.* 1973). The larva is similar in size to *A. rapae* and is a more general feeder compared to the latter (Harcourt 1963).

To move, *T. ni* crawls by doubling up to form a loop, then projecting the front end of the body forward. Hence, the common name, cabbage looper. When physically disturbed, *T. ni* larvae use this ability to display conspicuous postures to startle the intruder by projecting the front end of the body upward (F. Fournier, pers. comm.).

PREVIEW OF THE FOLLOWING CHAPTERS

The third chapter, "Differential prey exploitation by the generalist predator *Coleomegilla maculata lengi* according to prey age and species" (submitted to Entomologia Experimentalis & Applicata) deals with aspects of prey exploitation, voracity and predation efficiency by this generalist predator when it is confronted to eggs and larvae of three sympatric lepidopterous species in laboratory conditions.

The fourth chapter, " An image analysis system developed for evaluation of *Coleomegilla maculata* larvae's behavior" (published in Canadian Agricultural Engineering 40: 55-60). deals with the modification of an automated system initially developed for parasitoids, in order to evaluate behavioral parameters of prey selection in *C. maculata* larvae. This system was especially modified to replace visual observations required in chapter 5.

The fifth chapter, "Discrimination of parasitized prey by the generalist predator *Coleomegilla maculata lengi* (Coleoptera: Coccinellidae): mechanisms and implication on intraguild predation" (submitted to Oecologia), deals with evidence of discrimination capacity and prey selection according to intrinsic prey quality and how it may influence parasitoid populations.

The sixth chapter, "Learning affects prey selection in a generalist coccinellid predator" (to be submitted to Behavioral Ecology and Sociobiology), deals with the presence of behavioral plasticity in the egg rejection behavior observed in chapter 5 as well as the influence of hunger and the quality of the food ingested on the coccinellid larvae response.

All literature cited in these chapters is listed at the end of the thesis.

CHAPTER 3

Differential prey exploitation by the generalist predator *Coleomegilla maculata lengi* Timb. (Col., Coccinellidae) according to prey age and species

CAROLINE ROGER, DANIEL CODERRE & GUY BOIVIN

Submitted to Entomologia Experimentalis & Applicata

ABSTRACT

Prey exploitation by the generalist predator *Coleomegilla maculata lengi* Timb. (Col., Coccinellidae) of three sympatric lepidopterous species was quantified in relation with prey size (age) and prey species. Based on the optimal foraging theory, we argued that costs associated with the exploitation of small and large prey are higher than those of intermediate prey size. As a result, we expected a higher prey exploitation rate on intermediate prey size leading to a convex prey exploitation curve. Laboratory experiments showed that, within a given prey instar, *C. maculata* preyed more on the smallest species *Plutella xylostella* (L.) than on larger *Artogeia rapae* (L.) and *Trichoplusia ni* (Hübner). Generally, predation by *C. maculata* on the three prey species decreased with increasing immature size (age). The predation efficacy of *C. maculata* adults and fourth instar larvae was higher compared to second instar larvae. Although, *C. maculata* showed a higher level of predation on smaller immature prey, we demonstrated that it is not the optimal size range for this predator. As predicted, prey weight consumption rate by *C. maculata* was higher at intermediate prey size leading to a convex prey exploitation curve. The beneficial impact on the host plant of *C. maculata* predation was also estimated by using a Protection Index that considers the differential predation caused by the coccinellids and the relative importance of each pest species in terms of plant injury. *C. maculata* has a more significant beneficial impact when it preys on *T. ni* immatures.

Key words: *Coleomegilla maculata*, predation, prey size, prey species, predator-prey interactions, *Artogeia rapae*, *Plutella xylostella*, *Trichoplusia ni*, crucifer pests

INTRODUCTION

Generalist predators are confronted with different prey types which differ in energy values and costs associated with their capture and ingestion. Optimal foraging theory (Stephens & Krebs 1986) assumes that predators should select and exploit prey types that will allow them to maximize their instantaneous rate of energy gain. If prey quality is a function of prey size, the theory then predicts that generalist predators should preferentially exploit larger prey. This prediction is based on the fact that profitability (ratio of energy gain to costs associated with handling time) of small prey is lower than larger prey. However, prey-predator and host-parasitoid models have shown that capture success rate decreases with increasing prey size (Pastorok 1981; Chau & Mackauer 1997) and that it is often more advantageous to exploit smaller prey even if larger prey offer higher instantaneous energy gain. The lower capture rate of larger prey has been attributed to their better defense responses or escape ability (Evans & Schmidt 1990). Factors that strongly influence the capture success rate of predators include predator-prey size ratio (Sabelis 1992), prey detection, mobility and rapidity of predator response following prey contact (Malcolm 1992) and aspects of prey vulnerability related to prey size and prey species (Pastorok 1981).

Even if the costs associated with the exploitation of small and large prey are different, we hypothesize that, at both ends of the size spectrum, the insect predator experiences a decrease in prey exploitation efficacy. We therefore predict that prey exploitation will be higher on intermediate prey size leading to a convex prey exploitation curve. Because costs associated with handling time and risk of injury decrease with increasing predator size (Sabelis 1992), we also predict that larger predators will exploit large prey more efficiently. As the effectiveness of behavioural defense responses also varies with prey species (Dixon 1958; Hajek & Dahlsten 1987; Chau & Mackauer 1991), we further hypothesize that interspecific differences in morphology, mobility and behavioural defense capacity will modulate the prey exploitation response of the predator.

The predaceous coccinellid *Coleomegilla maculata lengi* Timb. is a neartic polyphagous species (Hodek & Honek 1996). All four instars, as well as the adult, are predaceous and may attack the prey type. Although many coccinellids are generalist feeders, predation studies indicate that they may also be selective in their prey choice (Mills 1981; Obrycki & Orr 1990). *C. maculata* has been reported feeding on aphids (Mack & Smilowitz 1980; Coderre *et al.* 1987), eggs of the European corn borer (Andow 1990; Coll & Bottrell 1991) and eggs and young larvae of Colorado potato beetle (Groden *et al.* 1990; Giroux *et al.* 1995). It may also complete its development on pollen of several plants (Smith 1960; Hodek *et al.* 1978). This species could be used as a biological control agent based on its capacity to rely on alternate prey during periods of low density of the target prey (Hodek 1993).

Predatory coccinellids, while searching for prey, orient themselves through taxes (phototaxis and geotaxis) and plant structure (Hodek 1993). Adult respond to visual cues (Meredia *et al.* 1992; Lambin *et al.* 1996) but only from very short distances (Stubbs 1980; Hatting & Samways 1995) whereas prey location in larvae occurs only upon physical contact (Dixon 1959; Storch 1976). Compared to larvae, adult coccinellids are known to have higher mobility (Wratten 1973), higher efficiency for prey detection (Lambin *et al.* 1996) and higher capture success rate (Dixon 1959). These factors often vary according to prey species (Dixon 1959; Hajek & Dahlsten 1987). Hence, predator age, prey size and their escape or defense responses may play an important role in prey exploitation for a coccinellid facing different prey types in a habitat. Although much information is available on the behaviors related to searching, pursuing, capturing and eating, very little is known about the behaviors related to prey size or prey species.

In southwestern Québec, *C. maculata* was found to be the most abundant in crucifers (Roger *et al.* 1995.) but little is known on its prey range and possible impact on the lepidopterous species occurring on crucifers. In a study realized in New York state, Pimentel (1961a) also noted that *C. maculata* was a major aphid predator on crucifers and suggested that it contributed to the control of the caterpillars (Pimentel 1961b) but the predation efficacy of the coccinellid was not quantified.

Three sympatric lepidopterous species can be found in crucifers in southwestern Québec, the imported cabbageworm, *Artogeia* (= *Pieris*) *rapae* (L.), the diamondback moth, *Plutella xylostella* (L.) and the cabbage looper, *Trichoplusia ni* (Hübner). These species are of different sizes, have different morphologies and exhibit different locomotion behaviours. These differences in sizes and feeding habits translate into differences in the level of damage these pests can cause (Shelton *et al.* 1982). Injury equivalencies for the three species have been established (Harcourt *et al.* 1955) and they are expressed in Cabbage Looper Equivalents (CLE). This index has been used to quantify the impact of each lepidopterous species on different plants in order to calculate more precise intervention levels (Shelton *et al.* 1982; Doman *et al.* 1994). In a tritrophic perspective, the combination of the CLE index and the different prey exploitation rates of these pests by the predator *C. maculata* could allow a better understanding of the effect of predator-prey interactions on primary production of the host plant.

The purpose of this investigation was to determine the influence of prey size, prey species and predator age on prey exploitation by the polyphagous predator *C. maculata* under laboratory conditions. The beneficial effect of predation on the host plant was also evaluated using a Protection Index (PI) that considered the differential mortality caused by *C. maculata* on the three lepidopterous species and the relative importance of each pest species in terms of plant injuries (CLE).

METHODOLOGY

Insects

Adult *C. maculata* were collected in early May from hibernation sites near corn fields in Saint-Hyacinthe (72°56'W,45°39'N), Québec, Canada. They were kept on a fresh liver-based artificial diet (Coderre, unpubl.) and on wild flower pollen at 22°C, 70% r.h., and a photoperiod of L16:D8. Eggs were collected twice a week and put in Petri dishes until hatching. Larvae were also fed with the liver-based diet and pollen. The predatory larvae used in the experiments had molted two days before the tests were conducted. Before a test, adults, second and fourth instar larvae were placed individually in 50-mm Petri dishes and starved for 24 h to standardize hunger level.

Eggs and larvae of the three lepidopterous species were reared at 25°C, 60% r.h. and a photoperiod of L14:D10 on an artificial diet specific to the needs of each species. These artificial diets were developed by Webb & Shelton (1988) for *A. rapae* , by Shelton *et al.* (1991) for *P. xylostella* and, by Shelton (pers. comm.) for *T. ni*. All eggs used in the experiments were less than 24 h old. All larvae were used 24 h after entering a specific instar and were identified using morphological characteristics described by Richards (1940) and Harcourt (1957; 1962).

Differential predation

Prey exploitation was measured using non-choice tests in which second and fourth instar larvae as well as adults *C. maculata* were individually placed in the presence of either eggs, first-, second-, or third- instar larvae of each lepidopterous species. Preliminary tests have indicated that neither larvae or adult *C. maculata* were able to consume fourth-instar larvae of *A. rapae* and *T. ni*. Consequently, this instar was not included in the experiment. Twenty replicates were conducted for every predator-prey combination. Each coccinellid was offered a number of prey, determined in preliminary tests, that varied between 30 and 200 eggs or between 3 and 35 larvae according to predator and prey sizes. To minimize potential interference on prey exploitation due to the decrease of prey availability, prey were offered in excess. The appropriate number of prey was placed on a leaf of cabbage (Prime blue Y.R. 65-3540) of approximately 7 cm in diameter in a plastic container (11 cm in diameter and 2 cm in depth). The stem of the leaf was inserted in the side of the container, the hole was sealed with plasticine and a wet piece of cotton was placed around the stem to prevent leaf dessication. The prey were allowed to settle before a predator was placed on the cabbage leaf. A piece of muslin held by a rubber band was used to close the system. The leaf was not in contact with the muslin nor the bottom and sides of the container allowing the prey and the predator to move freely without permitting prey access to a refuge but the prey could elude predator attacks by using escape responses. Five containers without predators were included in each predator-prey combination as experimental controls. A complete randomized block design was used which included all predator-prey combinations with replications in time. Experiments were held at 22°C, 70% r.h. and a photoperiod of L16:D8.

After 24h, mortality as determined by broken chorion for eggs or by death of larvae, was evaluated. To correct for mortality of prey unrelated to predation, mean mortality observed in controls was subtracted from mean mortality in the corresponding experimental treatments. Partial consumption of prey was included in the evaluation of fresh weight consumption. Square root transformed data were subjected to a 3-way ANOVA (SuperAnova, Abacus Concepts 1989) to test the influence of prey species, prey age and predator age. When interactions between factors occured, simple contrasts within the global model were performed. Prey capture efficacy of *C. maculata*, defined as the percentage of predators that were successful in attacking and consuming at least one prey, was also evaluated. Chi-square tests (StatView, Abacus Concepts 1993) were used to verify the influence of prey size, prey species and predator age on C. *maculata* capture efficiency.

Weight of C. *maculata* was determined by weighting 10 live individuals of each predator stage tested. Average weights obtained were submitted to a One-way ANOVA to evaluate the differences between predator stages.

Prey weight consumption

Mean weight of each stage of the three lepidopterous species was evaluated by weighting live individuals. Twelve replicates each containing 50 eggs, 20 first-, 10 second- or 5 third instar larvae of each prey species were conducted. Average weights obtained were analyzed using a 2-way ANOVA to determine if there were prey weight differences between prey species and prey stages.

In order to determine the food exploitation rate by coccinellids larvae, the number of prey consumed was transformed to prey weight consumption by multiplying the average weight of each prey stage by the number of prey consumed by each predator. To evaluate the effect of prey body size on the exploitation success rate of C. *maculata*, we related the results of prey weight consumption for all three lepidopterous species to the weight of each prey stage offered. These data were submitted to linear and polynomial regressions (StatView, Abacus Concepts 1993) to evaluate the relation between the two variables.

The number of successful attacks needed for a coccinellid predator to consume 1 mg in prey weight was estimated using the data obtained in the predation experiments (number of prey killed in 24 H) divided by the prey weight consumption rate for each predator-prey combination (Table 3.1).

Impact of predation on plant damage

The potential impact of predation by *C. maculata* on the primary production of the host plant was evaluated by using a Protection Index (PI). This index considers the number of prey killed by *C. maculata* for each pest species as established in the prey exploitation test divided by the corresponding Cabbage Looper Equivalency (CLE) established by Harcourt *et al.* (1955). CLE has been determined based on the total larval foliar consumption of each lepidopterous species. The foliar consumption of *A. rapae* and *P. xylostella* larvae were standardized according to the consumption of a *T. ni* larva; (One CLE = One *T. ni*, 1,5 *A. rapae*, 5 *P. xylostella*) (Shelton *et al.* 1982). The CLE for *P. xylostella* was decreased to 1 CLE = 5 rather than 1 CLE = 20 as determined by Shelton *et al.* (1982), because it better represents its potential for qualitative damage (Doman *et al.* 1994). Data were subjected to a 3-way ANOVA (SuperAnova, Abacus Concepts 1989)) to test the influence of prey species, prey age and predator age on the average Protection Index. When interactions between factors occured, simple contrasts within the global model were performed.

RESULTS

Differential predation

All *C. maculata* stages tested successfully attacked the eggs and the first three instars of all three lepidopterous species (Fig. 3.1). In preliminary tests, the fourth instar larvae of *T. ni* and *A. rapae* were not attacked while coccinellid larvae and adults killed, in average, less than one individual of *P. xylostella* fourth instar larvae per day. The results obtained with the fourth instar larvae of these two prey species were not included in the test. Significant interactions between the factors tested were noted (F=2.59; df=12; P=0.0023) indicating that the three stages of *C. maculata* differed in their predation responses depending on prey age and prey species.

Generally, exploitation on all three prey species decreased with prey age (F= 477.97 df= 3; P<0.0001) (Fig. 3.1). *C. maculata* was more efficient against eggs and first instar larvae whereas older prey larvae were significantly less vulnerable to predation. Furthermore, more than 85 % of the predaceous coccinellids tested successfully attacked and consumed at least one egg, first or second prey instar in a 24h period (Fig. 3.2). However, when confronted to third instar larvae, capture efficacy significantly decreased with an average of 36% successful coccinellids (Chi^2=253.74; df= 3; P<0.0001).

Within a prey instar, *C. maculata* second instar larvae were more voracious toward the eggs and first-instar larvae of *P. xylostella* compared to those of the other two prey species (*A. rapae*: F= 30.96; df=1; P<0.0001; *T. ni*: F= 58.79; df=1; P<0.0001) (Fig. 3.1). However, they did not show any differences in their predation efficacy on second- and third-instar *P. xylostella* larvae compared to *A. rapae* and *T. ni* larvae of the same age (P>0.05). Furthermore, *C. maculata* second instar larvae had a low capture efficacy on late instar prey (Fig. 3.2A). When these small predators occasionally killed larger prey, they only consumed them by partially sucking the body fluids. Overall, *C. maculata* fourth instar larvae and adults also had a higher predation rate toward immature individuals of *P. xylostella* compared to those of the two other prey species (Fig. 3.1B) but they generally killed *T. ni* and *A. rapae* immature instars in the same proportion (P>0.05).

Weights of the three developmental stages of *C. maculata* differed significantly (F= 52.73; df= 2; P= 0.0002). Fourth instar larvae and adults were approximatively 9 times larger (9.93 mg ±2.21) than second instar larvae (0.84 mg ± 0.05).

Predator size (age) significantly affected the level of prey exploitation (F=43.10; df=2; P<0.0001). Overall, predation efficacy of second instar coccinellids was significantly lower compared to *C. maculata* adults (F=183.22; df=1; $P<0.0001$) and fourth-instar larvae (F=207.61; df= 1; $P<0.0001$) (Fig. 3.1). However, predation rate on third instar prey was similar between all predatory coccinellid stages ($P>0,05$) even if the percentage of predators which caught prey was significantly lower for *C. maculata* second instar larvae compared to the other two predaceous instars ($Chi^2=25.80$; $P<0.0001$) (Fig. 3.2). It is also interesting to notice that, when significant differences were observed in predation behaviour between *C. maculata* fourth instars and adults, the predaceous larvae always showed a higher predation rate on lepidopterous larvae (F=207.61; df= 1; $P<0.0001$) whereas coccinellid adults were more efficient on eggs (F=27.21; df= 1; $P<0.0001$).

C. maculata prey weight consumption

Prey weight differed significantly between prey species (F= 32.3; df=2; $P<0.0001$) and age classes (F= 199.4; df=3; $P<0.0001$) (Table 3.1). Overall, *P. xylostella* immatures had a significant lower weight (approximately 50%) than the immatures of the two other lepidopterous prey species. Within a prey species, weight increased with prey age (Table 3.1).

To test the influence of prey body size on prey exploitation by *C. maculata*, prey weight consumption rates were compared to the weights of each immature prey stage offered (Fig.3.3). Using data obtained with all *C. maculata* stages, it appeared that exploitation of prey immatures of intermediate sizes was higher compared to small or large prey which induced a prey exploitation curve that was convex to the prey weight axis ($R^2=0.23$; $P=0.0090$) and peaked at a prey weight of 5.5 mg. The shape of the curve varied mainly according to predator size (age). *C. maculata* second instar larvae had a low efficacy rate in exploiting prey of most sizes producing no significant concavity ($R^2=0.041$; $P>0.05$). Fourth instar larvae and adults had significant convex prey exploitation curves that peaked at a prey weight consumption rates of 12.0 and 7.2 mg /day, respectively (Fig. 3.3).

Effect of CLE on prey exploitation results

C. maculata had a higher predation level on *P. xylostella* eggs and larvae than other prey (Fig.3.1). However, when these data are combined with a measure of foliar consumption by the three lepidopterous pest species (CLE) in order to evaluate the beneficial impact of predation by *C. maculata* (PI), it appeared that the highest impact of predation was on *T. ni* immatures (F=49.72; df=2; P<0.0001) (Fig. 3.4). This Protection Index decreased with prey age for all prey species tested (F=500.71; df=3; P<0.0001). Fourth instar larvae as well as adult *C. maculata* were significantly more efficient compared to second instar larvae (F=132.12; df; 2; P< 0.0001).

DISCUSSION

The present study demonstrates that immatures of the three lepidopterous species are acceptable prey for the generalist predator *C. maculata*. The predaceous larvae and adults consumed all prey instars except for the last instar of *T. ni* and *A. rapae*. There was an inverse relation between the number of prey killed and prey size (Fig. 3.1) which is consistent with other laboratory studies conducted with *C. maculata* in similar laboratory conditions (Groden *et al.* 1990; Giroux *et al.* 1995).

Even if predation rate was higher on small prey, they are not necessarily the most suitable prey for this predator. According to optimal foraging theory, predators are expected to exploit large prey in an effort to maximize energy return (Schoener 1969). On the other hand, consuming smaller prey may be adaptive if large prey are costly in terms of injury risks. Hence, even if the nature of costs associated to exploitation of small or large prey are different, they both could result in a lower net energy gain than intermediate sized prey. Based on these assumptions, our main prediction was that, all other factors being equal, a generalist predator like *C. maculata* should have a better exploitation rate on prey of intermediate size because they represent the best trade-off in terms of predation costs and instantanous rate of energy gain. If we assume that the rate of weight consumption is a good indication of the instantanous rate of energy gain, the results obtained support this prediction. As expected, prey weight consumption by fourth instar larvae and adult *C. maculata* was higher on prey immatures of intermediate size leading to a convex prey weight consumption curve (Fig. 3.3). Thus, even if *C. maculata* consumed a higher number of small prey (Fig. 3.1), they were not of high energetic value for this predator because it had to attack and consume many small prey to obtain sufficient energy (Table 3.1). Results showed that they obtained more total biomass from intermediate sized prey than from either small or large prey.

The predatory behaviour of a coccinellid is mainly composed of three major components: searching, prey capture and consumption (Nakamuta 1983). This sequence, defined as a feeding bout, has to be entirely followed for each prey encounter and costs associated with each of the predatory components can vary and consenquently influence the optimal prey exploitation level. When foraging in an environment containing only small prey, a predator has to spend more time searching because of the large number of prey it has to subdue to gain sufficient energy. In this case, search time can be viewed as placing an upper limit on the prey consumption rate of small prey and this could have obvious implications for predator fitness, as there is a negative correlation between time invested in searching (and the risk associated with this activity) and net energy gain (Crawley & Krebs 1992). Higher investment in searching time and low energetic value of small prey compared to larger prey may increase lost opportunity time on larger prey for *C. maculata*.

Coccinellid larvae usually locate prey by physical contact (Storch 1976; Carter & Dixon 1984) while adults may use visual contact but only at a very short distance (Stubbs 1980; Hattingh & Samways 1995). Because of this poor visual acuity, predaceous cocinellids must usually take the decision to attack and subdue or to give up upon encounter. Hence, for coccinellids, prey exploitation is related mostly to prey availability (Carter & Dixon 1982) and to the relative vulnerability of the food items (Wratten 1973). Because defense responses of large prey are usually more efficient than those of smaller sized prey (Dixon 1959; Hajek & Dahlsten 1987), their exploitation increases the probability of injury for the predators. These differences in prey vulnerability could have reduced the capture efficacy of *C. maculata* on late instars (Fig. 3.2) and increased the time allocated to their manipulation (handling time), consequently provoking a decrease in prey weight consumption (Fig. 3.3). Thus, despite the fact that the instantaneous energy gain per individual is higher on large prey, the time lost and the risk of injury associated with their capture and consumption could also have placed an upper limit on the prey consumption rate observed.

The complexity of the environment in which a predator forages can influence the magnitude of the costs and benefits associated with this activity. The simplified experimental system used in these experiments simulated a clumped distribution of lepidopterous prey not normally observed in the field (Harcourt 1962). Predaceous coccinellids have a searching behavior adapted to prey with a clumped distribution. After a prey encounter, a decrease in speed and an increase in turning rate (intensive foraging) augment the probability of locating another prey individual (Dixon 1959; Nakamuta 1982). The artificial prey aggregation possibly reduced the searching time and resulted in a higher consumption rate than should normally be expected in a natural and more complex environment. Furthermore, in an environment where prey individuals are scattered, because of its poor visual capacities, coccinellids could overlook small prey and show lower encounter rates. Frazer *et al.* (1981) showed that larvae of three predaceaous coccinellid species often failed to detect small aphid instars consequently increasing the time spent searching for potential prey. The simplified plant structure used here may also have reduced the potential for escape or refuge. We assume that costs involved to obtain a sufficient amount of energy are minimized in a simplified environment which favors a higher exploitation rate of prey at both ends of the size spectrum. Consequently, in nature, we should expect a steeper prey weight consumption bell shape curve with a prey consumption rate that still peaks at an intermediate prey size range.

Defense responses do not only increase with prey size (age) but can also be different between prey species (Dixon 1958; Hajek & Dahlsten 1987; Chow & Mackauer 1991; Brodeur *et al.* 1996). The three lepidopterous species we studied have different morphology, mobility capacity and defense behaviors that can influence their level of vulnerability to natural enemies. All other aspects of prey availability and suitability being equal, these variations in the nature and effectiveness of prey aggressive behavior might determine the outcome of prey exploitation by *C. maculata*. Behavioral observations realized during the tests showed that *T. ni* and *P. xylostella* larvae exhibited an array of active defensive responses such as violent wriggling in the presence of a predator, a behavior also observed by Harcourt (1957). These caterpillars also threw themselves off the cabbage leaf to which they remained attached by a silk thread. Whenever a coccinellid approached, *T. ni* larvae also displayed conspicuous postures to startle the coccinellid predators (projecting the front end of the body upward). When confronted to such behaviors, many coccinellids did not pursue their attack. In contrast, *A. rapae* usually stayed rather stationary and, as also observed by Ohsaki & Sato (1990), displayed virtually no active defensive behavior. However, this relative immobility can act as a passive defense system. In fact, Hajek & Dahlsten (1987) showed that in 50% of encounters with stationary aphids, *Adalia bipunctata* larvae walked over the aphids without exhibiting intensified searching behavior.

However, even if these lepidopterous prey exhibited different defense reponses, it did not result in consistent differences in the capture efficacy of *C. maculata* between prey species. Within a prey instar, prey exploitation was higher on individuals of the smallest prey species (*P. xylostella*). We suggest that this higher efficacy on *P. xylostella* was induced by differential prey size within a prey instar (Table 3.1) and not by differences in defense responses. For instance, *P. xylostella* third instar larvae were more exploited than *A. rapae* and *T. ni* of the same age but of different size.

The upper limit ot prey size exploitation is generally determined by the ability of the predator to search, capture and subdue the prey items (Malcolm 1992). Generally, second instar *C. maculata* consumed a lower number of prey of all sizes compared to adults and fourth instar larvae (Fig. 3.1), a situation also observed in other coccinellid species (Dixon 1959; Wratten 1976; Hajek & Dahlsten 1987). This resulted in a rather flat curve in prey weight consumption for these young and small coccinellids (Fig. 3.3). Usually predatory arthropods tend to attack prey smaller than themselves (Sabelis 1992). Most of the prey offered to the second instar coccinellids were of similar size or larger, whereas adults and fourth instar larvae were usually larger than the prey encountered. Hence, these differences in the size ratio were detrimental to second instar larvae not only with large prey but also with prey of intermediate size. The few encounters that we observed between young coccinellid larvae and prey larger than themselves, suggest that some attacks might result in injury or fail entirely. Furthermore, second instar coccinellids are less mobile compared to late instars larvae and adults (Wratten 1973; Hajek & Dahlsten 1987), and less effective in manipulating prey (Dixon 1959) consequently reducing the prey exploitation level on most prey sizes.

Even if the overall predation efficacy of adults and fourth instar *C. maculata* was similar, egg exploitation was higher for adults whereas prey larvae were more vulnerable to fourth instar larvae. Dixon (1959) proposed that the lower efficiency of adults on prey larvae could be caused by the fact that adults are more conspicuous (colored with an hemispherical shape) than the coccinellid larvae. Hence, a larval prey has more warning and can react more rapidly to the approach of a coccinellid adult. Better visual acuity of coccinellid adults (Lambin *et al.* 1996) can explain their higher efficacy on lepidopterous eggs compared to fourth instar larvae.

Overall, our results clearly indicate that the generalist predator *C. maculata* prey differentially according to predator/prey size ratio. Because prey size is positively correlated with prey age, the age structure of the lepidopterous population in the field can be expected to influence the level of predation. The outcome of these predator-prey interactions can have a determining effect on the level of plant damage induced by the pest. The Protection Index (PI) that we developed quantifies the reduction by *C. maculata* of plant damage caused by the lepidopterous complex. We showed in this study that *C. maculata* was more efficient in exploiting *P. xylostella* immatures. However, because of the higher level of damage caused by *T. ni* larvae, the PI indicates that *C. maculata* should have a more significant beneficial impact when it preys on *T. ni* immatures (Fig. 3.4). Furthermore, because of their high efficacy on eggs and small lepidopterous larvae, adults or fourth instar *C. maculata* should be released early when the first lepidopterous eggs or young larvae are observed.

Predation will significantly influence the population dynamics of the prey species and the primary production of the cruciferous crops depending on 1) the predation behavior of *C. maculata* in the presence of alternative prey, 2) the relative proportion of the lepidopterous pest complex present, 3) the influence of other natural enemies and, 4) the specific conditions of the agricultural system.

ACKNOWLEDGMENTS

We would like to thank William Vickery for useful comments on the manuscript and Bertrand Fournier for helpful advices concerning statistical analysis as well as Nathalie Dauphinais for technical assistance. This is the contribution no XXXXX of the Horticultural Research and Development Centre of Agriculture and Agri-food Canada at St-Jean-sur-Richelieu.

Fig. 3.1. Predation (number of prey killed per day ± s.e.) by (A) second instar, (B) fourth instar and, (C) adult *C. maculata* on immature instars of three lepidopterous species. Different letters within a same prey instar indicate significant differences at *P*=0.05 by pairwise comparisons among least square means.

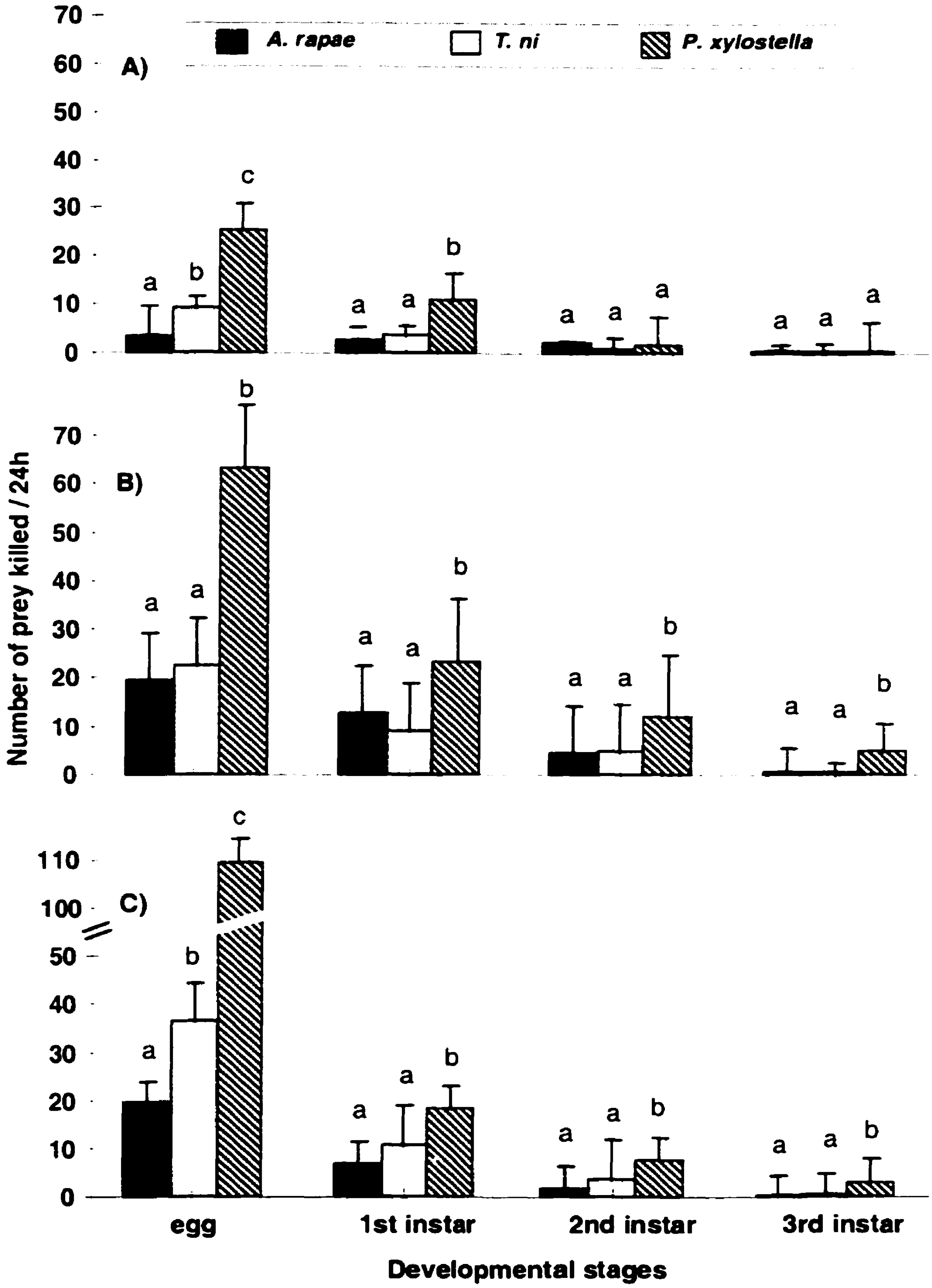
A. rapae
T. ni
P. xylostella
A)
B)
C)
Number of prey killed / 24h
0
10
20
30
40
50
60
70
100
110
a
b
c
egg
1st instar
2nd instar
3rd instar
Developmental stages

Fig. 3.2. Percentage of (A) second instar, (B) fourth instar and, (C) adult *C. maculata* that successfully attacked at least one prey over a period of 24h.

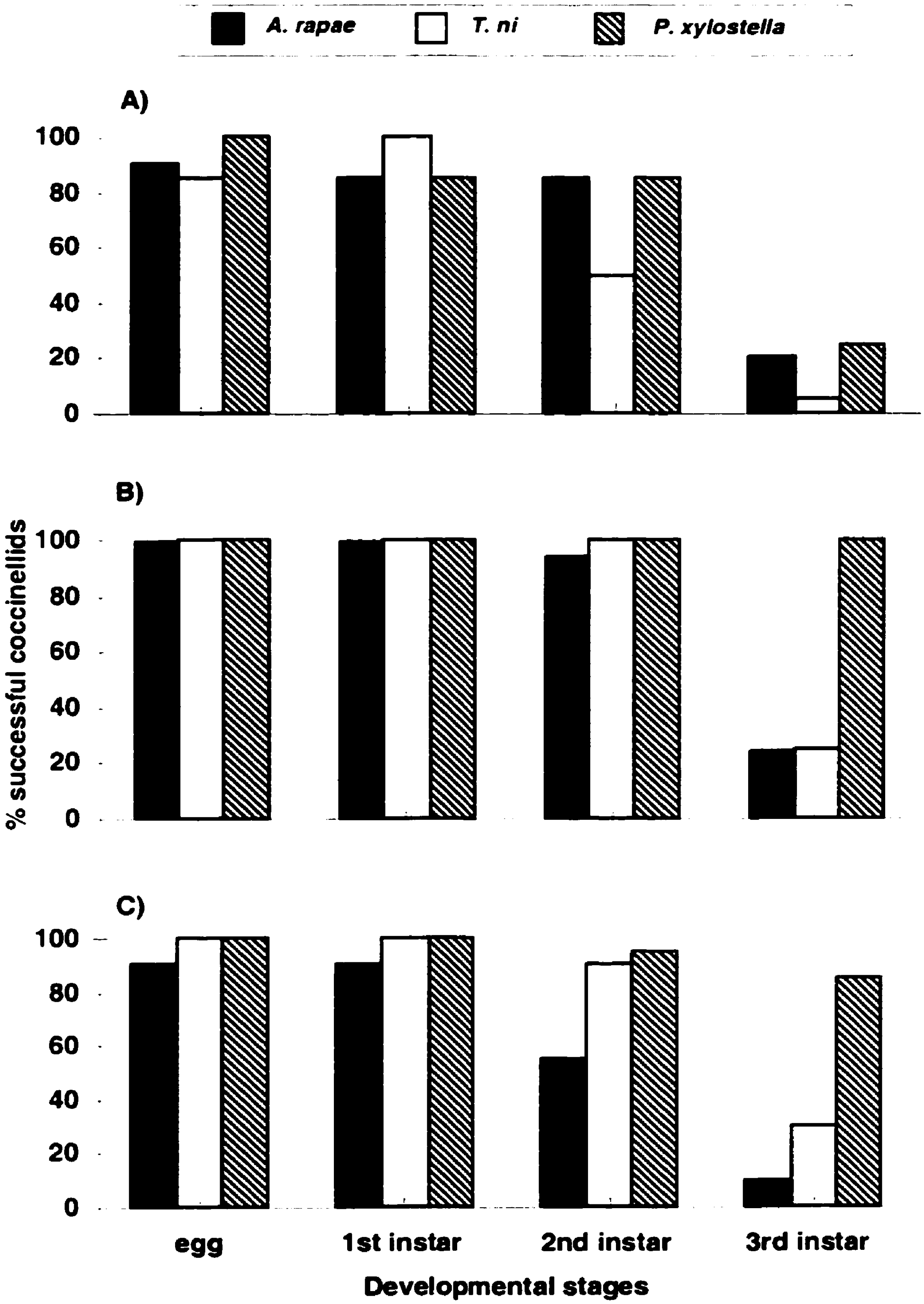

A. rapae
T. ni
P. xylostella
A)
B)
C)
100
80
60
40
20
0
% successful coccinellids
egg
1st instar
2nd instar
3rd instar
Developmental stages

Fig. 3.3. Prey weight consumption by (A) second instar, (B) fourth instar and, (C) adult *C. maculata* in a 24h period in relation to the weight of each prey stage offered. Results for eggs, first-, second- and third instar larvae are presented for *A. rapae* and *T. ni* and eggs, first-, second-, third- and fourth instar larvae are presented for *P. xylostella*.. Data on immatures of all three lepidopterous species are pooled for each *C. maculata* stage.

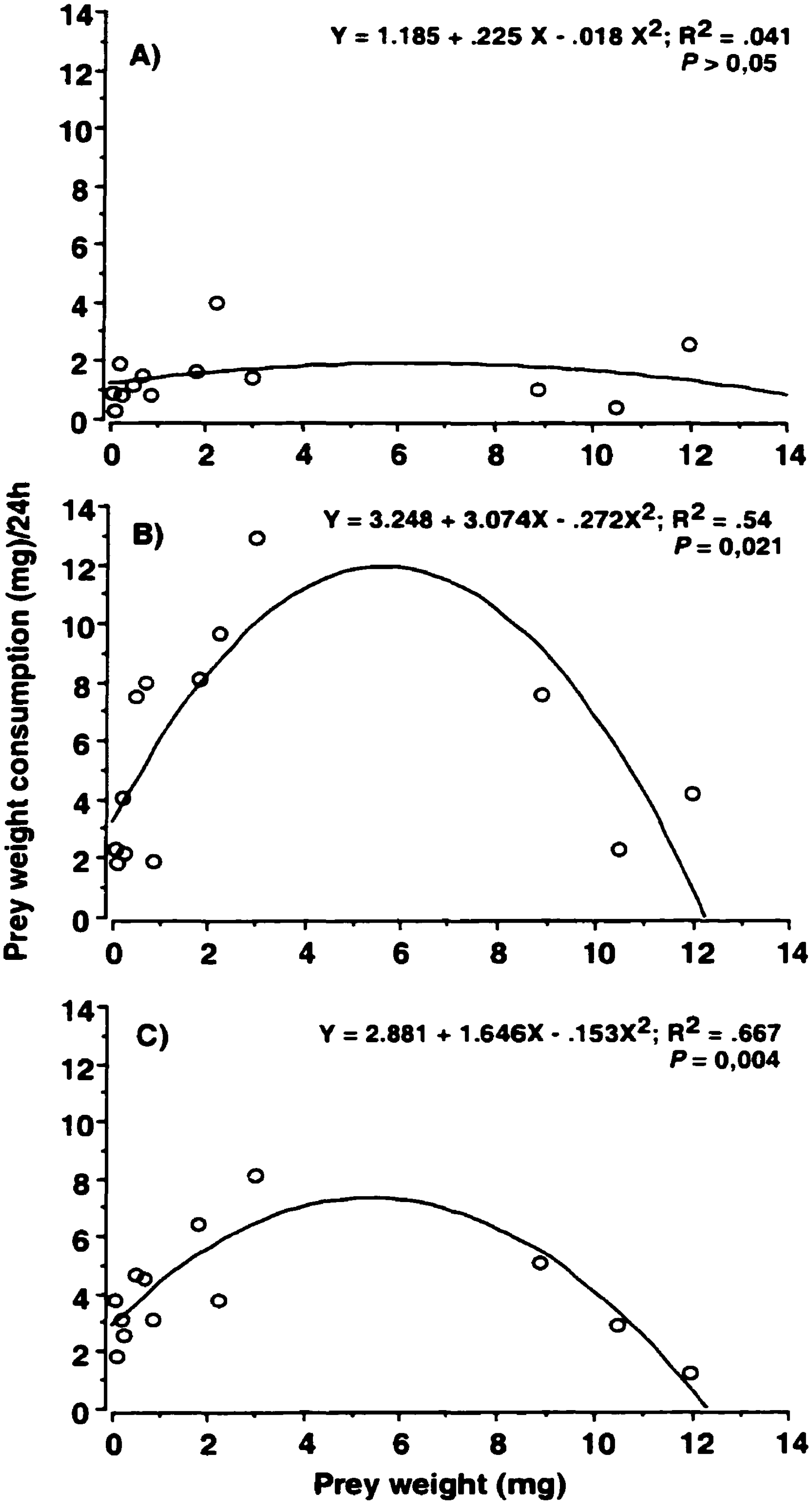

A)
Y = 1.185 + .225 X - .018 X²; R² = .041
P > 0,05
B)
Y = 3.248 + 3.074X - .272X²; R² = .54
P = 0,021
C)
Y = 2.881 + 1.646X - .153X²; R² = .667
P = 0,004
Prey weight consumption (mg)/24h
Prey weight (mg)
0
2
4
6
8
10
12
14

Fig. 3.4. Protection Index (PI) ± s.e. for (A) second instar, (B) fourth instar and, (C) adult *C. maculata*. The PI is the number of prey killed divided by the corresponding Cabbage Looper Equivalency (CLE). Different letters within a same prey stage indicate significant differences at P=0.05 by pairwise comparisons among least square means.

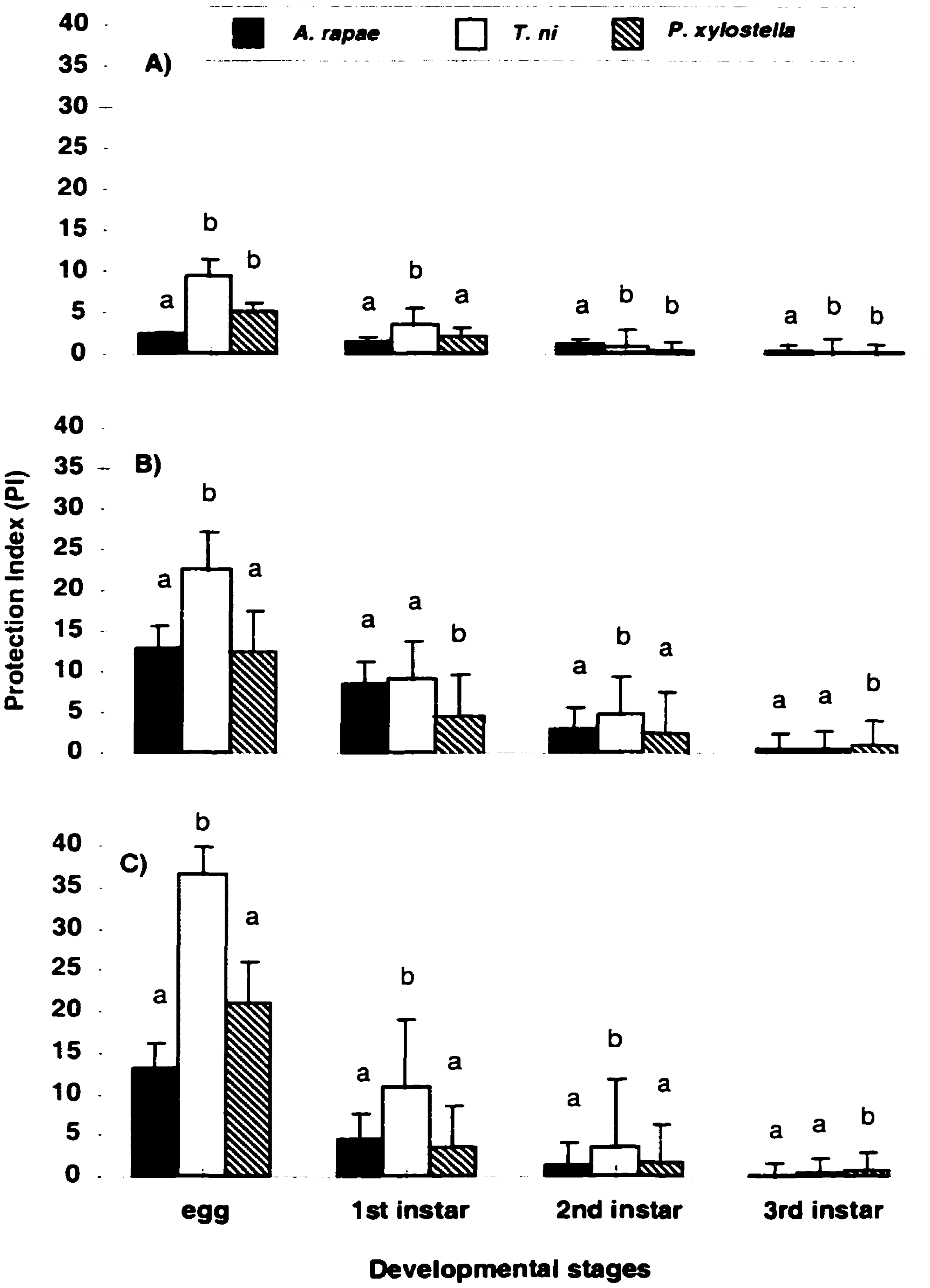

A. rapae
T. ni
P. xylostella
A)
B)
C)
Protection Index (PI)
0
5
10
15
20
25
30
35
40
a
b
egg
1st instar
2nd instar
3rd instar
Developmental stages

Table 3.1: Comparative fresh weight (mean fresh weight [mg ± s.d.]) of immature stages of the lepidopterous prey species and number of successful attacks by *C. maculata* (needed to obtain 1 mg in prey weight consumption).

	IMMATURE STAGES			
PREY	egg	1st instar	2nd instar	3rd instar
A. rapae	0.094 ± 0.005^{a} (10.6)*	0.649 ± 0.087^{a} (1.6)	2.238 ± 0.346^{b} (0.48)	11.996 ± 5.042^{c} (0.12)
T. ni	0.084 ± 0.005^{a} (11.9)	0.237 ± 0.040^{a} (4.2)	1.777 ± 1.000^{b} (0.56)	10.450 ± 1.801^{c} (0.12)
P. xylostella	0.036 ± 0.003^{a} (27.8)	0.172 ± 0.015^{a} (5.8)	0.466 ± 0.104^{a} (1.6)	2.967 ± 0.741^{b} (0.36)

Means followed by different letters within the same row are significantly different (Fisher's protected LSD test; $P<0.05$).

* Number of successful attacks needed to obtain 1 mg in prey weight consumption

In chapter 3, we observed that *C. maculata*, in non-choice tests, preyed differentially on eggs and larvae of three lepidopterous species and that prey weight intake was different according to prey and predator size. Based on these results, we were interested in evaluating whether *C. maculata*, which is one of the most generalist coccinellid species known, could discriminate and select their food according to prey profitability (chapter 5).

To reach this objective, we needed a tracking device that could automatically follow the insect movements and could quantify these specific behavioral parameters; (1) the number of encounters with eggs; (2) the number of egg rejections; (3) the number of eggs consumed (4) the time needed to consume each egg (handling time). The next chapter describes the modifications brought to an image analysis system develop for parasitoids, in order to evaluate the behavioral parameters described above.

CHAPTER 4

An image analysis system developed for evaluation of *Coleomegilla maculata* larvae's behavior

C. VIGNEAULT, C. ROGER, K.P.C. HUI & G. BOIVIN.

1998. Canadian Agricultural Engineering 40: 55-60.

ABSTRACT

A method based on machine vision was developed to replace manual observations required in a study of prey discrimination of the twelvespotted ladybird beetle *(Coleomegilla maculata)* larvae. The system recorded the movement of the II or IV instar larva while the beetle was in contact with a group of 36 cabbage looper *(Trichoplusia ni)* eggs, half of which had been parasitized by *Trichogramma evanescens* before the test. Since the beetle larvae are approximately 20 to 35 times larger than the eggs, the system was programmed to locate the eggs and to follow the movement of the larval head. A three step procedure was developed to detect the larval head position with less than 0.1% of error. Results recorded by the system were more objective than those obtained visually. The developed method and different parameters used within this method are presented in this paper.

Key words: machine vision, insect tracking, biological control, *Trichogramma evanescens*, *Trichoplusia ni*, prey discrimination, host discrimination.

INTRODUCTION

Most beneficial insects cannot reduce all by themselves a pest population below the economic threshold. In biological control, the simultaneous utilization of several entomophagous insects may provide a better control of pests. Nevertheless, introduction of multiple-beneficial insects may increase the number of interactions between these species. Some of these interactions might become harmful if the species are not complementary to each other in resource exploitation. This type of competition between beneficial insects has been frequently invoked to explain why some species failed to control pest(s) successfully (Mackauer 1990). Therefore, it is necessary to evaluate interactions between species before any release.

In North America, three lepidopterous pests are found in cruciferous crops, namely *Artogeia rapae*, *Plutella xylostella* and *Trichoplusia ni*. Losses caused by these pests are significant. For example, the cabbage looper (*Trichoplusia ni*) can eat up to 65 cm^2 of foliage during its development (Stewart & Jacques 1994). In a recent research project, two beneficial insects were chosen to reduce the cabbage looper population. The eggs of this pest can be controlled by *Trichogramma evanescens*, a small wasp that oviposits in lepidopterous eggs and consequently kills them. In addition, the twelvespotted ladybird beetle (*Coleomegilla maculata*) was also chosen to reduce eggs and larvae populations of the cabbage looper. *C. maculata* is a generalist predator which exhibits prey preferences. If *C. maculata* prefers eggs which are not parasitized, this could provide a more complete control on the cabbage looper population. The ability of the twelvespotted ladybird beetle to distinguish between unparasitized cabbage looper eggs and those parasitized by *T. evanescens* was therefore studied.

The tedious work of studying insect behavior has generally been achieved by collecting data manually (Vet *et al.* 1983; Frazer & McGregor 1994). Geers *et al.* (1991) used an image analysis system to examine animal behavior, which demonstrated the great potential inborn within machine vision to replace manual observations. However, only a very few computerized systems developed for insect tracking have been reported (Allemand *et al.* 1994). Vigneault *et al.* (1997b) examined several commercially available object tracking systems, and concluded that they were not well-adapted for insect behavior evaluation. Therefore, an image analysis system aimed at studying minute insects was developed by Vigneault *et al.* (1997a). The same system was modified and used to evaluate the prey preference of the twelvespotted ladybird beetle larvae.

The objective of the present work was to develop an image analysis system to evaluate some behavioral parameters of the twelvespotted ladybird beetle larvae required in the evaluation of this organism for a biological control program. This system has to be able to monitor the head position of a ladybird beetle larva in a matrix of eggs. It has to be programmed to automatically record the sequence of egg visits done by the larva, the number of contacts each egg received and the cumulative time of contacts for each egg.

MATERIALS AND METHODS

Preliminary preparation of eggs

Both II and IV instars larvae of the ladybird beetle were studied in the laboratory. They measured approximately 4200 x 1300 µm and 6300 x 1400 µm, respectively. In each test, a larva was placed on a glass plate within a group of 36 cabbage looper eggs. The cabbage looper eggs have shapes of ellipsoids and each egg measures approximately 430 µm in length and 590 µm in diameter. Half of the eggs had been parasitized by *T. evanescens* eight days before the test The 36 eggs were disposed vertically and their circular surface areas were captured by the camera. The cabbage looper eggs were initially placed on a moist glass plate, and once the water evaporated a thin sticky layer composed of proteins was formed between the eggs and the plate. The 36 cabbage looper eggs thus remained slightly glued. In addition, the eggs were arranged as a matrix, having 6 eggs on each row and column (Fig. 4.1). Positions of the parasitized and unparasitized eggs inside the matrix were pre-defined and were recorded by an operator before the test A distance of 4 mm and 8 mm was maintained between the eggs for the II and IV instars larvae, respectively (Fig. 4.2). These distances were determined based on the insect searching behavior observed during preliminary tests (unpublished data ; CR). Each test lasted 60 minutes.

The image analysis system

The image analysis system consisted of a background light source, a light diffuser, a CCD video camera, a VHS video cassette recorder, two video monitors and a IBM-AT compatible microcomputer. The light source, diffuser and camera were covered by an opaque curtain to eliminate the effects of ambient light on larvae and images captured by the camera. Every component of this system is commercially available.

The light source consisted of a circular neon light tube which provided light from the back of the image. An acrylic plate was used as a diffuser to distribute light evenly inside the field of vision. Thirty images were captured by the video camera each second. The original image was displayed on a video monitor which allowed the operator to center the matrix of eggs properly inside the field of vision. The processed image was then shown on the second video monitor. The video signal produced by the camera was recorded by a cassette recorder, permitting analyses of images to be processed either immediately or later. In the study of the ladybird beetle, all images were analyzed in real time. Video signals were also transmitted to the computer and were digitized at a rate of 30 images•s^{-1}.

Digitization of the images was accomplished by an Oculus-300 board (Coreco Inc., St. Laurent, Quebec) installed inside the computer. The digitizing board divided an image of 83 by 79 mm into 512(H) x 484(V) square pixels, measuring 163 μm on each side. Each pixel was described by its horizontal and vertical position coordinates and its light intensity measured in gray level. With 8 bits of resolution per pixel, gray level was ranked from 0 to 255 (black to white respectively).

Contrast stretching for captured images

After the glass plate containing moth eggs was centered inside the field of vision, the system was activated. The computer first determined the average background gray level by scanning a section of the original image which contained no eggs. The system then increased the contrast of the subsequent images by making use of this average background gray level (Jain 1989). Without affecting the results obtained in the analyses, this procedure allowed the operator to better distinguish the different objects presented on the second monitor. The average background gray level of the original image before contrast stretching was equal to 110 ± 4.

Identification of eggs

The operator specified the following parameters to the system: number of eggs, test duration, threshold gray level, minimal projected surface area of an object to be recognized as an egg, length of the border used to surround the eggs and minimal difference in gray level between the larva and the background used to recognize an object as larva. Next, the computer started to determine the position of each egg. Threshold gray level used to identify an egg was fixed to 150 after the contrast stretching. This threshold value corresponded to the gray level value located at half-way between the background gray level and the minimum gray level of the egg images. The system first identified all objects which had a gray level below this threshold level, traced the contour and calculated the projected surface area of each object by using a method developed by Vigneault *et al.* (1992). Objects which had a projected surface area larger than 3 pixels were then considered as eggs by the system and displayed on the second monitor. Finally, the computer located the four extremities (X*min*, X*max*, Y*min* and Y*max*) of each egg contour. A border was added to these four extremities to create a rectangular zone around each egg (Fig. 4.2). When the head of an insect entered into an egg zone, the larva was considered in contact with the egg. Depending on the size of the larval body, different borders were used. A border of 7 pixels was added to each extremity of the II instar larva whereas a border of 10 pixels was used for the IV instar larva during preliminary tests (unpublished data ; CR). The lengths of these borders in which an insect was considered to be in contact with an egg were determined. All eggs displayed on the second monitor were replaced by small rectangles. The operator could verify the positions of each egg and correct any existing error. The 36 egg zones were then numbered by the computer. A value of 0 was allocated to empty areas between the eggs, and values of 99 were used to define the exterior zone of the square eggs matrix (Fig. 4.2). The limits of each of the 38 zones were memorized by the system. Egg contacts made by a larva were counted by comparing the larval head position and the boundaries of the egg zones.

Image subtraction

Before placing the larva into the camera's field of vision and initiating actual insect tracking, a square fence was placed and centered around the matrix of eggs to restrain the insect to the interior of the field (Fig. 4.2). Square fences of 60 x 60 mm and 80 x 80 mm were used for the II and IV instars larvae, respectively.

A reference image was then scanned by the system. Subsequent digitized images were subtracted pixel by pixel from this reference image (Vigneault *et al.* 1997b). Only subtracted images were used for insect tracking. This subtraction procedure allowed the system to discriminate the insect from the eggs and the fence. After image subtraction, everything shown on the second monitor, including the matrix of eggs and the fence, became black. Bright points were presented only if a great difference in gray level existed between pixels of the reference image and the current image. Consequently, when a larva was introduced into the field, a brilliant object corresponding to the position of the insect appeared on the second monitor. Image subtraction proceeded at a rate of 30 images•s^{-1}. However, the computer could analyze only six subtracted images•s^{-1} in real time. This limitation was due to the calculation time required to identify and verify the larval head position.

Insect tracking

When a larva was released inside the square fence, the system's chronometer started running. The larval position was established by scanning from left to right at each 5 rows on full screen. The computer stopped tracking once it encountered a pixel with a gray level $140; this gray level was approximately half way between the screen background gray level and the maximum gray level of the pixels forming an insect. The technique of Vigneault *et al.* (1992) was used to trace the contour of this object and to calculate its perimeter. If the object had a perimeter ≥ 5 pixels, it was considered to be an insect and all the pixels which delimited the contour (contour pixels) of this insect were then memorized by the system.

A visit was counted only when the head of a larva entered in one of the 36 egg zones, because the projected body surface areas of the II and IV instars larvae were approximately 20 and 35 times larger than the cabbage looper egg. Localization of the larval head therefore became essential for this study. The insect head had to be located at one of the extremities of the larval body. However, the presence of legs increased the number of body extremities from 2 to 8 depending on how the legs were positioned.

Three steps were involved in the localization of the larval head (Fig. 4.3). First, after the presence of the insect was detected and all its contour pixels were traced by the system, the computer calculated the sum of all the distances between one particular contour pixel and the other contour pixels. This calculation was done for each contour pixel. Since the beetle larval tail is narrower than the head, the contour pixel which had the largest sum of distances generally corresponded to the insect's tail position. When the larval tail was located, the computer identified the contour pixel which was located at least 10 pixels (≈ 1/2 length of the larval body) away from the larval tail and had the largest sum of distances. This pixel was considered to be the larval head by the system.

The second step involved a reduction in the number of extremities, by elimination of insect legs on the larval image using an erosion technique. This technique consisted of erasing the original insect contour by giving a gray level value of zero to all the contour pixels and retracing another new contour around the insect. This erosion technique was developed specifically for this application and is much more efficient to erase larvae legs, than the erosion technique described by Jain (1989), which slowly attenuates the gray contrast of an object contour. One and two erosions were required for the II and IV instar larvae, respectively, because of the difference in body size. An example of the different shapes of contours before and after two erosions are shown in Figure 4.4, using an IV instar larva. After one or two erosion(s), a new insect contour was then traced. New larval tail and head positions were also identified using the same sum of distance technique.

The third step consisted of comparing the head and tail positions identified in step two, with the last five recorded larval head and tail positions. This procedure was used to eliminate the error of inversion between the larval head and tail. If the insect head and tail identified by the system was located at point H and B respectively, the larval head was actually located at H only if equation (1) was valid or the larval head was located at B.

$$\sum_{i=1}^{5}(TAIL_i\ H - HEAD_i\ H) \geq \sum_{i=1}^{5}(TAIL_i\ B - HEAD_i\ B) \qquad (1)$$

where

$TAILi\ H$ = distance between the *i*th last recorded tail and point H

$HEADi\ H$ = distance between the *i*th last recorded head and point H

$TAILi\ B$ = distance between the *i*th last recorded tail and point B

$HEADi\ B$ = distance between the *i*th last recorded head and point B

After the larval head position was determined with the 3 steps procedure, an **X** indicating the identified head position was placed on the image of the larval body on the second monitor. The system determined in which zone the head of the insect was located by comparing the **X** position with the boundaries of the 38 zones. The zone number and the time of entrance into that zone were then registered by the computer.

The computer retrieved a new image from the digitizing board, from which it searched for the insect in a square of 60 x 60 pixels centered on the previous larval head position. By scanning column by column at every 5 rows in this smaller research area, the computer could relocate the insect faster. In cases where the insect body was not found in this square, the system retrieved another new image and proceeded to scan inside the new square. After two new images, if the insect body position was still unknown, the system retrieved a third new image and searched for the insect on the full screen. Once the insect body was found, the computer retraced the insect body contour, re-determined the head larval position and relocated the insect among the 38 zones.

By comparing the previous zone numbers where the insect was located, the system determined if the insect had changed its location. If the two zone numbers were different, the new zone number where the insect head was located and the time of entrance in that zone were recorded. A sonic signal was also sent by the system to notify the operator that a change of zone had occurred. During the test, a list indicating the order of zone visits and the time of entrances in different zones were given by the computer.

At the end of each test, the computer calculated the number of contacts each egg received and the cumulative time of contact for each egg. A final report containing the sequence of zone visits, the time of entrance into each zone, the total number of egg contacts and the cumulative contact time was presented to the operator. Several options were also added to the program, which allowed the operator to choose another new reference image or to terminate the analysis at any moment during the test.

Evaluation of system performance

A series of tests was performed during the development of the system, to verify the performance of each of the three steps involved in the larval head localization procedure. For each step, a total of 10,000 images (1000 images/insect x 10 insects) were retrieved by the system to quantify its percentage of error. During each test, the processed image containing the larval body was displayed on the second monitor. An **X**, representing the larval head position determined by the computer, was shown on the same monitor simultaneously. An operator compared the position of the **X** visually with the real larval head position, and counted the number of errors. The system stopped automatically after every 1000 images in this series of tests.

RESULTS AND DISCUSSION

In the first step of the larval head localization procedure, the system was able to position approximately 90% of the larval heads accurately. Two types of errors were observed. Most often, the system assigned another insect body extremity as the larval head, which was generally due to the presence of a leg located near the larval head. In other cases, the positions of the head and tail were inverted by the system.

In the second step, utilization of the erosion technique reduced significantly the number of errors due to the presence of legs. Less than 13 errors per 1000 images were found and the head was correctly identified in 98.7% of the occasions. Inversion of larval head and tail was the most common error observed, due to the fact that the shape of the insect body changed in some of its displacements. This gave the system an impression that the larval head was narrower than the tail and hence errors occurred.

The verification process of step three increased the rate of success to greater than 99.9%. Errors occurred solely when the insect moved very rapidly in circles and the identified head position came across the recorded tail positions in less than 0,83 sec.. The error was automatically corrected on the following analyzed image, which was 1/6 of a second later. This means that any egg contact done during this type of displacement would be considered as lasting only 1/6 of a second. Omission of such a short egg visit did not affect the results obtained in the insect behavior analyses, since only egg visits which lasted more than 3 seconds were considered (Roger *et al.* submitted; Chapter 5). Thus, less than 0.1% of error was considered negligible. Results obtained at the end of step three were considered more than satisfactory.

CONCLUSION

A method based on an image analysis system was adapted to automate observations required in a study of the twelvespotted ladybird beetle's behavior. The objectivity of observations was increased by eliminating human intervention. A three step procedure was developed to detect the head position of insect on its larval body and the performance of each step was evaluated separately by a series of tests. The system was able to locate accurately the larval head with an acceptable rate of error, smaller than 0.1%. Several parameters were automatically calculated and recorded by the system: the order of zone visits done by a beetle larva, the time to enter in each zone, the number of contacts each egg received and the cumulative time of contacts for each egg, based on the positions of the larval head and the egg zones. This system successfully assisted in the evaluation of *Coleomegilla maculata* larvae's behavior.

Fig. 4.1. A IV instar larva of the twelvespotted ladybird beetle within a matrix of 36 cabbage looper eggs. The value 0 represents the empty areas between the eggs and the value 99 represents the exterior zone of the square egg matrix.

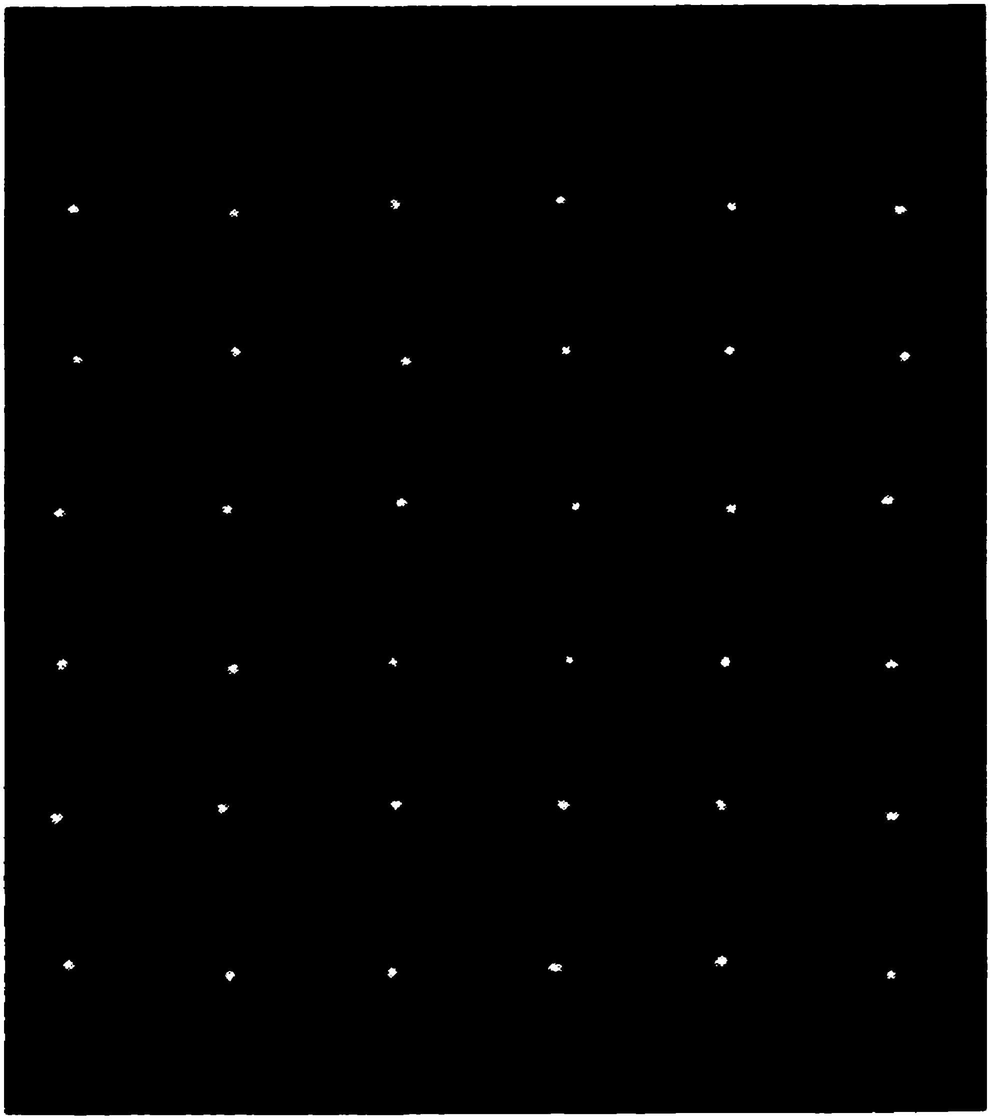


Fig. 4.2. Arrangement of the egg matrix and zones in the study of the IV instar larvae's behavior.

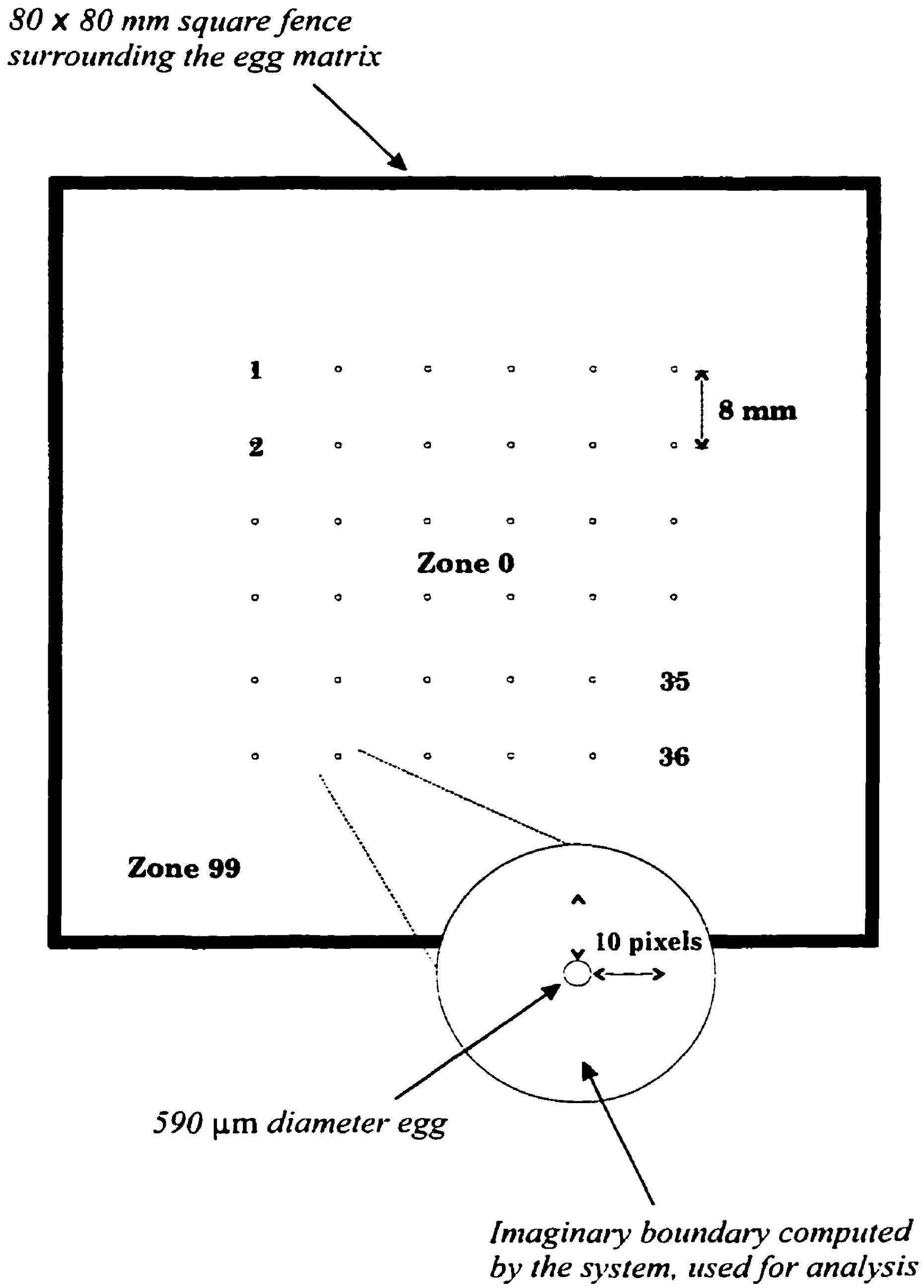

80 x 80 mm square fence surrounding the egg matrix
1
2
8 mm
Zone 0
35
36
Zone 99
10 pixels
590 µm diameter egg
Imaginary boundary computed by the system, used for analysis

Fig. 4.3. Basic steps involved in the insect tracking process.

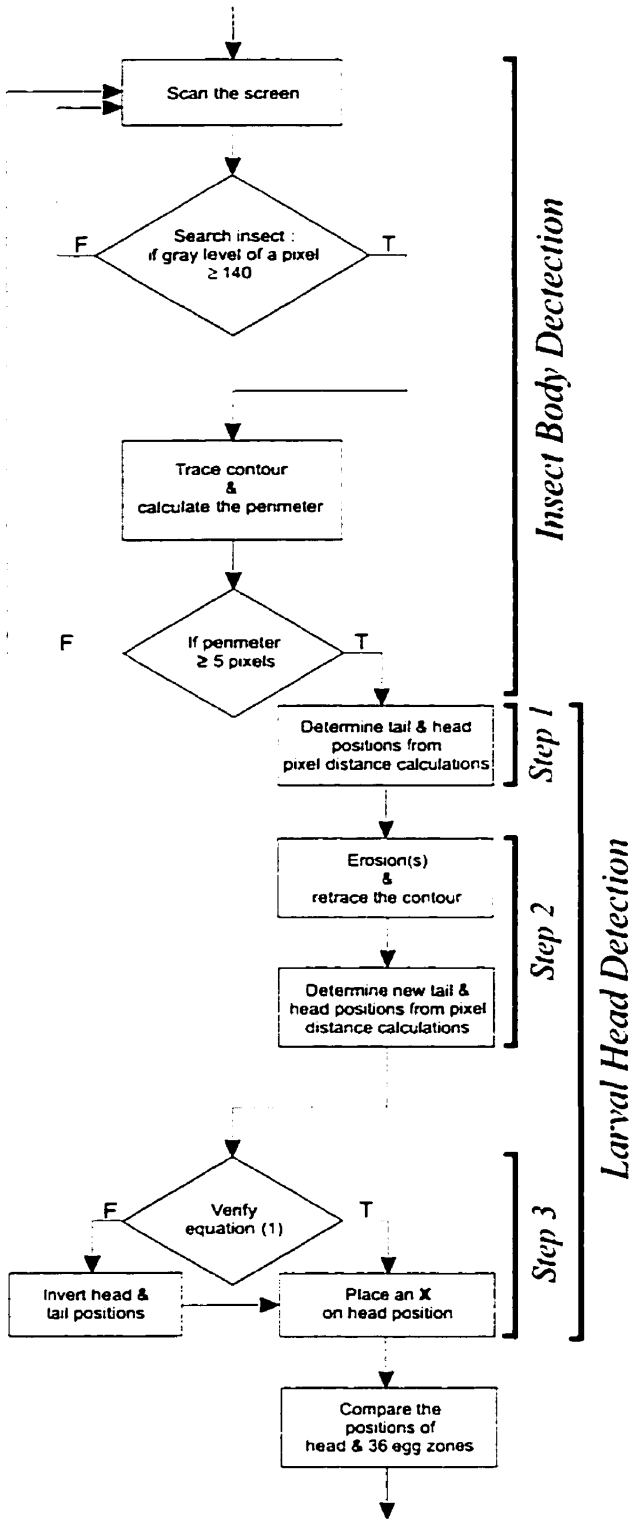
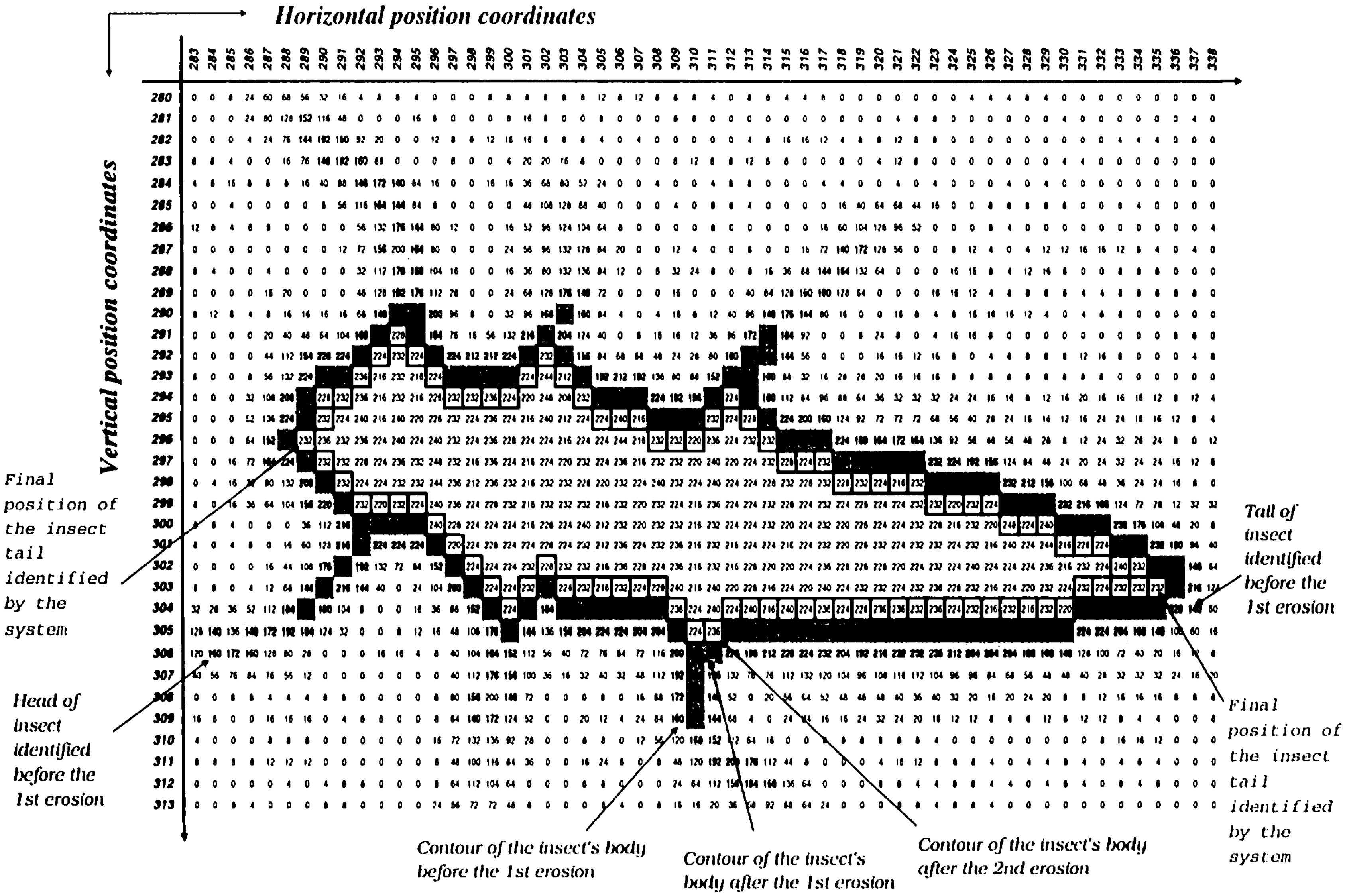

Scan the screen
Search insect : if gray level of a pixel ≥ 140
F
T
Trace contour & calculate the perimeter
If perimeter ≥ 5 pixels
F
T
Insect Body Dectection
Determine tail & head positions from pixel distance calculations
Step 1
Erosion(s) & retrace the contour
Determine new tail & head positions from pixel distance calculations
Step 2
Verify equation (1)
F
T
Invert head & tail positions
Place an X on head position
Step 3
Larval Head Detection
Compare the positions of head & 36 egg zones

Fig. 4.4. Erosion technique used on a digitized image of a IV beetle instar larva retrieved from a video cassette.


Horizontal position coordinates
Vertical position coordinates
Final position of the insect tail identified by the system
Head of insect identified before the 1st erosion
Contour of the insect's body before the 1st erosion
Contour of the insect's body after the 1st erosion
Contour of the insect's body after the 2nd erosion
Tail of insect identified before the 1st erosion
Final position of the insect tail identified by the system

In chapter 3, it was shown that, in a non-choice situation, the fourth instar larvae and the *C. maculata* adults exploited more efficiently intermediate-sized prey. It was argued that factors such as better defense responses could increase the costs associated to the capture of larger prey whereas higher searching time and low energetic value resulted in lower gains on smaller prey. Hence, factors affecting profitability could be influenced by different morphological and behavioral characteristics of prey and limit the quantity of prey weight that a predator can consume. Experiments in chapter 3 allowed us to evaluate a few mechanisms that could influence prey selection.

If *C. maculata* can forage optimally, we expect that it will select the most profitable prey when different prey qualities are present. This prey selection behavior was, therefore studied, in laboratory choice experiments. Apart from the factors described earlier, physiological modifications provoked by parasitism and ageing may also affect prey profitability. In order to keep costs related to prey size and prey defense responses relatively constant, lepidopterous eggs were chosen as prey in the following experiments. Hence, using the automated system modified in chapter 4, we examined the ability of *C. maculata* to discriminate and select between *T. ni* eggs that were physiologically different (unparasitized, parasitized, young or old).

CHAPTER 5

Discrimination of parasitized prey by the generalist predator *Coleomegilla maculata lengi* (Coleoptera: Coccinellidae): mechanisms and implication on intraguild predation

C. ROGER, D. CODERRE, C. VIGNEAULT & G. BOIVIN

Submitted to Oecologia

ABSTRACT

Generalist predators may perform intraguild predation (IGP) on parasitized hosts, consuming both the prey and the developing immature parasitoid. Prey discrimination could have strong selective advantages for a predator if it could allow an intraguild predator to eliminate a direct competitor or if parasitism-induced physiological changes alter prey suitability. Under laboratory conditions, we studied unidirectional IGP and prey discrimination in the predatory coccinellid *Coleomegilla maculata lengi* Timb. (Coleoptera: Coccinellidae) toward eggs parasitized by *Trichogramma evanescens* Westwood (Trichogrammatidae), a potential competitor of lepidopterous eggs in similar habitats. Choice experiments were used where second or fourth instar coccinellids had the choice between *Trichoplusia ni* (Hübner) eggs of different categories (unparasitized, parasitized, young or old). *C. maculata* larvae did not exhibit any preference between parasitized and unparasitized eggs of the same physiological age. However, when presented with parasitized and unparasitized eggs of different physiological ages, coccinellid larvae always preferred the younger eggs despite parasitism. Furthermore, the percentage of rejection and handling time were always higher on the less preferred egg type. The preimaginal development, the food intake to reach adulthood and the survival of coccinellid immatures were altered when the coccinellid larvae were fed with parasitized and old unparasitized old eggs. Overall, our results indicate that *C. maculata* larvae select eggs based on their physiological state regardless of parasitism and that IGP is epiphenomenal.

Moreover, fourth instar coccinellid larvae spent less time in patches containing solely parasitized eggs and their level of exploitation was greatly reduced compared to homogeneous patches containing unparasitized young eggs (which reflect the fact that parasitized eggs have been physiologically altered). This suggests that *C. maculata* larvae respond to variable patch quality by using flexible decision rules that reflect the pay-off of the patch. The results are discussed further in the context of optimal foraging theory.

Key words: generalist predator; *Coleomegilla maculata*; intraguild predation; prey discrimination; prey quality; predator-parasitoid interaction.

INTRODUCTION

In most ecosystems, the natural enemy complex is diverse and thus the potential for interspecific interactions is large. In this context, intraguild predation (IGP), defined as the killing and eating of species that use a common resource and thus are potential competitors (Polis *et al.* 1989), is a widespread ecological interaction. Predators and parasitoids, even if they do not use the same tactics in their acquisition of resources, may be present in the same guild and can therefore be involved in IGP (Rosenheim *et al.* 1995). As reported in several studies, predators may attack parasitized hosts, consuming both the prey and the developing immature parasitoid and thus directly interfere with parasitoids (Jones 1987; Brower & Press 1988; Ruberson & Kring 1991; Hoelmer *et al.* 1994). In these cases, IGP could be a simple act of predation directed at nutritionally profitable prey and decreased competition would then be an indirect consequence. However, IGP is likely to have a competitive basis when consuming parasitized hosts is suboptimal according to the criteria of standard optimal diet models (Polis *et al.* 1989).

In some of the studies exploring predator-parasitoid interactions, the impact of predators has been greater on parasitized than on unparasitized prey (Tostowaryk 1971; Jones 1987). This was observed mostly on mobile prey in which parasitism affected mobility, consequently provoking differential probability of encounter. However, in the case of predators attacking sessile prey like eggs or whiteflies, recently parasitized and unparasitized prey were consumed in the same proportion but prey containing late larval and pupal stages of parasitoids were avoided (Al Rouechdi & Voegelé 1981; Hoelmer *et al.* 1994). This avoidance may be due to parasitism-induced physiological changes that rendered the parasitized prey more difficult to exploit by the predator. In most of the studies on prey discrimination by predators, the mechanisms underlying such ability and their possible influence on IGP have received little attention.

Host discrimination provides parasitoids with useful information about host selection, patch time allocation and progeny and sex allocation (van Alphen & Visser 1990). Discrimination has an important adaptive value for parasitoids because it prevents wastage of eggs, time and energy by the female when rejection time is shorter than handling time and when it induces the parasitoid to leave a patch after a few encounters with parasitized hosts (van Lenteren 1981). The ability of a parasitoid to recognize hosts that have already been parasitized by themselves, by conspecific or even by another parasitoid species has been well studied (van Lenteren 1981; van Alphen & Visser 1990; van Baaren *et al.* 1994). For predators, even if foraging and reproduction are not as closely linked as in parasitoid insects, the evolution of interspecific discrimination ability (i.e. to distinguish parasitized from unparasitized prey) may also be adaptive. It could allow a predator to avoid or eliminate potential competitors (IGP), especially under conditions of local resource competition or under conditions of possible mutual predation (Polis *et al.* 1989). Discrimination ability could also allow the predator to evaluate prey suitability and reject less suitable ones.

Parasitism can provoke external or internal host modifications (Strand 1986; Vinson 1994) that could influence suitability of a prey for a predator. If a predator has the ability to detect a parasitized prey, it can choose to consume or the reject the prey, depending on the relative benefits and costs associated to both situations. According to optimal foraging theory, prey should be ranked by prey energetic value per unit of handling time, and should be added to the diet of a predator in decreasing order of instantaneous rate of energy gain per prey (Stephens & Krebs 1986). In contrast to host discrimination, the adaptive value of interspecific prey discrimination for predators has received little attention and is rarely mentioned.

The predatory coccinellid *Coleomegilla maculata lengi* Timb. which is widely distributed east of the Rocky Mountains in North America (Gordon 1985) was used to test the influence of parasitism on prey discrimination. It is one of the most abundant coccinellid species found in herbaceous crops and one of the most generalist coccinellid known (Hodek & Honek 1996). It feeds on many aphid species (Mack & Smilowitz 1980; Coderre *et al.* 1987) and various non-aphid prey such as Colorado potato beetle (Groden 1990; Giroux *et al.* 1995) and European corn borer (Andow 1990; Coll & Bottrell 1991) immatures. Both larval and adult stages are predaceous and attack the same prey type (Hodek & Honek 1996). Although many coccinellids are generalist feeders, studies indicate that they are selective in their prey choice (Mills 1981; Obrycki & Orr 1990), can forage in an optimal way (Hemptinne *et al.* 1993) and can be involved in bi-directional IGP with other generalist predators such as Chrysopidae and Cecidomyiidae larvae (Lucas *et al.* 1998). In southwestern Québec, *C. maculata* was found to be the principal predator in cruciferous fields and laboratory studies showed that it can consume relatively large numbers of eggs and small larvae of the lepidopterous species attacking these crops (Roger *et al.* 1995; Chapter 3).

Trichogramma spp. are egg endoparasitoids used worldwide for biological control of lepidopterous pests (Smith 1996). In Québec, the use of *Trichogramma evanescens* (Westwood) for the control of the lepidopterous pests of cruciferous crops is being investigated (Boivin & Fournier 1993). In this context, the probability of interactions and competition for egg resource between the predator *C. maculata* and the parasitoid *Trichogramma* is relatively high. Therefore, *T. evanescens* was used as the parasitoid and the cabbage looper *Trichoplusia ni* (Hübner) as the prey/host. This system has several favorable attributes for the study of prey discrimination from a functional perspective. During the acceptance behavior, *T. evanescens* females oviposit and inject a venom responsible for the cessation of host development and necrosis. After the oviposition phase, the female deposits an external chemical mark (Strand 1986). As a parasitoid larva develops within the host egg, it exploits the resources and secretes a surrounding membrane (Al Rouechdi & Voegelé 1981). These physiological changes harden the egg chorion and change the egg coloration that becomes gradually black. At the end of parasitoid larval development, most of the host resources are used up and air spaces form inside the egg (Al Rouechdi & Voegelé 1981). Parasitized *T. ni* eggs take approximately 9 to 10 days at 25°C to complete their development whereas unparasitized eggs take 4 days. Hence, in nature, parasitized eggs are vulnerable to predation for a longer period of time compared to unparasitized eggs.

Parasitism-induced modifications can alter prey suitability and change the energy and time payoffs for a foraging predator. In these circumstances, it can be advantageous even for a generalist coccinellid to discriminate against parasitized prey. The ability to discriminate was examined in the whitefly specialist coccinellid predator *Delphastus pusillus* (LeConte). Fourth instar larvae and adults equally attacked recently parasitized and unparasitized whiteflies but increasingly avoided those containing third instars and pupal parasitoids (Hoelmer *et al.* 1994).

In this paper, the discriminatory capacity of the generalist predator *C. maculata* was explored in a choice situation when presented *T. ni* eggs both unparasitized and parasitized by *T. evanescens*. The influences of prey quality on prey selection and patch exploitation was also studied. All other constraints being equal, if parasitized prey are less suitable than healthy ones, predator should have developed discrimination capacity to reduce loss of time and energy on parasitized prey. If a predator selects the most suitable prey, the elimination of the immature parasitoid is epiphenomenal. However, if the predator selects preferentially parasitized prey despite suboptimal suitability of such prey, IGP is likely to have a competitive basis. Furthermore, we will test the prediction included in optimal models, which predicts that an optimal forager should invest more time in patches with higher profitability than those with lower profitability. Therefore, the presence of discrimination should reduce the time spent in sites containing parasitized eggs. Rejection of parasitized hosts or patches containing parasitized eggs after recognition of prey and patch state could decrease the potential of direct competition between predators and parasitoids and consequently IGP.

MATERIAL AND METHODS

Insects

Insectary colonies of *C. maculata* were started with adults collected in spring from hibernation sites near corn fields in Saint-Hyacinthe (72°56'W,45°39'N), Quebec, Canada. They were maintained on a fresh liver-based artificial diet (Coderre, unpubl.) and on wild flower pollen at 22°C, 70% R.H., and a 16L:8D photoperiod. The predatory larvae used in all behavioral experiments had molted two days before the tests were conducted. Before a test, larvae were placed individually in 50 mm Petri dishes and starved for 24 h to standardize hunger level. In all experiments, coccinellid larvae were unexperienced with the prey used.

T. evanescens strain initially originating from Egypt was obtained from the collection maintained at the Department of Entomology in Wageningen (Pak & van Lenteren 1984) and was reared on eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) at 25°C, 30% RH and 16L:8D photoperiod. *T. ni* larvae were reared on a pinto beans based-artificial diet elaborated by Shorey (1963) and maintained at 25 °C 30% RH and 16L:8D photoperiod. It has been shown in laboratory studies that *T. ni* eggs are a preferential host of *T. evanescens* (Parker & Pinnell 1974) and are readily preyed upon by *C. maculata* (Roger *et al.* 1995; Chapter 3).

Influence of parasitism and prey age on prey discrimination

Discrimination experiments were conducted by offering a combination of equal densities of two egg types to a single second (L2)- or fourth-instar (L4) coccinellid larva without replacing consumed eggs. Eggs were alternately arranged on a 14 x 14 cm glass plate in a 2 x 2 cm grid for L2 and a 4 x 4 cm grid for L4 (six columns and six rows) and surrounded by a Fluon®-coated ring. A single coccinellid larva was observed for 1 h after the larva began to consume the first egg.

Five different combinations of eggs were used (Table 5.1). Combination 1 was realized to determine if *C. maculata* could detect the presence of early external or internal modifications of egg provoked by the ovipositing female or by the developing parasitoid larva. Combination 2 was performed to test if *C. maculata* could detect the presence of a parasitoid larva inside an host egg. Combination 3 was done to evaluate if *C. maculata* could detect the presence of a parasitoid pupae and it was conducted with L4 only. Combination 4 was performed to determine if parasitism, regardless of age, can influence prey discrimination. In both egg types of combination 4, the resources contained inside the eggs had been transformed by the developing parasitoid immature in parasitized eggs and by the *T. ni* embryo in unparasitized eggs. Combination 5 tested if age, regardless of parasitism, can influence prey discrimination. Each combination was replicated 20 times except for experiment 1 which was replicated 10 times.

The behavior of each coccinellid larva was video-recorded and automatically tracked using an image analysis system (Vigneault *et al.* 1998). For each combination and egg type: (1) the number of encounters with eggs; (2) the number of egg rejections; (3) the number of eggs consumed (4) the time needed to consume each egg (handling time) were recorded. An encounter was noted when the coccinellid larva head stayed in contact with the egg for more than 3 sec. An egg was considered rejected when the encounter with the latter did not result in egg consumption (no alteration). An egg was considered accepted when it was partially or totally consumed. The number of eggs consumed of each type was counted at completion of each trial. Partially consumed eggs were transferred to a growth chamber and allowed to develop, but none survived.

In order to determine whether the predators preferentially accepted one of the two prey types offered, Manly's preference index (Manly *et al.* 1972) were calculated on the number of eggs consumed. This index was used because it is the only method that takes into account the prey density depletion by predation during experiments (Cock 1978; Sherratt & Harvey 1993). For all combinations, the Manly's index of preference, the percentage of rejected eggs and handling time were submitted to Wilcoxon's matched-pairs signed rank tests (abbreviated as Wilc. MPSR) (StatView, Abacus Concepts 1993).

Influence of parasitism and age on prey suitability

To determine whether there is a cost to eat parasitized or old *T. ni* eggs, we evaluated the pre-imaginal developmental time and the number of eggs needed for *C. maculata* larvae to complete their pre-imaginal development. *C. maculata* eggs were obtained from insectary colonies and incubated at 22°C, 60% r.h., and a photoperiod of L16:D8 until hatching. Neonate larvae used in this experiment were of the same age. Each larva was individually kept in a 5-cm Petri dish with a wet cotton and with either *T. ni* unparasitized young eggs (1 day-old), *T. ni* unparasitized old eggs (3 days-old), or *T. ni* parasitized eggs (8 days-old) containing one or more *Trichogramma* pupae. Each larva was fed daily with an excess of eggs (>100) for all the duration of its immature development. This was repeated twenty-five times for each egg diet. Data obtained on daily consumption and duration of preimaginal development on each egg diet were analyzed with a one-way ANOVA followed by multiple comparisons tests (Fisher's Protected LSD) (SuperAnova, Abacus

Concepts 1989). Chi-square tests (StatView, Abacus Concepts 1993) were realized on the percentages of survival to adulthood.

Influence of patch quality on foraging

The influence of patch quality on the foraging behavior of L4 was investigated. The experimental design was composed of a raised 14 x 14 cm glass plate on which eggs were placed in a 7.5x7.5 cm grid (six rows and six columns). The glass plate was raised by 2.5 cm to allow the coccinellid to leave the arena. The plate was placed in a 50x50x25 cm plastic box to diffuse light in order to minimize the influence of phototaxis on the searching behavior of *C. maculata* and was open on one side to permit the experimentator to add eggs and to observe the coccinellid behavior. Three different patch types were used: 1) 36 unparasitized *T. ni* eggs (1 day-old), 2) 36 parasitized *T. ni* eggs (8 days-old), 3) a mixed patch containing 18 eggs of these two categories.

Larvae were individually placed within a Fluon® -coated ring and, when the first egg was encountered and feeding had started, the ring was carefully removed. Consumed eggs were immediately replaced by an egg of the same treatment to avoid influence of depletion rate on patch quality. The behavior sequences of 20 larvae for each patch type was recorded using The Observer software (Noldus Information Technology, Version 2.0, 1989). In every experiment; (1) the number of encounter, (2) the number of consumed eggs, (3) the number of rejected eggs, (4) the patch residence time (PRT), (5) the giving up time (GUT) and, (6) the handling time, were noted. Patch residence time began when a coccinellid larva had started consuming its first egg and was terminated when the larva left the glass plate. Giving up time was the period between the end of the feeding bout on the last egg encountered and patch emigration (Krebs *et al* 1974). Experiments were conducted at 22 ± 1.0 °C and 60% RH. Each data set was subjected to a one-way

ANOVA (SuperAnova, Abacus Concepts 1989)) to test the influence of patch quality followed by a multiple comparison tests (Fisher's Protected LSD).

RESULTS

Influence of parasitism and prey age on prey discrimination

In all experiments, the number of encounters with the two egg categories was similar (Wilc. MPSR; $P \geq 0.05$) indicating that the experimental arena did not induce bias in the searching behavior of the coccinellid larva and that no pre-contact discrimination occurred.

Both L2 and L4 consumed unparasitized eggs (U1) and eggs that were recently parasitized (P1) in the same proportion (Wilc. MPSR; L2; z=-0.67, $P>0.05$; L4 z=-0.20, $P>0.05$) (Fig. 5.1a,b) which indicates that coccinellid larvae showed no preference between these prey types. Therefore, they are no indications that *C. maculata* larvae are capable of interspecific discrimination using the egg alterations provoked by the female parasitoid.

Once embryogenesis is initiated, the unparasitized *T. ni* eggs undergo more rapid physiological changes and complete their development faster (4 days) at 25°C than do parasitized eggs (9 to 10 days). When confronted to 4 days-old unparasitized eggs containing a mature lepidopterous embryo (U4) and parasitized eggs containing a parasitoid larva (P4), both L2 and L4 accepted significantly more the parasitized eggs (Wilc. MPSR; L2; z=-3.45, $P \leq 0.001$; L4 z=-3.42, $P \leq 0.001$). However, when L4 had to choose between young unparasitized eggs (U1) and parasitized eggs containing a fully developed parasitoid pupa (P8), they preferred the unparasitized ones (Wilc. MPSR; z=-2.37, $P \leq 0.001$). Furthermore, we noticed that L4 frequently opened these parasitized eggs and pulled away the parasitoid pupa without attempting to consume it or the egg chorion.

To eliminate the age factor, 4 days-old unparasitized eggs (U4) and 8 days-old parasitized eggs (P8) (i.e. both at the end of their development) were proposed to coccinellid larvae. They were both consumed in equal proportion by L2 and L4 (Wilc. MPSR; L2; z=-1.82, $P>0.05$; L4 z=-0.91, $P>0.05$) (Fig. 5.1a). To evaluate the influence of prey age regardless of parasitism, young (U1) and old (U4) unparasitized *T. ni* eggs were offered to coccinellid larvae and the old eggs were significantly less frequently eaten by both L2 and L4 (Wilc. MPSR; L2; z=-3.42, $P\leq 0.001$; L4 z=-2.95, $P\leq 0.001$). These results show that the embryonic development of a parasitized or unparasitized egg is the determining factor in prey discrimination by *C. maculata* larvae regardless of parasitism.

In all combinations where discrimination was observed (Fig 5.1a,b), the percentage of rejected eggs was always significantly higher on the less preferred egg type (Wilc. MPSR; L2, U4/P4 z=-3.11 $P\leq 0.01$; L2, U1/U4 z=-2.98 $P\leq 0.01$; L4 U4/P4 z=-3.68 $P\leq 0.001$; L4, U1/U4 z=-2.85 $P\leq 0.01$;) (Fig. 5.1c,d) whereas in combinations where no preference has been shown, the percentage of egg rejection was equivalent for both egg types (Wilc. MPSR; L2, U1/P1 z= -0.11 $P>0.05$; L2 U4/P8 z=-1.27 $P>0.05$; L4 U1/P1 z= -0.94 $P>0.05$; L4 U4/P8 z=-1.78 $P>0.05$). Similarly, in all combinations with differential egg acceptance, handling time of the less preferred eggs was always significantly longer (Wilc. MPSR; L2, U4/P4 z=-2.41 $P\leq 0.01$; L2, U1/U4 z=-2.94 $P\leq 0.01$; L4 U4/P4 z=-2.80 $P\leq 0.01$; L4, U1/U4 z=-2.80 $P\leq 0.01$;) (Fig. 5.1e,f) whereas in combinations where no preference was detected, handling time was similar on both egg types (Wilc. MPSR; L2 U1/P1 z=-1.84 $P>0.05$; L2 U4/P8 z=-0.28 $P>0.05$; L4 U1/P1 z=-0.15 $P>0.05$). However, in the U4/P8 combination where the handling time of L4 was shorter on unparasitized eggs (Wilc. MPSR; z=-2.60 $P\leq 0.01$), even if the two egg types were eaten in the same proportion (Fig. 5.1b). Generally, the discrimination ability of young (L2) and old (L4) *C. maculata* larvae was similar for all egg combinations.

Influence of parasitism and age on prey suitability

To determine whether the energetic returns associated to eating parasitized or old unparasitized eggs, the preimaginal developmental time, the number of eggs needed for coccinellid larvae to complete their immature development and their survival were evaluated (Table 5.2). *C. maculata* required more parasitized eggs (P8) to complete its preimaginal development and took significantly longer on this diet to reach the adult stage compared to immatures provided with unparasitized young *T. ni* eggs (U1). Duration of preimaginal development was similar for immatures reared on young (U1) or old (U3) unparasitized egg. However, mean number of old unparasitized eggs (U3) needed to complete larval development was significantly higher compared to young eggs (U1), but lower compared to parasitized eggs (P8). The percentage of individuals that reached adulthood was not significantly different for the three diets even if a higher survival was noted on the diet of young *T. ni* eggs (U1). Therefore, young *T. ni* eggs (U1) are more suitable than are older unparasitized eggs (U3) or parasitized ones (P8) for the development of *C. maculata*.

Influence of patch quality on foraging

When it detects parasitized prey, a coccinellid larva should be able to reduce its searching effort to a degree that reflects the reduced payoff of the patch. L4 stayed for a longer period of time (PRT) (ANOVA; $F=6.367$, $P\leq0.01$) and gave up (GUT) less rapidly after the last egg encounter ($F=3.85$, $P\leq0.05$) in a patch containing only unparasitized eggs or in a mixed patch of unparasitized and parasitized eggs compared to larvae searching in a patch containing only parasitized eggs (ANOVA; $F=6.01$, $P\leq0.01$) (Fig. 5.2). Consequently, the level of patch exploitation was significantly reduced for patches containing only parasitized eggs (Fig. 5.2c). The mean number of encounter with eggs (ANOVA; $F=4.10$, $P\leq0.05$) and the mean number of consumed eggs (ANOVA; $F=3.93$, P≤0.05) was 50% lower in parasitized patches. However, L4 searching in mixed patches rejected significantly more eggs than L4 confronted to eggs in homogenous patches (ANOVA; F=6.86, P≤0.01) (Fig. 5.2c). Most of the rejected eggs in mixed patches were parasitized (>90%).

Another factor that could play an important role in patch exploitation by coccinellid larvae is the differential handling time observed on both egg types in mixed patches compared to the time allowed to egg manipulation in homogenous patches (Table 5.3). When confronted to both egg types simultaneously, L4 spent more time handling parasitized eggs compared to unparasitized eggs whereas in homogenous patches, handling time was similar for both. Nevertheless, longer handling times of parasitized eggs and time lost in egg rejection by L4 in mixed patches did not significantly affect their level of exploitation compared to homogenous unparasitized patches (ANOVA; F=3.93, P>0.05 (Fig. 5.2c).

DISCUSSION

In nature, even if most coccinellids feed on a wide range of prey types (Hodek & Honek 1996), their prey are not always of equivalent values (Smith 1965; Mills 1981; Obrycki & Orr 1990). As predicted by optimal foraging models, predators searching for prey should select the most profitable prey type and reject unprofitable ones (Crawley & Krebs 1992). Such decisions minimize loss of opportunity time and maximize energy return (Stephens & Krebs 1986). However, some environmental constraints such as the presence of a direct competitor may influence the decisions underlying prey selection. If this competitor is also a potential prey, such as in the case of oophagous immature parasitoids, it may be more beneficial for a predator to eliminate this direct competitor despite a lower prey profitability. We expect to observe IGP that results in immature parasitoid elimination in cases where both competitors i.e., the predator and the parasitoid, occur frequently together, attack the same prey/hosts and show mutual predation and seasonal overlap

(Polis *et al.* 1989). Therefore, the detection and recognition of a mark left by a parasitoid female or of morphological and physiological changes provoked by a developing parasitoid immature would have strong selective advantages for a predator if 1) it could allow an intraguild predator to eliminate a competitor or if 2) suitability and profitability had been altered by parasitism.

Our results showed that *C. maculata* larvae did not exhibit any preference between parasitized and unparasitized eggs which have reached a similar developmental stage, whether parasitized eggs had been recently parasitized or were at the end of their development. Hence, even if *C. maculata* larvae detected that its prey had been parasitized, it did not affect prey choice. In contrast, most studies conducted on the subject had showed that egg predators had a higher level of predation on unparasitized prey than on parasitized prey containing parasitoid larvae or pupae (Al Rouechdi & Voegelé 1981; Brower & Press 1988; Ruberson & Kring 1991; Hoelmer *et al.* 1994). It has been suggested that the differential preference observed was a function of the physiological changes provoked by the developing parasitoid (Fritz 1982). These changes harden the chorion and decrease the availability of the resource making it more difficult to penetrate the egg and obtain food (Al Rouechdi & Voegelé 1981; Ruberson & Kring 1991). However, despite these changes, handling time of parasitized eggs containing late parasitoid stages was not longer than the handling time of unparasitized eggs near eclosion. This suggests that the physiological changes occurring within old parasitized egg did not render these eggs more difficult to manipulate compared to old unparasitized eggs and that it did not stop both L2 and L4 from gaining access to the egg resource. However, the larvae frequently left aside parts of the eggs and often removed the parasitoid pupae without attempting to consume it, a behavior also observed by Wheeler *et al.* (1968) in a study involving *C. maculata* attacking parasitized aphids.

The lack of preference for parasitized eggs clearly demonstrates that IGP does not have a direct competitive basis. Discrimination in favor of parasitized eggs would have been adaptive for *C. maculata* under conditions of frequent local resource competition with *Trichogramma* or if these parasitoids had the potential to directly attack *C. maculata* by successfully parasitizing their eggs (bi-directional IGP). In southwestern Québec, natural populations of *Trichogramma* spp, whose species are more specialized than *C. maculata*, were rarely found in cruciferous crops (Boivin Unpubl.) whereas *C. maculata* was found to be the principal predator of eggs and larvae of the lepidopterous complex (Roger *et al.* 1995). In addition, no studes reported direct attacked of Trichogramma on predatory coccinellid species (Hodek & Honek 1996). Therefore, frequent local resource competition and bi-directional IGP between these potential competitors are unlikely to happen.

The present results strongly suggest that *C. maculata* larvae select prey based on the embryonic modifications caused by the developing embryo regardless of parasitism. In combinations 1 and 2, both egg types were of similar physiological age and no preference was observed. However, when confronted to unparasitized eggs at the end of their development and parasitized eggs at an intermediate stage of their development, the predaceous larvae consumed a greater proportion of parasitized eggs. This indicates that the developmental stage (age) could be the determinant factor influencing prey selection. To evaluate this assumption, we offered to coccinellid larvae unparasitized eggs at different development stages (combination 5) and young eggs were preferentially eaten compared to eggs at the end of their development.

Generally, the quality of the egg resource decreases with age (Vinson 1994). As the embryo develops, the stored resources within the egg decreases and the embryonic tissues become more complex (Pak 1986; Vinson 1994). Ruberson *et al.* (1987) suggested that, in the case of developing parasitoid larvae, this complexification of egg resource may reduce their accessibility and suitability. We hypothesized that age may also alter egg suitability for coccinellid larvae and that reduced suitability may explain the prey choice observed. Furthermore, we expected that both parasitoid larvae and *T. ni* embryo alter egg suitability in a similar way because they transform all egg resources to complete their development. Our findings corroborate our hypothesis since old parasitized and unparasitized eggs were less suitable for the development of *C. maculata* immatures. Longer developmental time was observed when coccinellid immatures were reared on old parasitized eggs rather than on young unparasitized eggs. In addition, more eggs were needed for *C. maculata* to complete its development on both old parasitized and old unparasitized eggs and survival to adulthood was greatly reduced on both egg types. Similarly, many studies concerning host suitability for oophagous parasitoids indicate that ovipositing parasitoid females usually select younger eggs and that it corresponds to higher suitability for parasitoid immature development (Juliano 1982; Hintz & Andow 1990; Ruberson & Kring 1993). They observed a longer developmental time and a decrease in survivorship as well as a production of smaller individuals in older eggs indicating an influence of host age on parasitoid fitness.

Egg profitability decreases with age as the developing embryo uses gradually the resources within the egg. As described in the optimal diet model (Crawley & Krebs 1992), profitability is defined as a ratio of the prey food energetic value to the time required to pursue and consume that prey (handling time). In our experimental design, all eggs were at a same distance and we therefore assumed that searching time on both egg type was similar. However, we expected longer handling time on older eggs due to the alterations provoked by the developing *T. ni* embryo or by the parasitoid immature. Our results support this prediction as handling time increased with egg age. Such phenomenon has been frequently observed with ovipositing parasitoid females (van Huis *et al.* 1991; Ruberson & Kring 1993). Hence, even if energetic value of young and old eggs is equal, it is probable that profitability of old eggs decreased due to higher handling time. Overall, egg preference by the generalist predator *C. maculata* was related to egg suitability and to their costs in terms of lost opportunity time. Therefore, IGP is a simple act of predation directed at nutritionally acceptable prey and the elimination of parasitoid immatures is only epiphenomenal, as Polis *et al.* (1989) have already suggested in similar situations.

An important role of prey discrimination is to perceive patch quality and adjust patch residence time to exploit the patch according to its relative quality. Many functional models make unrealistic assumptions on patch leaving behavior because they use simple fixed rules that simplify the constraints brought by the complexity of the environment and by the foraging activities of predators or parasitoids (Gibb 1962; Krebs 1973). For generalist predators that are confronted to a highly variable environment and differential prey suitability, those fixed rules are not optimal because they do not allow them to adapt their foraging strategy according to the quality of the resource contained in patches. However, other models, principally elaborated for parasitoids, propose that in a patch perceived as being of good quality, a female parasitoid should allocate more time to its exploitation (Waage 1979; McNair 1982). Similarly, one can expect that a predator with discrimination ability would invest its patch searching and exploitation time according to patch profitability. In such cases, flexible PRT and GUT would determine the pay-off of different patch qualities (van Alphen & Galis 1983). Coccinellid larvae left the patch and gave up more rapidly after the last egg encounter when the patch contained only parasitized eggs compared to homogeneous unparasitized patches resulting in a patch exploitation 50% lower in parasitized patches. These results strongly suggest that *C. maculata* larvae respond to variable patch quality by using flexible decision rules based on discrimination ability instead of fixed rules and that the decisions reflect the different patch quality .

After the consumption of the first egg, larvae of most coccinellid species usually adopted an intensive search pattern which involved slow and sinuous movements (Dixon 1959). The leaving of the patch was preceded by a switch to extensive search that involved faster linear movement, a searching behavior observed in many other cocinellid species (Dixon 1959; Nakamuta 1982). For such predators that use a searching pattern adapted to patchily distributed prey (Dixon 1959; Nakamuta 1982), it may not be optimal to be highly selective in prey choice when prey are relatively scattered like *T. ni* eggs usually are in nature (Harcourt 1962; Shorey *et al.* 1962). Hence, costs associated to travel and the increased risks of predation in doing so may outcome the benefits associated to the finding of a better patch (Charnov 1976; Stephen & Krebs 1986). With such constraints, it may be optimal for a predator like *C. maculata* to spend more time in patches containing prey of lower quality instead of leaving the patch to search for a higher quality patch. Our findings corroborate these assumptions since *C. maculata* larvae allocated similar periods of time in prey search in both heterogeneous patches and patches containing only good quality prey. There was no significant differences between the exploitation rates of the two patch types even though coccinellid larvae had to discriminate and reject parasitized eggs. The frequency of adequate hosts discovery in mixed patches was probably above the threshold at which a larva would switch from intra-patch intensive search to inter-patch extensive search.

Little is known on the mechanisms underlying prey discrimination by coccinellids. Rejection of parasitized eggs occurred only after *C. maculata* larvae had contacted and probed the egg, as in the case of other egg predators (Al Rouechdi & Voegelé 1981; Brower & Press 1988; Ruberson & Kring 1991). This recognition ability is adaptive for a forager because it allows the predator to locate and exploit suitable eggs and to save time on less suitable ones. Morphological and physiological egg modifications caused by age are exploitable cues for parasitoids that can be used to recognize and assess host quality (Strand 1986; Vinson 1994). Similarly, in studies involving predators confronted to sessile parasitized prey, it has been suggested that host modifications provoked by the developing parasitoid immature can change the texture, size and shape of the prey (Al

Rouechdi & Voegelé 1981; Hoelmer *et al.* 1994) and that a predator, as well as a parasitoid, could detect these egg modifications and select prey accordingly.

In both types of homogeneous patches, the percentage of rejected eggs and handling time was similar whereas in heterogeneous patches, a higher percentage of rejected eggs and longer handling times were observed on parasitized eggs. This could indicate that in heterogeneous patches *C. maculata* larvae could associate physiological and morphological characteristics to the corresponding egg type. These changes may signal to the predator that egg resource quality has decreased to an unacceptable level (Vinson 1994) consequently provoking a decrease of motivation to consume the less suitable prey type. This phenomenon has also been observed in parasitoid females that adjust the sex ratio of their progeny according to different host sizes present in the patch, whereas in patches containing only one host size, the progeny allocation was held constant (Kring 1993).

In heterogeneous patches, the coccinellid larvae consumed relatively high proportion of parasitized eggs. This may be because the *C. maculata* larvae used in our experiments were inexperienced with the food types offered and that recognition of the morphological and physiological changes has to be learned. Such evidence have been brought forward in many studies involving host location and selection by parasitoids (Vet & Groenewold 1990; Turlings *et al.* 1993) but has rarely been mentioned for generalist predators (Prokopy & Lewis 1993) and more specifically in Coccinellidae (Houck 1986). In *C. maculata* larvae, the percentage of rejected parasitized eggs increases with the number of encounters with parasitized eggs, suggesting that experienced larvae could recognize and assess favorable patches more rapidly and maximize prey exploitation accordingly (Roger *et al.* Unpubl.; Chapter 6).

The behaviors described in this study were performed in a simplified laboratory arena and may partially reflect the whole behavioral response of *C. maculata* in a natural and more complex habitat. In nature, variability in several habitat components in addition to prey quality and profitability can modify the benefits gained in being selective. Because of this higher complexity, we predict that selectivity would decline in a natural habitat and that *C. maculata* would be more inclined to consume parasitized or old unparasitized eggs despite long-term effect on its development. IGP would therefore be more frequent especially because parasitized eggs have a longer developmental time that increases their vulnerability. Age structure of both prey and parasitoid populations should play a determinant role in prey selection by *C. maculata* in the field.

AKNOWLEDGEMENTS

This paper has greatly benefited from criticisms by Bernard D. Roitberg. We thank Josée Delorme for assisting in the laboratory work. This research was supported by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) to D. Coderre. Caroline Roger acknowledges support from the Fonds pour la formation de Chercheurs et l'aide à la Recherche (FCAR) from the Ministère de l'Éducation du Québec. This is contribution no XXXXX of the Horticultural Research and Development Centre of Agriculture and Agri-food Canada at St-Jean-sur-Richelieu.

Fig. 5.1. Comparisons of (a,b) Manly's index of preference (mean ±SEM), (c,d) % of rejected eggs and, (e,f) handling time (sec.) between different combinations of unparasitized (U) and parasitized (P) eggs at different developmental times (number of days follows the letter of the combination). Experiments were performed with *C. maculata* L2 and L4 instars. NS non significant difference, * $P \leq 0.05$, ** $P \leq 0.01$ (Wilcoxon MPSR test).

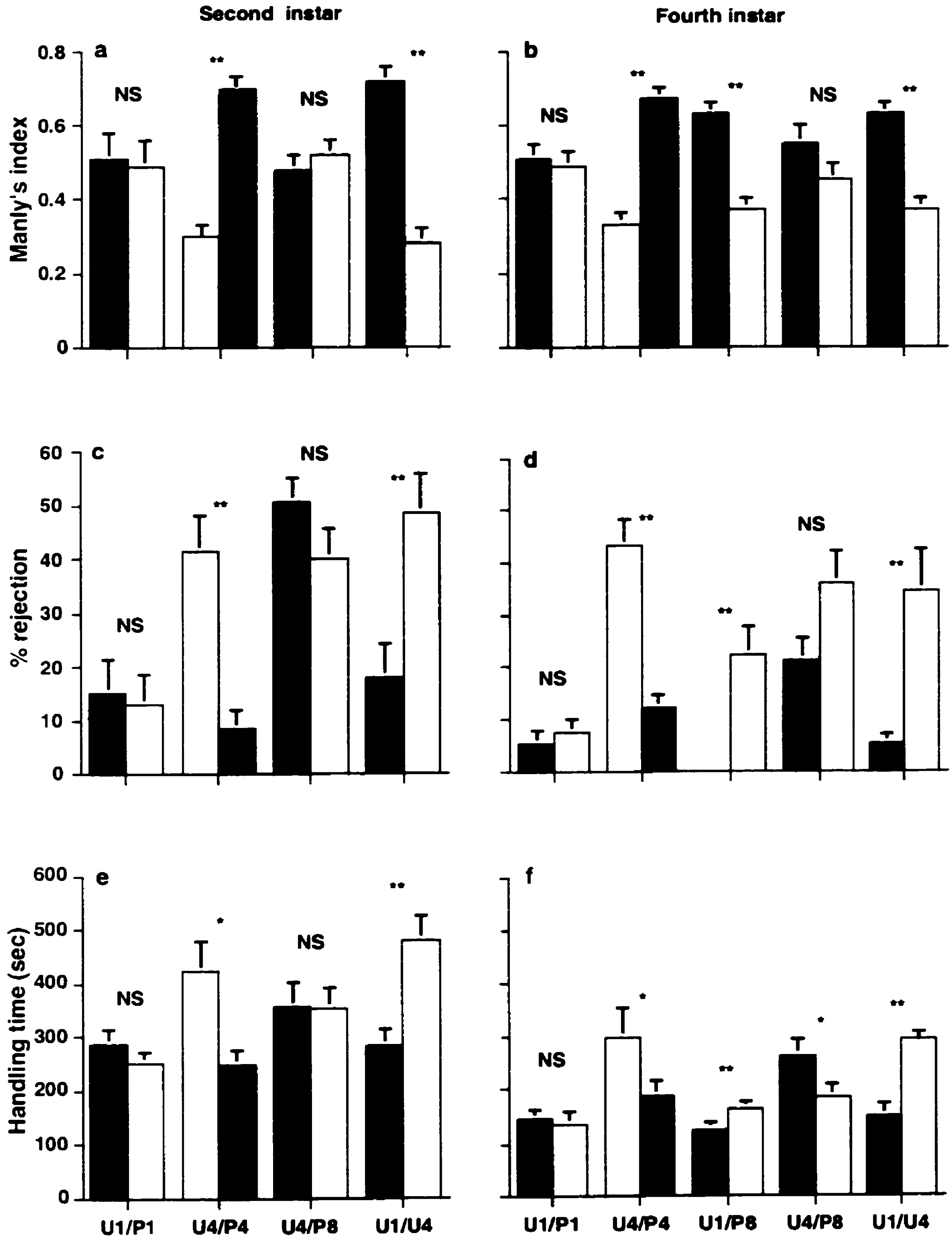
Second instar
Fourth instar
a
b
c
d
e
f
Manly's index
0.8
0.6
0.4
0.2
0
% rejection
60
50
40
30
20
10
0
Handling time (sec)
600
500
400
300
200
100
0
NS
**
*
U1/P1
U4/P4
U4/P8
U1/U4
U1/P1
U4/P4
U1/P8
U4/P8
U1/U4

Fig. 5.2. Mean (±SEM) (a) patch residence time, (b) giving-up time (GUT) and (c) number of encounters and % of rejection of eggs by *C. maculata* L4 in patches containing only unparasitized eggs (U1) or parasitized eggs (P8) or in patches with an equal density of both egg types (U1/P8). Different letters indicate significant difference at $P \leq 0.05$, (ANOVA followed by a Fisher's PLSD test).

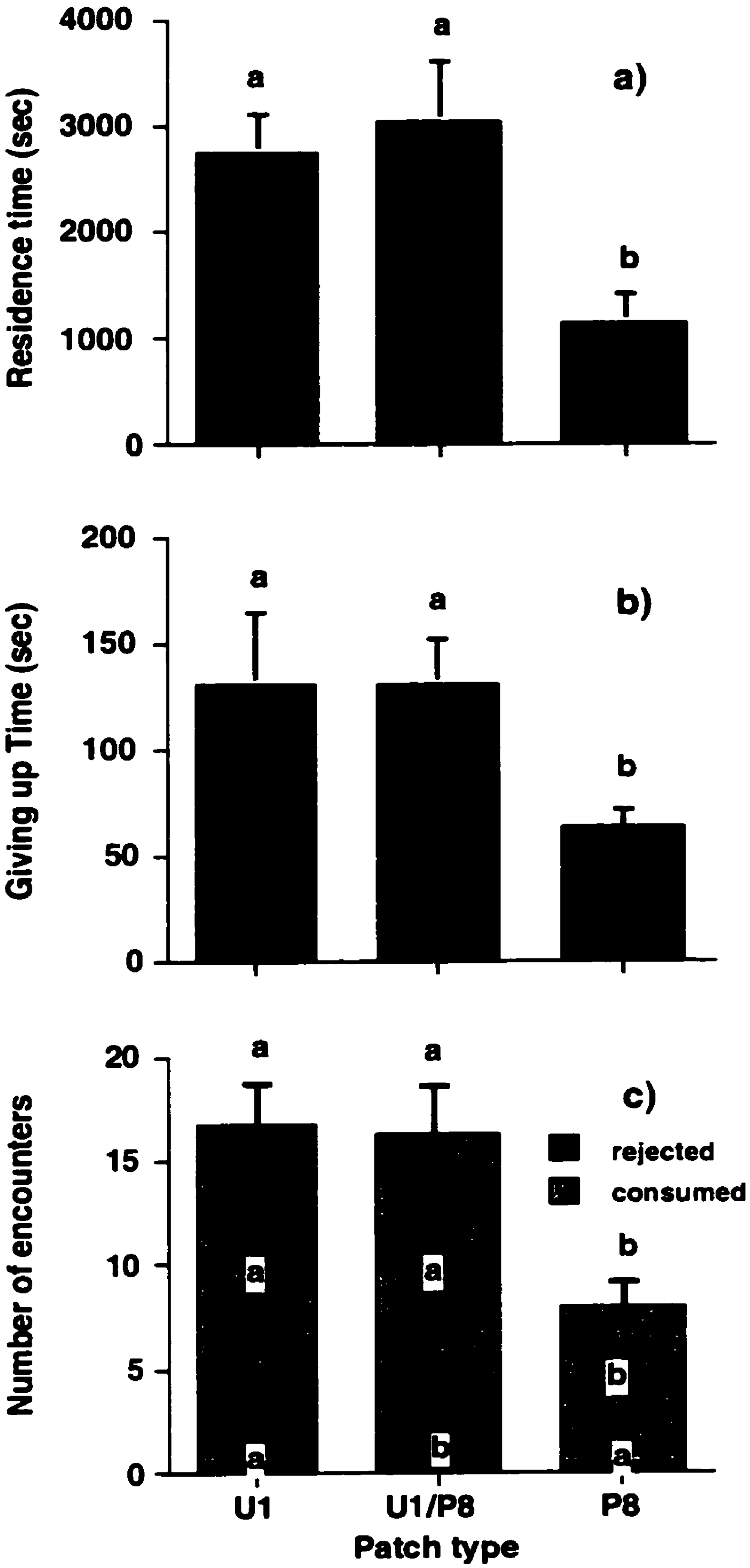

a)
Residence time (sec)
0
1000
2000
3000
4000
a
a
b
b)
Giving up Time (sec)
0
50
100
150
200
a
a
b
c)
Number of encounters
0
5
10
15
20
a
a
b
a
a
b
a
b
a
rejected
consumed
U1
U1/P8
P8
Patch type

Table 5.1. *T. ni* egg combinations offered to *Coleomegilla maculata* second and fourth instar larvae.

Combinations	Egg age (days)	Developmental stage
1) 18 parasitized eggs (P1)[1]	1	- Physiological age was equivalent for both egg types
18 unparasitized eggs (U1)	1	
2) 18 parasitized eggs (P4)[2]	4	- Parasitoid immature; larval stage
18 unparasitized eggs (U4)	4	-*T. ni* embryo; fully developed
3) 18 parasitized eggs (P8)[2]	8	- Parasitoid immature; pupal stage (fully developed)
18 unparasitized eggs (U1)	1	- Early in the development of *T. ni* embryo
4) 18 parasitized eggs (P8)[2]	8	- Parasitoid immature; pupal stage (fully developed)
18 unparasitized eggs (U4)	4	- *T. ni* embryo; fully developed
5) 18 unparasitized eggs (U1)	1	- Early in the development of *T. ni* embryo
18 unparasitized eggs (U4)	4	- *T. ni* embryo; fully developed

[1] Parasitism by *T. evanescens* females occurred 1h before observations began

[2] Parasitism occurred within 12 h of oviposition

Table 5.2. Mean duration (±SEM) of preimaginal development, mean number of consumed eggs by *C. maculata* larvae and percentage of survival to adulthood according to different egg diets; unparasitzed 1day-old (U-1), unparasitzed 3 days-old (U-3), parasitized 8 days-old (P-8). In a column, means followed by the same letter are not significantly different at $P \leq 0.05$ (Fisher's PLSD tests)

Diet	Mean duration (days)	Mean number of consumed eggs	Percentage of survival
U-1	24.65±0.31a	83.72±2.47a	72.0a
U-3	23.83±0.35a	100.20±2.54b	48.0a
P-8	25.75±0.39b	120.80±1.43c	48.0a
Tests	ANOVA; F=6.84 ($P \leq 0.01$)	ANOVA; F=61.80 ($P \leq 0.0001$)	Chi square*; $\chi 2=3.00$ ($P=0.08$)

* Survival values were compared among themselves

Table 5.3. Mean handling time (±SEM) of unparasitized and parasitized eggs in a mixed patch and in a patch containing only one of the two egg types. In a row, means followed by the same letter are not significantly different at $P \leq 0.05$ (Fisher's PLSD tests).

	Mean duration (sec)		
Patch	Unparasitized	Parasitized	ANOVA
Homogenous	112.14±4.01a	117.12±3.54a	F=0.645 ($P>0.05$)
Mixed	129.23±6.50a	172.70±12.65b	F=10.976 ($P \leq 0.05$)

In chapter 5, it was shown that *C. maculata* had discrimination abilities and could select prey according to their profitability. For example, coccinellid larvae rejected in a greater proportion old parasitized eggs compared to young unparasitized ones. During these experiments, we also noticed, that in a feeding sequence, coccinellid larvae increasingly rejected parasitized eggs whereas unparasitized eggs were nearly always accepted.

In patches containing prey of different qualities, learning abilities could allow the generalist predator *C. maculata* to improve its prey selection behavior and, consequently, to forage more optimally. In the next chapter, we demonstrate that the change in the egg rejection behavior observed in patches containing equal densities of parasitized and unparasitized eggs, resulted from learning.

CHAPTER 6

Learning affects prey selection in a generalist coccinellid predator

C. ROGER, D. CODERRE, E. WAJNBERG & G. BOIVIN

To be submitted to Behavioral Ecology and Sociobiology

ABSTRACT

In nature, generalist predatory insects are confronted to a variety of potential prey species, not all equally suitable and living in diverse habitats. Under those conditions, behavioral plasticity may be adaptive if it allows a generalist predator to adjust to variations in resource quality and availability. Under laboratory conditions, we examined the learning process and memory in prey selection in the predatory coccinellid *Coleomegilla maculata lengi* Timb. (Coleoptera: Coccinellidae). In choice tests, we studied prey rejection behavior of *C. maculata* fourth instar larvae toward eggs of *Trichoplusia ni* (Hübner) either unparasitized or parasitized by *Trichogramma evanescens* Westwood (Trichogrammatidae). We also tested the influence of hunger and prior experience with other food types on the egg rejection behavior of coccinellid larvae. *C. maculata* larvae gradually changed their behavior and more frequently rejected parasitized eggs whereas unparasitized eggs were nearly always accepted. After 48h, the learned behavior had been partially forgotten. Hunger and experience with other food types prior to the test had little effect on the gradual change of behavior but the quality of the food ingested influenced the initial level of egg rejection. Our results demonstrate that, (1) with experience, *C. maculata* larvae can adjust their prey selection behavior to select the more suitable prey and that (2) previous experience with other prey types could influence their initial preference.

Key words: Insecta; generalist predator; Coccinellidae; learning; physiological state; prey discrimination

INTRODUCTION

Optimal foraging models predict that a forager should maximize its encounter rate with the most suitable and profitable prey or hosts and avoid lesser quality individuals (Stephens & Krebs 1986). For generalist predatory insects whose successive generations are exposed to different prey species living in various microhabitats, recognition of specific cues to identify the most suitable prey is nearly impossible (Papaj & Lewis 1993). Behavioral plasticity (learning), as opposed to genetically fixed behavior, may be adaptive for such generalist insect because it allows an individual to gather information and adapt to a variety of situations that may be encountered (Lewis *et al.* 1990). Learning may also be adaptive for long-lived species, because it allows them to switch from microhabitats that decrease in profitability to more profitable ones (van Alphen & Vet 1986).

Insects use olfactory and visual cues to detect resource-rich habitats and, with experience, they can learn to associate these cues to the target habitat, prey or host (Lewis & Tumlinson 1988; Papaj & Prokopy 1989; Turlings *et al.* 1993). Learning is thus an important strategy to increase overall foraging efficiency. Pre-imaginal and adult learning appears to be widespread among phytophagous (Papaj & Propoky 1989), parasitoid (Turlings *et al.* 1993) and social insects (Menzel *et al.* 1993) (see Papaj & Lewis 1993 for a review). However, the adaptive value of learning in prey selection by generalist predators has received little attention. Most research report improvement in searching behavior (Ettifouri & Ferran 1993) and prey recognition in adults (Pasteels & Grégoire 1984; Blois & Cloarec 1985; Henaut *et al.* 1997), as a result of conditioning process or of influence of experience on prey capture behavior (Déjean *et al.* 1990).

Learning has been defined as a change in behavior as a consequence of experience (Kimble 1961). As suggested by Papaj & Prokopy (1989), an individual's behavior should (1) change in a repeatable way as a consequence of experience, (2) change gradually with continued experience and, (3) wane in the absence of continued experience of the same type, or as a consequence of a novel experience or trauma. The occurrence of learning is determined by the predictability of the environment within- or between generations (Stephens 1991) and is more likely to occur when the environment changes between generations but is rather constant within a generation.

Coleomegilla maculata lengi Timb. (Coleoptera: Coccinellidae) is one of the most generalist coccinellid species known (Hodek & Honek 1996). It feeds on a wide array of prey occurring in many habitats (Gordon 1985). In Canada, few generations per year are observed and longevity of the adults is approximately one year (Hodek 1973). Both larval and adult stages are predaceous and attack the same prey type (Hodek & Honek 1996). Because of their lower mobility compared to adults, coccinellid larvae generally search and exploit prey within one microhabitat (Wratten 1973). Prey choice in larvae is greatly influenced by the habitat preference of the ovipositing female (Hodek 1973). Most prey species exploited by coccinellids are ephemeral in time and space (Hodek & Honek 1996) and coccinellid adults must frequently disperse and move from one microhabitat to the other to find suitable prey. Therefore, a coccinellid immature may or may not forage in the same habitat as its mother did. Hence, *C. maculata* experiences a high predictability through its larval development and a low predictability between immatures generations and thus meets the conditions set forth by Stephens (1991) for the evolution of learning. It has been shown that prey preference (Houck 1986) and searching behavior (Ettifouri & Ferran 1993) of predatory coccinellids can be influenced by conditioning on a specific prey type.

Parasitism provokes external and internal host modifications (Strand 1986; Vinson 1994) that may influence suitability of a prey for a predator. If a predator has the ability to discriminate i.e. to detect if a prey is parasitized, it can choose to consume or reject the prey, depending on the relative benefits and costs associated with both situations. In *C.*

maculata, larvae can discriminate between eggs of *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) parasitized by *Trichogramma evanescens* (Westwood) (Hymenoptera: Trichogrammatidae) and unparasitized *T. ni* eggs (Roger *et al.* submitted; Chapter 5). At the beginning of a feeding sequence, coccinellid larvae readily accepted most of parasitized eggs but, after a few encounters, they increasingly rejected them whereas rejection of unparasitized egg was nearly null. In that study, parasitized eggs were less suitable prey for *C. maculata* than young unparasitized *T. ni* eggs. Pre-imaginal developmental time and food intake to reach adulthood increased and survival decreased when coccinellid larvae were fed with parasitized eggs.

In the present paper, we demonstrate that the change in egg rejection behavior resulted from learning. We also tested whether this learned behavior could be forgotten and we determined the influence of hunger and of the quality of food ingested prior to experiment on the egg rejection behavior.

METHODS

Rearing

Colonies of *C. maculata* were initiated with adults collected in spring from hibernation sites near corn fields in Saint-Hyacinthe (72°56'W,45°39'N), Québec, Canada. They were maintained on a fresh liver-based artificial diet (Coderre, unpubl.) and on wild flower pollen at 22°C, 70% R.H., and a 16L:8D photoperiod.

T. evanescens, initially originating from Egypt, was obtained from the collection maintained at the Department of Entomology in Wageningen (Pak & van Lenteren 1984) and was reared on eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) at 25°C, 30% RH and 16L:8D photoperiod. *T. ni* and *Artogeia rapae* (L.) (Lepidoptera: Pieridae) were reared on artificial diets (Shelton, pers. comm; Webb & Shelton 1988), and maintained at 25 °C, 30% RH and 16L:8D photoperiod. It has been shown in laboratory studies that *T. ni* eggs are preferential hosts for *T. evanescens* (Parker & Pinnell 1974) and are readily preyed upon by *C. maculata* (Roger *et al.* 1995; Chapter 3).

The potato aphid, *Macrosiphum euphorbiae* (Thomas) (Homoptera: Aphididae) was collected from potato fields near St-Hyacinthe, Canada and maintained in the laboratory on potato plants, cv. Norland at 20°C, 60-70% RH and 16L:8D photoperiod.

Experimental procedures

C. maculata fourth instar larvae used in all tests had molted one day before the tests were conducted and were inexperienced with the prey used. All experiments were realized by offering an equal number of two egg types to a single coccinellid larva. Eggs were arranged alternately on a 14 x 14 cm glass plate in a 4 x 4 cm grid (six columns and six rows) and surrounded by a Fluon® -coated ring. Prey offered to *C. maculata* larvae were 18 *T. ni* eggs parasitized since 8 days by *T. evanescens* and 18 one day-old unparasitized *T. ni* eggs. Parasitism occurred within the first 12 h of egg life. The experiment was terminated when the larva stopped searching for a period of 15 min. This foraging sequence was considered as one observation period. Consumed eggs were immediately replaced by an egg of the same type to keep the patch quality constant. A total of five different tests were performed to verify hypothesis included in the three experiments described below.

In each test, we counted the number of encounters, consumed eggs and rejected eggs. An egg was considered rejected when 1) the encounter lasted at least 3 sec. and did not result in egg consumption (no alteration) or, 2) resulted in a small alteration of the egg chorion (i.e. hole less than one mm in diameter). The larvae did not attempt to consume the resource within the egg. An egg was considered accepted when it was partially or totally consumed. For all tests in experiment 2, an egg was considered rejected when it was not or partially consumed (i.e. the chorion and part of the resources), in order to control food intake and consequently, the influence of satiation. This had the consequence of increasing the egg rejection response observed.

For each test, we used non-parametric statistics, as described by Wajnberg (1993), to determine if encounters with each egg type in a sequence were random. These statistics are derived from standard rank test procedures (Hajek & Sidak 1967). We computed the rank positions of parasitized and unparasitized eggs within each sequence produced by a larva and determined; 1) whether parasitized or unparasitized eggs were encountered at the beginning of a sequence (SR; Sum of the parasitized egg rank position), 2) if parasitized eggs were encountered in the middle of the sequence (VR; Variance of the parasitized eggs rank position), 3) if there was pooling of parasitized egg encounters within the sequence (CGP; Centre-group of parasitized egg encounter) or 4) of unparasitized egg encounters within the sequence (CGU) and 5) if there was autocorrelation of parasitized or unparasitized egg encounters within the sequence (NR; Number of runs of parasitized and unparasitized egg encounter). For all statistics performed in each test, a global statistical analysis (Chi^2) combining all coccinellids tested (Wajnberg 1993) was done (Table 6.1). For each test, the total number of encounters on each egg type was submitted to a Wilcoxon's matched-pairs signed rank test (Wilc. MPSR) (Abacus Concepts, StatView 1992).

In each experiment, we described by linear regressions (Snedecor & Cochran 1989), the changes in the proportion of egg rejection in relation to the order of encounter for parasitized and unparasitized eggs. Data were submitted to an arcsin square root transformation and were weighted by the number of repetitions for each encounter. The intercept of the regression represents the initial rejection of the egg type whereas the slope represents the speed in the change of behavior. For each experiment, the intercepts and slopes were compared among themselves (PROC GLM; SAS Institute 1985) to detect differences in the rejection behavior according to the rank of encounter. In experiment 3, we used a Student t-test (Snedecor & Cochran 1989) to compare the intercept value of time T2 to the point reached after 23 encounters in the first experiment (time T1), to evaluate if coccinellid larvae had forgotten the learned behavior.

Experiment 1

The objective of this experiment was to examine the learning process of coccinellid larvae by comparing the gradual rise in the proportion of rejection of unsuitable prey (parasitized eggs) compared to suitable ones (unparasitized eggs). This experiment tested for the repeatability of the individual's behavior changes, and the gradual change in behavior with continued experience. In this experiment, we used 19 coccinellid larvae starved for a period of 24h before the beginning of the test (test 1).

Experiment 2

To evaluate the influence of hunger and food quality on the change of behavior, we used three treatments: 14 larvae fed with 5 third-instar nymphs of *M. euphorbiae* (average of total weight; 0.75 mg ± 0.37) (test 2), 13 fed with 10 *A. rapae* eggs (average of total weight; 0.74 mg ± 0.16) (test 3) and 19 starved (for 24h) coccinellid larvae (test 4). For fed larvae, the tests began after all prey were consumed. *C. maculata* is principally an aphidophagous coccinellid species and this prey type is known to be highly suitable for them (Hodek 1973). However, in a choice situation between *T. ni* and *A. rapae* eggs, *C. maculata* always prefered *T. ni* eggs (Roger *et al.* Unpubl.) which may indicate that *A. rapae* is not a preferential prey *for C. maculata*. Therefore, we assume that aphids are a better quality prey *for C. maculata* than *A. rapae* eggs. The regression lines of the proportion of rejection of parasitized eggs of each treatment were pooled in a same plot and compared to determine if hunger and food quality had an influence on the rejection behavior of parasitized eggs.

Experiment 3

To test if coccinellid larvae could forget a learned behavior, larvae used in experiment 1 were fed, after the test, with artificial diet for 24h, starved for another 24h period and replaced in the same experimental conditions (18 parasitized and 18 unparasitized eggs) (48h after the first test; time T2) (test 5). The regression lines of the proportion of rejection of parasitized eggs at T1 and T2 were pooled and compared to determine if the learned behavior have changed after a 48h time lap.

RESULTS

The probability of encountering a parasitized egg following the encounter of an unparasitized egg (and vice versa) was higher than predicted (Table 6.1). Because of the regular square distribution of the two egg types within the patch, the shortest distance between an egg and its neighbors was with the eggs of the other type. This experimental bias increased the probability of encountering a different egg type at each visit. However, this autocorrelation did not cause an increase in the number of visit to a specific egg type (Wilc. MPSR, $P>0.05$) indicating that experience with each egg type was similar and that no pre-contact discrimination occurred. Furthermore, in the other four statistical analyses, the encounter of parasitized or unparasitized eggs happened at random (Table 6.1), indicating that the sequence of encounter did not influence the change of behavior observed.

Experiment 1

C. maculata larvae rejected unparasitized eggs in a proportion varying from 0 to 12% (Fig. 6.1) but this proportion did not increase with encounters (Fig. 6.1). However, larvae gradually changed their behavior and, compared to parasitized eggs, rejected more parasitized eggs with increasing encounter with this egg type (slope; $t= 2.38$, $P= 0.0218$) (Table 6.2) with 38% of the larvae rejecting the parasitized eggs after 23 encounters. The proportion of larvae that initially rejected parasitized eggs (first encounter; 5.5%) was significantly higher than the proportion observed on unparasitized eggs (0%) (Intercept; $t= 3.27$, $P= 0.0021$) indicating that some larvae could discriminate parasitized eggs on first contact.

Experiment 2

Larvae that were fed prior to the experiment were tested to determine if the gradual rejection of parasitized eggs with encounter could be caused by satiation. The pattern of rejection was similar for fed and starved larvae (slope; fed with *A. rapae* eggs vs starved, $t= 1.63$, $P> 0.05$; fed with *M. euphorbiae* vs starved, $t= 0.47$, $P> 0.05$) (Fig. 6.2) indicating that a decrease in hunger is unlikely to explain the increasing number of parasitized eggs rejected with encounter. The quality of the food ingested before the experiment influenced the initial number of larvae (first encounter) that rejected parasitized eggs (Fig. 6.2, Table 6.2). The proportion of larvae that initially rejected parasitized eggs when fed with aphids was significantly higher than for larvae fed with *A. rapae* eggs (intercept; $t=2.25$, $P= 0.0293$) or for larvae starved for 24h (intercept; $t=2.27$, $P= 0.0280$). After 18 encounters, the percentage of rejection was of 75% for larvae fed with aphids, 63% for starved larvae and 43% for larvae fed with *A. rapae* eggs.

Experiment 3

To test if, with time, the larvae would forget the experience acquired with encounters, the larvae used in experiment 1 were tested again after 48h (time T2). The regression slopes at time T1 and T2 were similar (t=0.87, $P>0.05$) which indicates that the gradual change of behavior was not influenced by experience (Fig. 6.3, Table 6.2). However, the percentage of larvae that rejected parasitized eggs on the first encounter was significantly higher after 48h (18% of egg rejection) than after 24h (intercept; t=2.32, $P=0.0252$) but was significantly lower (t=2.98, $P< 0.05$) than the point reached after 23 encounters in the first experiment (38%) (Fig. 6.3). This indicate that the coccinellid larvae had at least partially forgotten the learned behavior after a period of 48h.

DISCUSSION

When confronted to a mixed patch of parasitized and unparasitized lepidopteran eggs, *C. maculata* larvae can discriminate and select the most profitable prey (Roger *et al.* submitted; Chapter 5). The results obtained here indicate that this discrimination ability can be improved with experience. *C. maculata* larvae displayed a change in their rejection behavior, the magnitude of which increased with successive contacts with parasitized eggs. Encounters with unparasitized eggs did not induce such change. We also demonstrated that this change of behavior was reversible. Therefore, the three critera of learning suggested by Papaj & Prokopy (1989) are respected.

The rejection behavior changed linearly with no asymptote, suggesting that, as for other generalist insects (Maldonado *et al.* 1979; Johnson 1991), learning is a rather slow process in *C. maculata* compared to parasitoid species (van Baaren & Boivin 1998). Because generalist predators are frequently confronted to a diversity of food types (Hodek

& Honek 1996), instantaneous recognition of specific cues associated to each food type is unlikely (Papaj & Lewis 1993). *C. maculata* larvae probably need several periods of exposure to the same egg type before they can adequately discriminate and adjust their foraging strategy accordingly. Furthermore, in nature, if the differences in the quality of encountered prey are too small, the fitness gain when discrimination occurs would be to small to be adaptive. We have previously shown that, compared to unparasitized eggs, the developmental time was longer on parasitized eggs, but that *C. maculata* immatures could reach adulthood (Roger *et al.* submitted; Chapter 5). Hence, even if parasitized eggs are less suitable than unparasitized eggs, it could be more costly to learn to discriminate than to prey upon them. This may explain why a proportion of parasitized eggs was readily consumed.

Repeated exposures could also allow a coccinellid larva to evaluate if patch quality is relatively constant. Hence, if patch quality decreases rapidly and density of suitable prey becomes too low, it may be too costly to learn to recognize and select a specific prey type. It has been shown in parasitoids that rejection of parasitized hosts is not always adaptive for an ovipositing female (van Alphen & Visser 1990: van Alphen 1993). In patches containing a high proportion of parasitized hosts, it may be more optimal to superparasitize. Similarly, *C. maculata* larvae searching and consuming only unparasitized eggs in patches containing a high proportion of parasitized eggs could obtained a lower net energy gain compared to larvae searching and eating indiscriminately both egg types. In that case, it may be more advantageous not to learn.

In the absence of prey contact for 48h, *C. maculata* larvae partially forgot what they had learned. With experience, even generalist insects tend to specialize on certain food type (Fox & Morrow 1981; Papaj & Rausher 1983) but this specialization could become detrimental if the resource becomes scarce. Forgetting previous experience could allow to adjust to fluctuations in food availability in a changing environment. It is likely that the duration of retention can also be influenced by the duration of the exposition period and by the ranking preference of prey as shown in parasitoids (Kaiser *et al.* 1989; Bjorksten & Hoffmann 1998). In generalist species, such as desert seed-harvester ants and preying mantis, it takes several days to completely forget a learned behavior (Johnson 1991; Maldonado *et al.* 1979).

It has been demonstrated that the physiological state and previous oviposition experience of parasitoids could influence their learning response (Takasu & Lewis 1993) and preference (Bjorksten & Hoffmann 1995). In the present study, hunger and experience on other food types had little effect on the rate of change of behavior but rather changed the initial level of egg rejection. When previously fed with aphids, the initial level of parasitized eggs rejection was higher than the rejection level observed with starved coccinellids but the opposite response was obtained when coccinellid larvae were previously fed with *A. rapae* eggs. Aphids are known to be the preferential prey for most predatory coccinellids and are highly suitable for the development of most coccinellids (Hodek 1973). However, *C. maculata* always preferred *T. ni* eggs compared to *A. rapae* eggs (Roger *et al.*unpubl.) in choice tests, suggesting that *A. rapae* is not a preferential prey for *C. maculata* larvae. Hence, coccinellid larvae that had acquired experience on their preferential prey type became more selective in their prey choice afterward. These results indicate, as suggested by Vet *et al.* (1990), that previous experience may alter prey ranking of different prey types and consequently affect food selection.

This study demonstrates the importance of learning in improving prey selection in a generalist predator. The results obtained suggest that, in an heterogeneous patch, lost opportunity time on less profitable prey could be reduced. Hence, our results provide support that experienced larvae could select their resource more effectively in mixed patches, as long as these resources remain available and, that learning should improve their overall foraging efficiency as shown in other insects (Punzo & Garman 1989; Papaj

& Vet 1990; Johnson 1991). Furthermore, we have shown that previous experience on other prey types could influence the initial preference of *C. maculata*. Therefore, it may be possible, through experience or conditioning on a target prey, to change the ranking preference of a generalist insect species and to consequently increase their performance as biological control agents (van Alphen & Vet 1986; Lewis *et al.* 1990).

ACKNOWLEDGMENTS

We sincerely thank Sophie Demougeot for technical assistance. This research was supported by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) to D. Coderre. Caroline Roger acknowledges support from the Fonds pour la formation de Chercheurs et l'aide à la Recherche (FCAR) from the Ministère de l'Éducation du Québec. This is contribution no XXXXX of the Horticultural Research and Development Centre of Agriculture and Agri-food Canada at St-Jean-sur-Richelieu.

Table 6.1. Chi^2 computed for each of the five statistics used to describe the sequences of encounter with parasitized and unparasitized eggs by *C. maculata* larvae. *: $P < 0.05$; NS: Non-significant.

Tests	df	SR**	NR	VR	CGP	CGU
Test 1	38	38.44 NS	117.38 *	22.30 NS	23.68 NS	23.81 NS
Test 2	28	20.52 NS	42.57 *	26.33 NS	30.02 NS	24.02 NS
Test 3	26	10.53 NS	53.26 *	16.25 NS	14.71 NS	15.24 NS
Test 4	38	35.58 NS	65.65 *	33.87 NS	25.70 NS	35.90 NS
Test 5	38	28.79 NS	110.05 *	19.19 NS	21.42 NS	22.98 NS

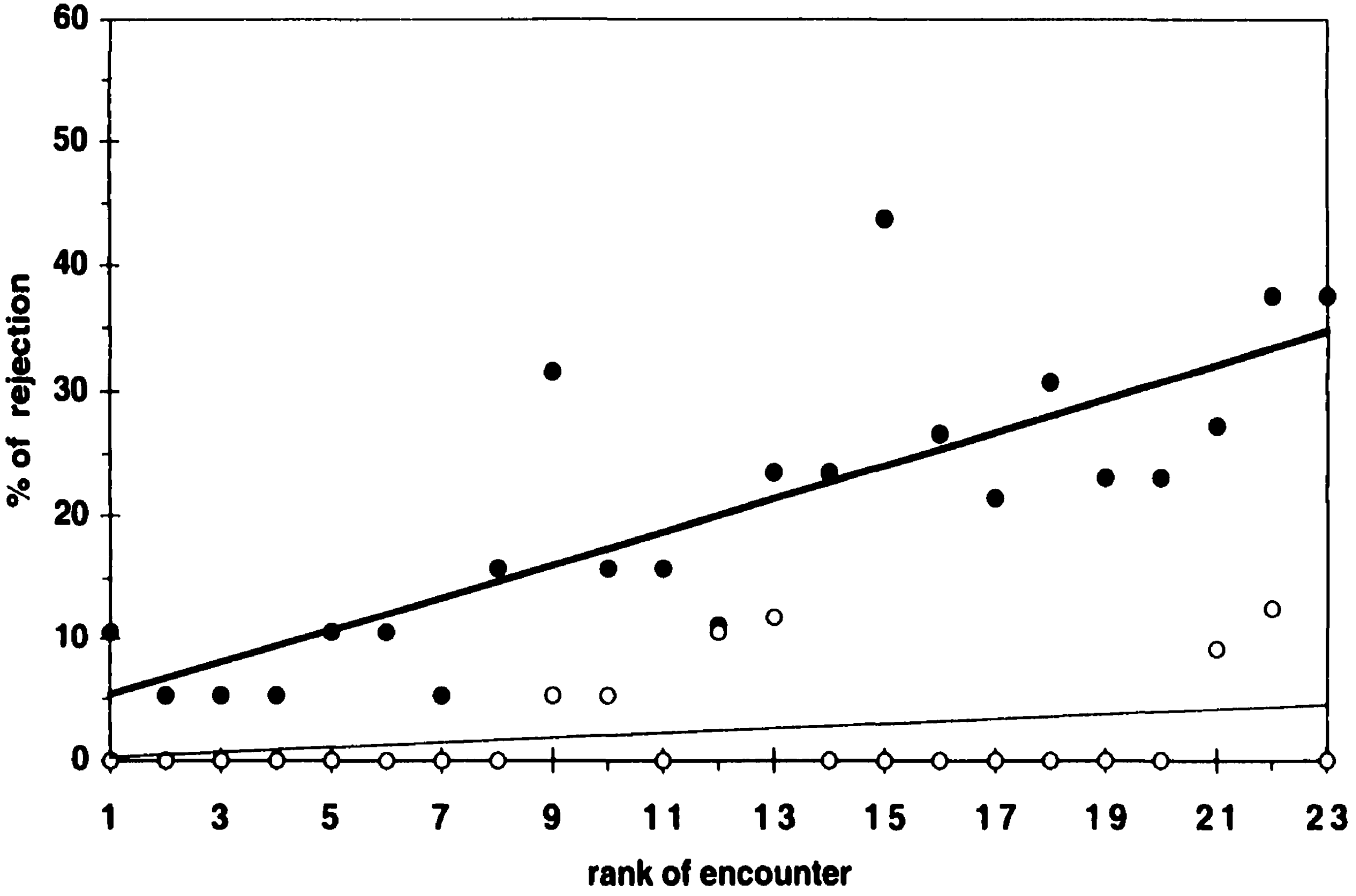
**For the signification of the test's abbreviations; see the methods section

Table 6.2. Statistics of regression analysis on transformed data that describe the changes in the proportion of egg rejection in relation to the order of encounter for each experience.

	Food intake	n	Intercept	Slope	R^2	Anova	P
Experience 1							
Unparasitized	Starved	19	0.015[1]	0.006	0.07	1.55	0.2267
Parasitized	Starved	19	0.231	0.018	0.63	36.49	0.0001
Experience 2							
Parasitized	Starved	19	0.322	0.036	0.69	36.41	0.0001
Parasitized	*M. euphorbiae*	14	0.510	0.032	0.45	12.89	0.0024
Parasitized	*A. rapae* eggs	13	0.306	0.023	0.67	32.76	0.0001
Experience 3							
Parasitized (T1)	Starved	19	0.231	0.018	0.63	36.49	0.0001
Parasitized (T2)	Starved	19	0.398	0.013	0.26	7.36	0.0130

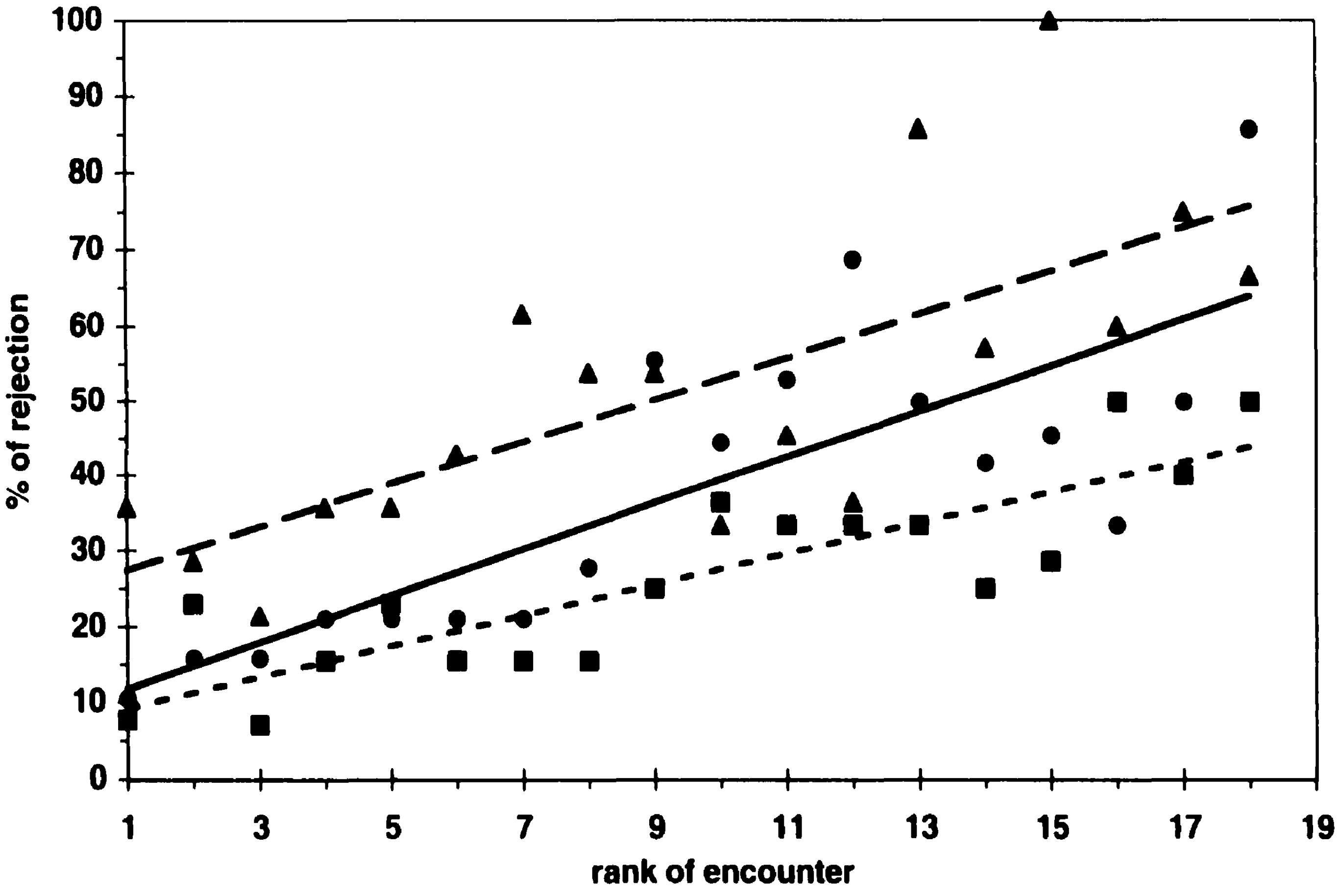
[1] All data on the proportion of rejection were submitted to sqrt arcsin transformation and were given a weight that corresponded to the number of larvae observed at each encounter.

Fig. 6.1. Experiment 1. Change in the proportion of rejection of unparasitized eggs (*thin line, open circle*) and eggs parasitized by *T. evanescens* (*thick line, dark circle*). 18 eggs of each type were offered to fourth instar *C. maculata* larvae.



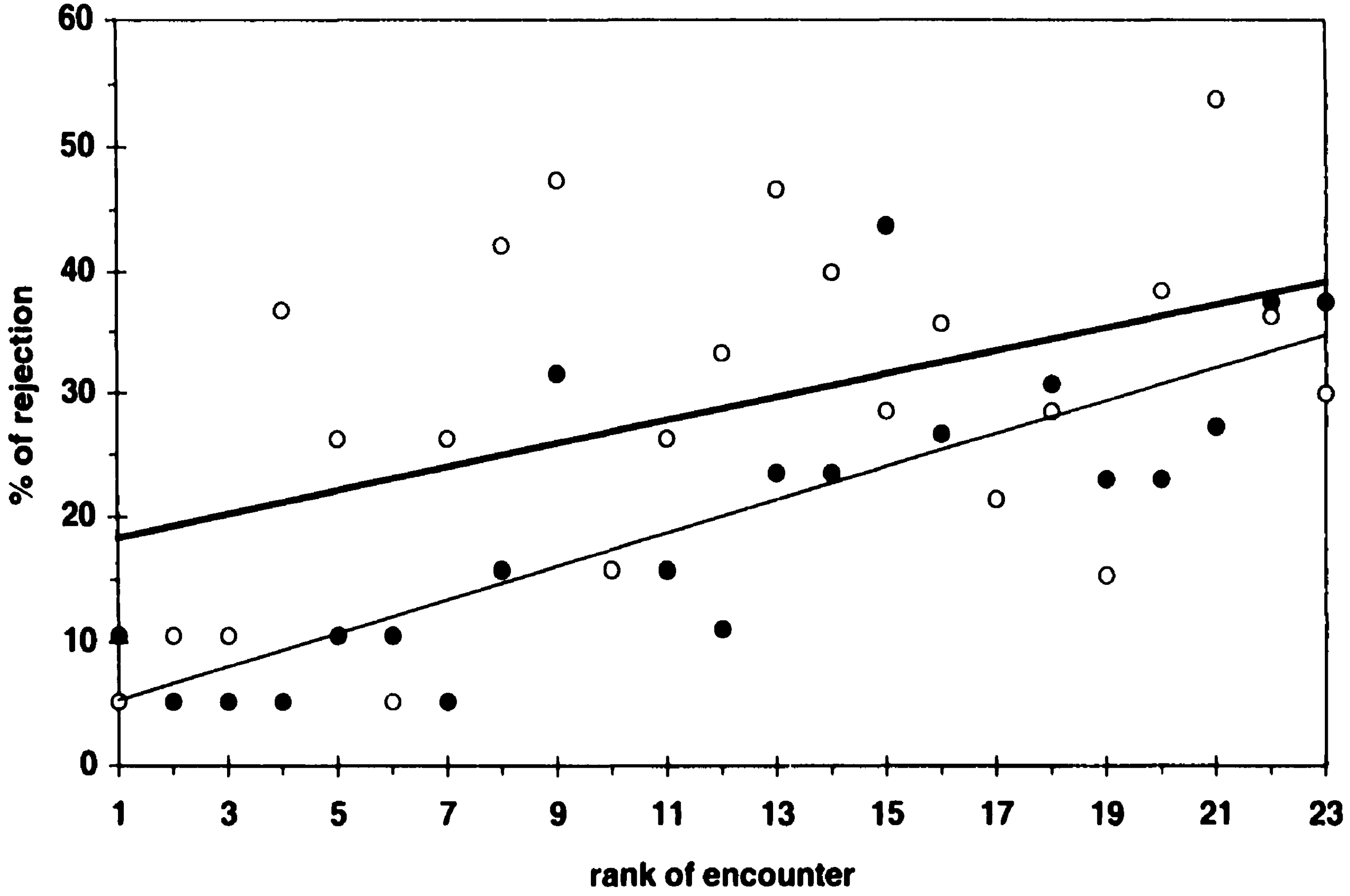
60
50
40
30
20
10
0
% of rejection
1
3
5
7
9
11
13
15
17
19
21
23
rank of encounter

Fig. 6.2. Experiment 2. Influence of starvation (continuous line, circle) and food intake (*M. euphorbiae*; long dashed line, triangle: *A. rapae*; short dashed line, square) on the proportion of rejection of parasitized eggs by fourth instar *C. maculata* larvae in the presence of 18 unparasitized eggs and 18 eggs parasitized by *T. evanescens*.



100
90
80
70
60
50
40
30
20
10
0
% of rejection
1
3
5
7
9
11
13
15
17
19
rank of encounter

Fig. 6.3. Experiment 3. Change in the proportion of rejection of parasitized eggs by fourth instar *C. maculata* larvae at time T1 (*thin line, dark circle*) and T2 (*thick line, open circle*) in the presence of 18 unparasitized eggs and 18 eggs parasitized by *T. evanescens*.


60
50
40
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10
0
% of rejection
1
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rank of encounter

CHAPTER 7

SUMMARY AND CONCLUSION

The subject of this research was to determine the mechanisms underlying prey selection in the generalist predator *Coleomegilla maculata lengi* Timb. (Coleoptera: Coccinellidae). The four main objectives were; (1) to determine the influence of prey species, prey size and predator age on prey exploitation by *C. maculata;* (2) to modify an image analysis system developed for parasitoids in order to evaluate behavioral parameters of prey selection in *C. maculata* larvae; (3) to evaluate the discriminatory capacity and the prey selection behavior of *C. maculata* larvae when given a choice between prey of different qualities; (4) to demonstrate that the behavior leading to prey selection involve a learning process.

The modifications brought to the image analysis system were accurate for the quantification of the specified parameters and well adapted for the observation of the second and fourth larval instars. The system was easy to use, efficient and greatly reduced the time needed for behavioral observations.

It was initially believed that highly generalist predators such as *C. maculata* had very low selectivity for their prey compared to more specialist species (Hodek 1973), even if prey individuals are not always of equivalent values (Smith 1965; Mills 1981; Obrycki & Orr 1990). However, optimal foraging models predict that predators, including generalist species, should select the most profitable prey types and reject unprofitable ones (Crawley & Krebs 1992).

This study revealed that, despite its wide polyphagy, the generalist predator *C. maculata* has the ability to discriminate and to select the most suitable and profitable prey. It was also clearly demonstrated that this predator could use flexible decision rules and adjust its foraging behavior according to patch quality. With experience, *C. maculata* could also improve its prey selection behavior within a patch. Furthermore, it was shown that the response of *C. maculata* to prey age, size and quality could be explained by optimal foraging strategies.

Hence, when coccinellid larvae were given a choice between eggs of different categories (unparasitized, parasitized, young or old), they did not exhibit any preference between parasitized and unparasitized eggs of similar development stage. However, when development stages differed, coccinellid larvae always preferred the younger eggs whether parasitized or not. The preferred egg types were always the ones with lower costs related with their intake. Costs were expressed through higher handling time and detrimental effects on immature developmental time and survival. Thereby, intrinsic physiological modifications within a prey can influence prey profitability and predatory coccinellid larvae could detect these modifications and select prey according to prey profitability.

Sessile prey such as lepidopterous eggs do not exhibit active defense or escape responses and are usually more or less similar in size. Therefore, factors affecting their profitability are mainly related with differences in energetic value or in differences provoked by physiological or morphological alterations, as it was clearly demonstrated earlier. However, coccinellid larvae may also attack mobile lepidopterous larvae of the three lepidopterous species occurring in cruciferous crops. Depending on their age, lepidopterous larvae may differ in size, mobility capacity and defense behaviors. These morphological and behavioral differences may affect prey profitability for coccinellid larvae and limit their prey consumption rate. This research revealed that, in a non choice situation, *C. maculata* had a higher consumption rate on intermediate-sized prey compared to smaller or larger prey even though they killed a higher number of small prey. It was suggested that this higher consumption rate on intermediate-sized prey was caused by higher costs associated with the exploitation of prey at both ends of the size spectrum. Exploiting large prey may request longer handling time because of the difficulty of capture attributed to their better defense or escape responses, whereas lower exploitation rates of small prey may be provoked by higher searching time and low energetic value per individual. This research have also shown that young and old coccinellid larvae have similar discrimination abilities in a choice situation but that young larvae are generally less efficient in exploiting prey of any sizes. Therefore, in biological control programs against lepidopterous pests populations, it should be preferable to favour the release of coccinellid late instars which are more voracious.

Furthermore, especially in inundative control programs, the simultaneous use of both *Trichogramma* and *C. maculata* may increase the probability of unidirectional intraguild predation on parasitized prey by coccinellids and may therefore reduce the additive effect normally expected in such programs. However, the presence of alternative prey types for coccinellids, such as lepidopterous larvae or aphids, may possibly reduce interference with the action of the parasitoid populations and their development. Therefore, complementary control should be expected in situations where both natural enemies possess different nutritional and spatial niches. This is more likely to occur in more heterogenous environments containing prey populations with different age structures.

The compatibility of the predator *C. maculata* and the parasitoid *Trichogramma* may also depend on the timing of utilization. Since *C. maculata* fed less frequently on *T. ni* eggs in the late stages of parasitism, this predator should be released a few days after *Trichogramma* (1 or 2 weeks) in order to allow the development of parasitoid immatures to the adult stage where predation is unlikely to happen. It would also be preferable to delay parasitoid releases depending on the seasonal peak of natural coccinellid populations.

This study also revealed that coccinellid larvae could improve their discrimination abilities with experience and learn to reject less suitable prey. We have shown that this learned response was gradual and relatively slow compared to many parasitoid species and that it did not reach an asymptote in one feeding sequence. Therefore, these results suggest that coccinellid larvae possibly need several periods of exposure to adequately discriminate and reach their optimal potential.

Learning abilities were demonstrated in *C. maculata* using lepidopterous eggs which are sessile and that do not exhibit active defense responses. As it was shown, the individuals of this species may also attack the larvae of the three lepidopterous species and could be confronted to different species or lepidopterous individuals that may differ in size. In a choice situation involving different prey types that differ in profitability, learning to discriminate should also be expected. Similarly, improvement of predation efficacy with experience is also expected in naive or young coccinellid individuals facing aggressive and bigger lepidopterous prey.

These learned abilities may be useful for coccinellid larvae foraging in many habitats containing patches with prey of different quality. Optimal foraging theory assumes that a predator has the ability to estimate patch profitability before entering a patch, but this assumption is unrealistic for coccinellid larvae that present poor visual acuity. However, discrimination and learning abilities may allow a foraging larva to gather information on variations in resource quality and availability in a patch and to adjust its behavior accordingly. Therefore, a coccinellid larva may use the gathered information to decide if it shoud stay and exploit more extensively a patch or decide to leave immediately. Thereby, discrimination and learning may be involved, at least partially, in the flexible patch time allocation process observed in coccinellid larvae.

The results obtained in the learning experiments also indicate that previous experience on other prey types could influence the initial preference of coccinellid larvae and strongly suggest that learning could improve their foraging efficiency. Therefore, in a biological control context, it could be possible to enhance their predation efficiency by giving them appropriate experience or by rearing them on target prey.

However, we have demonstrated that learned behaviors are at least partially forgotten. For an individual, the advantage of forgetting rapidly in a fluctuating environment is obvious. However, in biological control programs, a generalist predator such as *C. maculata* can exploit other food types when the target prey becomes scarce and consequently partially or completely forget the training previously received. It would be thus interesting: (1) to evaluate the time needed for coccinellid larvae to completely forget in the absence of continued experience with the target prey and, (2) to determine if a learned behavior is retained in subsequent larval or adult stages to maintain an adequate predation efficacy for a longer period of time.

This research unraveled a small part of the complexity of coccinellid behavior and how they behave under their own immediate limitations and constraints imposed by their environment. It was shown that, when confronted to a diversity of prey types that differ in quality, *C. maculata* can exploit the resource according to their relative costs and benefits using discrimination abilities. Learning abilities may serve to improve this insect discrimination capacity. Hence, a vast array of fixed and plastic behaviors may be used by coccinellids searching for food resources and these behaviors may change depending on the encountered prey and the environmental constraints. Because of all these different behavioral variables, the outcome of coccinellids predation activities is often very difficult to predict and may at least partially explain the lack of consistency in the results obtained in biological control programs using coccinellid species.

All experiments included in this study were performed in simplified experimental systems with high prey aggregation. This clumped distribution may have increased predation efficiency and prey selectivity compared to what could be expected in nature. In a more complex environment, prey patches are more dispersed and frequently change or deplete rapidly. Therefore, the costs associated to travel between prey patches are often higher. In this context, it would be less advantageous for *C. maculata* to be highly selective and avoid parasitized or aged prey. Moreover, because coccinellid larvae respond to variable patch quality by using flexible decision rules, they could decide to stay in the patch for a longer period of time and exploit more intensively parasitized eggs. Therefore, the evaluation of prey selectivity in a more complex environment and in natural conditions is the next logical step.

Hence, complementary field work will be essential to understand which selective forces are the main determinants in prey selection and to determine whether *C. maculata* is foraging optimally or not on lepidoperous populations with different species and age structures.

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