Host selection behavior of the adult parasitoid *Microctonus hyperodae* Loan (Hymenoptera : Braconidae; Euphorinae) and the egg parasitoid *Anaphes victus* Huber (Hymenoptera : Mymaridae), parasitoids of the carrot weevil, *Listronotus oregonensis* LeConte (Coleoptera : Curculionidae)

by

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Host selection behavior by insect parasitoids

Abstract

The carrot weevil, *Listronotus oregonensis* LeConte (Coleoptera: Curculionidae), is an important pest of carrot in northeastern North America. The objective of this study was to determine the host selection stimuli used by two *L. oregonensis* parasitoids: the adult parasitoid *Microctonus hyperodae* Loan (Hymenoptera: Braconidae; Euphorinae) and the egg parasitoid *Anaphes victus* Huber (Hymenoptera: Mymaridae). *Microctonus hyperodae* is a parasitoid native to South America and has been recently associated with *L. oregonensis* in laboratory in Québec while *A. victus* is an indigenous egg parasitoid of *L. oregonensis* in northeastern North America.

In the first chapter, the response of *M. hyperodae* females to various odor components from potential hosts and host plants was tested with a Y-olfactometer. *Microctonus hyperodae* females responded only to odor components of *L. oregonensis* adults suggesting that laboratory selection and/or previous experience with *L. oregonensis* odor components have enhanced the response of *M. hyperodae* females to this odor component.

In the second chapter, the effect of *L. oregonensis* adult sex, feces and movement on host selection behavior of *M. hyperodae* females was evaluated by quantifying *L. oregonensis* adult movement and various *M. hyperodae* female behaviors in an arena. *Listronotus oregonensis* adult sex had no significant effect on host selection behavior by *M. hyperodae* females. However, *L. oregonensis* adult feces decreased the number of weevil antennations done by *M. hyperodae* females suggesting that host feces play a role in host recognition by *M. hyperodae* females. Moreover, weevil antennations decreased as the intensity of *L. oregonensis* adult movement increased suggesting that host movement is also involved in host recognition by *M. hyperodae* females. Since *M. hyperodae* females also stopped more frequently in front of a moving *L. oregonensis* adult than in front of an immobile one, and that most oviposition attempts were directed to a moving *L. oregonensis* adult, it appears that host movement attracts *M. hyperodae* females and it is the major oviposition stimulant for this parasitoid. Most of the oviposition attempts were directed to the mouth or the anus of *L. oregonensis* adults, but there were more oviposition attempts directed to the mouth when the anterior part of *L. oregonensis* body

was moving suggesting that *M. hyperodae* females aimed the moving part of their host body when trying to oviposit.

In the third chapter, the response of *A. victus* females to different *L. oregonensis* related odor components was evaluated using a filter paper of which half was moistened with odor components and the other half with water. *Anaphes victus* females responded to *L. oregonensis* adult feces and egg plugs odor components suggesting that *A. victus* females use these two odor components as host location cues.

The adults of Curculionidae are the most susceptible stage to parasitism because eggs, larvae and pupae are hidden in plant tissue and soil. It appears from this study that both the adult parasitoid *M. hyperodae* and the egg parasitoid *A. victus* use the stimuli produced by their adult host during host selection. Moreover, as the reliability of the adult stimuli differs according to the host stage attacked by the parasitoid, it appears that each parasitoid has evolved to use different facets of the host adult biology during host selection. Thus, stimuli highly related to the adult itself were attractive to *M. hyperodae* and those highly related to the eggs were attractive to *A. victus*

Résumé

Le charançon de la carotte, *Listronotus oregonensis* LeConte (Coleoptera : Curculionidae), est un important ravageur des cultures de carottes dans le Nord-Est de l'Amérique du Nord. Le but de cette étude était de déterminer quels sont les stimuli utilisés par le parasitoïde du stade adulte *Microctonus hyperodae* Loan (Hymenoptera : Braconidae; Euphorinae) et le parasitoïde oophage *Anaphes victus* Huber (Hymenoptera : Mymaridae) lors de la sélection de leur hôte. *Microctonus hyperodae* est un parasitoïde originaire d'Amérique du Sud nouvellement associé avec *L. oregonensis* en laboratoire au Québec, alors que *A. victus* est un parasitoïde indigène des œufs de *L. oregonensis* dans le Nord-Est de l'Amérique du Nord.

Dans le premier chapitre, la réponse des femelles de *M. hyperodae* à différentes odeurs d'hôtes et de plantes hôtes a été évaluée à l'aide d'un olfactomètre en Y. Les femelles de *M. hyperodae* ont uniquement répondu à l'odeur des adultes de *L. oregonensis*, ce qui semble attribuable à une sélection en laboratoire ou à une expérience précédente avec des odeurs reliées à *L. oregonensis.*

Dans le deuxième chapitre, les effets du sexe, des fèces et du mouvement des adultes de *L. oregonensis* sur le comportement de *M. hyperodae* lors de la sélection de son hôte ont été mesurés en quantifiant le mouvement de *L. oregonensis* et plusieurs comportements de *M. hyperodae*. Le sexe de *L. oregonensis* n'a pas eu d'impact significatif sur le comportement de *M. hyperodae*. Par contre, la présence de fèces de *L. oregonensis* réduisait le nombre d'antennations effectuées par *M. hyperodae* sur *L. oregonensis*. Ce résultat indique que la détection de fèces par *M. hyperodae* favorise la reconnaissance de leur hôte. De plus, l'augmentation de l'intensité du mouvement de *L. oregonensis* entraînait aussi une diminution du nombre d'antennations sur *L. oregonensis.* Ce résultat suggère que la détection du mouvement de l'hôte par *M. hyperodae* favorise également la reconnaissance de son hôte. Par ailleurs, *M. hyperodae* s'arrêtait plus fréquemment devant un *L. oregonensis* en mouvement que devant un *L. oregonensis* immobile, et la quasi-totalité des tentatives d'oviposition de *M. hyperodae* avait lieu sur un *L. oregonensis* en mouvement. Ainsi, le mouvement de l'hôte semble attirer *M. hyperodae* et déclencher leur ponte. La majorité des tentatives d'ovipositions de *M. hyperodae* était dirigée vers la bouche ou l'anus de *L. oregonensis*. De plus, la plupart des tentatives d'ovipositions de *M. hyperodae* étaient dirigées vers la bouche lorsque *L. oregonensis* bougeait uniquement les parties antérieures de son corps. Ceci suggère que lorsqu'elles tentent de pondre, les femelles de *M. hyperodae* visent une partie du corps en mouvement.

Le dernier chapitre consistait à mesurer la réponse des femelles de *A. victus* à différentes odeurs reliées à *L. oregonensis*. Pour se faire, un papier filtre dont une moitié était imbibée d'une odeur et l'autre d'eau a été utilisé. Les femelles de *A. victus* ont répondu à l'odeur de fèces de l'adulte de *L. oregonensis* et à l'odeur des bouchons qui recouvrent les œufs de *L. oregonensis*. Ceci semble indiquer que *A. victus* se sert de ces odeurs pour trouver les œufs de *L. oregonensis*.

Chez les Curculionidae, le stade adulte est le plus susceptible d'être parasité puisque l'œuf, la larve et la pupe sont cachés dans le tissu végétal de la plante hôte ou le sol. Il apparaît en effet que les deux parasitoïdes, le parasitoïde de l'adulte *M. hyperodae* et le parasitoïde oophage *A. victus*, utilisent les stimuli émis par l'adulte de l'hôte lors de la sélection de leur hôte. Selon le stade attaqué par le parasitoïde, les stimuli de l'adulte de l'hôte ne fournissent pas la même information quant à la présence et la localisation de leur hôte. Par conséquent, les deux parasitoïdes ont évolué en utilisant différentes facettes de la biologie de l'adulte de l'hôte. Ainsi, les stimuli reliés spécifiquement à l'adulte sont utilisés par *M. hyperodae* alors que ceux reliés aux œufs sont utilisés par *A. victus*.

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Table of contents

List of Tables

List of Figures

I. INTRODUCTION

Natural selection tends to increase the fitness of an organism by keeping behaviors that increase the chances of survival of an organism and eliminating those that are harmful (Becquemont 1992). Because of the direct link between the Darwinian fitness of parasitoids and their hosts, host selection behavior of parasitoids is expected to be under strong natural selection pressure (Hubbard & Cook 1978). The optimal foraging theory assumes that hosts are distributed in patches of different quality in the environment, and that hosts within a patch have different quality values. Therefore, female parasitoids are expected to use host related stimuli during host selection process in order to find the most profitable patches and the most profitable hosts within a patch (Waage & Hassell 1984).

As parasitoids are the most important biotic factor of insect mortality (Hawkins *et al.* 1997; Boivin 1999a), studies about parasitoid host selection have also a great interest for biological control purposes. *Listronotus oregonensis* LeConte is an important pest of carrot crops in northeast North America (Boivin 1999b). The major biotic mortality factors of this species are egg parasitoids (*Anaphes listronoti* Huber and *Anaphes victus* Huber), and parasitism by *Anaphes* spp may sometimes exceed 70% in the province of Québec (Boivin 1986, 1992). However, there is a delay in early summer before egg mortality reaches a level at which damage caused by *L. oregonensis* larvae is kept below an economic level, and consequently, insecticide treatments are still used in carrot fields (Boivin 1999b).

In order to increase the biological control of *L. oregonensis*, a new host-parasitoid association between *L. oregonensis* and *Microctonus hyperodae* Loan was created in the laboratory in Canada in 1996 (Boivin 1999b), and this parasitoid has been recently released in fields (Boivin unpublished). *Microctonus hyperodae* is a parasitoid native to South America that attacks the adult stage of Brachycerinae Curculionidae (Goldson *et al.* 1992; Barratt *et al.* 1997). New host-parasitoid associations may give better results than old associations in biological control programs because the two species have not coevolved, and therefore are not in ecological homeostasis (Pimentel 1963; Hokkanen & Pimentel 1984, 1989). However, interactions between hosts and parasitoids in such associations are poorly documented.

A promising avenue to increase the biological control efficiency of *M. hyperodae* and *Anaphes* spp. on *L. oregonensis* populations is to document the host selection stimuli used by these parasitoids and manipulate them in carrot fields.

In this biological control context, the following objective was defined: determine the host selection stimuli used by *M. hyperodae* and *A. victus*.

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« Candidates have the option of including, as part of the thesis, the text of a paper(s) submitted or to be submitted for publication, or the clearly-duplicated text of a published paper(s). 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 the objectives of the study, a comprehensive review of the literature, a final conclusion and a summary, and 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 the 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 defence. 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. Under no circumstances can a co-author of any component of such a thesis serve as an examiner for that thesis. »

All chapters are co-authored papers submitted. I have done all experiments, and written all papers. The co-author, Dr. Guy Boivin, has revised and corrected the papers before their submission. The third chapter "Infochemicals used by the parasitoid *Microctonus hyperodae* Loan (Hymenoptera: Braconidae, Euphorinae) when searching for its adult weevil hosts" has been submitted to Entomologia Experimentalis et Applicata. The fourth chapter "Short distance cues used by the adult parasitoid *Microctonus hyperodae* Loan (Hymenoptera: Braconidae, Euphorinae) for host selection" has been submitted to Journal of Insect Behavior and the fifth chapter "Kairomones used by the egg parasitoid *Anaphes victus* Huber (Hymenoptera: Mymaridae) when searching for its hosts" has been submitted to The Canadian Entomologist.

II. LITERATURE REVIEW

1. Terminology

As in most specialized fields, the study of insect parasitoids has a number of specific concepts and a specialized terminology. In this first section, the terms and concepts discussed in this thesis are defined. First, a parasitoid is: « an organism which develops on or in another single (« host ») organism, extracts nourishment from it, and kills it as a direct or indirect result of that development » (Eggleton & Gaston 1990). According to this definition, parasitoids are an impressive group of diversified organisms that include insects, nematodes, protists, fungi, crustaceans, turbellarians, bacteria, viruses and some green plants (Eggleton & Gaston 1990). However, only the more « classical » parasitoids, the insect parasitoids and especially the hymenopterous parasitoids, are treated in this thesis.

An important aspect that contributes to parasitoid diversity is that all stages of development of hosts can be attacked by parasitoids. Depending of the stage attacked, the parasitoid is named egg, larval, pupal or adult parasitoid (Doutt 1959; Godfray 1994). Some parasitoids lay their eggs in one stage, but the adult emerges and kills the host at a subsequent stage, such as in egg-larval or larval-pupal parasitoids (Doutt 1959; Godfray 1994). The larval development of parasitoids may occur on or in a host; we name ectoparasitoids those that develop on the host and endoparasitoids those that develop in the host (Doutt 1959; Godfray 1994). In solitary parasitoids, only one parasitoid emerges from a host while in gregarious parasitoids two to several thousand parasitoids may emerge from a host (Doutt 1959; Godfray 1994). In some species, one or several parasitoids may emerge from the host and they are termed facultative gregarious parasitoids (Godfray 1994). When the host remains alive and mobile during the parasitoid larval development, the parasitoid is considered as a koinobiont parasitoid and when the larval development occurs in a dead or paralyzed host, it is considered as an idiobiont parasitoid (Godfray 1994).

2. Host selection

Successful parasitism may be divided into five successive parts: host habitat location, host location, host acceptance, host suitability and host regulation (Doutt 1959; Vinson 1976). Host habitat location, host location and host acceptance are defined as the host selection process of parasitoids (Doutt 1959; Vinson 1976), and are generally achieved by the adult female parasitoid, although in some species the female parasitoid oviposits or larviposits in the soil, and therefore part of the host selection process is achieved by the larva (Allen *et al.* 1999). Host suitability and host regulation involves the larval development process in the host (Vinson 1976). In this paper, only the host selection process is treated.

2.1 Optimal foraging theory

Since hosts are essential for larval development of parasitoids, host selection is expected to be under strong natural selection pressure (Hubbard & Cook 1978). The optimal foraging theory assumes that hosts are distributed in patches of different quality throughout the environment, and that hosts within a patch have different quality (Waage & Hassell 1984). Female parasitoids are expected neither to search the environment randomly for hosts or parasitize any hosts encountered because it is expensive in foraging time to explore areas without potential hosts, and expensive to parasitize hosts of low quality when hosts of high quality are available. Instead, female parasitoids should use foraging strategies that provide the maximum net rate of energy gain, endowing the parasitoid with the greatest fitness (Pyke *et al.* 1977). Thus, female parasitoids are expected to use environmental stimuli that permit them to find host patches that contain the most valuable hosts and to select the most valuable hosts within the patch (Waage & Hassell 1984). The female parasitoid is also expected to stay in the patch until the profitability of the patch falls to a level equal to the average of all patches in the habitat, afterward the female parasitoid should leave the patch and search for a more profitable patch (Pyke *et al.* 1977; Charnov & Skinner 1985).

2.2 Host selection stimuli used by parasitoids

2.2.1 Infochemicals

Infochemicals are the most important stimuli used by parasitoids for host selection (Doutt 1959; Vinson 1976; Godfray 1994). Infochemical is a general term that refers to « a chemical that, in the natural context, conveys information in an interaction between two individuals, evoking in the receiver a behavioral or physiological response » (Vet & Dicke 1992). Infochemicals can be divided into two broad categories: pheromones and allelochemicals (Dicke & Sabelis 1988; Vet & Dicke 1992). Pheromones refer to infochemicals that are emitted and received by individuals of the same species, and are at least favorable for the biology of one of the individuals (Dicke & Sabelis 1988; Vet & Dicke 1992). On the other hand, allelochemicals are infochemicals interacting between individuals of two different species, and evoke in the receiving individuals a behavioral change (Dicke & Sabelis 1988; Vet & Dicke 1992).

According to the the relative benefits of the emitter and receiver, an allelochemical may be considered as a kairomone, an allomone, an antimone or a synomone (Dicke & Sabelis 1988). Kairomones are chemical compounds that evoke adaptively favorable behavioral changes in the receiver but is harmful to the emitter (Nordlund 1981; Dicke & Sabelis 1988). For example, chemical compounds released by the host and its by-products, and used as cues by their parasitoids for host selection, are kairomones (Vet & Dicke 1992). Among the kairomones used by parasitoids are host feces, cuticle, exuviae, secretions of mandibular and accessory glands, host pheromones, honeydew, body scales and hemolymph (Vet & Dicke 1992). Inversely, a chemical compound is considered as an allomone when it is favorable to the emitter and harmful to the receiver, such as herbivore repulsive chemicals emitted by plants (Nordlund 1981; Dicke & Sabelis 1988). However, these chemical compounds may also be repulsive for natural enemies of the herbivore, and the negative effect on the natural enemies may be superior to the positive effect on the herbivore. These chemical compounds are considered as an antimone because both the emitter and the receiver are affected negatively by the emission of the chemical compounds (Dicke & Sabelis 1988). Finally, when an allelochemical favors both the emitter and the receiver, it is considered as a synomone (Nordlund 1981; Dicke & Sabelis 1988; Vet & Dicke 1992). For example, when attacked by herbivores, several plants produce chemical compounds that are used by parasitoids to find their hosts (herbivores), and in this situation the chemical compounds are considered as synomones because they favor both the plants (emitters) and the parasitoids (receivers).

Infochemicals can be volatile and used by female parasitoids as long-distance host selection cues (Vinson 1981; Weseloh 1981; Godfray 1994), or can be non or less volatile and used as short-distance host selection cues (Arthur 1981; Weseloh 1981; Godfray 1994). Depending on the type of infochemicals, they can be detected by chemoreceptors located on the antennae (van Lenteren 1981; Loke & Ashley 1984; Meyhöfer *et al.* 1997), the ovipositor (van Lenteren 1981) and the tarsi (Vinson 1976; Meyhöfer *et al.* 1997). Volatile infochemicals are detected in the air with the antennae (Vinson 1976; Weseloh 1981) while non volatile infochemicals, also named contact kairomones, are generally detected by antennal contacts with the infochemicals (Vinson 1976), but also by ovipositor or tarsal contacts (van Lenteren 1981).

When compared to visual stimuli, infochemicals generally give more information on host identity and suitability (Dippel & Hilker 1998). However, the detection of infochemicals is influenced by several factors such as the rate of emission of odor molecules, the release area, the distance between insect and odor source, wind speed, turbulence, and contrast against background odors (Turlings *et al.* 1993), and therefore, infochemicals are generally less precise then visual cues in indicating host location (Bell 1990).

2.2.2 Visual stimuli

Visual stimuli such as color (Arthur 1966; Hollingsworth *et al.* 1970; Schmidt *et al.* 1993), host movement (Monteith 1956; Richerson & DeLoach 1972; Dippel & Hilker 1998) and shape (Turlings *et al.* 1993) are frequently involved in host selection behavior of several parasitoid species. Visual detection is achieved by the compound eyes of the parasitoid, and one advantage of visual cues compared to infochemicals is that visual signals supply more reliable information on the direction of and the distance to the source than infochemicals (Turlings *et al.* 1993). However, physical barriers, such as dense vegetation, may obstruct the parasitoid vision and limit the use of visual cues to only a short distance from a host (Turlings *et al.* 1993).

2.2.3 Other stimuli

In addition to infochemicals and visual cues, some parasitoids use physical stimuli such as wind, light, humidity and temperature for host selection. However these stimuli are generally used when no stimulus from the host plant and the host are detected (Vinson 1976).

Several parasitoids of singing hosts such as cricket and cicada parasitoids also use the sound produced by their host to attract a sexual partner as a host selection cue (Cade 1984; Soper *et al.* 1976 respectively). The advantage of sound cues is in their high detectability, and therefore, they may serve as long-distance searching cues.

In contrast, tactile cues such as host texture and shape have low detectability and usually serve in the last steps of host selection once the host has been contacted (Vinson 1998). Tactile cues are detected by antennal drumming on the host, and are frequently used by parasitoids of sessile hosts such as egg and pupal parasitoids (Vinson 1998).

Vibrations produced by hidden hosts such as leaf-miner larvae (Meyhöfer *et al.* 1997) or dipteran larvae living in galls or in decaying fruits (Vet & Bakker 1985; Sokolowski & Turlings 1987), may also be detected by their parasitoids, and used as host selection cues. In the case of *Sympiesis sericeicornis* Nees (Eulophidae), the vibrations of its host, the apple leafminer *Phyllonorycter malella* Gerasimov, are detected by tarsal mechanoreceptors (Meyhöfer *et al.* 1997).

2.3 Factors influencing the responses of parasitoids to host related stimuli

2.3.1 The reliability-detectability problem

Parasitoids have evolved in a tritrophic context in which the first level is represented by host plants, the second by hosts and the third by parasitoids (Vet & Dicke 1992). Both host plants and hosts are important sources of stimuli that orient parasitoids in their host selection process (Vet & Dicke 1992). How parasitoids use these stimuli for host selection depends on the reliability and the detectability of the stimuli (Doutt 1959; Vet & Dicke 1992; Godfray 1994). The reliability of a stimulus indicates the host presence, its accessibility and its suitability while its detectability indicates the degree to which it can be perceived (Vet & Dicke 1992). Stimuli from the host level are generally the most reliable stimuli because the host itself or the products of its activities emit them.

However, they are less detectable because hosts are only small components of a complex environment, and inconspicuous stimuli have been selected through natural selection to avoid parasitoid detection (Vet & Dicke 1992; Vinson 1998). On the other hand, stimuli from the host plant are generally less reliable because they may bring parasitoids to areas without any hosts. However, they are more detectable because plants represent a larger biomass than hosts (Vet & Dicke 1992).

To solve this reliability-detectability problem, parasitoids may use a hierarchic search in which more detectable stimuli, such as physical stimuli (wind, light, humidity, temperature) and plant synomones, are used for host habitat location, and more reliable stimuli, such as host kairomones, are used for host location in the habitat (Vinson 1976; Vet & Dicke 1992). The advantage of such a hierarchic search is to always reduce the area to be covered before finding a host (Vinson 1976; Vet & Dicke 1992). However, in some species the hierarchic search can be by-passed by using stimuli that are both highly detectable and highly reliable, such as herbivore-induced synomones and host pheromones (Vet & Dicke 1992).

2.3.2 Host habitat location

Although female parasitoids may be already in the host habitat and ready to search for a host when emerging, sometimes they may be far from potential hosts upon emergence (Vinson 1976, 1981). Three reasons may explain the need for host habitat location by female parasitoids. The first is that hosts may have completed their development and migrated through another habitat, and therefore the suitable host stage is no longer available when the female parasitoid emerges (Vinson 1981). The second is that, even in presence of hosts, a female parasitoid may have a tendency to disperse and find another habitat for food, mate and shelter, or because hosts are already parasitized (Vinson 1981). The third is that adverse conditions, such as high winds, may bring the female parasitoid or the parasitized host outside its usual habitat (Vinson 1981). In these situations, it is more adaptive for a female parasitoid to have efficient host habitat location behaviors enabling it to find habitats containing high quality hosts than to stay in a habitat containing no or few high quality hosts (Vinson 1981).

When searching for host habitat, female parasitoids may first use physical stimuli such as wind, light, humidity and temperature, or visual stimuli such as color and shape

(Vinson 1976, 1981). However, these stimuli are generally used when no plant synomone is detected (Vinson 1976, 1981). Thus, green color (560 nm) elicited a locomotory response by the larval parasitoid *Campoletis sonorensis* Cameron (Ichneumonidae), and the parasitoid is more active when illuminated by that color suggesting that green color could be an important cue for host habitat location of this species (Hollingsworth *et al.* 1970). However, *C. sonorensis* females also use host plant synomones when orienting through host habitat, and in their presence, visual stimuli play a lesser role (McAuslane *et al.* 1991).

Host plant synomones are the most important cues used by parasitoids when searching for host habitat (Vinson 1976, 1981; Vet & Dicke 1992). Although uninfested host plants are known to be attractive to some parasitoid species (Drost *et al.* 1988), this type of synomone gives little information on host presence and identity (Vet & Dicke 1992; Steinberg *et al.* 1993). An efficient way to solve this reliability-detectability problem is to respond to synomones released by plants when damaged (Vet & Dicke 1992). These synomones are more reliable than undamaged plant synomones because the damage is generally achieved by the host (Vet & Dicke 1992) and highly detectable because they are emitted by the whole plant and not only by the damaged parts (Dicke *et al.* 1990). Accordingly, several parasitoid species are more attracted by herbivore-damaged host plants than by undamaged host plants (Monteith 1964; McAuslane *et al.* 1990, 1991; Turlings *et al.* 1991a; Steinberg *et al.* 1993).

However, synomones released by damaged plants are sometimes not highly reliable, though more than undamaged plant synomones, because the emission of the volatile chemicals result from mechanical disruption of plant cells, and therefore are non-specific for herbivores (Vet & Dicke 1992). *Campoletis sonorensis* is a generalist parasitoid of noctuid larvae and the chance that any type of wound on a cotton plant was caused by a potential host is higher than for a specialist parasitoid (McAuslane *et al.* 1991). Accordingly, *C. sonorensis* females are equally attracted by any type of damage on cotton plants, mechanical or natural (McAuslane *et al.* 1991). Nevertheless, the *Heliothis* larvae specialist *M. croceipes* is attracted by both host damaged leaves and artificial damaged leaves of cowpea (Eller *et al.* 1988; Turlings *et al.* 1993). Therefore, the risks of exploring plants containing only unsuitable hosts for this species is higher than for *C. sonorensis.*

Plant synomones may be more reliable by being herbivore specific (Vet & Dicke 1992). This kind of synomone, termed herbivore-induced synomones, is released by some plants only when damaged by specific herbivores, and therefore acts as an indicator of herbivore identity for parasitoids (Vet & Dicke 1992). Thus, when damaged by oral secretions of *Spodoptera* larvae, corn seedlings release a large amount of terpenoids, otherwise not produced, that attract the *Spodoptera* larval parasitoid *Cotesia marginiventris* Cresson (Turlings *et al.* 1990, 1991b). Similarly, the egg parasitoid *Oomyzus gallerucae* Fronscolombe is attracted by elm leaves damaged by egg depositions of its host, *Xanthogaleruca luteola* Muller, but not by elm leaves carrying non host species eggs (Meiners *et al.* 2000).

Infochemicals from decaying materials may also be a major source of infochemicals for parasitoids of saprophagous insects such as *Alysia manducator* Panzer and *Nosonia vitripennis* Walker. These parasitoids are attracted by decomposing meat in which their larval hosts feed (Doutt 1959; Vinson 1981). Similarly, *Biosteres longicaudatus* Ashmead is attracted by the fungus *Monolinia fructicola* Winter that is responsible for the decaying of fruits in which its Tephritidae larval hosts feed (Vinson 1976, 1981).

Although not frequently observed, stimuli from the host level may serve as host habitat location cues in some parasitoid species. Generally, these cues are sexual signals emitted by the host when attracting a sexual partner. Thus, *Trichopoda pennipes* Fay (Tachinidae) is attracted from a long distance by male pheromones of its host *Nezara viridula* L. (Mitchell & Mau 1971) while several Ormininae (Tachinidae) parasitoids locate their cricket hosts by using the sound produced by male crickets when attracting females (Cade 1984; Robert *et al.* 1992). Similarly, the parasitoid *Colcondamyia auditrix* Shewell (Sacrophagidae) locates its cicada hosts by using the sound produced by male cicadas when attracting females (Soper *et al.* 1976). In these cases, host pheromones and host sounds are highly detectable and reliable, and therefore may serve as host habitat and host location cues.

2.3.3 Host location

Once in the host habitat, a female parasitoid must locate a potential host. This part of the host selection process is named host location (Vinson 1976). Generally, host location starts when the parasitoid is walking on the host plant, and ends with the encounter of the host (Weseloh 1981). Stimuli used for host location are generally less detectable but more reliable than those used for host habitat location. Most of the time, these stimuli are low volatile kairomones or contact kairomones emitted by the host itself or by the products of its activities (Weseloh 1981 Vet & Dicke 1992). Usually, when a parasitoid encounters contact kairomones, they provoke a change in the locomotory behavior (ortho-klinokinesis) of the parasitoid, keeping it on the infested host plant and increasing its probabilities of discovering hosts (Hendry *et al.* 1973; Bragg 1974; Weseloh 1981; Loke & Ashley 1984; van Alphen & Vet 1986; Vinson 1998; Meiners *et al.* 2000).

However, host location strategies may differ according to the host stage attacked by the parasitoid. In contrast with egg and pupal hosts, larval and adult hosts are active and produce kairomones. Accordingly, many larval and adult parasitoids use kairomones emitted by the host itself or by the products of its activities as host location cues. These kairomones may come from the host integument (Schmidt 1974), honeydew (Shaltiel & Ayal 1998), feces (Hendry *et al.* 1973; Bragg 1974; Weseloh 1981; Eller *et al.* 1988; Lewis & Tumlinson 1988; Lewis & Martin 1990) or mandibular and labial gland secretions (Weseloh 1981; Lewis & Martin 1990).

Furthermore, parasitoids of larval and adult stages may also use host movement as host location cue. Thus, *Drino bohemica* Mesn. (Tachinidae) is attracted by the movement of a feather in an olfactometer suggesting that movement of its larval sawfly hosts could serve as host location cue (Monteith 1956). However, the moving feather is attractive only in presence of host kairomones suggesting a hierarchy in the signal in which host kairomones act as long-distance searching cues and host movement as a short-distance searching cue (Monteith 1956). In contrast, any moving objects attract *Perilitus coccinellae* Schrank (Euphorinae) and *Drino inconspicua* Meigen (Tachinidae), while host kairomones have little or no effect on host location of these species (Richerson & DeLoach 1972; Dippel & Hilker 1998 respectively). Accordingly, these two parasitoids are generalist species, and the possibility that a moving « object » is a potential host is higher for these species than for more specialized species such as *D. bohemica.*

Though host movement is frequently detected by vision, in several Eucoilidae parasitoids it is detected through vibrations produced by the movement of their Drosophilidae larval hosts in the substrate (Vet & Bakker 1985; Sokolowski & Turlings 1987). Similarly, the parasitoid *S. sericeicornis* (Eulophidae) locates its leaf-miner larval host by the vibrations produced by the movement of the larva in its mine (Meyhöfer *et al.* 1997)

In contrast with larval and adult hosts, host eggs are poor emitters of stimuli because they are inactive (Vinson 1998). Therefore, egg parasitoids have to use other stimuli than those emitted by the egg itself for host location. Thus, the egg parasitoid *Telenomus remus* Nixon is attracted by volatile pheromones of *Spodoptera frugiperda* Smith females in olfactometer (Nordlund *et al.* 1983) while in *O. gallerucae*, contacts with *X. luteola* adult feces increase the host searching behavior of female parasitoids (Meiners *et al.* 2000) suggesting that adult host kairomones are indicating egg presence for these parasitoids. Egg parasitoids also use stimuli related to the host female oviposition, these stimuli being more reliable than adult pheromones or feces. In *Trichogramma* spp, contacts with scales of the adult moth that have fallen on the substrate during oviposition of the female moth, increase the host searching behavior of female parasitoids (Vinson 1976; Weseloh 1981) while in *Ibalia* spp the symbiotic fungus introduced in the wood hole by their *Syrex* spp hosts when laying their eggs is used as an indicator of host egg presence by female parasitoids (Weseloh 1981). In the egg-larval parasitoid *Halticoptera rosae* Burks (Pteromalidae), the marking pheromone of its host, *Rhagoletis basiola* Osten-Sacken, is used as a trail that guides female parasitoids to the oviposition site of *R. basiola* (Hoffmeister *et al.* 2000).

2.3.4 Experience

Previous experiences of female parasitoids are known to affect their host selection behavior by changing their response to host related stimuli. Although experiences during the immature stages may affect subsequent host selection behaviors of female parasitoids in some species (Hérard *et al.* 1988; Bjorksten & Hoffman 1995), generally experiences at the adult stage have more impacts on host selection behaviors of female parasitoids

(Turlings *et al.* 1993). These behavioral changes can occur during a sensitive period lasting a few hours after female emergence or most of the life of the female parasitoid (Turlings *et al.* 1993; Godfray 1994).

The physiological mechanisms causing changes in the parasitoid behaviors following experiences are poorly known, but evidence indicates that previous experiences cause sensitivity changes in the chemoreceptors of the parasitoid antennae (Vet & Dicke 1992; Turlings *et al.* 1993). Experiences can also change the response of female parasitoids to visual stimuli, but physiological mechanisms causing these changes are still unknown (Turlings *et al.* 1993).

The capacity to change behaviors following experiences is adaptive in situations where the environment is unpredictable between generations but predictable during the parasitoid lifetime, or when a parasitoid lives in an environment that varies in a regular way (Vet & Dicke 1992; Turlings *et al.* 1993). In this situation, demonstrating flexibility in its host selection behavior is adaptive because the parasitoid may modify its host selection behavior according to environment changes (Vinson 1976).

Generally, changes in response to host related stimuli of female parasitoids occur through a learning process (Turlings *et al.* 1993). Learning is defined as a reversible change of behavior that occurs with experience (Papaj & Prokopy 1989). There are three criteria for a behavioral change to be considered as learning; 1- the behavior change needs to be always observed and predictable, 2- the behavior change needs to be gradual, 3- the behavior change needs to be forgotten with time (Papaj & Prokopy 1989). There are four types of learning in parasitoids: associative learning, sensitization, habituation and imprinting.

Associative learning. Associative learning occurs when a parasitoid innately recognizes a host-related stimulus (unconditioned stimulus) upon contact, and associates it to surrounding stimuli (conditioned stimuli) to which it originally showed no or limited responsiveness (Vet & Dicke 1992; Turlings *et al.* 1993; Godfray 1994). Subsequently, the parasitoid becomes responsive to the newly learned stimuli (conditioned stimuli) and uses them for host selection (Turlings *et al.* 1993).

Most of the work on associative learning in parasitoids concerns larval parasitoids and has been demonstrated in several species such as in the Braconidae *Bracon mellitor* Say (Vinson *et al.* 1977), *Cotesia marginiventris* Cresson (Godfray 1994) and *Microplitis croceipes* Cresson (Lewis & Tumlinson 1988), the Ichneumonidae *V. canescens* (Arthur 1971), and the Eucoilidae *Leptopilina heterotoma* Thomson (Papaj & Vet 1990; Godfray 1994). All these species can associate a surrounding odor with the presence or the possible presence of a host, and subsequently use this odor as a host selection cue. In addition to olfactive stimuli, *M. croceipes* is also able to associate visual stimuli with the presence of host, and can avoid a previously visited site following visual recognition (Turlings *et al.* 1993). Similarly, the Ichneumonidae *Itoplectis conquisitor* Say (Arthur 1966) and *Exeristes roborator* Fay (Turlings *et al.* 1993; Godfray 1994) can associate the color of a microhabitat to the presence of hosts while *D. bohemica* (Tachinidae) can associate the moving of part of its cage to the presence of hosts (Monteith 1963).

Sensitization. Sensitization implies the increase of the female parasitoid response to a stimulus innately recognized following a previous contact with the stimulus (Turlings *et al.* 1993). When the response to a stimulus is increased following a contact with another stimulus without any association between the stimuli, it is named priming, and may be viewed as a kind of sensitization learning (Turlings *et al.* 1993). Sensitization has been demonstrated in *C. sonorensis* in which contacts with its host larva in the absence of host plants increase its subsequent response to host plant odors (McAuslane *et al.* 1991). Similarly, the response of *M. croceipes* females to host feces is increased following an oviposition into host larvae (Turlings *et al.* 1993).

Habituation. In contrast with sensitization, habituation is the waning of the response to a stimulus after repeated exposure to that stimulus (Papaj & Prokopy 1989). Habituation is an important mechanism that permits dispersion of parasitoids when foraging in a patch that is no longer profitable (Vet & Dicke 1992). Inversely, it can also keep parasitoids in a patch by not responding to warning stimuli when these are repeated without unfavorable consequences (Monteith 1963). For example, *D. bohemica* is innately frightened by the movement of an artist's paint brush, but after five or more exposures without harmful effect or reward, it loses its fear and becomes habituated (Monteith 1963).

Imprinting. Imprinting may not be considered as learning because it involves irreversible changes of behavior that occur during a short sensitive period of the parasitoid development (Gould 1993). Nevertheless, it can change the host selection behavior of parasitoids. According to the chemical legacy hypothesis (Corbet 1985), these changes occur when larval conditioning is carried over from the larval to the adult stage or when the teneral adult encounters the larval environment before emerging from its pupa. Although larval conditioning seems relatively rare (Jermy *et al.* 1968; Jaenike 1983), there is evidence that contact with larval environment by the teneral adult prior to or just after emerging from its pupa may influence subsequent host selection behavior of the adult parasitoid. Thus, when *Microplitis demolitor* Wilkinson (Braconidae) has antennal contacts with its pupa, it subsequently increases its response to the host-plant complex *Heliothis zea*-cowpea suggesting that larval environment is an important source of host infochemicals for *M. demolitor* (Hérard *et al.* 1988). Moreover, the behavioral change lasted for at least seven days suggesting that the behavioral change was irreversible (Hérard *et al.* 1988). Similarly, *Opius dissitus* Muersebeck female parasitoids are more attracted by leafminer-infested lima bean than by leafminer-infested eggplant when reared on the former, but there is no difference in the response if *O. dissitus* females are removed from their pupae before eclosion suggesting that imprinting may occur in the parasitoid pupa (Turlings *et al.* 1993).

2.3.5 Genetic variability

Parasitoid genetic variability may be an important factor affecting the response of parasitoids to host related infochemicals, and especially during the laboratory rearing process (Simmonds 1944). Indeed, laboratory rearing may select for individuals more adapted to the rearing conditions, and subsequently change the host selection behavior of the parasitoid (Doutt 1959). Thus, Powell and Wright (1988) concluded that successful laboratory transfers of the Aphidiidae parasitoids *Aphidius ervi* Haliday and *Aphidius rhopalosiphi* De Stefani Perez between host species was the result of a laboratory rearing selection. Similarly, after being reared for seven years on *Corcyra cephalonica* Stainton, the larval parasitoid *Microbracon gelechiae* Ashmead responded to the odor components of *C. cephalonica* larvae over the odor components of its natural host, *Gnorimoschema operculella* Zeller, suggesting that a laboratory selection was involved in this host change preference (Weseloh 1981).

2.3.6 Physiological state

The internal state of a female parasitoid may also influence its foraging behavior (Doutt 1959; Bell 1990; Lewis & Martin 1990; Vet & Dicke 1992). Generally, the interest of a female parasitoid for host searching decreases when deprivation of other basic needs increases (Vet & Dicke 1992). The deprivation may be caused by a lack of nutrition reserves (Godfray 1994), a lack of eggs (Lewis & Martin 1990) or a lack of fertilized eggs (Bragg 1974). Thus, a hungry female parasitoid tends to respond to stimuli associated to food while a food-satiated female parasitoid tends to respond to host related stimuli (Lewis & Martin 1990; Turlings *et al.* 1993). Similarly, an unmated female searches for a mate before a host, and therefore responds to host related stimuli only after a period allocated to mating (Bragg 1974; Loke & Ashley 1984).

2.3.7 Variability of the sources of infochemicals

Hosts and host plants genetic variability can be an important source of variability in the quantity and quality of the infochemicals released in the environment, and consequently, affects the host selection behavior of female parasitoids (Vet & Dicke 1992). Although genetic variation in both host kairomones and host plant synomones emission is not well documented, glanded cultivars of cotton produce 100 times more synomones than nonglanded cultivars (Vet & Dicke 1992). As these synomones are used by *C. sonorensis* during host selection (Vet & Dicke 1992), it is possible that hosts living on glanded cotton cultivars are more parasitized by *C. sonorensis* than those living on nonglanded cultivars. Similarly, in a first experiment, *Eucelatoria bryani* Sabrosky (Tachinidae) responded to cotton leaves of the variety Deltapine 15 (Neetles 1980), but did not respond to the variety Stoneville 213 in another experiment (Martin *et al.* 1990) suggesting that the type of cotton cultivars can influence the host selection of this parasitoid.

Nongenetic variations in infochemicals emission are documented more than genetic variations (Vet & Dicke 1992). Many parasitoids that respond to host feces are affected by the host diet because it changes the constitution of host feces (Vet & Dicke 1992). Within the same host stage, the age of the host may also affect the internal constitution of the host, and subsequently its acceptance by the parasitoid (Reznik & Umarova 1990).

As discussed in a previous section, the host plant condition (undamaged vs herbivore-damaged) is an important factor influencing the emission of synomones by plants. In addition to its condition, the age of the plant may also influence its emission of synomones, and consequently the host selection behavior of parasitoids using these synomones (Vet & Dicke 1992). Thus, *M. croceipes* females made more sustained flights toward a mature cowpea leaves-*Heliothis zea* complex than toward a cowpea seedling-*H. zea* complex suggesting that the age of the plant affects the type and/or the quantity of volatiles released by the plant, and subsequently its attractiveness to *M. croceipes* (Drost *et al.* 1988).

2.3.8 Dietary specialization

According to Vet & Dicke (1992), the degree of specialization of both the parasitoid at the host level and the host at the plant level should influence the type of stimuli used by female parasitoids when searching for host. Thus, specialist parasitoids are expected to use more host specific stimuli such as host kairomones or herbivore-induced synomones while generalist parasitoids are expected to use non-host specific stimuli, such as vibration or visual cues (Vet & Dicke 1992). For example, *M. croceipes* is a specialist parasitoid of *Heliothis* larvae, and uses a variety of specific host kairomones such as feces, hemolymph and salivary secretions when searching for *Heliothis* larvae (Vet & Dicke 1992). In contrast, *Aphaerata minuta* Fischer is a generalist larval parasitoid of Drosophilidae, and parasitizes any larvae found using non-specific vibrotaxis cues (Vet & Dicke 1992).

Furthermore, the dietary specialization of the parasitoid and its host should also set the degree to which responses to host-plant system infochemicals may be modified by experiences. Thus, strong innate responses to infochemicals from both the host and the plant levels should be obtained when the parasitoid is specialized at both levels while the effect of previous experiences on infochemical responses from host and plant levels should increase as the number of hosts and host plants increase respectively (Vet & Dicke 1992).

2.4 Host acceptance

2.4.1 Host evaluation

After a female parasitoid has found a host, depending on the host quality, it can reject or accept it. The host quality evaluation is achieved by the detection of diverse stimuli that can be the same or different as those used for host location (Godfray 1994). These stimuli are detected during an external and/or an internal examination of the host. Generally, external examination is achieved following antennal drumming on the host (Godfray 1994). After external examination, a parasitoid may reject the host or attempts to oviposit into or on it. If the parasitoid tries to oviposit into the host, an internal examination with its ovipositor may occur. Then, the parasitoid can reject the host or parasitizes it. Thus, for the egg-larval *Ascogaster reticulatus* Watabe (Braconidae), a specific external kairomone on *Adoxophyes* sp. egg surface serves as host recognition cue while the mixture of several amino acids in the egg serves as an internal cue for inducing oviposition (Kainoh *et al.* 1982, 1989).

As in host location, the strategies employed by female parasitoids for host acceptance depend on the host stage attacked. Mobile hosts such as larval or adult hosts have better mechanisms of defence and escape than immobile hosts such as egg and pupal hosts (Harvey & Thompson 1995). Accordingly, the external host evaluation by their parasitoids occurs generally by a quick antennal drumming on the host (Bragg 1974; Schmidt 1974; Harvey & Thompson 1995). Thus, for the larval parasitoid *C. sonorensis*, whose host (*Heliothis zea* larvae) shows aggressive behaviors when disturbed, the duration from the antennal examination to oviposition takes only 1,8s in average (Schmidt 1974). In contrast with *C. sonorensis*, when *Leiophron uniformis* Gahan (Braconidae) attacks its *Lygus* spp nymphal host, it grasps the nymph with its legs and immobilizes it avoiding any chance of escape of the nymph (Debolt 1989). Then, *L. uniformis* inserts its ovipositor into the host and an internal host evaluation follows. The internal examination serves for host species recognition, and depending on the mirid species, the nymph is parasitized or liberated (Debolt 1989).

In other parasitoid species of mobile stages, host movement serves as an oviposition stimulant instead of being a physical constraint. Thus, the oviposition of *Microctonus aethiopoides* Nees (Euphorinae) occurs usually when its adult weevil host is active and if the weevil stops, *M. aethiopoides* also stops until the weevil resumes its activities (Loan & Holdaway 1961). Similarly, *Microctonus vittatae* Muesebeck attacks principally moving hosts while immobile ones are frequently examined by antennal drumming suggesting that host kairomones are also important in the last steps of host selection of this species (Doutt 1959; Vinson 1976). Combination of infochemicals and host movement in the last steps of host selection is observed in many parasitoid species of mobile stages such as in *M. croceipes* (Turlings *et al.* 1993), *V. canescens* (Harvey & Thompson 1995), *C. sonorensis* (Schmidt 1974), *D. bohemica* (Monteith 1956) and *P. coccinellae* (Richerson & DeLoach 1972). All these species are koinobiont parasitoids, and therefore have to develop in a living host. As an immobile host may be sick, dead or already parasitized, host movement for these parasitoids may serve as an indicator of a high quality host (Godfray 1994) while infochemicals may serve as a host recognition cues (Vinson 1998). Similarly, other less specific stimuli such as color and shape are also used in combination with infochemicals in the final steps of host selection of several parasitoids of mobile stages (Wilson *et al.* 1974; Michaud & Mackauer 1995)

Contrary to mobile hosts, egg and pupal hosts are immobile and have neither aggressive nor escape behaviors. Therefore, their parasitoids may mount and examine them with their antennae before oviposition (Godfray 1994). In egg parasitoids, these antennal drummings serve for the detection of contact kairomones on the surface of the egg chorion (Godfray 1994). When investigated, these contact kairomones originate from the reproductive system of the adult host female (Kainoh *et al.* 1982; Strand & Vinson 1983; Nordlund *et al.* 1987; Takasu & Nordlund 2001) and are emitted by adhesives used for attachment of eggs to oviposition sites (Strand & Vinson 1982; Nordlund *et al.* 1987; Bin *et al.* 1993).

However, in some egg parasitoid species, host evaluation is achieved by detection of tactile stimuli instead of host kairomones. Thus, in *T. evanescens*, a generalist parasitoid of lepidopteran eggs, the size of the host is evaluated by walking and antennal drumming on the egg. The critical size for host egg is between about 0,25 and 4,5 mm in diameter, and an oviposition attempt is often observed in any globular objects of that size suggesting that host kairomones are less important than tactile cues for host evaluation of this species (Arthur 1981; Godfray 1994). Moreover, egg size determines the number of eggs that will be laid in the host; larger eggs have larger clutch size and smaller eggs have smaller clutch size (Arthur 1981; Godfray 1994).

Nevertheless, non-specific cues such as tactile cues are generally used in combination with host kairomones in egg parasitoids. Thus, the egg parasitoid *Telenomus heliothidis* Ashmead (Scelionidae) attempts to oviposit only in glass beads between 0,5 and 0,6 mm, but only if the glass beads are coated with the kairomones of its host, the moth *Heliothis virescens* F. (Strand & Vinson 1983). Similarly, the egg parasitoids *Chelonus insularis* Cresson and *Anaphes iole* Girault use contact kairomones on the surface of their host egg as host recognition cues while tactile cues such as shape, and surface texture stimulate the oviposition of the female parasitoids (Conti *et al.* 1996; Takasu & Nordlund 2001).

2.4.2 Host discrimination

Host discrimination is the ability for a female parasitoid to distinguish between parasitized and unparasitized hosts (van Lenteren 1981; van Alphen & Visser 1990) while superparasitism is the action to oviposit in a host already parasitized (Vinson 1976; van Alphen & Visser 1990; Godfray 1994). Because a female can avoid wasting eggs, host discrimination has a selective advantage when the survival in a superparasitized host is less than in an unparasitized host (Bakker *et al.* 1985; Godfray 1994), and when the rejection of a parasitized host is faster than ovipositing (Bakker *et al.* 1985; van Baaren *et al.* 1994; van Baaren & Boivin 1998). Accordingly, host discrimination has been observed in between 150 and 200 species (van Lenteren 1981).

There are three types of discrimination: self, conspecific and interspecific discrimination (van Baaren *et al.* 1994; van Baaren & Boivin 1998). Self-discrimination occurs when a female parasitoid has the ability to differentiate between an unparasitized and a host parasitized by itself while conspecific discrimination occurs when a female parasitoid has the ability to differentiate between an unparasitized host and a host parasitized by a female of the same species. Less frequently observed, interspecific discrimination occurs when a female can distinguish between an unparasitized host and a host parasitized by another species (Bakker *et al.* 1985; van Alphen & Visser 1990; van Baaren *et al.* 1994).

Host discrimination is generally achieved by detection of external and/or internal pheromones on and/or in the host (Vinson 1976; van Alphen & Visser 1990; Godfray 1994). Though in some species, physiological changes into the host, host behavioral changes, oviposition holes in the host and pheromones on the surroundings of the host may be used as host discrimination cues (van Lenteren 1981). The advantage of using external pheromones is that the parasitoid can discover a parasitized host faster than using internal signals (van Lenteren 1981). Thus, *Caraphractus cinctus* Walker (Mymaridae) and *Trissolcus basalis* Wollaston (Scelionidae) use only external pheromones to detect prior parasitism of their hosts, the eggs of submerged water beetle and the eggs of the *Nezara viridula* F. (Pentatomidae) respectively (Godfray 1994).

However, external pheromones may be water soluble and washed off by rain (van Lenteren 1981). Therefore, in several parasitoid species, only internal or both external and internal marks are used. In *T. evanescens* the external pheromones deposited by the first female on the host egg deter subsequent females from attacking the host while physiological changes caused by the developing parasitoid in the egg can be detected by the parasitoid female ovipositor (Vinson 1976; Godfray 1994). Similarly, *A. victus* uses antennal and oviposition discriminations (van Baaren *et al.* 1994; van Baaren *et al.* 1998), but when a late instar larva is detected by the female parasitoid ovipositor, the female parasitoid kills the larva by repeated stinging, and then oviposits (van Baaren *et al.* 1995).

Host discrimination is also common in parasitoids of mobile stages, and antennal discrimination is observed in several species such as in the aphid parasitoids *A. ervi* and *Aphidius smithi* Sharma & Subba Rao (McBrien & MacKauer 1991), in the larval parasitoid *C. sonorensis* (Schmidt 1974), and in the larval-pupal parasitoid *Phaeogenes cynarae* Bragg (Bragg 1974). However, mobile hosts often contact other individuals, and the risk that external pheromones on parasitized individuals are transferred on unparasitized hosts may be high (van Lenteren 1981). Accordingly, internal discrimination is also observed in many parasitoid species of mobile stages such as in the larval parasitoids *V. canescens* (Hubbard *et al.* 1987; Marris *et al.* 1996), *Asobara tabida* Nees and *L. heterotoma* (Sokolowski & Turlings 1987), and in the aphid parasitoids *A. smithi* (McBrien & MacKauer 1991), *A. ervi* and *Aphelinus asychis* Walker (Godfray 1994). In the case of *V. canescens*, the internal mark is a pheromone produced by Dufour's gland, and injected into the host by the female parasitoid when ovipositing (Hubbard *et al.* 1987; Marris *et al.* 1996). In addition to pheromones, parasitoids of mobile stages may also use a reduction of host mobility as a signal that the host is already parasitized (Godfray 1994).

3. Biological control applications

3.1 Parasitoid releases

Biological control using parasitoids is often achieved by the releases of natural parasitoids of a pest in the environment. These parasitoids may be mass-reared in the laboratory and released in order to control the pest rapidly (inundative releases). If pest densities have not reached a critical threshold, the amount of parasitoids released may be less important and the control of the pest relies on the auto-propagation of the parasitoids in the environment long-term (inoculative releases) (Cloutier & Cloutier 1992).

The parasitoids used in biological control may also be exotic species. In classical biological control, these parasitoids have generally evolved with the pests in its native habitat, and consequently, form an old association with the pests (Hokkanen & Pimentel 1984; Simberloff & Stiling 1996a). The success of this old association approach is based on the assumption that parasitoids in old associations are adapted to find and successfully parasitize the host with which they have evolved in the native habitat (Pimentel 1963). Although the old association approach has succeeded in many situations (Pimentel 1963; Howarth 1991), ecological homeostasis may occur in old associations and may limit the effectiveness of the parasitoids in pest control (Pimentel 1963; Hokkanen & Pimentel 1984, 1989). For example, several parasitoids and predators from the native habitats of the gypsy moth *Lymantria dispar* L. and the Japanese beetle *Popillia japonica* Newman were introduced in North America, but did not control the targeted pest at a satisfactory level, though some of these parasitoids and predators successfully colonized the new habitat (Pimentel 1963).

In contrast with the old association approach, the new association approach, also termed neo-classical biological control approach, consists of introducing parasitoids that have not evolved with the hosts (Hokkanen & Pimentel 1984, 1989), and have attack strategies different from those used by the natural parasitoids of the host (Hokkanen & Pimentel 1989). New associations can give better results than old associations in biological control because contrary to old associations, in a new association, the parasitoid may exploit the lack of host defence mechanisms against it, resulting in a more virulent exploitation by the parasitoid and a more efficient control of the host (Pimentel 1963; Hokkanen & Pimentel 1984, 1989). Thus, several exotic pests have been successfully controlled following the introduction of parasitoid species that had not evolved with them (Pimentel 1963; Hokkanen & Pimentel 1989).

New associations may serve not only to control exotic pests but also native pests (Pimentel 1963; Hokkanen & Pimentel 1989). Thus, the coconut moth *Levuana iridescens* B.-B., the coconut leaf-mining beetle *Promecothera reichei* Baly, the coconut hispid beetle *Brontispa mariana* Spaeth, the sugar cane beetle *Oryctes tarandus* Olivier, the pyralid moth *Tirathaba trichogramma* Meyr. and the sweet potato leaf-miner *Bedellia orchilella* Walsm. are examples of native pests that were successfully controlled by the introduction of parasitoids that had not coevolved with them (Pimentel 1963).

Although generally much safer than chemical pesticides (Pimentel *et al.* 1984; Frank 1998), the introduction of parasitoids in a new area may sometimes be harmful for the environment because the parasitoid may attack non-target native species and eliminate them (Howarth 1991; Simberloff & Stiling 1996a, b). Indeed, many parasitoids that were introduced in several Pacific islands have become pests themselves in attacking and sometimes eliminating non-target native species (Howarth 1991; Simberloff & Stiling 1996a, b). Furthermore, the introduced parasitoid may interfere with a previously introduced parasitoid or a native parasitoid, and subsequently worsen the situation in decreasing the control achieved by the first parasitoid (Pimentel *et al.* 1984). This occurred when the parasitoid *Quaylea whittieri* L. was introduced in California to control the black scale, *Saissetia oleae* Olivier, *Q. whittieri* became a hyperparasitoid of *Metaphycus lounsburyi* Howard, an important parasitoid of *S. oleae*, and subsequently diminished the biological control achieved by *M. lounsburyi* instead of increasing the general control of *S. oleae* (Pimentel *et al.* 1984).

Manipulating genotypic and phenotypic variations of the parasitoids before releasing them in the environment can enhance the effectiveness of parasitoids in pest control. For example, before releasing parasitoids in fields, a selection of the best strain and the physiological state may be done (Lewis & Martin 1990; Vet & Dicke 1992). A more frequently suggested application is to increase host searching behavior of parasitoids in fields by giving them an experience with host related stimuli before the release (Vinson 1976; Lewis & Martin 1990; Vet & Dicke 1992; Turlings *et al.* 1993). Thus, when baits of mushroom or apple yeast both infested with Drosophilidae larvae were arranged in the forest, two-hour long experience on a apple yeast or a mushroom diet by *L. heterotoma* increased by three times their host foraging abilities compared to naive females because they found their hosts more often and more rapidly (Papaj & Vet 1990). Moreover, *L. heterotoma* females experienced on apple yeast found apple yeast baits more often than mushroom baits while *L. heterotoma* females experienced on mushroom baits found mushroom baits more often than apple yeast baits (Papaj & Vet 1990). Similarly, when *M. croceipes* females and *Trichogramma pretiosum* Riley females are exposed to host feces and host scales respectively, the rate of parasitism in the area where they were released was higher compared to the rate of parasitism in the area where only naive females were released (Lewis & Martin 1990).

3.2 Manipulating stimuli sources

The manipulation of stimuli sources most frequently used is the dissemination of a previously identified kairomone that increases host searching behavior of parasitoids (Vet & Dicke 1992). Thus, when *Heliothis* spp kairomones were artificially released in fields, it increased the rates of parasitism of *Trichogramma* spp and *M. croceipes* on *Heliothis* spp suggesting that contact with host kairomones have stimulated the host searching behavior of these parasitoids (Vinson 1976; Weseloh 1981; Lewis & Martin 1990). However, this practice has been attempted many times without success on other parasitoid species, and one possible explanation for these unsuccessful attempts, is that host kairomones have kept the parasitoids in area without hosts (Vet & Dicke 1992).

Host plant synomones may also be used as host searching stimulants for parasitoids in fields. Thus, when water extracts of *Amaranthus* spp or corn were applied in soybean, cowpea, tomato and cotton fields, it increased rates of parasitism of *Trichogramma* spp on *H. zea* suggesting that *Amaranthus* spp and corn produce synomones that increase host searching behavior of *Trichogramma* spp (Altieri *et al.* 1981; Lewis & Martin 1990). Another promising method is to select plant cultivars that are the most attractive to parasitoids, and especially those that produce herbivore-induced synomones at damaged sites (Vet & Dicke 1992).

4. *Listronotus oregonensis* LeConte

4.1 Reproduction and development

Listronotus oregonensis is a Curculionidae (Brachycerinae) native to northeast North America and associated with umbelliferous plants (Boivin 1999b). Adults are elongate-oblong and the body is covered with tan scales (Martel *et al.* 1976). *Listronotus oregonensis* females are longer and heavier than males; females have an average of 6,5 ± 0,3 mm in length and 0,29 mg in weight while males have an average of 6,0 ± 0,3 mm in length and 0,23 mg in weight (Martel *et al.* 1976). Adults can be sexed on the basis of the first ventral segment; the segment is depressed on males and swollen on females (Martel *et al.* 1976). This species has sexual reproduction and mating occur about two weeks (min. 11 days at 25°C) after emergence at 25°C during the scotophase (Martel *et al.* 1975; Baudoin & Boivin 1985). Only one mating is enough to fertilize all female eggs, and one male can fertilize at least four females (Baudoin & Boivin 1985). Sexually immature females contain no eggs (Boivin 1999b).

On carrot, *L. oregonensis* adults feed and lay their eggs on the vegetative part (Boivin 1988, 1999b). Adults feed predominantly on the crown, petioles and leaves of plants while eggs are laid principally on the petiole and the crown, and to a lesser extend on leaves (Boivin 1988). Females can oviposit between 200-250 eggs in their lifespan (Boivin 1999b). Before laying eggs, *L. oregonensis* females chew a hole in the plant, lay their eggs, and cover them with a plug made of feces and saliva (Martel *et al.* 1975, 1976). A female makes between two to four egg cavities per carrot depending on the weevil density, and an average of two eggs per cavity are deposited (Martel *et al.* 1976;

Boivin 1999b). Eggs are creamy white in the first 48h, and then become brownish and black prior to larval hatching (Martel *et al.* 1976; Boivin 1999b); sterile eggs remain creamy white (Baudoin & Boivin 1985).

Damage caused by *L. oregonensis* adults feeding and ovipositing on the vegetative part of the carrot has little economic importance (Whitcomb 1965). However, upon emergence, first instar larvae crawl to the surface of the foliage or burrow down a leaf petiole toward the root, and then make tunnels in the carrot root (Martel *et al.* 1976; Boivin 1988, 1999b). Carrots with tunnels have no economic value and can increase the risk of infestation by microorganisms such as the bacterial soft rot (Boivin 1999b). Larvae feed in the carrot root until the fourth instar, then stop feeding, and leave the carrot as prepupae (Martel *et al.* 1975, 1976; Boivin 1999b). The prepupa then builds a pupal chamber in the soil near the carrot plant (Martel *et al.* 1976; Boivin 1999b). The development of the carrot weevil, from egg to adult, takes 130 days at 12,7°C and 27 days at 30-32°C (Simonet & Davenport 1981) and adults may live over 392 days at 25°C (Baudoin & Boivin 1985; Boivin 1999b).

4.2 Population dynamics in carrot fields

In carrot fields, feeding activities start when cotyledons appear, and are almost finished when carrots reach the six true-leaf-stage (Boivin 1985, 1988, 1999b), while most of the ovipositing activities occurs between the four and the eight true-leaf-stage of carrot (Boivin 1988, 1999b). *Listronotus oregonensis* has one to three generations per year depending on the region (Boivin 1999b). Adults overwinter in carrot fields and/or in ditchbanks in the top 50 mm of soil (Stevenson 1976; Boivin 1999b). As they are poor fliers, migration between fields and ditchbanks occurs generally through walking (Boivin 1999b). *Listronotus oregonensis* females have a reproductive diapause characterized by a lack of ovarian development (Boivin 1985, 1999b). The diapause is regulated by both temperature and photoperiod; diapause occurs when both photoperiod and temperature decrease (Boivin 1999b).

4.3 Host plants

Listronotus oregonensis lives in low vegetation, and especially on umbelliferous plants in commercial fields and ditchbanks (Boivin 1999b). This species is generally associated with organic soils where carrots (*Daucus carota* L. var. *sativa*) are grown (Martel *et al.* 1975), and of which it is a major pest (Stevenson 1976; Boivin 1999b). *Listronotus oregonensis* is also a major pest of celery (*Apium graveolens* L.) (Stevenson 1976; Boivin 1999b) and parsley (*Petroselinum hortense* Hoffm.) (Boivin 1999b), and a minor pest of parsnip (*Pastinaca sativa* L.), turnip (*Brassica rapa* L.), and dill (*Anethum graveolens* L.) (Boivin 1999b). *Listronotus oregonensis* is also found on several wild plants such as wild carrot (*Daucus carota* L.), parsley (*Apium petroselinum* L.), wild parsnip (*Pastinaca sativa* L.), water parsnip (*Sium suave* Walt.), common plantain (*Plantago major* L.), lance-leafed plantain (*Plantago lanceolata* L.), and several *Rumex* species (Boivin 1999b).

4.4 Control

4.4.1 Chemical control

Chemical treatments are applied principally against young larvae and adults because eggs and late instar larvae are protected by plant tissue, and pupae are protected by soil (Boivin 1999b). In Canada, chemical treatments applied against *L. oregonensis* consist of one or two foliar treatments of phosmet depending on monitoring results (Boivin 1999b).

4.4.2 Integrated Pest Management

In 1981, an Integrated Pest Management (IPM) was started in the province of Ontario (Boivin 1999b). The first step of the program was the elaboration of an efficient monitoring method for *L. oregonensis* adult populations. Among the methods suggested, two were selected. The first method is the carrot root section and was used in Ontario (Boivin 1999b). Carrot root sections are inserted vertically in the soil between carrot rows. At 3-4 day intervals, the number of oviposition punctures made by *L. oregonensis* females is counted. Then, the number of punctures is transformed into *L. oregonensis* egg cavities per root section per day (CSD) (Boivin 1999b). The CSD observed determines the need for an insecticide treatment. The threshold established is of 0,3 CSD or, when less than 50 % of the carrot sections bears an oviposition scar, 0,5 CSD (Boivin 1999b).

The second method of monitoring is the wooden plate trap and was used in Québec (Boivin 1985). The wooden plate trap consists of an assemblage of wooden plates in which a carrot root section is inserted; the carrot root section acts as a bait (Boivin 1985). Wooden plate traps are deposited in the field in early May. Twice weekly, the number of *L. oregonensis* adults is counted (Boivin 1999b). The economic threshold was established at a mean cumulative capture per trap of 1,5 *L. oregonensis* adults (Boivin 1999b). Using this threshold, damage is kept below 1 % in commercial fields (Boivin 1999b).

When results of sampling suggest a chemical treatment, it is important to synchronize the treatment with the oviposition of *L. oregonensis* females. The critical period for treatment is when adult females start to oviposit. This occurs between 147 and 456 $DD_{7°C}$, and when carrots have reached the four true-leaf-stage (Boivin 1988).

Before 1981, one granular insecticide and an average of five foliar treatments were used annually against *L. oregonensis* (Boivin 1999b). With the establishment of the IPM program, the granular insecticide has been abandoned (Boivin 1988a), and foliar treatments have decreased from an average of five to a maximum of two per year which result in substantial reductions of chemical insecticide released in the environment and cost production for growers (Boivin 1999b).

4.4.3 Biological control

Most of the biological control against *L. oregonensis* in northeastern North America is caused by egg parasitoids of the Mymaridae family: *Anaphes listronoti* Huber, *A. victus* and an unidentified species of *Anagrus* (Zhao *et al.* 1991; Boivin 1999b). The percentage of egg mortality caused by these species can reach 60 % in Québec (Boivin 1986, 1992). To a lesser extent, *L. oregonensis* populations can be also controlled by the action of nematodes (*Steinernema* spp and *Heterorhabditis bacteriophora* Oswego), predators (Carabidae beetles) (Boivin 1999b), and microorganisms (*Beauveria bassiana* Bals., *Metarhizium anisopliae* Metsch. and *Bacillus thuringiensis* Berliner) (Boivin 1999b).

5. *Microctonus hyperodae* Loan

5.1 Reproduction and development

In 1996, a new host-parasitoid association between *L. oregonensis* and *M. hyperodae* was created in the laboratory in Canada (Boivin 1999b). *Microctonus hyperodae* is an oligophagous solitary endoparasitoid that attacks adult Curculionidae of the Brachycerinae subfamily (Goldson *et al.* 1992; Barratt *et al.* 1997). It is a thelytokous parthenogenetic parasitoid, and males are extremely rare (Loan & Llyod 1974; Goldson *et al.* 1990a; McNeill *et al.* 1993). The reproductive tract of a *M. hyperodae* female has 6,1 ± 0,2 ovarioles and contains a total of 40-60 oocytes (Goldson *et al.* 1995). Under optimal conditions, a female lives approximately 20 days and can oviposit between 30 to 60 eggs (McNeill *et al.* 1993; Goldson & McNeill 1994; Goldson *et al.* 1995; Philips & Baird 2001). In these conditions, almost half of the eggs can be laid within three days (Goldson & McNeill 1994; Goldson *et al.* 1995) and the minimum temperature for oviposition is 5°C; beyond this, the rate of increase is linear until 30°C whereafter the rate decreases abruptly (Goldson *et al.* 1995). After the parasitoid's single egg has been deposited in the haemocoel of the host, its volume increases by 205 times prior to hatching (Goldson *et al.* 1995). Despite superparasitism in this species, only one larva may develop per host (Loan & Llyod 1974; Goldson *et al.* 1998).

Microctonus hyperodae is a koinobiont parasitoid, and therefore, the parasitized weevil stays alive and mobile during the development of the parasitoid larva (Loan & Llyod 1974). Larval development is influenced by the quality of the host diet, and some compounds such as diterpenes and alkaloids can retard the parasitoid larval development (Barker & Addison 1996). Early in its development, the larva eats the reproductive system of the weevil, which rapidly sterilises it, and is completed when the larva has reached the fourth instar (Loan & Llyod 1974; McNeill *et al.* 1996, 2000). Then, the larva emerges from the weevil, killing it, and spins a pupa in the soil near the host plant (Loan & Llyod 1974; Barratt *et al.* 1997). The adult parasitoid emerges from the pupa in 10-19 days (Loan & Llyod 1974).

Microctonus hyperodae has two to three generations per year depending on the region (Goldson *et al.* 1998; Barlow *et al.* 1994), and overwinters as an egg or a first instar larva in a state of photoperiodically induced diapause (Loan & Llyod 1974;

Goldson & McNeill 1992; McNeill *et al.* 1993). The larval development continues in spring when temperature increases (McNeill *et al.* 1993).

5.2 Origin and habitat

Microctonus hyperodae has evolved with the Argentine stem weevil (*Listronotus bonariensis* Kuschel) in South America (Loan & Llyod 1974; Goldson *et al.* 1990b). The original habitat of *L. bonariensis* consists of humid ecosystems in temperate zones, characterized by stream-fed and swampy areas dominated by Juncaceae, Cyperaceae and some Graminae such as *Poa* spp and *Deyeuxia* spp (Goldson *et al.* 1990b). In these habitats, *L. bonariensis* feeds on *Poa* spp and *Deyeuxia* spp (Goldson *et al.* 1990b). However, *L. bonariensis* has recently colonized dryland ecosystems, such as ornamental and pasture areas in South America (Loan & Llyod 1974; Goldson *et al.* 1990b); these areas comprise principally introduced ryegrasses such as *Lolium multiflorum* Lamarck (Goldson *et al.* 1990b).

Listronotus bonariensis was introduced in New Zealand at the beginning of the 20th century, and became a major pest of pasture (*Lolium* spp) in that country (Loan & Llyod 1974; Barker 1989). In order to reduce the population of *L. bonariensis* in New Zealand pastures, a biological control program with *M. hyperodae* was undertaken in 1989. *Microctonus hyperodae* was collected from eight South American locations comprising Brazil (Porto Alegre), Uruguay (Colonia), Argentina (Ascasubi, General Roca, Bariloche, Mendoza) and Chile (Conception, La Serena) (Goldson *et al.* 1990b). With the exception of the progeny from the Mendoza's female, approximately equal numbers of parasitoids derived from each of these locations, for a total of c. 270 000 parasitoids (Goldson *et al.* 1995), were released in New Zealand (Goldson *et al.* 1993). Release sites were agricultural pasture habitats consisting principally of Graminae species such as ryegrasses (*Lolium* spp), white clover (*Trifolium repens* L.) and *Poa* spp. (Barker 1989; Goldson *et al.* 1998). *Microctonus hyperodae* is now established in New Zealand, and parasitism levels in some locations exceed 80% (Goldson *et al.* 1994). Morphometric analysis of *M. hyperodae* collected from the field show that populations derived from east of the Andes (Argentina, Brazil and Uruguay) have out-competed those originating from Chile (Philips *et al.* 1994; Phillips & Baird 1996; Philips *et al.* 1997). Molecular data also indicate that *L. bonariensis* populations established in New Zealand originate from the east coast of South America (Williams *et al.* 1994).

5.3 Host selection

Most studies on *M. hyperodae* host selection concern its host range. Since its introduction in New Zealand, *M. hyperodae* has been recovered only twice (once on *Irenemus aequalis* Broun, and once on *Sitona lepidus* Gyllenhal) on species other than *L. bonariensis* (Barratt *et al.* 1997). However, under laboratory conditions, *M. hyperodae* has a wider host range than under field conditions. Seven other Brachycerinae species (*Nicaeana cinerea* Broun, *Nicaeana cervina* Broun, *Irenimus aemulator* Broun, *Irenimus egens* Broun, *Irenimus stolidus* Broun, *Irenimus* sp.3 and *Catoptes robustus* Sharp) were parasitized (Goldson *et al.* 1992; Barratt *et al.* 1997). However, these hosts did not sustain *M. hyperodae* development as well as *L. bonariensis*, and considerable levels of larval encapsulation and melanization were observed (Goldson *et al.* 1992; Barratt *et al.* 1997). Similarly, recent studies with *Listronotus maculicollis* Kirby in USA showed that *M. hyperodae* is also able to parasitize this species, but *L. maculicollis* was attacked less than *L. bonariensis* and did not sustain *M. hyperodae* larval development as well as *L. bonariensis* (McNeill *et al.* 1999, 2000). Under similar laboratory tests in Canada, four other species (*Listronotus sparsus* Say, *Gymnetron tetrum* Fabricius, *Ceutorhynchus irysimi* Fabricius and *Nedyus flavicaudis* Boheman) were parasitized by *M. hyperodae* in addition to *L. oregonensis* and *L. maculicollis* (Boivin unpublished).

There is evidence that learning could influence the host selection behavior of *M. hyperodae*, as rates of parasitism on *L. bonariensis* and *L. maculicollis* were increased following a previous experience with these hosts (Barker & Addison 1997; McNeill *et al.* 1999).

Host acceptance by *M. hyperodae* is not influenced by the sex of the weevil under both laboratory and field conditions (Goldson *et al.* 1995, 1998). The oviposition behavior of *M. hyperodae* females is typical to those observed within the Euphorinae subfamily (Tobias 1965; McNeill *et al.* 1996). Prior to oviposition, a *M. hyperodae* female may stalk its host for a long period. When ready to oviposit, the female extends its ovipositor beneath its abdomen in front of its head, and then makes quick thrusts in the weevil direction (McNeill *et al.* 1996). These oviposition attempts may be aimed at the apex of the abdomen, the thorax or the head (McNeill *et al.* 1996). Furthermore, when in the presence of both *L. bonariensis* and *L. maculicollis*, *M. hyperodae* stalks and tries to oviposit in the most active weevils suggesting that host movement is an important cue used by *M. hyperodae* in the last steps of host selection (McNeill *et al.* 1999).

6. *Anaphes victus* Huber

6.1 Reproduction and development

Anaphes victus is a solitary endoparasitoid of Curculionidae eggs (Huber *et al.* 1997). As the majority of *Anaphes* species, *A. victus* is arrhenotokous; virgin females produce males and mated females produce both sexes (Boivin *et al.* 1993). *Anaphes victus* is a short-lived parasitoid with a mean longevity of four days and fecundity of 50 eggs (van Baaren *et al.* 1994). Mating and oviposition occur rapidly following adult emergence, and most of the eggs are oviposited in the first 48h (van Baaren *et al.* 1994). Parasitoid development occurs entirely in the egg, and it takes 11-12 days at 23 ± 1°C from oviposition to adult emergence (Boivin *et al.* 1993). This parasitoid has four larval instars, the first being a mymariform larva (Boivin *et al.* 1993; van Baaren *et al.* 1997). The mymariform larva is mobile and has a hook, which can serve to attack other mymariform or older larvae when superparasitism occurs (Boivin *et al.* 1993; Nénon *et al.* 1995; van Baaren *et al.* 1997; Boivin & van Baaren 2000). The mymariform larva presents sexual dimorphism principally in the number of setae and the morphology of the caudal hook (Boivin *et al.* 1993; van Baaren *et al.* 1997) that could be responsible for the larval female advantage when competing in a host (van Baaren *et al.* 1999). *Anaphes victus* older instar larvae are sacciform; these larvae are motionless and devoid of offensive structures (Boivin *et al.* 1993; Nénon *et al.* 1995).

6.2 Population dynamics in carrot fields

Anaphes victus adults emerge approximately at 105 DD $_{7°C}$ in late April and early May (Cormier *et al.* 1996). This species has more than 10 generations per year and for the first two or three generations, *A. victus* lives on *L. oregonensis* eggs laid on alternate host plants and on eggs of other weevil species (Cormier *et al.* 1996; Huber *et al.* 1997; Boivin 1999b). *Anaphes victus* adults migrate in carrot fields when *L. oregonensis* starts oviposition (Cormier *et al.* 1996), and parasitize *L. oregonensis* eggs throughout its oviposition period (Boivin 1992; Cormier *et al.* 1996). Parasitism follows a temporal density-dependant relationship during most of the summer, but in late summer, parasitism remains high even with low host densities suggesting a high searching efficiency by *A. victus* (Boivin 1992). Parasitism rates can exceed 70 % from early July to late summer in Québec (Boivin 1986, 1992). However, there is a delay of 10-15 days in early summer before the mortality on carrot weevil populations reaches a level at which damage caused by weevil larvae is kept below an economic level (Boivin 1999b). *Anaphes victus* is a parasitoid that overwinters in the host egg as a larva, and the larva can survive in freezing temperatures by changing the host egg concentration of cryoprotectants, glycerol and sugar (Hance & Boivin 1993).

6.3 Host selection

Anaphes victus is known to parasitize four Curculionidae species other than *L. oregonensis*: *Nedyus flavicaudis* Boheman on *Urtica dioica* L., *Auleutes epilobii* Paykull on *Epilobium angustifolium* L., *Conotrachelus anaglypticus* Say on *Thalictrum pubescens* Pursh. and *Listronotus sparsus* Say on *Plantago major* L. (Huber *et al.* 1997).

However, most of the work done on host selection of *A. victus* concerns its capacities to discriminate between parasitized and unparasitized hosts. *Anaphes victus* can discriminate between eggs parasitized either by itself, by another *A. victus* female or by *A. listronoti* (van Baaren *et al.* 1994; van Baaren & Boivin 1998), though superparasitism is frequently observed (Boivin *et al.* 1993; van Baaren *et al.* 1994; van Baaren & Boivin 1998). Host discrimination is achieved through antennal rejection or ovipositor (sting) rejection, and can be enhanced through learning (van Baaren *et al.* 1994; van Baaren & Boivin 1998); self-discrimination occurs mostly by antennal rejection, conspecific discrimination by both antennal and ovipositor rejection, and interspecific discrimination mostly by ovipositor rejection (van Baaren *et al.* 1994; van Baaren & Boivin 1998). Furthermore, *A. victus* is also able to discriminate between hosts parasitized at different time intervals, and changes its behavior accordingly (van Baaren *et al.* 1995). Indeed, superparasitism is higher on newly parasitized eggs than on eggs containing a mymariform or sacciform larva (van Baaren *et al.* 1995), and when a female superparasitizes a host containing a sacciform larva, the female kills the larva by repeated stinging with its ovipositor before laying its egg (van Baaren *et al.* 1995).

While parasitized eggs are not readily accepted by *A. victus*, host age does not affect the degree of acceptance of *A. victus*. Eggs of *L. oregonensis* are accepted by *A. victus* during most of their development (Picard *et al.* 1991; Boivin 1999b), but development in sterile eggs is impossible and parasitoid mortality is higher in newly oviposited (18h) and old (162h) eggs (Picard *et al.* 1991).

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III. INFOCHEMICALS USED BY THE PARASITOID *MICROCTONUS HYPERODAE* LOAN (HYMENOPTERA : BRACONIDAE, EUPHORINAE) WHEN SEARCHING FOR ITS ADULT WEEVIL HOSTS.

Abstract

Infochemicals are the most important cues used by parasitoids for host location. The attractiveness of the infochemicals in a tritrophic context is expected to be determined by the degree of specialization of the parasitoid and host(s). *Microctonus hyperodae* Loan is an oligophagous parasitoid that attacks adult Curculionidae of the Brachycerinae subfamily, but especially *Listronotus bonariensis* Kuschel, on Graminae. In 1996, a new host-parasitoid association between the carrot weevil *Listronotus oregonensis* LeConte and *M. hyperodae* was created in the laboratory. In this study, the infochemicals used by *M. hyperodae* when searching for its adult weevil hosts were determined using a Y-shaped olfactometer. Three Curculionidae species (*L. oregonensis*, *Listronotus sparsus* Say and *Nedyus flavicaudis* Boheman) and one Bruchidae species (*Callosobruchus maculatus* Fabricius) with their feces were tested. It was expected that hosts phylogenetically and ecologically close to *L. bonariensis* would be more attractive than species less related. However, *M. hyperodae* responded only to *L. oregonensis* with its feces. When separate tests were conducted, *M. hyperodae* responded significantly to *L. oregonensis* but did not respond to its feces suggesting that most of the kairomones came from the host itself. Host plants were also tested, but *M. hyperodae* responded neither to *Lolium multiflorum* Lamarck (Graminae) nor to *Daucus carota* L. (Umbelliferous) leaves. It is likely that the response to *L. oregonensis* kairomones was due to laboratory selection and/or a previous experience with *L. oregonensis* infochemicals.

Key words: host location, host range, kairomone, olfactometer, adult parasitoid, Curculionidae, *Listronotus oregonensis,* new association

Introduction

Infochemicals (chemical cues) are the most important cues used by parasitoids when searching for hosts (Godfray 1994). Sources of infochemicals may come from the host plant, the host itself and host activities (Godfray 1994). Thus, infochemicals have to be considered in a tritrophic context in which the first level is the host plant, the second is the host and the third is the parasitoid (Vet & Dicke 1992). How parasitoids use infochemicals from the first and second levels depends on their reliability and detectability. The reliability of a stimulus indicates the host presence, its accessibility and its suitability while its detectability indicates the degree to which it can be perceived (Vet & Dicke 1992). Infochemicals from the host level are highly reliable because they are released by the host itself or by the products of its activities. However, they are poorly detectable because hosts are small components of a complex environment, and natural selection has favored inconspicuous stimuli (Vet & Dicke 1992). On the other hand, infochemicals from plants (synomones) are generally less reliable than infochemicals from the host level, but are more detectable because plants have a bigger biomass than hosts (Vet & Dicke 1992). To solve the reliability-detectability problem, parasitoids may use a hierarchic search, in which the more detectable infochemicals from the host plants serve for host habitat location, and the more reliable infochemicals from the host level serve for host location in the habitat (Vinson 1976). Another solution is that parasitoids may use infochemicals that are both highly detectable and highly reliable such as herbivore-induced synomones (Vet & Dicke 1992).

The response of a parasitoid to host-plant infochemicals may be innate (Jones *et al.* 1971; Hendry *et al.* 1973; Hérard *et al.* 1988) or the result of a previous experience (Turlings *et al.* 1993). The degree to which responses to host-plant infochemicals may be modified by experience depends on the dietary specialization of the parasitoid and its host (Vet & Dicke 1992). Strong innate responses to infochemicals from both the host and the plant levels should be obtained when the parasitoid is specialized at both levels. Inversely, the effect of previous experience on the response to infochemicals from host and plant levels should increase as the number of hosts and host plants increase respectively.

In 1996, a new host-parasitoid association between the carrot weevil (*Listronotus oregonensis* LeConte) and *Microctonus hyperodae* Loan was created in the laboratory in Canada (Boivin 1999). *Microctonus hyperodae* reproduces by thelitokous parthenogenesis (Loan & Lloyd 1974), and is a solitary koinobiont endoparasitoid that attacks adult Curculionidae of the Brachycerinae subfamily (Loan & Lloyd 1974; Goldson *et al.* 1992; Barratt *et al.* 1997). *Microctonus hyperodae* is native from South America where its only known host is the Argentine stem weevil (*Listronotus bonariensis* Kuschel) (Loan & Lloyd 1974). The original South American habitat of *M. hyperodae* and *L. bonariensis* consists of humid habitats such as low-lying damps and swamps (Goldson *et al.* 1990), and are similar to those described for *L. oregonensis* (Stevenson 1976). In its original habitats, *L. bonariensis* feeds on Graminae species such as *Poa* spp and *Deyeuxia* spp (Goldson *et al.* 1990). However, *L. bonariensis* has recently colonized ornamental grass and pasture areas in South America; these areas comprise principally introduced ryegrasses such as *Lolium multiflorum* Lamarck (Goldson *et al.* 1990).

Listronotus bonariensis was introduced in New Zealand at the beginning of the 20th century (Loan & Lloyd 1974), and became a major pest of pasture (*Lolium* spp) in that country (Barker 1989). In an attempt to reduce the population of *L. bonariensis* by biological control, *M. hyperodae* was introduced to New Zealand in 1991, and was successfully established in 90% of the sites where it was released resulting in parasitism levels reaching 93% in some sites (Goldson *et al.* 1995). In addition to *L. bonariensis*, *M. hyperodae* has been reported to parasitize *Irenimus aequalis* Broun and *Sitona lepidus* Gyllenhal under field conditions (Barratt *et al.* 1997). In the laboratory, *M. hyperodae* showed a wider host range than under field conditions. Seven other Brachycerinae species (*Nicaeana cervina* Broun, *Nicaeana cinerea* Broun, *Irenimus aemulator* Broun, *Irenimus egens* Broun, *Irenimus stolidus* Broun, *Irenimus* sp.3, and *Catoptes robustus* Sharp) were parasitized, but again, parasitism was less important than for *L. bonariensis* (Goldson *et al.* 1992; Barratt *et al.* 1997). Under similar laboratory tests with North American species, *M. hyperodae* parasitized five other weevil species (*Listronotus sparsus* Say, *Listronotus maculicollis* Kirby, *Neydus flavicaudis* Boheman, *Ceutorhynchus irysimi* Fabricius and *Gymnetron tetrum* Fabricius) associated with low vegetations in addition to *L. oregonensis* (Boivin unpublished data).

Host changes may influence subsequent host selection behaviors of a parasitoid by selecting individuals more adapted to their new host (Simmonds 1944; Powell & Wright 1988) or by increasing the response of the parasitoid to the new host following a previous experience on that host (Monteith 1955; Kudon & Berisford 1980). Host change may be achieved for biological control purposes by creating new host-parasitoid associations. New host-parasitoid associations are frequently used in biological control programs and may give better results than old associations (Hokkanen & Pimentel 1984, 1989). However, to our knowledge, there is no study that has treated the host location behaviors of parasitoids in such associations. We hypothesized that a specialist parasitoid in the first steps of a new association should use infochemicals from the host-plant system in which it has evolved.

In this paper, the infochemicals used by *M. hyperodae* when searching for its weevil hosts were investigated using a Y-shape olfactometer under laboratory conditions. Since *M. hyperodae* has evolved on *L. bonariensis*, it was expected that hosts phylogenetically close to *L. bonariensis* and living in low vegetation habitats as *L. bonariensis* would be more attractive than species less related. Similarly, since *M. hyperodae* has evolved on Graminae, Graminae were expected to be more attractive than other plants.

Materials and Methods

Insect rearing and plants.

Listronotus oregonensis was reared at 25 ± 1°C, with a photoperiod of 16L : 8D and 40-60 % R. H. following the technique of Martel *et al.* (1975). *Microctonus hyperodae* was reared in an incubator at 25 ± 1°C, with a photoperiod of 16L : 8D and 60-90 % R. H. At emergence, each *M. hyperodae* female was placed in a Solo cup (250 ml) with ten sexually mature *L. oregonensis* of both sexes from the laboratory rearing. A piece of carrot root 10 cm long and two size 3 (ca. 300 µl) Beem ™ polyethylene embedding capsules filled with cotton, one with a honey solution (50% honey 50% water) and one with water, were placed in the Solo cup. A filter paper in the bottom of the Solo cup absorbed moisture and feces. The Solo cups were placed in an incubator at 25 ± 1°C, with a photoperiod of 16L : 8D and 60-90 % R. H. for one week. The *L. oregonensis* adults were then removed and placed on screened-bottom Solo cup containers (225 ml) in which a piece of carrot root was added. The screened-bottom Solo cup was fitted into another plastic container (600 ml Genpak) in which a thin layer of moist soil and a filter paper were added. Fifteen to 30 days later, *M. hyperodae* final instar larvae emerged from *L. oregonensis*, passed through the screen, and dropped to the soil to pupate. The pupae were removed from the plastic container and placed on filter papers in Petri dishes (35 X 10 mm). One or two pupae were placed per Petri dish, and the filter paper was moistened with water to prevent pupae from drying out. Petri dishes were sealed with Parafilm®, and placed in an incubator at 25 ± 1°C, with a photoperiod of 16L : 8D and 60-90 % R. H. until adult emergence. Female *M. hyperodae* were supplied with water at emergence and kept separately in Petri dishes until the day they were tested. All *M. hyperodae* used in the experiments were naive females, less than three days old and daughters of 20 females of the strain UR21 that originates from Colonia in Uruguay (Goldson *et al.* 1990). Female *M. hyperodae* used in the experiments have completed between 30 and 40 generations on *L. oregonensis.*

Hosts used in the experiments were selected in a manner that they could be ranked as a function of their similarities with *L. bonariensis*, and consequently, a function of their potential attractiveness for *M. hyperodae*. Three criteria were used for host selection: their capacity to sustain *M. hyperodae* development, their taxa, and their biology. Thus, three Curculionidae, *L. oregonensis* (Rhytirhinini), *L. sparsus* (Rhytirhinini), *N. flavicaudis* (Ceutorhynchini), and one Bruchidae, *Callosobruchus maculatus* Fabricius, were selected for the experiments. *Listronotus bonariensis* was not tested because of restrictions on the importation of exotic pests into Canada.

Listronotus oregonensis used in the experiments came from the laboratory rearing while *C. maculatus* were reared in 9-liter glass containers on peas of *Vigna ucaguiculata* L. *Listronotus sparsus* and *N. flavicaudis* were collected in the field during the summer on *Plantago major* L. and *Urtica procera* Mühlenberg respectively. These were overwintering adults, and were kept in plastic containers along with two cups filled with cotton, one with a honey solution (50% honey 50% water) and one with water. *Listronotus sparsus* and *N. flavicaudis* were regularly supplied with leaves of *P. major* and *U. procera* respectively. All species were kept at 20-25 °C, with a photoperiod of 16L : 8D and 40-70 % R.H. until the day they were tested.

Plants were also tested to determine whether *M. hyperodae* used plant volatiles for host selection. Two species were used, the Graminae *Lolium multiflorum* (ryegrass) and the Umbelliferous *Daucus carota* L. var Caropak (carrot). *Lolium multiflorum* and *D. carota* are important hosts of *L. bonariensis* and *L. oregonensis* respectively. Both plants were grown in peat soil in a greenhouse, and were not treated with chemical pesticides.

Olfactometer tests.

The experiments were performed in the laboratory at 22 ± 2°C and at 60-90 % R. H. The olfactometer is a glass Y-tube, 1,3 cm in diameter, made of two arms 23 cm long, and a common tube 9 cm long. A line was traced at 19 cm from the end of each arm. The olfactometer was connected to an airflow system; the airflow of pressured medical air was set at 150 ml/min. Before arriving in the olfactometer, the air passed through a 1-liter flask containing distilled water, an airflowmeter, and two 250 ml flasks; one containing odor components and one containing clean air. All pieces of the system were connected together by plastic tubing (Masterflex® 06409-17 Tygon®). Because *M. hyperodae* were attracted by light, the olfactometer was deposited in a black box that had one side made of translucent white plastic. Once lighted, the white plastic produced a diffused light that stimulated *M. hyperodae* movement. The light was placed beside the box.

Microctonus hyperodae females were used once. For each replication, a plastic capsule containing one *M. hyperodae* female was opened and inserted into the common tube of the olfactometer. Then, the top of the box was closed, and the position of the *M. hyperodae* female in the olfactometer was established each minute for a maximum of five minutes. The *M. hyperodae* female was considered to have made a choice when it crossed the line indicated on an arm within the five minutes. As most of the female *M. hyperodae* made a choice within five minutes, those that stayed in the capsule in the common tube or at the junction of the two arms after five minutes were considered as non-responding individuals. For each treatment, 40 replications (e.i. 40 *M. hyperodae* females that made a choice) were conducted, and odor sources were compared with clean air. To avoid bias, the odour sources were tested 20 times on the left side and 20 times on the right side. A maximum of 15 replications was performed before washing the olfactometer. The olfactometer and flasks were washed with Sparkleen Soap Fisherhand ®, and plastic tubing were changed when treatments changed. Results were statistically analyzed with X^2 tests and preference was considered significant when the test indicated a distribution significantly deviating from 20:20 at $P < 0.05$.

Odors tested.

In a first series of experiments, we tested the response of *M. hyperodae* to different species of Coleoptera adults along with their feces: *L. oregonensis*, *L. sparsus*, *N. flavicaudis* and *C. maculatus*. Depending of the species, between five and 16 adults (for a total of 0,05 g) of both sexes were used. The insects were inserted in a 250 ml flask along with a cup filled with a cotton soaked with water for approximately 72 h; a screen covered the top of the flask to prevent any escapes. Then, the flask containing the insects with their feces was connected to the olfactometer system and compared with clean air. Each species with their feces was tested separately. After a day of experimentation, insects were reintegrated in the rearing; therefore, insects may have been used more than once, as several days of experimentation were needed to complete 40 replications.

Since *M. hyperodae* responded to *L. oregonensis* with its feces, two additional treatments were tested to determine whether *M. hyperodae* responded to the kairomones emitted by *L. oregonensis* adults or by its feces. For the treatment with *L. oregonensis* adults, between 5-7 *L. oregonensis* adults of both sexes were kept unfed in a Petri dish 1-4 h before the experiment. They were then inserted in a 250 ml flask and tested against clean air. During the experiment, the flask containing *L. oregonensis* adults was regularly changed to prevent any accumulation of feces in the flask. For the treatment with *L. oregonensis* feces without *L. oregonensis* adults, the manipulations were the same as in the treatment with *L. oregonensis* adults along with their feces, except that *L. oregonensis* adults were removed from the flask before the experiment.

When testing plant odors, only the vegetative part of the plants was used. For *L. multiflorum*, 1 g and 4 g of leaves were used in two series of experiments. Leaves of *L. multiflorum* were wrapped in Parafilm®. For *D. carota*, 1 g of a leaf of 4-8 true-leaf stage was used. Because the *D. carota* leaves wilted rapidly, they were inserted in a cup filled with a soaked cotton instead of being wrapped in a Parafilm®.

Results

Microctonus hyperodae responded significantly to the odor of *L. oregonensis* with its feces ($X^2 = 6,4$; $p < 0,05$), but did not respond to the odors of *L. sparsus* ($X^2 = 0$; $p > 0,05$), *N. flavicaudis* ($X^2 = 0,1$; $p > 0,05$) and *C. maculatus* with their feces ($X^2 = 0,1$; $p > 0,05$) (Fig 3.1). When *L. oregonensis* and its feces were tested separately, *M. hyperodae* responded to the odor of *L. oregonensis* adults without feces ($X^2 = 6,4$; $p < 0,05$), but did not respond to the odor of *L. oregonensis* feces alone ($X^2 = 0$; $p > 0,05$) (Fig 3.1). No significant response was obtained with 1 g of *L. multiflorum* leaves ($X^2 = 0,4$; $p > 0,05$), 4 g of *L. multiflorum* leaves ($X^2 = 0,9$; $p > 0,05$) and 1 g of *D. carota* leaf ($X^2 = 0$; $p > 0,05$) (Fig 3.1).

Discussion

Microctonus hyperodae females responded only to odor components of *L. oregonensis* and did not respond to odor components of *L. sparsus* and *N. flavicaudis*, although these species are parasitized in small arena tests (Boivin unpublished). Similarly, in small arena tests with New Zealand Curculionidae species, *M. hyperodae* attacked nine species including *L. bonariensis* (Goldson *et al.* 1992; Barratt *et al.* 1997), but with the exception of a single parasitoid found once on *I. aequalis* and once on *S. lepidus*, under field conditions it only parasitized *L. bonariensis* (Barratt *et al.* 1997). Host movement is an important cue used by many euphorine parasitoids in the final stage of host selection (Tobias 1965; Richerson & Deloach 1972; Shaw 1988), and there is evidence that *M. hyperodae* also uses host movement as a host selection cue (McNeill *et al.* 1999; Cournoyer & Boivin unpublished). As host movement is probably less specific than host kairomones (Dippel & Hilker 1998), it is likely that the wider host range observed in small arena tests compared to those observed in olfactometer tests and in fields is due to the artificial conditions of the arena tests, in which long-range host location processes are by-passed, and only short range cues such as host movement are involved.

Species phylogenetically close to each other are expected to have similar odor components because they have evolved from a common ancestor, and generally have similar ecology. However, it appears that phylogenetic and ecological distances between hosts species play a minor role in host location by *M. hyperodae*. Indeed, *L. oregonensis* and *L. sparsus* are two phylogenetically close species with a similar ecology (Huber *et al.* 1997; Boivin 1999), but *M. hyperodae* only responded to *L. oregonensis* kairomones and did not respond to *L. sparsus* kairomones. Moreover, in South America, *M. hyperodae* parasitizes *L. bonariensis*, but not the three other species that co-exist with *L. bonariensis* and are phylogenetically close to *L. bonariensis*: *Listronotus ruber* Hustache, *Listronotus minutus* Blanchard and *Listronotus cyrticus* Desbrochers (Loan & Llyod 1974). Similarly, many Brachycerinae species that co-exist with *L. bonariensis* in New Zealand are not parasitized by *M. hyperodae* (Goldson *et al.* 1992; Barratt *et al.* 1997).

However, contrary to *L. sparsus*, *L. oregonensis* was the rearing host of the *M. hyperodae* individuals used in the tests. Laboratory rearing may select for parasitoids more adapted to the rearing conditions, and subsequently increases the response of the parasitoid to the rearing host (Simmonds 1944; Weseloh 1981; Powell & Wright 1988). Thus, laboratory rearing may have selected for *M. hyperodae* that were able to locate *L. oregonensis*, as the *M. hyperodae* used in the experiments have completed between 35 and 45 generations with *L. oregonensis*. During that period, the parasitism rate of *M. hyperodae* on *L. oregonensis* increased from less than 5% in the first few generations to near 15% from the 10th until the 45th generation (Boivin unpublished data).

Since *M. hyperodae* was reared on *L. oregonensis*, we cannot rule out the possibility that the response of *M. hyperodae* to *L. oregonensis* kairomones has been increased by larval or early adult experiences. Although larval conditioning is not well supported by empirical data, there is evidence that early adult experiences with larval environment can change host selection behaviour of parasitoids (Corbet 1985; Hérard *et al.* 1988; Bjorksten & Hoffmann 1995; van Emden *et al.* 1996; Bjorksten & Hoffmann 1998; Vinson 1998). According to the Chemical legacy hypothesis (Corbet 1985), this may occur when chemical traces from the larval stage modify the adult chemosensory responsiveness and therefore its response to host related stimuli.

When *L. oregonensis* and its feces were tested separately, *M. hyperodae* responded to *L. oregonensis* adults only, suggesting that most of the kairomones that attracted *M. hyperodae* came from the integument of *L. oregonensis* or from sexual pheromones released by *L. oregonensis* adults. The use of host integument kairomones has been demonstrated in the Euphorinae *Perilitus coccinellae* Schrank, in which coxal secretions of Coccinellidae applied on artificial host models increased the number of pursuits and stances made by the parasitoid (Richerson & DeLoach 1972). Other parasitoids of mobile stages also use host integument kairomones for host selection (Jones *et al.* 1971; Arthur 1981). However, these kairomones have generally a low volatility, and therefore, a low detectability at long distance (Schmidt 1974; Vet & Dicke 1992). For parasitoids of adult stage, this reliability-detectability problem could be solved by using more detectable cues such as host pheromones. Although no example of a parasitoid using pheromones of Curculionidae as kairomones exists, the Tachinidae *Trichopoda pennipes* Fay is attracted by male pheromones of its host *Nezara viridula* L. (Mitchell & Mau 1971). Moreover, sexual pheromones are common in Curculionidae species (Smart *et al.* 1994; Eller *et al.* 1994; Innocenzi *et al.* 2001; Leskey & Prokopy 2001), and therefore, could have been involved in the response of *M. hyperodae* to *L. oregonensis* adults.

Since hosts represent only a small component of a complex environment, host kairomones may be difficult to detect for a parasitoid. Plant synomones are the stimulus most frequently used by parasitoids when no host kairomone is detected (Vinson 1976; Vet & Dicke 1992). Moreover, as *L. bonariensis* is specialized on Gramineae, it was expected that *M. hyperodae* would respond innately to Gramineae, but *M. hyperodae* did not respond to any plant synomones in the experiments. As the *M. hyperodae* used in the experiments were naive females, it is possible that *M. hyperodae* did not respond to plant cues because these have to be learned or because naive females respond to volatiles from damaged plants; such behaviors have been observed in several parasitoid species (McAuslane *et al.* 1990; Du *et al.* 1996; Steidle & Schöller 1997; Morgan & Hare 1998). Another possibility is that *M. hyperodae* is guided by Gramineae synomones only when flying, and did not respond to *L. multiflorum* because it was walking in the olfactometer, and consequently, was in a host location mode rather than in a host habitat location mode. Further experiments with a flight tunnel could confirm this.

Listronotus oregonensis is an important pest of carrots in North America (Boivin 1999). Therefore, the response of *M. hyperodae* to *L. oregonensis* kairomones can be considered as an encouraging result for its use in biological control because one may expect that *M. hyperodae* will locate *L. oregonensis* in the field using host kairomones.

Moreover, as most of the parasitism pressure on *L. oregonensis* is achieved by egg parasitoids (Boivin 1999), one may expect that *L. oregonensis* defence mechanisms against parasitoids of adult stages are not efficient, and therefore, *M. hyperodae* should take advantage of this situation.

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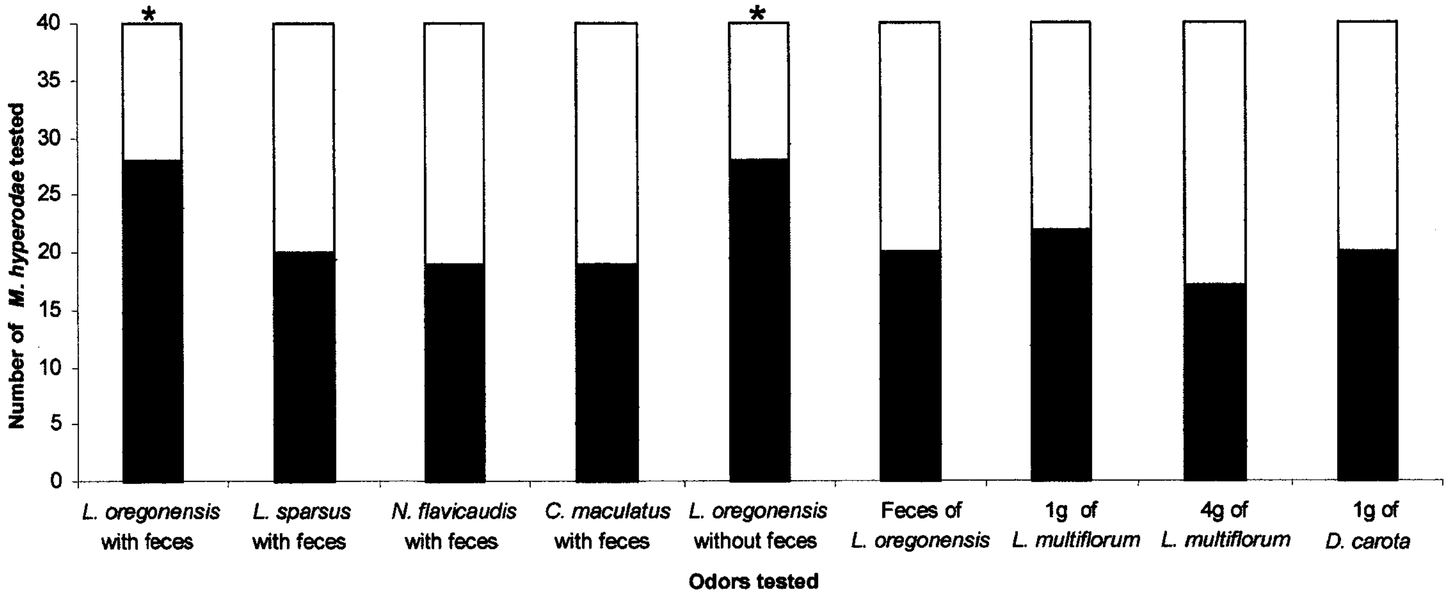

Figure 3.1 : Choice between clean air (white columns) and various odor components (black columns) by *M. hyperodae* females in a Y-tube olfactometer. For each treatment, 40 *M. hyperodae* females were used. The asterisk indicates a response significantly different from 20:20 (X^2 tests, $p<0,05$).

Connecting text

In the preceding chapter, the response of *M. hyperodae* females to volatile infochemicals from different potential hosts and host plants was evaluated using an olfactometer under laboratory conditions. *Microctonus hyperodae* females responded to *L. oregonensis* adult kairomones suggesting that host kairomones are important cues for host selection of *M. hyperodae* females.

However, Curculionidae adults are mobile hosts, and even if host kairomones are detected, mobile hosts may walk away and escape from their parasitoids. Therefore, it was hypothesized that, in addition to host kairomones, *M. hyperodae* females should use host movement as a host selection cue. In the following chapter, this hypothesis was tested using a small arena under laboratory conditions.

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IV. SHORT DISTANCE CUES USED BY THE ADULT PARASITOID *MICROCTONUS HYPERODAE* LOAN (HYMENOPTERA : BRACONIDAE, EUPHORINAE) FOR HOST SELECTION.

Abstract

Host selection by female parasitoids can be divided into three steps: host habitat location, host location and host acceptance, and is generally achieved following the detection of host related infochemicals (chemical cues). Female parasitoids use these infochemicals in a hierarchic way that brings them always closer to their hosts. When they are on a host plant of their host, female parasitoids generally use host by-product kairomones for host location and host kairomones for host acceptance. However, adult hosts may be far from their by-products and, even if host kairomones are detected, they can walk away and escape from their parasitoids. It was hypothesized that in addition to using host kairomones, parasitoids of the adult stage should use host movement for host selection. In this paper, the effect of *Listronotus oregonensis* LeConte (Coleoptera : Curculionidae) adult sex, feces and movement on host selection behavior of *Microctonus hyperodae* Loan (Hymenoptera : Braconidae; Euphorinae) females was evaluated using a small arena under laboratory conditions. The *L. oregonensis* sex did not affect the host selection behavior of *M. hyperodae*. However, the presence of host feces in the arena decreased the number of weevil antennations suggesting that host feces play a role in host recognition of *M. hyperodae*. Furthermore, since *M. hyperodae* stopped less frequently near immobile *L. oregonensis* than near walking ones and the latter were pursued by *M. hyperodae*, it is likely that host movement is used as a short distance host location cue by *M. hyperodae*. It also appears that host movement is the stimulus that elicits oviposition by *M. hyperodae* females, as most oviposition attempts were when *L. oregonensis* adults were moving. The adaptive implications of these results are discussed.

Key words : host selection, host acceptance, host movement, host feces, kairomone, new association, ethogram, Curculionidae, Euphorinae, adult parasitoid

Introduction

Host selection behavior of female parasitoids is divided into three steps: host habitat location, host location and host acceptance (Doutt 1959; Vinson 1976), and is achieved following the detection of various host related stimuli. How female parasitoids use these stimuli for host selection depends on their reliability and detectability (Vet & Dicke 1992). The reliability of a stimulus indicates the host presence, its accessibility and its suitability while its detectability indicates the degree to which it can be perceived (Vet & Dicke 1992). Generally, host plant stimuli (synomones) have low reliability but high detectability while host stimuli (kairomones) have high reliability but low detectability (Vet & Dicke 1992). To solve this reliability-detectability problem, female parasitoids use a hierarchic search in which the more detectable stimuli, such as host plant synomones, are used for host habitat location, and the more reliable stimuli, such as host kairomones, are used for host location and acceptance (Vinson 1976).

When female parasitoids are on host plants of theirs hosts, they generally use kairomones from host by-products and from the host itself for host location and acceptance (Arthur 1981; Weseloh 1981). Encounters with host by-product kairomones provoke a change in the locomotory behaviors of female parasitoids, keeping them on the infested plant and increasing their probabilities of discovering hosts (Vinson 1998) while kairomones emitted by the host itself are used as contact kairomones for host acceptance (Vinson 1998).

Female parasitoids may also use visual cues such as color, shape and host movement when at close distance from a host (Arthur 1981; Weseloh 1981; Godfray 1994). Host movement is frequently reported in the literature as an important cue used by Euphorinae parasitoids in the last steps of host selection (Loan & Holdaway 1961; Shaw 1988; Godfray 1994; McNeill *et al.* 1999), but only one study (Richerson & DeLoach 1972) has quantified host movement. In contrast with egg and pupal parasitoids, Euphorinae parasitoids have to find mobile hosts that may be far from their nutrition sites and their by-products. Moreover, host acceptance of a mobile host may be difficult, and even risky, for a female parasitoid because the host may walk away and escape, or injure the female parasitoid when moving. It was hypothesized that, in addition to using host related infochemicals, parasitoids of mobile stage, such as Euphorinae, should use host movement for host location and host acceptance.

In 1996, a new host-parasitoid association between the carrot weevil (*Listronotus oregonensis* LeConte) and the Euphorinae *Microctonus hyperodae* Loan was created in the laboratory in Canada (Boivin 1999). *Microctonus hyperodae* reproduces by thelitokous parthenogenesis (Loan & Lloyd 1974), and is a solitary koinobiont endoparasitoid that attacks Curculionidae adults of the Brachycerinae subfamily (Loan & Lloyd 1974; Goldson *et al.* 1992; Barratt *et al.* 1997). *Microctonus hyperodae* is native to South America where its only known host is the Argentine stem weevil (*Listronotus bonariensis* Kuschel) (Loan & Lloyd 1974). In an attempt to control the population of *L. bonariensis* by biological control, *M. hyperodae* was introduced to New Zealand in 1991, where, in addition to *L. bonariensis*, it has on one occasion parasitized *Irenimus aequalis* Broun and *Sitona lepidus* Gyllenhal under field conditions (Barratt *et al.* 1997). Under laboratory conditions, *M. hyperodae* showed a wider host range than under field conditions. Seven other Brachycerinae species (*Nicaeana cervina* Broun, *Nicaeana cinerea* Broun, *Irenimus aemulator* Broun, *Irenimus egens* Broun, *Irenimus stolidus* Broun, *Irenimus* sp.3 and *Catoptes robustus* Sharp) were parasitized, but again, parasitism was lower than for *L. bonariensis* (Goldson *et al.* 1992; Barratt *et al.* 1997). Under similar laboratory tests with North American Curculionidae species, *M. hyperodae* parasitized five other species (*Listronotus sparsus* Say, *Listronotus maculicollis* Kirby, *Nedyus flavicaudis* Boheman, *Ceutorhynchus irysimi* Fabricius and *Gymnetron tetrum* Fabricius) in addition to *L. oregonensis* (Boivin unpublished), but responded only to the odor components of *L. oregonensis* adults and did not respond to odor components of *L. sparsus* and *N. flavicaudis* adults in olfactometer tests (Cournoyer & Boivin submitted). The wider host range observed in arena tests compared to those observed in olfactometer tests and in fields is probably due to the artificial conditions of the arena tests, in which long-range host location processes are by-passed, and only short range cues such as host movement are involved.

In this paper, the host selection behavior of *M. hyperodae* females when at close distance to a *L. oregonensis* adult was evaluated using a small arena under laboratory conditions.

Materials and Methods

Listronotus oregonensis was reared at 25 ± 1°C, with a photoperiod of 16L: 8D and 40-60 % R. H. following the technique of Martel *et al.* (1975). *Microctonus hyperodae* was reared in an incubator at 25 ± 1°C, with a photoperiod of 16L: 8D and 60-90 % R. H. At emergence, each *M. hyperodae* female was placed in a Solo cup (250 ml) with ten sexually mature *L. oregonensis* from the laboratory rearing. A piece of carrot root 10 cm long and two size 3 (ca. 300 µl) Beem ™ polyethylene embedding capsules filled with cotton, one with a honey solution (50% honey 50% water) and one with water, were placed in the Solo cup. A filter paper in the bottom of the Solo cup absorbed moisture and feces. The Solo cups were placed in an incubator at 25 ± 1°C, with a photoperiod of 16L: 8D and 60-90 % R. H. for one week. The *L. oregonensis* adults were then removed and placed on screened-bottom Solo cup containers (225 ml) in which a piece of carrot root was added. The screened-bottom Solo cup was fitted into another plastic container (600 ml Genpak) in which a thin layer of moist soil and a filter paper were added. Fifteen to 30 days later, *M. hyperodae* final instar larvae emerged from *L. oregonensis*, passed through the screen, and dropped to the soil to pupate. The pupae were removed from the plastic container and placed on filter papers in Petri dishes (35 X 10 mm). One or two pupae were placed per Petri dish, and the filter paper was moistened with water to prevent pupae from drying out. Petri dishes were sealed with Parafilm®, and placed in an incubator at 25 ± 1°C, with a photoperiod of 16L: 8D and 60-90 % R. H. until adult emergence. *Microctonus hyperodae* females were supplied with water at emergence and kept separately in Petri dish until the day they were tested. All *M. hyperodae* females used in the experiments were naive females, less than three days old and daughters of 20 females of the strain UR21 that originates from Colonia in Uruguay (Goldson *et al.* 1990).

General experimental procedures

The experiments were performed in the laboratory at 22 ± 2°C and 60-90 % R. H. The top of a Petri dish (35 X 10 mm), in which four cardboard walls were fixed to make a rectangle of 30 X 22 mm, was used as arena. The top of the Petri dish was placed on an inverted plastic cup on which a filter paper was fixed. For each replication, one *M. hyperodae* female and one *L. oregonensis* adult from the laboratory rearing were inserted together in the arena. As M.hyperodae for ten minutes. To insert the *M. hyperodae* female in the arena, a Petri dish containing a *M. hyperodae* female was opened in a plastic cup covered with a screen allowing the female parasitoid to exit the Petri dish, and to fly on a side of the container or on the screen. Then, the *M. hyperodae* female was inserted in a size 3 (ca. 300 µl) Beem ™ polyethylene embedding capsule and immediately deposited on the filter paper of the arena, along with a *L. oregonensis* adult, by tapping delicately on the capsule containing the *M. hyperodae* female. The top of the Petri dish was then replaced on the filter paper to avoid any escape of the insects, and the replication was started. A camera was placed above the arena, and each replication was recorded on a VHS videotape for further examination. *Listronotus oregonensis* adults and *M. hyperodae* females were used once.

Effect of *L. oregonensis* adult sex and feces on host selection behavior of *M. hyperodae* females

A first series of experiments was performed to determine the effect of *L. oregonensis* adult sex and feces on host selection behavior of *M. hyperodae* females. Thirty replications without and 30 replications with *L. oregonensis* adult feces were performed. For the replications without feces, one *M. hyperodae* female was inserted in the arena along with one *L. oregonensis* adult. For the replications with feces, one *M. hyperodae* female was inserted in the arena along with one *L. oregonensis* adult and 0,004 g of *L. oregonensis* adult feces that were inserted in the arena by scratching the feces of a filter paper of a jar containing *L. oregonensis* adults.

Of the 60 *L. oregonensis* adults used in these experiments, 41 were sexed forming four treatments: *L. oregonensis* female without feces (n=10), *L. oregonensis* male without feces (n=5), *L. oregonensis* female with feces (n=12) and *L. oregonensis* male with feces (n=14). The effect of *L. oregonensis* adult sex and feces on host selection behavior of *M. hyperodae* females was determined by comparing the mean number of *M. hyperodae* female behaviors in each treatment using two-way ANOVA tests. The *M. hyperodae* female behaviors compared were:

Stop: when the *M. hyperodae* female stayed at the same place for more than one sec.
Walk: when the *M. hyperodae* female walked.

Fly: when the *M. hyperodae* female flew.

Front grooming: when the *M. hyperodae* female groomed its head or its front legs with its front legs.

Back grooming: when the *M. hyperodae* female groomed its ovipositor or its back legs with its back legs.

Feces antennation: when the *M. hyperodae* female touched *L. oregonensis* feces with its antennae.

Weevil antennation: when the *M. hyperodae* female touched the *L. oregonensis* adult with its antennae.

Wait: when the *M. hyperodae* female stopped near the *L. oregonensis* adult more than one sec with its antennae directed through the *L. oregonensis* adult.

Pursuit: when the *M. hyperodae* female followed the *L. oregonensis* adult, reversed when the *L. oregonensis* adult moved toward it, approached the *L. oregonensis* adult after a « wait » or went round the *L. oregonensis* adult.

Oviposition attempt: when the *M. hyperodae* female bent its abdomen beneath its thorax when close to the *L. oregonensis* adult.

The effect of host feces on the behavioral sequence of *M. hyperodae* females was further analyzed using two ethograms. Because host sex had no significant effect on the mean number of none of the *M. hyperodae* female behaviors measured, the first ethogram (n=15) was done by merging the data of the treatments *L. oregonensis* females without feces (n=10) and *L. oregonensis* males without feces (n=5) while the second ethogram (n=26) was done by merging the data of the treatments *L. oregonensis* females with feces (n=12) and *L. oregonensis* males with feces (n=14).

Effect of *L. oregonensis* adult movement on host selection behavior of *M. hyperodae* females

Oviposition attempts were observed in 11 of the 60 replications performed, and for these 11 replications, *L. oregonensis* adult movement was divided into four categories according to their intensity:

Immobile: no movement.

Small movement: when the *L. oregonensis* adult moved its antennae and/or its head and/or its leg(s) without walking; frequently observed when the *L. oregonensis* adult cleaned its antennae with its front legs.

Body movement: when the *L. oregonensis* adult moved its body entirely without walking; observed when the *L. oregonensis* adult rotated on itself or tried to climb on the side of the arena.

Walking: when the *L. oregonensis* adult walked.

Using X^2 tests, the effect of host movement on host selection behavior of *M. hyperodae* was determined by comparing the percentage of *M. hyperodae* waits, weevil antennations and oviposition attempts for each *L. oregonensis* movement to the percentage of *M. hyperodae* waits, weevil antennations and oviposition attempts expected for each *L. oregonensis* movement during the 11 replications. The percentages of *M. hyperodae* waits, weevil antennations and oviposition attempts expected were the percentages of time allowed by *L. oregonensis* to each category of movement during the 11 replications. Since waits were frequently observed after a pursuit or a weevil antennation, and therefore when *M. hyperodae* was already close to *L. oregonensis*, only waits done after a walk were used to verify if *M. hyperodae* was attracted from a distance by host movement.

To determine whether host movement influenced the part of the host body (head, abdomen-thorax, anus) aimed by the oviposition attempt, *M. hyperodae* oviposition attempts were noted according to the part(s) of the *L. oregonensis* body (immobile, head and/or front legs (anterior parts), all the body) that were moving during the oviposition attempt. The proportions of oviposition attempts aimed at each *L. oregonensis* body part when the weevil was immobile, was moving its anterior parts or was moving its body entirely were compared using X^2 tests. A preference for a part of the *L. oregonensis* body was considered significant when the test indicated a distribution significantly deviating from 1/3-1/3-1/3 at $P< 0,05$.

Results

Effect of *L. oregonensis* adult sex and feces on host selection behavior of *M. hyperodae* females

The host sex and feces had no significant effect on the mean number of most of the *M. hyperodae* female behaviors measured (p > 0,05), but there was significantly less weevil antennations in the presence of host feces than in the absence of feces (p < 0,05), and antennations on host feces were observed (Table 4.1). No interaction between host sex and feces was obtained for any of the *M. hyperodae* females behaviors measured (p > 0,05) (Table 4.1).

When the *M. hyperodae* female was inserted in the arena along with a *L. oregonensis* adult and without feces, the female parasitoid was generally very active and made a series of short walks and stops with its antennae moving in the air (Fig 4.1). *Microctonus hyperodae* rarely walked directly toward the *L. oregonensis*, but when close to it, *M. hyperodae* stopped and waited with its antennae moving in the air, or less frequently, antennated it, after which *M. hyperodae* generally left (walked). However, if *L. oregonensis* was walking, a series of pursuits and waits were engaged, in which antennations and oviposition attempts were also observed. After an oviposition attempt, the *M. hyperodae* female waited near the *L. oregonensis* and resumed pursuit.

Two types of grooming were observed: front and back grooming, and they were generally followed by a walk (Fig 4.1). Flights were also observed, and occurred generally when the *L. oregonensis* « charged » on *M. hyperodae*. After a flight, *M. hyperodae* walked or stopped.

The behavioral sequence of *M. hyperodae* females in presence of both a *L. oregonensis* adult and its feces (Fig 4.2) had a similar pattern, though some differences were observed. An important difference was that *M. hyperodae* antennated *L. oregonensis* feces and then walked or stopped. Also, there were more stops after a walk or a grooming and more groomings after a stop in presence of *L. oregonensis* feces. Furthermore, the propensity to leave (walk or fly) after a wait, a weevil antennation, or a pursuit was lower in presence of *L. oregonensis* feces.

Effect of *L. oregonensis* adult movement on host selection behavior of *M. hyperodae* females

L. oregonensis was immobile more than half the time and small movement, body movement and walking represented between 11 and 19% of its activity (Fig. 4.3A). When compared to the percentage of time allocated by *L. oregonensis* to each category of movement, there were no significant differences in the the percentages of waits when *L. oregonensis* were doing small movement ($X^2 = 2{,}21$; $p > 0{,}05$) or body movement ($X^2 = 1{,}16$; $p > 0{,}05$), but there were less waits than expected when *L. oregonensis* were immobile ($X^2 = 8{,}38$; $p < 0{,}01$) and more waits when *L. oregonensis* were walking ($X^2 = 14{,}57$; $p < 0{,}01$) (Fig 4.3B). There were no significant differences in the percentages of weevil antennations made on an immobile *L. oregonensis* ($X^2 = 0{,}07$; $p > 0{,}05$) and on a *L. oregonensis* doing a body movement ($X^2 = 0{,}28$; $p > 0{,}05$), but there were more weevil antennations than expected on a *L. oregonensis* doing a small movement ($X^2 = 5{,}95$; $p < 0{,}05$), and less weevil antennations on a walking *L. oregonensis* ($X^2 = 1{,}40$; $p < 0{,}05$) (Fig. 4.3C). There were significantly less oviposition attempts than expected when the *L. oregonensis* was immobile ($X^2 = 38{,}78$; $p < 0{,}01$), but more when the *L. oregonensis* was doing a small movement ($X^2 = 8{,}63$; $p < 0{,}01$) or a body movement ($X^2 = 19{,}51$; $p < 0{,}01$), and no significant difference when the *L. oregonensis* was walking ($X^2 = 1{,}59$; $p > 0{,}05$) (Fig. 4.3D).

The four oviposition attempts by *M. hyperodae* on immobile *L. oregonensis* were directed at their heads (Table 4.2). When only the anterior parts of the *L. oregonensis* body were moving, there was significantly more oviposition attempts directed to its head than to its abdomen-thorax or its anus ($X^2 = 13{,}5$; $p < 0{,}01$) (Table 4.2). When all the *L. oregonensis* body was moving, there were significantly more oviposition attempts directed at its head and its anus than at its abdomen-thorax ($X^2 = 11{,}64$; $p < 0{,}01$) (Table 4.2). An additional X^2 test was done to verify whether the proportion of oviposition attempts directed at the *L. oregonensis* head when all its body was moving was significantly different to the proportion of oviposition attempts directed at its anus, but there was no significant difference ($X^2 = 0{,}82$; $p > 0{,}05$).

Discussion

Effect of *L. oregonensis* adult sex and feces on host selection behavior of *M. hyperodae* females

When close to a *L. oregonensis*, the host selection behavior of *M. hyperodae* females is not affected by the sex of the adult. This confirms results obtained with *M. hyperodae* and *L. bonariensis* under laboratory and fields conditions in New Zealand (Goldson *et al.* 1995, 1998). Furthermore, as *L. oregonensis* females are slightly larger (6,5 ± 0,3 mm in length and 0,29 mg in weight) than *L. oregonensis* males (6,0 ± 0,3 mm in length and 0,23 mg in weight) (Martel *et al.* 1976), it appears that small difference in host size do not influence host selection of *M. hyperodae*.

Host by-products, such as host feces, have generally low volatility and are used as contact kairomones by female parasitoids when they are on the host plant of their hosts. When a female parasitoid encounters these kairomones, they provoke a change in its locomotory behaviors keeping the parasitoid female on the infested host plant and increasing its probability of discovering hosts (Vinson 1998). Accordingly, *M. hyperodae* antennated *L. oregonensis* adult feces in the arena but did not respond to this odor component in olfactometer tests (Cournoyer & Boivin submitted) suggesting that host feces has low volatility. Moreover, it is possible that host feces had an arrestment effect on the locomotory behavior of *M. hyperodae* females as, in presence of *L. oregonensis* feces, *M. hyperodae* females stopped more frequently after a walk and a grooming. However, more groomings were observed, and it is likely that *M. hyperodae* females were contaminated by feces particles and stopped more frequently to groom their body. Further experiments are needed to verify whether the host feces increase the probabilities of finding hosts by *M. hyperodae*.

Nevertheless, the presence of *L. oregonensis* feces in the arena decreased the number of weevil antennations and the propensity of *M. hyperodae* to leave after having waited near to, pursued or antennated a *L. oregonensis*. These results suggest that host feces serve as a host recognition cue for *M. hyperodae* and influence the host acceptance of this parasitoid. As *M. hyperodae* responded to *L. oregonensis* adult kairomones (Cournoyer & Boivin submitted) and sometimes stopped in front of *L. oregonensis* feces for several seconds after having antennated them, adult and feces of *L. oregonensis* may share common odor components and *M. hyperodae* may stop in front of host feces as if it was a host. A similar host acceptance behavior was observed in the larval parasitoid *Campoletis sonorensis* Cameron where several *Heliothis zea* by-products elicited oviposition of female parasitoids (Schmidt 1974).

Effect of *L. oregonensis* adult movement on host selection behavior of *M. hyperodae* females

Adult hosts may be far from their by-products, and even when kairomones from the host itself are detected, the host may walk away and escape the female parasitoid. Therefore, parasitoids of the adult stage rely on more directional cues when at close distance to their host (Richerson & DeLoach 1972; Shaw 1988; Godfray 1994). As predicted, walking *L. oregonensis* were frequently pursued by *M. hyperodae* females, and *M. hyperodae* stopped less frequently near immobile *L. oregonensis* than near walking ones, suggesting that host movement is used as a host location cue by *M. hyperodae*.

Furthermore, since weevil antennations decreased as the intensity of *L. oregonensis* movement increased, it is likely that host movement is also used as a host recognition cue by *M. hyperodae*. Thus, when *M. hyperodae* detected no *L. oregonensis* movement, it used *L. oregonensis* kairomones and antennated them. Such a hierarchy in the stimuli could be adaptive because in avoiding antennal contacts with moving hosts, *M. hyperodae* decreases the probability of being injured by host movement during host acceptance.

Antennations on immobile hosts may also provoke host movement, and expose an area on the host body for the parasitoid female ovipositor (Tobias 1965). However, only on one occasion a weevil antennation has been followed by an oviposition attempt suggesting that antennations on immobile *L. oregonensis* did not activate host movement, and subsequently, *M. hyperodae* ovipositions. Furthermore, when disturbed, Curculionidae immobilized and dropped on the soil instead of moving (Barratt *et al.* 1995). This behavior may have evolved in Curculionidae as an answer to parasitism risk, and therefore, when *M. hyperodae* antennated a *L. oregonensis*, it may increase the probability that the *L. oregonensis* stopped.

The *L. oregonensis* adult is covered by a cuticle probably impossible to pierce by the thin ovipositor of *M. hyperodae*, and accordingly, most oviposition attempts were directed to the mouth and the anus of *L. oregonensis*. Furthermore, the majority of these oviposition attempts occurred when the *L. oregonensis* was doing a small movement or a body movement, and according to the moving parts of the *L. oregonensis* body, *M. hyperodae* aimed the mouth or the anus of *L. oregonensis*. Thus, it is possible that, in nature, hosts that are feeding and ovipositing could be parasitized more by *M. hyperodae* than immobile and walking ones because the insertion of the ovipositor into the host mouth and anus is easier when the host is feeding and ovipositing respectively.

In addition to facilitating the insertion of the ovipositor into the host, host movement may also serve as a host quality criterion by *M. hyperodae*. Indeed, as a koinobiont parasitoid, larval development of *M. hyperodae* occurs in a living host, and therefore, one may expect that, by parasitizing moving hosts, *M. hyperodae* ensures that only healthy hosts able to support larval development of its progeny are parasitized.

Dietary specialization is an important factor influencing the use of host related stimuli by female parasitoids (Vet & Dicke 1992). Thus, the specialist larval parasitoid *Drino bohemica* Mesn. is attracted by a moving feather in olfactometer, but only in presence of host kairomones suggesting that host movement is used only when host kairomones are detected (Monteith 1956). In contrast, the generalists *Drino inconspicua* Meign (Dippel & Hilker 1998) and *Perilitus coccinellae* Shrank (Richerson & DeLoach 1972) are attracted by any moving object, and host kairomones have little or no effect on host selection of these species. As *M. hyperodae* is a specialist parasitoid of *L. bonariensis* and responds to *L. oregonensis* kairomones (Cournoyer & Boivin submitted), it is possible that host movement is used only in the presence of host kairomones. Indeed, when inserted in the arena, the *M. hyperodae* female rarely walked directly toward the moving *L. oregonensis*, and host movement seemed attractive only at close distance to its host when host kairomones were detected. Such a hierarchic search could be adaptive for *M. hyperodae* because it decreases the probabilities of pursuing and parasitizing unsuitable hosts. Furthermore, it could explain why *M. hyperodae* has been found only twice on species other than *L. bonariensis* in New Zealand. Indeed, it is possible that these two individuals were parasitized « accidentally » by *M. hyperodae* because they were encountered in presence of *L. bonariensis* kairomones.

Microctonus hyperodae was recently released in Canada in order to control *L. oregonensis*, and the use of a hierarchic search, in which host movement serve only in presence of host kairomones, could reduce the risk that *M. hyperodae* enlarges its host range and attacks any moving Curculionidae encountered. Thus, it is possible that only Curculionidae species sharing common odor components with *L. oregonensis* and *L. bonariensis* or those encountered in presence of *L. oregonensis* kairomones should be parasitized by *M. hyperodae*.

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Table 4.1: Effect of *L. oregonensis* adult sex and feces on the mean number of *M. hyperodae* female behaviors; two-way ANOVA tests.

Microctonus hyperodae female behaviors	Treatments (mean number per replicate ± s.e.)				Main effects		Interaction Sex X Feces
	Female N = 22	Male n = 19	Feces n = 26	No feces n = 15	Sex p	Feces p	p
Walk	16,9 ± 3,3	21,2 ± 4,1	18,6 ± 3,6	19,3 ± 3,5	0,58	0,90	0,43
Stop	11,2 ± 2,7	12,8 ± 2,9	13,4 ± 2,8	9,5 ± 2,1	0,90	0,30	0,30
Fly	1,8 ± 0,8	2,2 ± 1,1	2,4 ± 1,0	1,2 ± 0,6	0,97	0,38	0,58
Front grooming	1,3 ± 0,3	1,0 ± 0,21	1,2 ± 0,2	0,9 ± 0,2	0,20	0,26	0,43
Back grooming	1,0 ± 0,3	1,1 ± 0,5	1,4 ± 0,4	0,5 ± 0,2	0,70	0,09	0,42
Feces antennation	0,3 ± 0,2	0,9 ± 0,5	0,9 ± 0,4	-	0,51	-	-
Weevil antennation	1,8 ± 0,7	1,7 ± 0,5	1,0 ± 0,3	3,0 ± 1,1	0,63	0,03	0,63
Wait	8,8 ± 1,4	12,2 ± 2,1	10,2 ± 1,6	10,7 ± 1,9	0,21	0,67	0,84
Pursuit/approach	2,7 ± 0,8	3,9 ± 1,2	3,6 ± 1,0	2,6 ± 0,5	0,63	0,51	0,50
Oviposition attempt	0,6 ± 0,3	0,6 ± 0,3	0,7 ± 0,3	0,5 ± 0,3	0,63	0,52	0,39

Table 4.2: Number of *M. hyperodae* oviposition attempts according to the part of the *L. oregonensis* body aimed by the oviposition attempt and the type of movement executed by *L. oregonensis* during the oviposition attempt. A $p < 0{,}01$ indicates that the proportions of oviposition attempts aimed to the different parts of the *L. oregonensis* body for a type of movement are significantly different of 1/3-1/3-1/3 at $p < 0{,}01$ using X^2 tests.

Listronotus oregonensis adult movement	Part of the body aimed by the oviposition attempt				
	Head	Abdomen -thorax	Anus	X^2	p
Immobile	4	0	0	-	-
Anterior parts	10	1	1	13,50	< 0,01
All the body	11	3	19	11,64	< 0,01

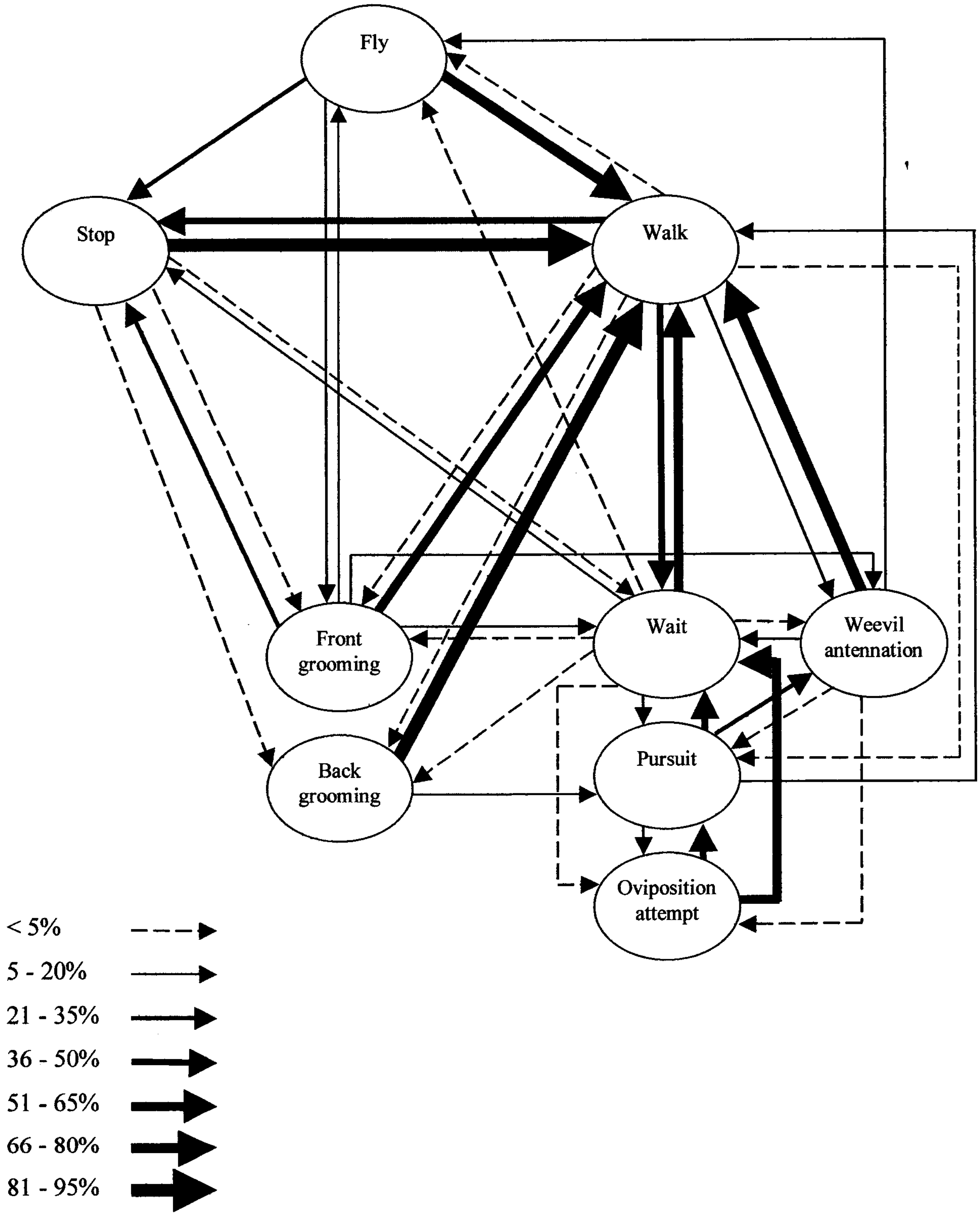

Figure 4.1: Ethogram illustrating the behavioral sequence of *M. hyperodae* females when in an arena with a *L. oregonensis* (female or male) without *L. oregonensis* adult feces (n = 15). For each behavior, the frequencies of the behaviors are represented in percentage by arrows.

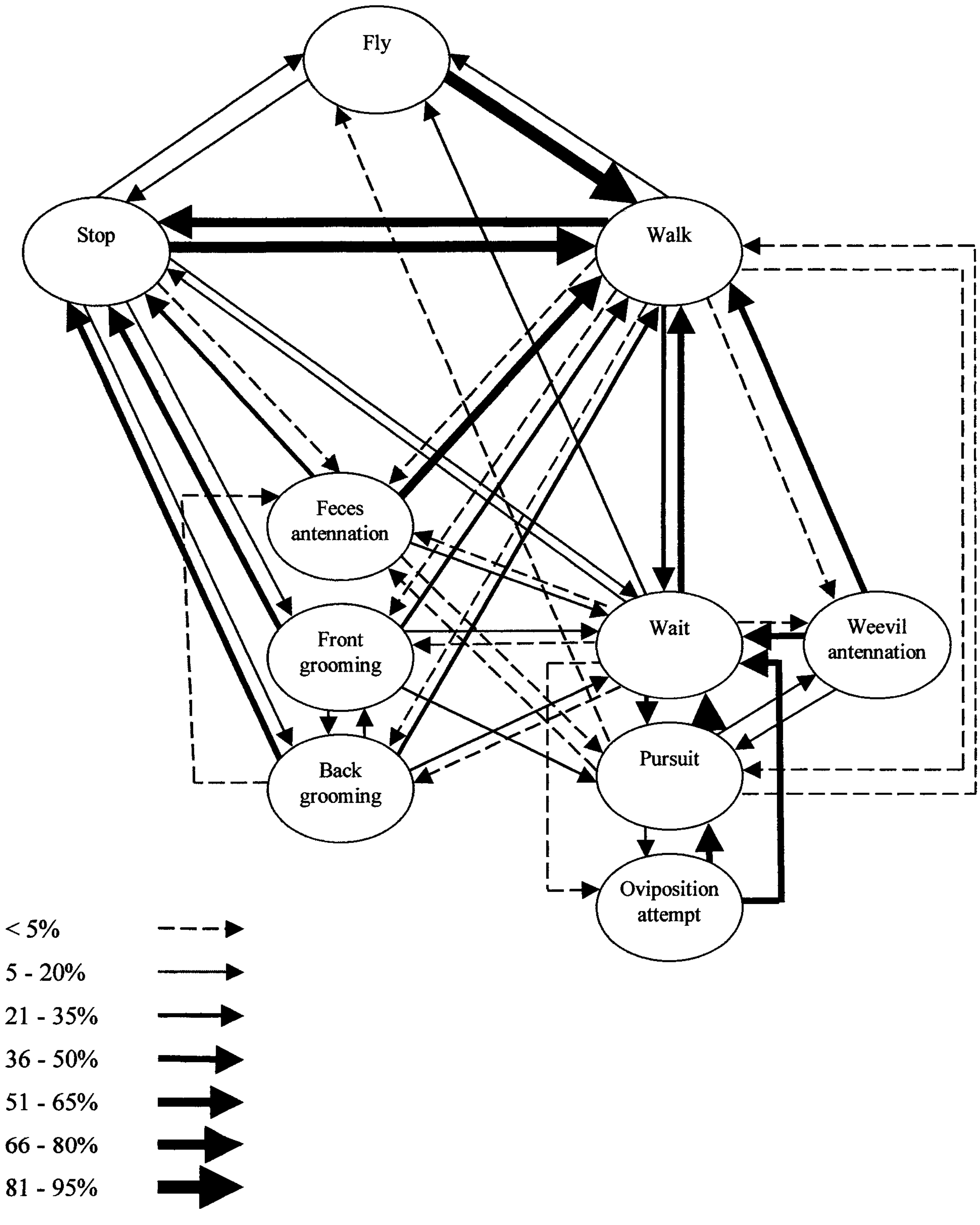

Figure 4.2 : Ethogram illustrating the behavioral sequence of *M. hyperodae* females when in an arena with a *L. oregonensis* (female or male) and *L. oregonensis* adult feces (n = 26). For each behavior, the frequencies of the behaviors are represented in percentage by arrows.

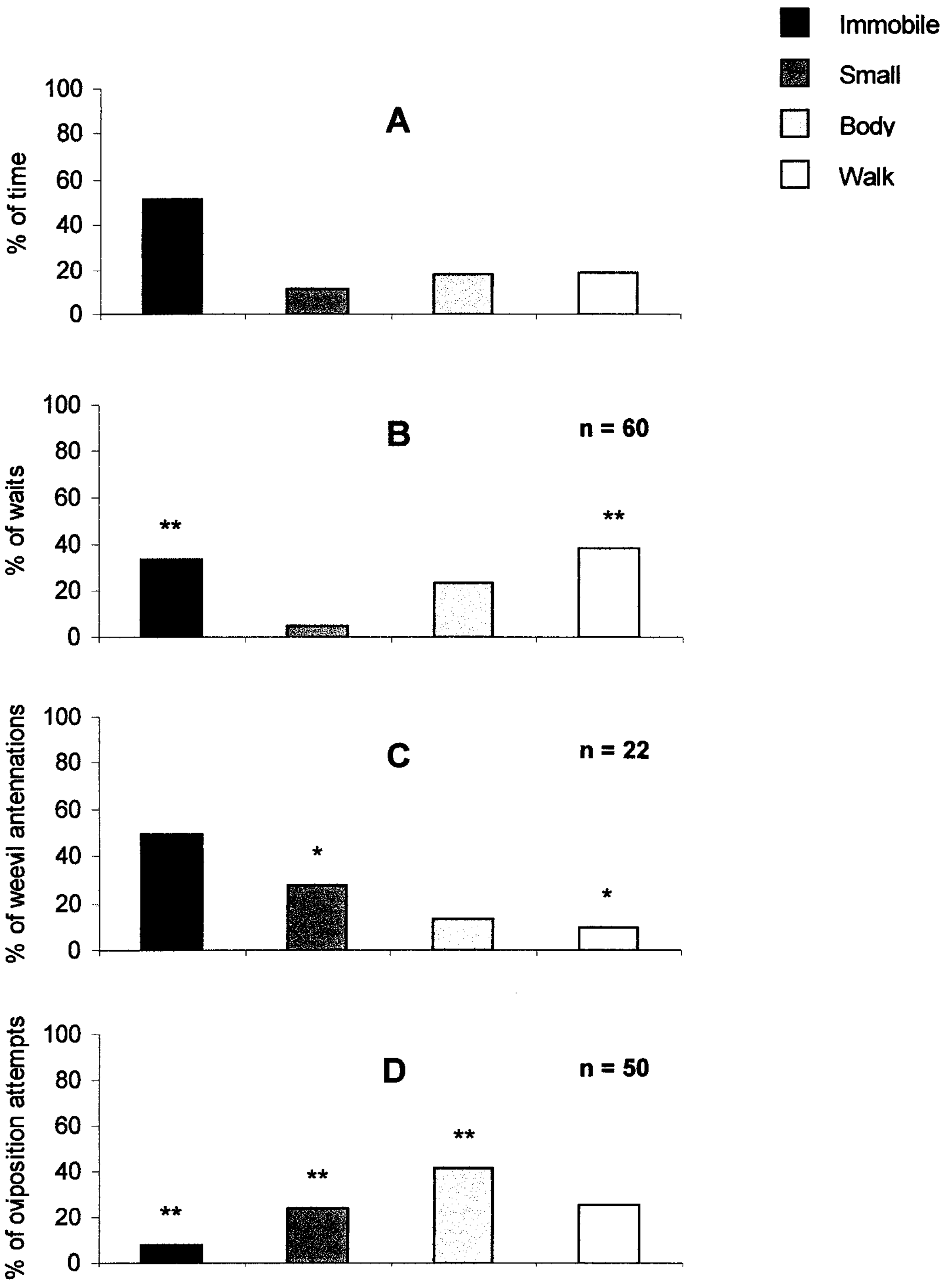

Figure 4.3 : Comparison between (A) the percentage of time allocated by *L. oregonensis* to different categories of movement and the distribution of (B) waits, (C) weevil antennations and (D) oviposition attempts executed by *M. hyperodae* for each of these categories of movement. Columns topped with an asterisk indicate that the percentage of the behavior observed for this category of movement is significantly different from the one expected in (A) using X^2 tests; * = $p < 0,05$; ** = $p < 0,01$.

Connecting text

In the two preceding chapters, the effect of different host-related stimuli on host selection behavior of *M. hyperodae* females was evaluated. It appeared from these two studies that host kairomones and host movement are important stimuli for host selection of *M. hyperodae* females.

However, in contrast with adult hosts, host eggs are inactive and emit few stimuli. Therefore, egg parasitoids should rely on more detectable cues than egg stimuli when searching for host eggs. As *L. oregonensis* eggs are hidden in plant tissue, we hypothesized that *A. victus* females should use kairomones from *L. oregonensis* adults to detect its eggs. In the following chapter, this hypothesis was tested using a small arena under laboratory conditions.

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V. KAIROMONES USED BY THE EGG PARASITOID *ANAPHES VICTUS* HUBER (HYMENOPTERA : MYMARIDAE) WHEN SEARCHING FOR ITS HOST.

Abstract

Host kairomones are frequently used by parasitoids for host location. However, egg kairomones have a low detectability, and therefore, many egg parasitoids rely on more detectable cues, such as host plant synomones and adult host kairomones, for host egg location and use egg kairomones only in the final stages of host selection. *Anaphes victus* Huber (Hymenoptera: Mymaridae) is a solitary egg parasitoid of the carrot weevil *Listronotus oregonensis* LeConte (Coleoptera: Curculionidae). *Listronotus oregonensis* eggs are deposited in cavities made by females in the plant tissue and are covered with a plug made of feces and saliva. As *L. oregonensis* eggs are hidden in plant tissue, we hypothesized that when on a carrot plant, *A. victus* females would use infochemicals related to *L. oregonensis* adults to locate *L. oregonensis* eggs. In this paper, the response of *A. victus* females to different infochemicals related to *L. oregonensis* eggs was evaluated using a small arena under laboratory conditions. Five treatments were tested: *L. oregonensis* eggs extracted from host plant, *L. oregonensis* eggs extracted from artificial substrate, *L. oregonensis* egg plugs, *L. oregonensis* feces and host plant. As expected, *A. victus* females responded to the odor components of *L. oregonensis* egg plugs and feces. Although *A. victus* females also responded to odor components of *L. oregonensis* eggs extracted from host plant, they responded neither to odor components of *L. oregonensis* eggs extracted from artificial substrate nor to host plant suggesting that residues of egg plugs on eggs extracted from host plant have elicited the response of *A. victus* females to this odor component.

Key words: host location, kairomones, egg parasitoid, *Listronotus oregonensis*, *Anaphes victus*, Curculionidae, Mymaridae

Résumé

Chez plusieurs espèces de parasitoïdes, les femelles localisent leurs hôtes en utilisant les kairomones émises par ces derniers. Les œufs des hôtes sont cependant difficiles à détecter, par conséquent, les femelles des parasitoïdes oophages utilisent les synomones émises par la plante hôte et les kairomones émises par l'hôte adulte pour repérer les œufs de l'hôte, et n'utilisent les kairomones émises par les œufs que lors de l'examination de ces derniers. *Anaphes victus* Huber (Hymenoptera : Mymaridae) est un parasitoïde solitaire des œufs du charançon de la carotte *Listronotus oregonensis* LeConte (Coleoptera : Curculionidae). Les œufs de *L. oregonensis* sont pondus dans des cavités creusées par la femelle et recouverts d'un bouchon constitué de fèces et de salive. Étant donné que les œufs de *L. oregonensis* sont cachés dans le tissu végétal de la carotte, nous avons émis l'hypothèse que les femelles *A. victus* devraient utiliser les kairomones émises par les *L. oregonensis* adultes afin de trouver ses œufs sur la carotte. La réponse des femelles *A. victus* à différentes odeurs reliées à *L. oregonensis* a été évaluée dans une arène en laboratoire. Cinq odeurs ont été testées : les œufs de *L. oregonensis* provenant de la plante hôte, les œufs de *L. oregonensis* provenant d'un substrat artificiel, les fèces de *L. oregonensis*, les bouchons de *L. oregonensis* et la plante hôte. Tel que prévu, les femelles *A. victus* ont répondu aux bouchons et aux fèces de *L. oregonensis*. Les femelles *A. victus* ont également répondu aux œufs de *L. oregonensis* extraits de la plante hôte, mais n'ont toutefois pas répondu aux œufs de *L. oregonensis* extraits du substrat artificiel ni à la plante hôte. Ceci semble indiquer que les résidus de bouchons recouvrant les œufs extraits de la plante hôte ont provoqué la réponse des femelles *A. victus* à l'odeur des œufs de *L. oregonensis* extraits du substrat artificiel.

Introduction

Because parasitoids need hosts to complete their life cycle, natural selection is expected to select individuals with abilities to find hosts. The majority of parasitoids find their host by using infochemicals (chemical cues) (Godfray 1994), and how they use these for host selection depends on the reliability and detectability of the infochemicals (Vet & Dicke 1992). The reliability of an infochemical indicates the host presence, its accessibility and its suitability while its detectability indicates the degree to which the infochemical can be perceived (Vet & Dicke 1992). Infochemicals from the host are the most reliable, but they have a low detectability because hosts are small components of a complex environment, and selection has favored hosts emitting inconspicuous infochemicals (Vet & Dicke 1992). To solve this reliability-detectability problem, parasitoids, when searching for hosts, generally rely on more detectable infochemicals, such as host plant synomones and host by-products, and use infochemicals in a hierarchic search that bring them always closer to the host (Vinson 1976).

Host eggs have a low detectability because of their small size and their inactivity. Accordingly, many egg parasitoids rely on more detectable cues, such as host plant synomones (Nordlund 1994; Meiners *et al.* 2000) and adult host kairomones (Nordlund *et al.* 1983; Nordlund 1994; Colazza *et al.* 1999; Meiners *et al.* 2000) for host egg location and use egg kairomones only in the final stages of host selection (Kainoh *et al.* 1982; Strand & Vinson 1982, 1983; Nordlund *et al.* 1987; Meiners *et al.* 2000; Takasu & Nordlund 2001). Generally, egg kairomones originate from the reproductive system of the adult host (Strand & Vinson 1982, 1983; Nordlund *et al.* 1987; Takasu & Nordlund 2001) and are emitted by the adhesives used for attachment of eggs to oviposition sites (Strand & Vinson 1982; Nordlund *et al.* 1987; Bin *et al.* 1993). However, egg kairomones are available only to parasitoids attacking exposed or partially exposed eggs (Kainoh *et al.* 1982; Strand & Vinson 1982, 1983; Nordlund *et al.* 1987; Conti *et al.* 1996; Meiners *et al.* 2000; Takasu & Nordlund 2001). Because embedded eggs are hidden in plant tissues, we hypothesized that when on host plant, parasitoids of fully embedded eggs should use infochemicals related to the adult host rather than egg kairomones to locate host eggs.

Anaphes victus Huber (Hymenoptera: Mymaridae) is a solitary egg parasitoid of the carrot weevil *Listronotus oregonensis* LeConte (Coleoptera: Curculionidae) (Boivin

1999). As the majority of *Anaphes* species, *A. victus* is arrhenotokous; virgin females produce males and mated females produce both sexes (Boivin *et al.* 1993). With *Anaphes listronoti* Huber, *A. victus* is the most important biotic factor of mortality of *L. oregonensis* (Zhao *et al.* 1991). In Québec, parasitism rates may exceed 70 % from early July to late summer, and parasitism remains high even with low host densities suggesting a high searching efficiency by these egg parasitoids (Boivin 1999). However, there is no study on the stimuli used by these species when searching for *L. oregonensis* eggs. *Listronotus oregonensis* live on a variety of umbelliferous plants, including the cultivated carrot (*Daucus carota* L. var. *sativa*), of which it is a major pest in northeastern North America (Boivin 1999). The eggs are oviposited in cavities made by the female in the leaf petiole or in the crown of the carrot, and are then covered with a plug made of feces and saliva (Boivin 1999).

In this paper, the response of *A. victus* females to infochemicals related to *L. oregonensis* eggs was evaluated using a small arena under laboratory conditions.

Materials and Methods

Insects

Listronotus oregonensis were reared at 25 ± 1 °C, with a photoperiod of 16L: 8D and 40-60 % R. H. following the technique of Martel *et al.* (1975) while *A. victus* were reared on eggs of *L. oregonensis* in an incubator at 25 ± 1 °C, with a photoperiod of 16L: 8D and 60-90 % R. H. following the technique of Boivin (1988). At emergence, *A. victus* females were mated and were given one oviposition experience on a 1-3 day old *L. oregonensis* egg extracted from a carrot root from the laboratory rearing. *Anaphes victus* females were then individually inserted in a size 3 (ca. 300 µl) Beem ™ polyethylene embedding capsule for a maximum of 3h until the experiments.

Experimental procedures

The experiments were performed in laboratory at 22 ± 2°C and 60-90 % R. H. Because *A. victus* is attracted by light, the tests were done in a cage with dark sides and top and with a translucent white plastic floor lighted with neon. A Petri dish (35 X 10 mm) placed over a filter paper was used as an arena. The arena was divided in two equal parts by a line traced in the middle of the Petri dish. One half of the filter paper was moistened with 20 ul of a solution containing odor components, and the other half was moistened with 20 ul of distilled water. The *A. victus* female was deposited on the filter paper by tapping delicately on a capsule containing the *A. victus* female just above the filter paper. Then, the Petri dish was immediately replaced on the filter paper avoiding any escape of the *A. victus* female. After being inserted in the arena, the *A. victus* female generally stayed on the top of the Petri dish for a few minutes, and the experiment started only when the female went down on the filter paper. From that moment, the time spent by the *A. victus* female on each half of the filter paper was measured during five minutes. During the five-minute test, only the time spent on the filter paper was taken into account, therefore the time spent on the sides and on the top of the arena was not calculated. After each replication, the filter paper and the *A. victus* female were changed, and the Petri glass was rinsed with distilled water.

Odors tested

Five treatments were tested: *L. oregonensis* eggs extracted from host plant, *L. oregonensis* eggs extracted from artificial substrate, *L. oregonensis* egg plugs, *L. oregonensis* feces and host plant. For each treatment, replications were individually prepared 24h before the experiments by inserting the odor components in a size 3 (ca. 300 µl) Beem™ polyethylene embedding capsule containing 30 ul of distilled water. For the treatment with *L. oregonensis* eggs extracted from host plant, ten 1-3 day old eggs extracted from a carrot root of the laboratory rearing were used. Before inserting eggs in the capsule, most of the egg plugs on the surface of the eggs were removed with forceps. To eliminate the possibility of contamination of the eggs by egg plugs and carrot root, another treatment (*L. oregonensis* eggs extracted from artificial substrate), using ten 1-3 day old eggs extracted from a Kimwipe paper tissue instead of a carrot root, was tested. For the treatment with *L. oregonensis* egg plugs, 0,0004g (approx. 10 egg plugs) of 1-3 day old egg plugs removed from a carrot root was used. For the treatment with *L. oregonensis* feces, 0,0004 g of 1-3 day old feces removed from a filter paper placed at the bottom of a jar containing a carrot root and *L. oregonensis* adults was used. To verify whether odor components of the carrot root were involved in the response of *A. victus* females to treatments with *L. oregonensis* eggs extracted from host plant, *L. oregonensis* egg plugs and *L. oregonensis* feces, an additional treatment (host plant) was tested with carrot root only. For this treatment, a section of 0,002 g of carrot root (it represented approximately the surface of the carrot root that was in contact with 10 *L. oregonensis* eggs) without eggs, egg plugs and feces was used. For each treatment, 20 ul of the water solution containing the odor components were compared with 20 ul of distilled water, and 20 replications were performed. Data were statistically analyzed with paired T-tests.

Results

Anaphes victus females spent significantly more time on the section of filter paper containing a solution of *L. oregonensis* eggs extracted from host plant (t = 2,50; p < 0,05), a solution of *L. oregonensis* egg plugs (t = 3,92; p < 0,001) and a solution of *L. oregonensis* feces (t = 5,04; p < 0,001) than on the section of the filter paper containing distilled water (Fig 5.1). There was no significant difference between *L. oregonensis* eggs extracted from artificial substrate and distilled water (t = 0,10; p > 0,05) and between host plant and distilled water (t = 1,05; p > 0,05) (Fig. 5.1).

Discussion

Anaphes victus females responded to odor components of *L. oregonensis* eggs extracted from host plant and *L. oregonensis* egg plugs, but responded neither to odor components of *L. oregonensis* eggs extracted from artificial substrate nor to host plant suggesting that residues of egg plugs on eggs extracted from host plant elicited the response of *A. victus* females to this odor component. Although egg kairomones are used by many egg parasitoids in the final stages of host selection, these parasitoids attack exposed or partially exposed eggs that can be mounted and antennated by the parasitoid (Kainoh *et al.* 1982; Strand & Vinson 1982, 1983; Nordlund *et al.* 1987; Conti *et al.* 1996; Meiners *et al.* 2000; Takasu & Nordlund 2001). As *L. oregonensis* eggs are hidden in carrot tissues, *A. victus* females cannot mount and antennate *L. oregonensis* eggs, and are therefore unlikely to respond to these odor components.

As predicted, *A. victus* females use kairomones emitted by *L. oregonensis* adult by-products when searching for *L. oregonensis* eggs. For *A. victus* females, it is likely that *L. oregonensis* feces and egg plugs are more detectable than its eggs because *A. victus* females may contact them when walking on the plant or may detect them in the air. In addition to being more detectable, *L. oregonensis* feces and egg plugs are also reliable kairomones. Indeed, the presence of *L. oregonensis* feces on host plant implies that there are or there were *L. oregonensis* adults on the plant and possibly eggs while the presence of *L. oregonensis* egg plugs is directly linked to *L. oregonensis* eggs.

Although not measured, intensive antennal drumming and changes in the locomotory behavior of *A. victus* females were observed when they were on the sections of filter papers containing *L. oregonensis* feces and egg plugs. Generally, host by-products are contact kairomones and used by female parasitoids as host searching stimulants on the host plant of the host (Vinson 1998). When the female parasitoid encounters these kairomones, they provoke a change in its locomotory behaviors, keeping it on the infested host plant and increasing its probabilities of discovering hosts (Vinson 1998). Thus, it is possible that contacts with *L. oregonensis* feces and egg plugs have a similar effect on *A. victus* female, but further experiments are needed to confirm this.

Host selection of *A. victus* females can be modified by learning (van Baaren *et al.* 1994) and as *A. victus* females used in the experiments had an experience with *L. oregonensis* eggs that probably had traces of egg plugs on their surface, we cannot rule out the possibility that the response to *L. oregonensis* feces and egg plugs was the result of an associative learning or previous experience with these odor components. However, eggs on which *A. victus* females were experienced had also traces of host plant, and *A. victus* females did not respond to this odor component.

Anaphes spp are the most important biotic mortality factor of *L. oregonensis* in northeastern North America (Boivin 1999). However, parasitism on *L. oregonensis* is not sufficient to keep *L. oregonensis* populations under an economic threshold, and chemical insecticides are still used in carrot fields (Boivin 1999). The response of *A. victus* females to *L. oregonensis* kairomones can be considered as a promising result for further research on the use of kairomones that could increase host searching efficiency of *Anaphes* spp in carrot fields and decrease the amount of chemical insecticides applied against *L. oregonensis*.

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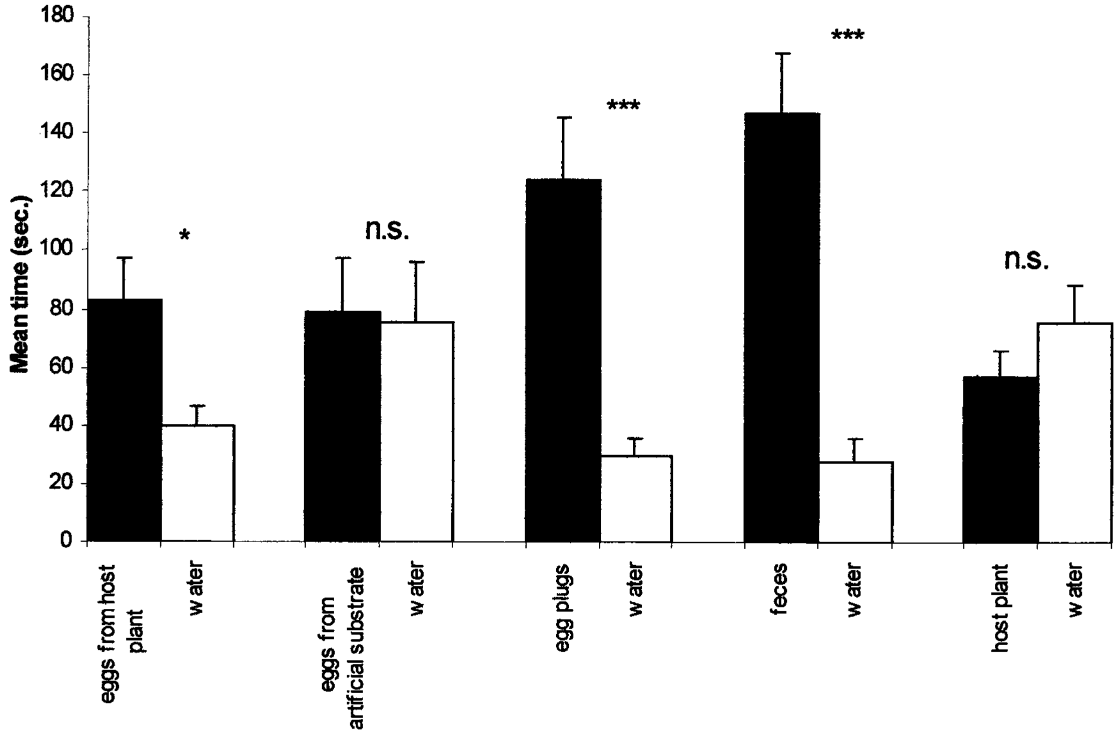

Figure 5.1: Mean ± SE time spent by *A. victus* females on a section of filter paper containing an odor component related to *L. oregonensis* (black columns) and a section of a filter paper moistened with distilled water (white columns). n.s. = non-significant difference; * p < 0,05; *** p < 0,001 (Paired t-tests).

VI. GENERAL CONCLUSION

The objective of this research was to determine the stimuli used by *M. hyperodae* and *A. victus* during host selection, as a basis to eventually manipulate the host selection behavior of these parasitoids in carrot fields and increase their effectiveness in controlling *L. oregonensis* populations.

In an attempt to determine the volatile infochemicals used by *M. hyperodae* females during host selection, it was hypothesized that parasitoids in new associations should use infochemicals related to their natural host(s) during host selection. However, *M. hyperodae* females responded only to *L. oregonensis* odor components suggesting that the response of *M. hyperodae* females to *L. oregonensis* kairomones was enhanced by laboratory selection and/or previous experiences with *L. oregonensis* related odor components.

Since Curculionidae adults are mobile hosts, in a second study, we hypothesized that, in addition to host kairomones, *M. hyperodae* should use host movement as a cue in the final steps of host selection. As predicted, *M. hyperodae* females stopped more frequently in front of a moving *L. oregonensis* adult than in front of immobile ones suggesting that host movement attracted *M. hyperodae* females. However, *M. hyperodae* females rarely walked directly toward moving *L. oregonensis* and most of the time host movement became attractive only when *M. hyperodae* was near the moving *L. oregonensis* adult. Therefore, it is likely that *M. hyperodae* females use host movement only at close distance to its host when host kairomones are detected. As *M. hyperodae* is a specialist parasitoid, such a hierarchic search could be adaptive because it decreases the probabilities of pursuing and parasitizing unsuitable hosts. The use of a hierarchic search in which host movement serves only in presence of host kairomones may limit the probabilities that *M. hyperodae* enlarges its host range in nature. Furthermore, as the detection of *L. oregonensis* adult feces kairomones and host movement decreased the number of weevil antennations done by *M. hyperodae* females, it is likely that both host feces and host movement play a role in host recognition of *M. hyperodae* females. Thus, it is possible that when a *M. hyperodae* female detects host feces kairomones or host kairomones it may then attack a species normally not attacked only because the individual moves. Such a behavior could be a cause of « accidental » parasitism on non-target species and may explain why *M. hyperodae* was found only on two occasions on species other than *L. bonariensis* in New Zealand.

Since most oviposition attempts observed were directed to *L. oregonensis* mouth and anus when *L. oregonensis* was moving, it is possible that when *L. oregonensis* moves, its mouth and anus are more exposed to the *M. hyperodae* ovipositor. Moreover, *M. hyperodae* had a tendency to aim at moving parts of the *L. oregonensis* body when trying to oviposit suggesting that, in nature, the insertion of the ovipositor into the host mouth or anus is easier when hosts are feeding and ovipositing respectively. In addition to facilitate the insertion of *M. hyperodae* ovipositor, host movement may also serve as a host quality criterion since *M. hyperodae* is a koinobiont parasitoid and larval development occurs in living host.

In contrast to adult hosts, host eggs have low detectability, and therefore egg parasitoids rely on cues more detectable than egg kairomones when searching for host eggs. In a third study we hypothesized that egg parasitoids should use stimuli from the adult host to detect host eggs. As predicted, host feces and egg plugs were important source of kairomones used by *A. victus* females during host selection. Whether egg plugs and host feces have different functions in host selection by *A. victus* females remains to be determined. Nevertheless, it is possible that host feces serve as host searching stimulant while egg plugs serve as an indicator of host oviposition sites. Although the effect of host feces on host selection by *M. hyperodae* females was less obvious than for *A. victus* females, one may expect that *L. oregonensis* feces contain odor components that are attractive to both parasitoids. From a biological control point of view, these results could orient the development of artificial kairomones increasing the searching efficiency of both parasitoids.

As predicted by the optimal foraging theory, it appeared from this study that both *M. hyperodae* and *A. victus* use host related stimuli during host selection. The adult of Curculionidae is the most susceptible stage to parasitism because the egg, the larva and pupa are hidden in plant tissue and soil. Accordingly, both the adult parasitoid *M. hyperodae* and the egg parasitoid *A. victus* use stimuli produced by adult Curculionidae during host selection. Although the stimuli from adult Curculionidae are the most detectable for their parasitoids, their reliability may vary according to the stage attacked by the parasitoid. Thus, it appears that each parasitoid has evolved to use different facets of the host adult biology during host selection. As an adult parasitoid, *M. hyperodae* uses host adult odor components and host mobility while, as an egg parasitoid, *A. victus* uses egg plugs. Moreover, the contact with host feces has probably an arrestment effect on *M. hyperodae* while it seems to increase the locomotory behaviors (ortho-klinokinesis) of *A. victus*. Since the adult Curculionidae is mobile, after having contacted host feces, *M. hyperodae* may stop and use visual cues to locate its adult host. In contrast, eggs of *L. oregonensis* are hidden under egg plugs in plant tissue, and therefore, the contact with host feces may increase the locomotory behaviors of *A. victus*, and subsequently, enhance its probabilities of encounter an egg plug.

Anaphes spp are the major biotic mortality factor of *L. oregonensis* in northeast North America. However, there is a delay in early summer before egg mortality reaches a level at which damage caused by *L. oregonensis* larvae is kept below an economic level, and consequently, insecticide treatments are still used in carrot fields. However, *M. hyperodae* was recently released in Québec, and the addition of an adult parasitoid in carrot agroecosystems could increase the mortality on *L. oregonensis* adults in early summer, and subsequently decreased the number of insecticide treatments applied against the carrot pest. Furthermore, since *L. oregonensis* has no parasitoid of adult stage, it is possible that its mechanisms of defence against such parasitoids are not developed, and therefore, *M. hyperodae* could take advantage of this weakness.