EFFECT OF ARTIFICIAL AND NATURAL PLANT STRUCTURES ON HOST SEARCHING BEHAVIOR OF THE EGG PARASITOID *TRICHOGRAMMA* SPP. (HYMENOPTERA:TRICHOGRAMMATIDAE)

BY

DANIEL GINGRAS

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

Department of Natural Resource Sciences
Macdonald campus of McGill University
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SHORT TITLE

Effect of plant structure on host finding by T*richogramma* spp.

Daniel Gingras

ABSTRACT

Ph. D. **Entomology**

Effect of artificial and natural plant structures on host searching behavior of the egg parasitoid *Trichogramma* spp. (Hymenoptera: Trichogrammatidae)

Differences in plant structure and host abundance, distribution and density within and between plants may affect host finding success of searching parasitoids. The main objective of this research consisted in developing and validating a model that can predict parasitism by *Trichogramma evanescens* on various plant structures. Also, we evaluated the effect of both artificial and natural plant structures on host encountering success and on searching behavior of two species of *Trichogramma*.

Size (S), heterogeneity (H) and connectivity (C) define plant structure. The development of the model of parasitism was based on laboratory experiments using three dimensional artificial plants of different combinations of S, H and C. The model was then validated with experiments, within greenhouse, using natural cruciferous plants of different structures. Significant regressions of observed values of parasitism as a function of those predicted by the model were obtained.

The effect of plant structure on two species of *Trichogramma* was studied by using three species of Lepidoptera and three structurally different but closely related crucifers. Also the distribution pattern of parasitized eggs according to leaf side and plant height was characterized. A repeated measure ANOVA in time where plant ages defined the repeated measures showed that all three main effects (plant, host, parasitoid) had significant effect on parasitism and only the parasitoid x plant structure interaction was significant. Mean percent of parasitism was higher on cabbage, intermediate on broccoli and lower on

Brussels sprouts whereas cabbage appeared to be intermediate in plant structure, broccoli appeared to be the most simple and Brussels sprouts the most complex plant structure. On simple plant structure, both wasp species performed well on the three varieties of plant but *T. evanescens* outperformed *T. pretiosum* more often on the various plant structures. A doubly repeated measures ANOVA in space revealed significant effects of leaf side and plant height on parasitism, being greatest under leaf surface and at the base of the plant. The interaction between those two variables was not significant.

The effect of plant structure on searching behavior of *T. evanescens* was determined by direct observation of individual females searching on simple and complex plant structures during 1 hour. Time, frequence and sequence associated to activities and plant parts explored were obtained and analyzed. Plant structure had significant effect on time budget associated to activities (walking, resting, flying) and plant parts explored.

This study demonstrates that plant structure mediates ecological interactions by affecting host finding success and this may have important implications in population dynamics, evolution of hosts and parasitoids but also in biological control programs.

RÉSUMÉ

Ph. D. **Entomologie**

Effet de la structure de plantes artificielles et naturelles sur la recherche d'hôte par les parasitoïdes d'oeufs *Trichogramma* spp. (Hymenoptera: Trichogrammatidae).

Les différences de structure et les différences d'abondance, de distribution et de diversité d'hôte sur une plante ou entre des plantes peuvent affecter la découverte d'hôtes. Le but principal de cette recherche a consisté à développer et valider un modèle prédictif de parasitisme pour *T. evanescens* lorsque cherchant sur des plantes de différentes structures. Aussi, nous avons évalué l'effet de la structure de plantes artificielles et naturelles sur le succès de rencontre d'hôtes et sur le comportement de recherche de deux espèces de parasitoïdes du genre *Trichogramma*.

La taille (T), l'hétérogénéité (H) et la connectivité (C) définissent la structure d'une plante. Le développement du modèle a été basé sur des résultats de parasitisme obtenus par des expériences en laboratoire sur des plantes artificielles utilisant différentes combinaisons de T, H et C. Le modèle a été validé par des expériences en serre sur des plantes naturelles, soit des crucifères de différentes structures. Des régressions significatives des valeurs observées de parasitisme en fonction des valeurs prédites par le modèle ont montré que le modèle performe bien.

Avec les plantes naturelles, nous avons déterminé l'effet de la structure de la plante sur deux espèces de Trichogrammes en utilisant trois espèces de Lépidoptères et trois variétés de crucifères de structures différentes. Aussi, la distribution des oeufs parasités selon la hauteur de la plante et le côté de la feuille a été caractérisée. Une analyse de variance sur des mesures répétées dans le temps, où l'âge de la plante a défini les mesures répétées, a montré que les effets principaux (plante, hôte, parasitoïde) influencent le parasitisme et seule l'interaction parasitoïde x plante structure a été significative. Comme avec les plantes artificielles, le parasitisme diminue avec l'augmentation de la structure de la plante, étant plus élevé sur le chou (structure simple) que sur le chou de Bruxelles (structure complexe) puis intermédiaire sur le brocoli. Sur les plantes de structure simple, les deux espèces de *Trichogramma* ont bien performé et ce pour les trois variétés de plante mais *T. evanescens* s'est avéré meilleur que *T. pretiosum* sur des plantes complexes. Une analyse de variance sur des mesures répétées dans l'espace a révélé des effets significatifs de la hauteur de la plante et du côté de la feuille sur le parasitisme; ce dernier était plus élevé sous la surface des feuilles et à la base de la plante. L'interaction entre ces deux variables n'a pas été significative.

L'effet de la structure de la plante sur le comportement de recherche de *T. evanescens* a été déterminé par des observations directes, pendant une heure, d'une femelle cherchant sur des plantes de structures simples et complexes. Des durées, des fréquences et des séquences associées aux activités et aux parties de plante explorées ont été obtenues et analysées. La structure de la plante a eu des effets significatifs sur le temps associé aux activités et aux parties de plante explorées.

Cette étude a démontré que la structure d'une plante affecte le succès de découverte d'hôte, ce qui a des répercussions sur la dynamique des populations, sur l'évolution des hôtes et parasitoïdes mais aussi sur l'élaboration de programmes de lutte biologique.

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I deeply and warmly acknowledge Ginette, Josée and my son Vincent for their love, care, support, encouragement, comprehension, patience and to whom I dedicate this thesis.

PREFACE

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

> *Candidates have the option of including, as part of the thesis, the text of one or more papers submitted or to be submitted for publication, or the clearly-duplicated text of one or more published papers. These texts must be bound as an integral part of the thesis.*
>
> *If this option is chosen,* ***connecting texts that provide logical bridges between the different papers are mandatory****. The thesis must be written in such a way that it is more than a mere collection of manuscripts; in other words, results of a series of papers must be integrated.*
>
> *The thesis must still conform to all other requirements of the "Guidelines for Thesis Preparation".* ***The thesis must include:*** *a table of contents, an abstract in English and French, an introduction which clearly states the rationale and the objectives of the study, a comprehensive review of the literature, a final conclusion and summary, and a thorough bibliography or reference list.*

Additional material must be provided where appropriate (e.g. in appendices) and in sufficient detail to allow a clear and precise judgment to be made of the importance and originality of the research reported in the thesis.

In the case of manuscripts co-authored by the candidate and others, ***the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent.*** *Supervisors must attest to the accuracy of such statements at the doctoral oral defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of all the authors of the co-authored papers.*

The thesis includes four manuscripts, the first three have been submitted to different journals while the fourth one has to be submitted. All manuscripts are co-authored.

THIRD CHAPTER

Gingras, D. and G. Boivin. 2001. Effect of plant structure, host density and foraging duration on host finding by *Trichogramma evanescens*. Submitted to Environmental Entomology.

FOURTH CHAPTER

Gingras, D., P. Dutilleul, and G. Boivin. 2001. Modelling the impact of plant structure on host finding behavior of parasitoids. Submitted to Oecologia.

FIFTH CHAPTER

Gingras, D., P. Dutilleul, and G. Boivin. 2001. Ecological constraints imposed by plant structure on foraging parasitoids. Submitted to Entomologia Experimentalis & Applicata.

SIXTH CHAPTER

Gingras, D. and G. Boivin. 2001. Effect of plant structure on searching strategy and searching efficiency of *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae). To be submitted to Journal of Insect Behaviour.

CONTRIBUTION OF CO-AUTHORS TO MANUSCRIPTS

Under the guidance of his supervisor, the candidate was in charge of experimental design, laboratory set-up, experimental execution and management, data collection and statistical analyses as well as preparation of manuscripts for publication.

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

Dr. Pierre Dutilleul provided valuable assistance and advice on the experimental design and statistical analyses on the manuscript of chapter four and five. He also read, revised and provided comments on these two manuscripts

CLAIMS OF CONTRIBUTION TO KNOWLEDGE

Major contributions of this research are summarized in this section.

1. Development of a predictive model of parasitism for *T. evanescens* when it forages on plants for which the size, the heterogeneity and the connectivity are known. The model was based on percentages of parasitism obtained from an experiment with three-dimensional artificial plants where one component (size or heterogeneity or connectivity) was varied at the time and by keeping plant surface area constant.

2. Validation of the model on three tritrophic systems utilizing natural organisms

3. Demonstrating that connectivity affected area searched per time unit and consequently affected the number of hosts encountered.

4. Klinokinesis, viewed as an increase in turning rate, appear to be predominant when a female *T. evanescens* encountered a connection between plant parts.

5. First study to vary and quantify the three components of plant structure: size, heterogeneity and connectivity and to apply such an approach to insects.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

GENERAL INTRODUCTION

Relationships between insects and plants are encompassed by several ecological theories that have not usually been viewed in a multitrophic context: theory of plant chemical defense, food web theory, general population dynamics and optimal foraging theory. In a determinant manuscript published in 1980, Price *et al.*, argued that theory on insect/plant interactions cannot progress realistically without considering the third trophic level. Since then, research on tritrophic interactions relating to arthropod herbivores has expanded rapidly (Price et al. 1980, 1990; Price 1991; Vet and Dicke 1992; Godfray 1994; van Lenteren et al. 1996; Verkerk and Wright 1998 and references therein; De Moraes and Mescher 1999). Numerous interactions between entomophagous arthropods and herbivores are mediated by plants and these may influence the efficiency of parasitism. Chemical and morphological plant attributes affect the efficacy of biological control agents by influencing their abundance, survival, rate of herbivore attack, fecundity and development. Moreover, such traits can affect qualities of a herbivore that, in turn, affect the physiology, behavior or development of natural enemies (De Moraes and Mescher 1999). Much of our current knowledge and theoretical considerations of multitrophic interactions are based on chemically mediated aspects (Sandlan 1980; Nordlund *et al.* 1981; Bell and Cardé 1984; Dicke *et al.* 1990; Schmidt and Carter 1992; Vet and Dicke1992). However, there is empirical evidence that plant structure influences herbivore diversity (Lawton 1983; Hawkins and Lawton 1987; Brown 1991), abundance (Fowler 1985; Basset and Burckhardt, 1992), distribution (Brodeur and McNeil 1991) and alters the capacity of natural enemies to exploit potential hosts/prey (Pimentel 1961; Smith 1972; Crowley 1978; Price and Clancy 1986; Gross and Price 1988; Andow and Prokrym 1990).

Among the natural enemies of herbivorous insects are the parasitoids. These are organisms whose larvae develop by feeding on or within a single arthropod host and kill it as a direct or indirect result of that development (Gauld and Bolton 1988; Eggleton and Gaston 1990). Larvae of parasitoids can be divided into two classes according to their feeding behavior. Those that feed from the inside and develop within the host body are named endoparasitoids. Ectoparasitoids, on the other hand, feed from the outside and develop externally (Godfray 1994). Parasitoids that allow hosts to continue to grow in size after parasitism are called koinobionts as opposed to idiobionts, where hosts stop growing and the parasitoid larvae must complete development with host resources present at oviposition (Godfray 1994). Adult insect parasitoids are free-living and feed on nectar and pollen (Waage and Greathead 1986). Adult parasitoids may attack and oviposit in any stage of the life cycle of herbivorous insects, like eggs, larvae, pupae and adults. Insect parasitoids are mainly found in 5 orders: Hymenoptera, Coleoptera, Diptera, Neuroptera, and Lepidoptera (Godfray 1994). The Hymenoptera encompass the great majority of parasitoids, 67% of the species of insects within the order show a parasitoid life style. It is also within this order that we find the greatest diversity of life styles and adaptations to different hosts and habitats. It is followed by the Diptera, with 18% of the species being parasitic. The importance of the parasitoid life style is minor within the other orders.

Interest in parasitoids has grown since the very first and most serious scientific studies on foraging behavior of these insects by Laing (1937, 1938) and Salt (1935, 1937). The proportion of scientific papers devoted to parasitoids is increasing with time in the scientific literature. By using "parasitoid" as a key word in a literature search it comes out that scientific papers concerned with parasitoids represented 0.26 % in 1980 while it represented 0.70 % in 1997 (Boivin 1999). The growing interest for parasitoids can be attributed to their important role in controlling and regulating populations of herbivores in natural and artificial environments. A study published by Hawkins et al. in 1997 revealed that parasitoids kill many more herbivores (proportion killed= 0.04) than predators (proportion killed= less than 0.005) or pathogens (proportion killed= 0). Parasitoids have also been important in many biological control programs in natural and artificial environments. One of the best known and successful example is provided by the hymenopteran parasitoid, *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae), which attacks and kills whiteflies, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) (van Lenteren et al. 1996). This parasitoid became a valuable ally for greenhouse producers and has now been used for more than twenty years. Finally, the direct link that exists between searching efficiency and fitness in parasitoids promotes interest in the insects and makes them predilected biological models to test hypotheses related to evolution, population dynamics and behavioral ecology.

The host location process is of crucial importance and this may explain why it is intensively and extensively investigated. From theoretical considerations, it is important for completion of the reproductive cycle. From practical considerations, it is important for regulation of pest insect populations. The process of host discovery and factors affecting it have been reviewed at different times by Vinson (1976, 1981, 1984, 1998). The host location process has been divided into five steps: host habitat location, host location, host acceptance, host suitability, host regulation. This study is concerned with the first three but mainly with the second and third steps.

To successfully complete its reproductive cycle, a parasitoid must develop an efficient strategy for locating hosts in a complicated heterogeneous environment and for overcoming host defenses and the obstacles presented by competitors and natural enemies (De Moraes and Mescher 1999). Such a strategy will likely involve multiple cues and foraging tactics employed at multiple spatial scales as well as the development of behavioral and physiological adaptations to the host's internal environment (De Moraes and Mescher 1999).

Parasitoids may thus forage at various scales, proceeding from the largest which are the ecosystem and macrohabitat to the smallest units, which are the microhabitat and the patch (Hassell and Southwood 1978). The structure of these different spatial units varies in time and space and may influence ecological interactions. Plants represent the first trophic level and may be viewed as a microhabitat. Their physical characteristics can affect bottom-up trophic interactions. One physical attribute of plants is their structure. Many terms have been used to define and qualify the structure of plants, which leads to confusion and makes difficult comparisons of results between studies (Bell et al. 1991). Rigorously defining the structure of a plant appears to be imperative. Plant structure influences interactions between parasitoid and host by acting on encountering success of parasitoids. Plant structure appears to affect movements and activities associated with searching and therefore affects searching efficiency. Little is known concerning the exploration of plant structure by parasitoid insects and if some components of plant structure have more effect on their movements and searching efficiency. Activities and strategies used by parasitoids to explore their environment may vary with the complexity of plant structure. Plant structure may affect differentially parasitoid species or it may have sweeping effects on numerous species. Clearly, plant structure affects benefits and costs associated with host searching behavior and quantification of plant structure may lead to the possibility of developing predictive models of the impact of plant structure on host finding success. It would then be possible to compare rate of host discovery resulting from searching on different plant structures and to gain insight on the causes and consequences of inter and intra-specific variability in host finding success by parasitoids. The effect of plant structure on host finding may have important repercussions in population dynamics, evolution and biological control.

Parasitoids are natural enemies of herbivores but natural populations of parasitoids may not be present in sufficient numbers to efficiently control sometimes explosive pest insect populations and to suppress the abundance of herbivores to a level at which they no longer cause economic damage. Introduction of parasitoids through inundative release in agro-ecosystems and forest ecosystems, to increase their distribution and abundance has been practiced for more than 50 years. Since then there have been more than 3600 purposeful introductions of parasitoids against more than 500 arthropod pests in almost 200 countries and islands around the world (Greathead and Greathead 1992).

Among all existing species of parasitoids, those that belong to the genus *Trichogramma* are extensively studied. *Trichogramma* are minute (less than 1 mm in length), generalist, idiobiont, egg endoparasitoids of the Trichogrammatidae family (Godfray 1994 ; Pinto and Stouthamer 1994). The advantage of using egg parasitoids over larval parasitoids in biological control is that the former prevent hatching (Li 1994). Inundative releases of *Trichogramma* parasitoids are being practiced against various pests on many crops and in many countries (Ridgeway et al. 1981; Voronin and Grinberg 1981; Hassan 1988; Voegelé 1988). China and Russia are still the leading countries for the introduction of these parasitoids in the field, mainly for the culture of cotton. In Canada, the use of *Trichogramma* is relatively recent and inundative releases are mostly practiced in corn fields (Neil and Specht 1990; Yu and Byers 1994). However, there have been studies for selecting suitable *Trichogramma* parasitoids to control various pest species occurring on different plant species (Smith et al., 1986, 1990; Smith and You 1990; Henderson and Myers 1994; McGregor et al. 1997). Fournier and Boivin (2000) evaluated 40 species/strains of Trichogrammatidae spp. and aimed to identify parameters that can help for the selection of biological control agents of cruciferous pests in Quebec, Canada.

The varieties of *Brassica oleracea* grown in Canada include cabbage, chinese cabbage, broccoli, Brussels sprouts, and cauliflower. These cole crops are typically grown in small plots that are plowed annually. The three most important Canadian production areas of *B. oleracea* are Quebec, Ontario, and British Columbia (Richard and Boivin 1994). In Quebec, the production of crucifers (cabbage, broccoli, Brussels sprouts) covered an area of approximately 3 600 hectares and provided 29 millions $ at harvest in 1997 (Statistique

Canada 1998). The surface area devoted to the culture of cabbage are greater than those of broccoli and Brussels sprouts.

For our study, we chose to work with cabbage, broccoli and Brussels sprouts. The use of these three varieties of *Brassica oleracea* has, from a functional perspective, several favorable attributes. First, as they belong to the same species, they share a similar chemistry, in particular the presence of glucosinolates (van Etten and Tookey 1979; Simmonds 1979). Second, they are all green, they all possess glabrous and waxy leaves. Third, they show very different structures that may lead to differences in searching efficiency by parasitoids.

These three plants may be seriously damaged by three species of lepidoptera, namely the imported cabbageworm, *Pieris rapae* (L.) (Lepidoptera: Pieridae), the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) and the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae).

The present project comes within the scope of a larger investigation for the selection of species and strains of *Trichogramma* for the control of a lepidopterous pest complex in cruciferous crops (Boivin and Fournier 1993). Currently, there exists more than 145 species of *Trichogramma*, and there is considerable inter and intra specific variability among the different species/strains (Pinto and Stouthhamer 1994), thus some species or strains may be more suitable than others as control agents. Because failures to control agricultural pests could be due to the use of less suitable *Trichogramma* species or strains

(Hassan 1989) we must be parsimonious and rigorous in the choice of a species. The species we wish to select should not show a strong preference for one plant, should deal efficiently with constraints imposed by the different structures of the three crop varieties and should accept to oviposit in eggs of the three lepidopters.

This Ph.D study will look at plant/insect relationships at three trophic levels (plants, hosts and parasitoids) and investigate how and why plant structure influences parasitoid/host interactions. The theoretical basis of this investigation relates to behavioral ecology, and theories of population dynamics, optimal foraging and evolution. The study has practical applications in biological control of lepidopteran pests of crucifers in agroecosystems but also in the manipulation of the first trophic level, either by the management and the choice of plant species or by genetic manipulation to develop new phenotypes.

General Objectives:

(A) To determine if plant structure affects host/parasitoid interactions in a tritrophic context.

(B) To quantify plant structure and to model the rate of parasitism

Specific Objectives:

(a) to quantify plant structure and to determine if it affects host finding success

(b) to develop and validate a model of parasitism using artificial plants

(c) to compare the rate of parasitism between two species of *Trichogramma* foraging on cruciferous plants of different structures and to examine the searching pattern of these parasitoids by characterizing the distribution pattern of parasitized of host eggs according to height and leaf side of cruciferous plants

(d) to examine the influence of plant structure on searching strategies and searching efficiencies of *Trichogramma* female parasitoids.

CHAPTER 2

LITERATURE REVIEW

Theoretical concepts debated in manuscripts included in the next chapters will be presented in this literature review. I will first briefly present behavioral aspects related to host searching by parasitoids, then report different models or definitions of plant structure. Afterwards, behavioral aspects related to host searching by parasitoids in a multitrophic context and its repercussions on optimal foraging, population dynamics and evolution will be discussed. Finally, a description of each insect and cruciferous plant composing the tritrophic system studied will be presented.

1. HOST SEARCHING BEHAVIOR BY PARASITOIDS

A female parasitoid may emerge far from potential hosts and therefore may have to search for suitable hosts in which she will lay one or more eggs to transmit its genome to complete its life cycle (Vinson 1981). According to Vinson (1998), initiation of host searching is influenced by internal factors such as egg load, age, physiological condition, cleaning, hunger, and experience. External factors include factors such as diseases, presence of predators and competitors, presence of refuges, environment (light, temperature, humidity, wind, and chemicals) and food.

1.1 From host searching to fitness

For parasitoids, there is a direct link between successful searching for hosts and reproductive success, which is a component of fitness. Thus, the better a parasitoid is at searching, the fitter it will be. Natural selection should therefore favor parasitoid females that optimize the search for, and exploitation of potential hosts (Cook and Hubbard 1977; van Alphen 1986; Godfray 1994). Optimal foraging theory predicts that a forager should maximize its encounter rate with the most suitable and profitable hosts, and when hosts are abundant, avoid individuals of lesser quality (Stephens and Krebs 1986). In parasitoid foraging studies, it is generally assumed that offspring produced per unit of foraging time is the currency in which gains are expressed (Van Alphen and Vet 1986). Females should try to maximize their fitness or total genetic contribution to future generations by optimizing sex ratio and maximizing oviposition rate. To locate a host, a female must use efficient strategies to explore the host's environment to minimize costs and maximize gains. Costs for female parasitoids are associated with time and energy spent in searching and handling hosts but also include risks associated to that action like hyperparastism and predation (Barnard 1983).

Parasitoid wasps are found in a variety of habitats, from swampy marshlands to hot dry deserts, and occur on both, low-lying vegetation and in strictly arboreal habitats (Nagarkatti and Nagaraja 1977). Consequently, they may forage within environments, habitats and plants whose structure varies in time and space. The definition of the structure of plant or habitat has until now been rather vague, intuitive and subjective. Examination of plant structure in more controlled, more readily quantifiable conditions may permit better comparison of results from different studies on plant structure using different organisms (Bell et al. 1991).

1.2 Different conceptualizations of plant structure

1.2.1 Qualitative approaches

Various qualitative approaches that aim to capture the essential of plant structure have been proposed. One of the first comprehensive studies on vegetative structure of plants was published by Hallé and Oldeman in 1970. Since then other studies, all on tropical trees, have been published on the subject (Hallé et al. 1978; Mueller 1985; Tomlinson 1987; Cremers and Edelin 1995). These works proposed basic models of plant structure based essentially on descriptions of structural and phenological characteristics and patterns of growth. Lawton (1983), who investigated the influence of plant complexity on the number of species of insects feeding on them developed an architectural concept for plants. He classified plant structure on the following attributes: size, growth form, seasonal development, persistence, and variety of above-ground parts. Moran (1980), used five criteria to derived an 'architectural rating' for the desertic plant species *Opuntia*. The criteria were height, mean number of cladodes, approximate area of an average cladode (cm^2), development of woody stems, cladode complexity. Ross (1981) defines plant structure as "the set of features delineating the shape, size, geometry and external structure of a plant". Lukianchuk and Smith (1997) suggested that the physical aspect of plant growth has two components, biomass and structural arrangement of biomass in space (structural complexity). While biomass is a known concept (Barbour et al. 1987), structural complexity is the result of branching patterns or connections between plant parts. Price et al (1995) considered shoots, leaves, buds, flowers as modules and utilized a plant modular structure approach to compare very different species of insects using very different plant hosts, both taxonomically and structurally. Using such approach they showed that plant structure was comparable and that responses of different species of herbivores were similar. These approaches found application in botany and ecology but remained descriptive, relative and subjective. The approaches of Hallé and other similar studies may hardly serve the goal of our study because proposed models are highly descriptive, thus hard to quantify and were developed for botanical applications. Some plant attributes in the definitions of Lawton, Moran and Ross, like seasonal development, persistence and variety of above-ground parts number of cladodes, development of woody stems, geometry and external structure of a plant may not be relevant to foraging parasitoids and may be difficult to quantify.

1.2.2 Quantitative approaches

Studies where some aspects of plant structure were quantified such as branching patterns and branching attributes (e.g. branch length, branch angles) have also been published. In these studies, plants, usually trees, are translated into mathematical models, defined as a branching system with only one possible pathway between any two points; such methodology is named a binary approach. These studies investigated the functional significance of branching patterns and are used for computer simulation of plant architecture (Honda 1971; Leopold 1971; McMahon 1975, 1976; Bell et al. 1979; Honda et al. 1981; Honda et al. 1982). A promising method allowing automatic measurement of the three dimensional architecture of trees was published by Sinoquet and Rivet (1997).

The three dimension digitising of foliage canopies is driven by a software and involved the technique of current induction in magnetic fields. It can estimate plant height from soil level to highest shoot, the tree radius, the volume of the canopy, the number of branches, leaves and fruits. Fractal mathematics have been used to quantify the structure of habitats and plants (Williamson and Lawton 1991; Foroutan-pour et al. 1999).

Casas and Aluja (1997), provided a framework for describing and understanding the geometry of insects foraging within complex plant canopies where the insect is exposed to varying stimuli. They used the apple maggot fly, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), foraging in apple trees. They concluded that canopy architecture influences insect movement not only by defining the set of locations that the insect can visit using predefined rules for movement but also by defining the rules of movement. To gain a better understanding of the tritrophic system, host plant-greenhouse whitefly-*Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae), van Roermund et al. (1997) developed a simulation model of the population dynamics of the pest insect-parasitoid interaction. Their simulation model was based on the developmental biology of both insect species, and on the parasitoid's searching and parasitizing behavior in relationship to host plant characteristics (number and size of leaflets) and greenhouse climate and may help to explain failure or success of biological control. Suverkropp (1997) developed stochastic Monte Carlo simulation models of the searching behavior of *Trichogramma brassicae* Bezdenco (Hymenoptera: Trichogrammatidae) that calculate the probability of encounter with hosts on maize leaves and plants but he did not quantify the structure of maize plants.

1.3 Definition and quantification of plant structure

Many terms have been used to refer to and to qualify the structure of plants and habitats (Bell et al. 1991). We will use here the word structure rather than architecture because architecture implies design and esthetic aspects that may not be relevant or important to animals. Also we adopted the approach of Andow and Prokrym (1990) and Bell et al. (1991) to define plant structure. These authors recognized three components to plant structure: size, heterogeneity and connectivity. Because these three components vary in nature and may interact, we may assume they represent the potential niche of herbivores and the potential searching area of parasitoids.

Size corresponds to the height of the plant and is quantified by measuring, in cm, the main stem, from the highest leaf to the collar. Heterogeneity corresponds to the relative abundance, per unit area or per unit volume, of structural components, such as leaves, buds, flowers and fruits, but also to components within the plant, with different characteristics (glabrous versus hairy leaves) or morphologies (male versus female flowers). Heterogeneity is calculated by using the formula of Shannon-Wiener's index of biodiversity (Barbour et al. 1987), which is, $[\, - \Sigma \;\; X_i / X_o \;\; x \;\; (\ln X_i / X_o)\,]$. In that equation, X_i denotes the abundance of each structural component (number of leaves or number of buds, etc ...) while X_0 corresponds to the sum of the abundance of all structural components (number of leaves + number of buds, etc ...). Connectivity corresponds to the absolute abundance, per unit area or per unit volume, of connections between plant parts.

Connections are the link between the plant components like the stem, the branches, the petioles, the pedoncules.

Connectivity can be independent of plant surface as demonstrated by the following analogy. Let's consider a hand with fingers close together. This hand is characterized by a certain surface area and the connectivity is 0 because no connection exists. Now let's consider the same hand with fingers spread. The surface area has not changed but connectivity did. Previous studies on the effect of plant surface area on searching behavior of parasitoids did not control and did not report any information relatively to variables that define plant structure (Knipling and McGuire 1968; Need and Burbutis 1979; Burbutis and Koepke 1981; Kanour and Burbutis 1984; Bigler and Brunetti 1986; Maini and Burgio 1990). More recent studies controlled plant surface area while varying variables defining plant structure (Andow and Prokrym 1990; Geitzenauer and Bernays 1996).

1.4 Host location process

Since the pioneer work of Laing (1937, 1938) and Salt (1935, 1937) the conceptual model of host location by parasitoids has been refined. Successful reproduction of a female parasitoid engaged in host location depends on appropriate habitat identification, host location, host acceptance, host suitability and frequently host regulation (Flanders 1953; Doutt 1964; Vinson 1976; Vinson 1998). As pointed out by Vinson (1998) the host selection process is not a fixed series of steps. In nature there is overlap, certain steps may be absent, modified or subdivided. Parasitoids are guided through these different steps by responding to a hierarchy of physical and/or chemical stimuli that may be attractant or arrestant. Attractant stimuli elicit orientation to areas containing hosts and can be produced by the host and by the immediate environment. Arrestant stimuli caused by visual or tactile stimuli from host or due to a contact with the host elicit a reduction in the distance or area covered per unit time by a parasitoid (Waage 1978). Searching for hosts typically involves responses to stimuli that females have the capacity to perceive and to interpret. As discussed by Lewis et al. (1990) and Vet et al. (1990), genetic variations, physiological status, experience and learning all provide additional variation and flexibility of responses to stimuli perceived. This may be the cause of variability in searching behavior between and within species of parasitoids but also with age of individuals.

1.5 Searching for a host in a multitrophic context

In addition to foraging at multiple scales (ecosystem, macro-habitat, micro-habitat, patch), parasitoids evolved within a multitrophic context characterized by dynamic interactions with several host species (Hassell and Waage 1984; Vet and Dicke 1992). During the various phases of the host searching process, the physico-chemical informations emanating from the different trophic levels may influence the foraging behavior of female parasitoids (Vinson 1981; Van Alphen and Vet 1986). Chemicals appear to play a major role at almost every level of the host location process (Vinson 1976, Van Alphen and Vet 1986). The important role of chemical cues in the host location process and the mediation of trophic interactions of entomophagous arthropods have been documented in more details than have physical ones (Nordlund et al 1981; Bell and Cardé 1984; Vinson 1984 a, b 1985, 1986; Lewis and Nordlund 1985; Van Alphen and Vet 1986; Noldus 1989; Dicke et al. 1990; Vet and Dicke 1992). Nevertheless, the influence of physical characteristics of plants may be significant in the mediation of host-parasitoid interactions.

1.5.1 Influence of host on searching behavior of parasitoids

1.5.1.1 Chemical cues

According to Cloutier and Bauduin (1990), information from the host is probably the most important cue to parasitoids engaged in a host location process. Searching within host habitat for hosts in the absence of host cues is assumed to be random for most parasitoids (Waage 1979, Vinson 1984a). It has been shown that direct chemical (cuticular secretions, kairomones) and indirect chemical or modified host signals (feces, scales from wings) aid several *Trichogramma* species to locate host eggs (Jones 1981; Weseloh 1981; Bell and Cardé 1984). Several species of *Trichogramma* parasitoids react to host sex pheromones (Lewis et al. 1982; Noldus and van Lenteren 1985a, b; Noldus 1988, 1989, 1991). Also response to kairomones from host scales or to extracts appears to be very common in *Trichogramma* species. Kairomones can modify searching behavior of hymenopteran parasitoids by increasing searching time or by restricting it to a limited area, a patch (Lewis et al 1971, 1975; Waage 1978; Vinson 1986) and in such a sense they act as search intensifiers rather than attractants (Gardner and van Lenteren 1986; Schmidt and Carter

1991). Lewis et al. (1972, 1975 a, b, 1979), Gross (1981) and Lewis and Martin (1990) demonstrated that kairomones could be used in a variety of ways to increase rates of parasitization by *Trichogramma* spp. in the field and greenhouse.

1.5.1.2 Physical cues

Physical cues such as sound, movement, mechanical vibrations elicit orientation responses to hosts (Jones 1981; Weseloh 1981; Vinson 1981, 1984; van Alphen and Vet 1986). Moreover, the acceptance of hosts by parasitoids has been attributed to a number of factors such as evaluation of physical characteristics like shape (Vinson 1968; Carton 1971, 1974), size (Richerson and Borden 1972), but also to perception of movement (Tothill et al 1930; Richerson and DeLoack 1972) and sound (Quednau 1967).

1.5.2 Influence of plant on searching behavior of parasitoids

Herbivorous insects are small, highly dispersed and have evolved adaptations to avoid predation and parasitization (Heinrich 1979; Vet and Dicke 1992). Such reality may drive the evolution of indirect host searching strategies by parasitoids, likely the exploitation of environmental cues indirectly associated with host presence such as those from plants (Vet and Dicke 1992). The quality of cues from plants depends on their reliable association with the presence of a host, their detectability, i.e. the ease of stimulus discovery (Vet et al. 1995) and the information content of the signal (e.g., its taxonomic specificity) (De Moraes et al. 1998). These cue qualities presumably enhance searching efficiency and hence fitness (Wäckers and Lewis 1994; De Moraes et al. 1998) Thus, members of the third trophic level must often rely on informations originating from plants to locate hosts. In a key paper, Price et al. (1980) discussed how plant characteristics influence tritrophic interactions by modifying, directly or indirectly, the herbivore's availability to their natural enemies (Kareiva and Sahakian 1990; Price 1991; Takabayashi et al. 1998). The plant can thus modify the dynamic interactions between parasitoid and host by operating on the host, the parasitoid, or both, through chemical and physical characteristics it possesses.

1.5.2.1 Chemical cues

Chemical informations emanating from undamaged plants help some parasitoid to locate hosts and host habitat (Price 1981; Elzen et al. 1983; Ma et al. 1992; Ngi-Song et al. 1996). Plant volatiles released in response to damage caused by herbivores have also been found to be attractive to parasitoids (Turlings et al. 1990; Turlings et al. 1991; Geervliet et al. 1994; Finidori-Logli et al. 1996; Takabayashi et al. 1998). Turlings et al (1990) discovered that parasitoids used volatiles released by caterpillar-damaged corn plants as searching cues and that caterpillar-damaged plants were significantly more attractive than artificially damaged plants. Cornelius (1993) found that foraging behavior of a paper wasp was influenced by chemicals released following caterpillar feeding damage on tobacco plants but not on tomato plants. Furthermore, her results suggest that modification of leaf shape may affect wasp detection of caterpillar feeding damage and thus detection of prey. Messing et al. 1996 showed that chemical stimuli associated with cucurbitaceae plant decay were used by *Pysttalia fletcheri* (Sylvestri) (Hymenoptera: Braconidae), a parasitoid of the melon fly, *Batrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) as cues for host-habitat finding when they were exposed to host-plant stimuli in a wind tunnel.

1.5.2.2 Physical cues

If chemical cues emanating from plants can be detectable, reliable and facilitate host location, there is empirical evidence that physical characteristics and spatial configurations of ecosystems, habitats, plants and flowers alter the capacity of natural enemies to exploit potential hosts or preys by affecting accessibility to hosts, movements and time to find hosts.

Ecosystems

Previous ecological studies showed that characteristics of ecosystems influence trophic interactions (Price et al. 1980, Price 1991). Roland and Taylor (1997) reported results of a many-year study at the ecosystem level that examines the interaction between levels of parasitism by parasitoids and anthropogenic forest fragmentation at large and multiple spatial scales. Forest fragmentation, viewed as a reduction in ecosystem complexity, was found to affect levels of parasitism, this being significantly reduced in fragmented forest.

Habitats

At the scale of the habitat, vegetation structure and density have been shown to influence predator-prey interactions in aquatic organisms (Main 1987; Diehl 1988; Pierce 1988; Heck and Crowder 1991; Greenberg et al. 1995), birds (Eiserer 1980; Barnard and Thompson 1985; Brodmann et al. 1997) and spiders (Uetz 1991). Coll and Bottrell (1996) found that plant assemblages, viewed as mono or di-specific plant stands, affected wasp emigration or within-habitat movement and wasp immigration of parasitoids. Crist and Wiens (1994) found that vegetation structure (seen as vegetation type: short grass, medium grass and shrub-grass and the % cover of each type) clearly affected features of ant movement pathways. They observed an increase in mean foraging distances and mean distance per step from medium grass (2.62 m, 0.09 m/step) to short grass (3.80, 0.11 m/step) to shrub-grass pastures. Thus, broad-scale differences in vegetation structure observed at the habitat level had important influences on forager movement and seed removal by ants

Flowers

Patt et al. (1997) evaluated the foraging performance of two parasitoid wasps on a variety of real and artificial flowers with disparate floral architectures viewed as the arrangement of petals, stamens and other floral parts in relation to nectar gland location. They found that foraging success relies on interplay of insect morphology, floral architecture and searching behavior. The observation by Cartar and Real (1997) of bumble bees collecting nectar from artificial flowers arranged in two spatial configurations revealed that the arrangement of flowers affects pollinator movement and consequently fitness of both pollinators and plants. The complexity of flowers (from six plant species representing a range of structural complexity) was found to influence foraging success of bumble bees (*Bombus* spp) (Laverty 1980).

Plants

At the plant level, the texture of plant parts was found to influence searching efficiency and hence rates of parasitism or of predation of entomophagous insects. On mutant leafless peas, coccinellids found and captured more pea aphids than they do on normal peas (Kareiva and Sahakian 1990). On waxy pea leaves coccinellid larvae captured fewer pea aphids because larvae fell more frequently compared to broad bean plants (Carter et al 1984). On the smooth upper surface of corn leaves, *Trichogramma* sp. parasitized a greater percentage of *Heliothis* sp. eggs than on any other plant part despite more eggs were present on corn silks (Phillips and Barber 1933). The density of hairs and trichomes at the surface of leaves influences many entomophagous species (Obrycki 1986; Treacy et al. 1986; Kauffman and Kennedy 1989; Kashyap 1991). Foliar pubescence of various plant species (cucumber, tomato) reduced walking speed and therefore searching efficiency and parasitism of the parasitoid *Encarsia formosa* (Juniper and Southwood 1986; van Lenteren et al. 1995; van Roermund and van Lenteren 1995; Sütterlin and van Lenteren 1997).

In addition to texture of plant surface, plant attributes such as topography, surface area or volume, size, and complexity were found to influence host-parasitoid interaction. The topography of plants and of leaves can strongly influence searching pattern of parasitoids (Evans 1976; Verkerk and Wright 1997). Finding success by different parasitoid species generally decreased with an increase in plant surface area or volume (Knipling and McGuire 1968; Need and Burbutis 1979; Burbutis and Koepke 1981; Kanour and Burbutis 1984; Bigler and Brunetti 1986; Maini and Burgio 1990), in size (Ables et al. 1980; Thorpe 1985; Wang et al. 1997), and in plant complexity (Ramsy 1977, Bond 1983, Andow and Prokrym 1990; Kareiva and Sahakian 1990; Grevstad and Klepetka 1992; Mackauer and Völkl 1993; Geitzenauer and Bernays 1996).

The effect of plant structure on searching behavior of parasitoids and predators has been studied with different experimental procedures, different natural plant species (Pimentel 1961; Gardner and Dixon 1985; Mackauer and Völkl 1993), and very often, artificial plant models. The use of artificial plants permits study of the effect of physical structure alone, without variability of chemical (e.g. volatiles) and physical (e.g. color, texture) characteristics that we would have with natural plants. Different types of material have served to test the effect of plant structure, like waxed paper (Andow and Prokrym 1990), rod (Bond 1983), paper (Lukianchuk and Smith 1997), artificial silk plants (Frazer and McGregor 1994; Geitzenauer and Bernays 1996).

So far, studies on the influence of physical characteristics of plants on searching behavior of entomophagous insects have focused on one aspect of plant structure, either size, heterogeneity or connectivity and very often, used only one species of predator or parasitoid and one herbivore species. In nature, searching insects may be submitted to variation of the three components of plant structure because they may search on plants of different species, varieties or age. Additionally, the diversity, density and distribution of hosts may vary between plants and within plant parts which adds to the challenge of finding hosts by such minute and short-lived parasitoids of the genus *Trichogramma*.

2. THE TRITROPHIC SYSTEM UNDER STUDY

2.1 The cruciferous plants - first trophic level

Members of the plant family Cruciferae represent a diverse, widespread, and important plant group that includes cabbage, broccoli, cauliflower, collards, rapeseed, mustard, and Chinese cabbage. Members of this diverse plant group are cultivated for various edible plant parts (Talekar and Shelton 1993).

2.1.1 Production of crucifers in Canada

Four species of *Brassica* are cultivated in Canada. The rutabaga and turnip (*B. rapa*) are grown for their root while rapes (*B. napus* and *B. campestris*) are grown for oilseed production (canola oil) and are increasing in importance. Finally, *B. oleracea* is grown in a multitude of varieties in which various plant parts are edible.

In Canada, crucifers are produced mostly in Quebec, Ontario, and British Columbia (Richard and Boivin 1994). In Quebec, the production of crucifers (cabbage, broccoli, Brussels sprouts) covered an area of approximately 3 600 hectares and provided 29 million $ at harvest in 1997 (Statistique Canada 1998). The surface area devoted to the culture of cabbage is greater than that for broccoli and Brussel sprouts.

2.1.2 Brassica oleracea varieties used for this study

Among the vegetables derived from *Brassica oleracea* (L.) are kale, broccoli, cauliflower, many forms of cabbage and Brussels sprouts. These plants are a clear example of the outcome of the process of artificial selection, in which, leaves, stems, roots, axillary buds, or inflorescences have been altered to give rise to strikingly different forms (Hawkes 1983).

For this study, we chose to work with cabbage, broccoli and Brussels sprouts because as they belong to the same species, they probably share a similar chemistry, in particular the presence of glucosinolates although there may exist variations in the type and concentration of these molecules (van Etten and Tookey 1979; Simmonds 1979). Also, even though the three varieties are similar in color and in leaf texture, they exhibit very different structures that may lead to differences in searching efficiency and searching activities by female parasitoids and consequently to differences in host finding success and fitness.

2.2 The hosts - second trophic level

The different varieties of *Brassica oleracea* can be damaged by numerous insect pests, such as different species of aphids, the cabbage root maggot, *Delia radicum* (Diptera: Anthomyiidae), and caterpillars of some species of lepidopters (Harcourt 1966).

2.2.1 Lepidopterous pest insects of crucifers in North America

Only three major lepidopterous pest species are known to do economic damage on *Brassica oleracea* (L.) varieties in North America: the imported cabbageworm, *Pieris rapae* (L.) (Lepidoptera: Pieridae), the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) and the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) (Pimentel 1961; Harcourt 1963; Shelton et al. 1982; Chagnon et al. 1990; Biever et al. 1992; Godin and Boivin 1998). The population dynamics of these three pest species have been studied in various regions of North America (Pimentel 1961; Harcourt 1963; Oatman and Platner 1969; Elsey and Rabb 1970; Ru and Workman 1979; Biever et al. 1992). Their abundance and the seriousness of the damage produced vary according to regions and years. For example, *T. ni* and *P. xylostella* are more abundant than *P. rapae* in the regions of Texas, California, and Louisiana (Edelson et al. 1988). In southern California, the most serious pests were found to be *T. ni* and *P. rapae* (Oatman 1966). In eastern Ontario, Harcourt (1960), reported that *P. xylostella* was more abundant than *P. rapae* and *T. ni* during 1952-1956. In Southwestern Quebec, Godin and Boivin (1998) reported that *P. xylostella* and *P. rapae* were the most important and abundant pests. But according to Biever et al. (1992) *P. xylostella* is considered to be the least injurious pest while *T. ni* can potentially be the most injurious species all over North America, in most years.

2.2.2.1 Biology of *T. ni*

The biology and behavior of *T.ni* have been described by Shorey et al. (1962). *T. ni* moths become active shortly after sunset, and usually feed and deposit their eggs at dusk (Shorey et al. 1962). The eggs, roundly shaped (0.6 mm diameter x 0.4 mm height), are creamy white and vertically stripped (Sutherland 1966). They are generally laid singly or in groups of 2 to 11, on the outer leaves of the plant and 91% of the eggs are laid on the under leaf surface (Harcourt 1962; Sutherland 1966). But Harcourt (1963) reported that eggs could be deposited on either side of the leaves, usually near the margins. The caterpillars feed on the foliage throughout their development, mostly on the lowest leaves of the plant, passing through five instars (Hoy et al. 1989). When mature, the larvae crawl to the lower leaves of the plant to pupate. There are three to four generations yearly in eastern North America. *T. ni* does not overwinter in Canada (Sutherland 1966; Elsey and Rabb 1970; Chamberlin and Kok 1986) and it is believed that populations observed in Canada originate from southern regions from where moths emigrate. When present, *T. ni* is considered a serious pest of various *Brassica* crops (Harcourt 1986; Richard and Boivin 1994).

2.2.2.3 Biology of *P. rapae*

P. rapae was first discovered in North America in 1860 in Quebec City but now occurs throughout most of North America (Harcourt 1963). Adult females are active during the day and lay their eggs singly on the outer leaves of the host plant and approximately 70 % of the eggs are laid under leaf epidermis surface (Harcourt 1962). The eggs of *P. rapae* are yellow, bullet-shaped and are 1.0 x 0.4 mm in size (Pak et al. 1986). According to Ives (1978), larger plants and older leaves are preferred for oviposition, though the oldest are sometimes avoided. Larger plants also receive more eggs than smaller ones of the same cultivar, but cultivar preferences may override the response to size (Ives 1978). *P. rapae* has five larval instars. Young larvae remain on the outer older leaves while instars three to five prefer young leaves and feed on the upper leaves of the plant (Harcourt 1962; Ives 1978), causing changes in both plant structure and plant growth (Samson and Geier 1983).

2.2.2.4 Biology of *P. xylostella*

P. xylostella moth adults become active at dusk and continue their activity into the night (Harcourt 1954). Female moths start laying eggs soon after mating, and the oviposition period lasts 4 days, during which the female lays 11-188 eggs (Harcourt 1954). The incubation period of eggs is influenced by temperature but lasts usually 5 to 6 days. According to Harcourt (1961), most of the *P. xylostella* eggs are laid on the upper leaf epidermis, singly, or in groups of 2 to 8. Less than 1% of the eggs are laid on the stems and petioles of cabbage. The eggs are very small and have a flat oval shape, 0.44 x 0.26 mm in diameter. Typically, they are laid in depressions of the leaf along the midrib and larger veins, or on the concave surfaces of the smaller veins. This habit, combined with their small size, makes them difficult to find. The duration of the four larval instars also depends on temperature but the average duration was between 4 and 5.6 days for the first through fourth instars. The duration of the pupal period varies from 4 to 15 days depending on temperature (Harcourt 1957). Adults feed on water drops or dew and are short-lived.

In southern Ontario, Harcourt (1986) reported four or five generations a year, depending on seasonal temperatures. The period required for a complete generation varies from 18 to 51 days, averaging 25 in July and August. The diamondback moth pest status is uncertain in Canada, and it is believed that most of the annual infestations arise from adults which disperse from winter breeding sites in U.S.A. (Harcourt 1986).

Although *P. xylostella* is believed to have originated from the Mediteranean area (Harcourt 1954), the source of some of our most important crucifers, *P. xylostella* now occurs wherever crucifers are grown, and this insect is believed to be the most universally distributed of all Lepidoptera (Meyrick 1928). The diamondback moth continues to present one of the greatest threats to crucifer production in many parts of the world (Wührer and Hassan 1993). In recent years, *P. xylostella* has become the most destructive insect of cruciferous plants throughout the world. The larvae inflict more than 1 billion dollars in losses per year on cruciferous crops worldwide (FAO 1992; Talekar 1992). In the United States only, the annual cost for managing *P. xylostella* is estimated to be U.S. $1 billion (Talekar and Shelton 1993).

2.3 The Parasitoids - third trophic level

2.3.1 Parasitoids of the genus Trichogramma

The Trichogrammatidae family represents a large group of minute parasitic wasps that attack eggs of various insects, many of which are of economic importance (Nagarkatti and Nagaraja 1977). Nagarkatti and Nagaraja (1977) and Pinto and Stouthamer (1994) made comprehensive reviews on the Trichogrammatidae family.

The genus *Trichogramma* was created by Westwood with *T. evanescens* designated as the type species (Nagarkatti and Nagaraja 1977; Pintureau 1993). Prior to the 1970s, only a few species of the genus *Trichogramma* were described because of taxonomic difficulties (Nagarkatti and Nagaraja 1977). Differences in the structure of male genitalia are now used to describe new species (Nagaraja and Nagarkatti 1969).

2.3.2 A generalist gender

There exist 145 species in the genus *Trichogramma* (Pinto and Stouthamer 1994) and these are known to attack a wide variety of field crop moths (Salt 1935, 1938, 1940) and several lepidopteran species of stored-product pests are suitable hosts (Boldt et al. 1973; Voegelé et al. 1974; Benoit and Voegelé 1979; Brower 1983). Some species of *Trichogramma*, like *T. evanescens*, appear to be truly polyphagous since they can develop successfully in at least 65 different lepidopteran hosts (Salt 1935). Eggs of lepidopters appear to be the only suitable hosts for *Trichogramma*.

Differences in host preference have been observed and these may be based on egg size, egg shape, thickness of the chorion, and chemical characteristics of the host (Salt 1935, 1938; Laing 1937). The study of Brower (1983) confirmed that large spherical eggs appear to be most preferred while small cylindrical eggs were least preferred. *Trichogramma* spp. usually shows a significant preference for young (less than 2 days old) over old (less than three days before hatching) host eggs (Reznik et al. 1997). Godin and Boivin (1999) tested 42 species/strains of *Trichogramma* and Trichogrammatidae and found that most of the females prefer young eggs of *P. rapae*, *T. ni*, and *P. xylostella*; age of eggs varied between 2 and 72 hours. Some *Trichogramma* females refuse to parasitize old eggs (Pak 1986; Pak et al. 1986; Hintz and Andow 1990; Reznik and Umarova 1990; Li and Henderson 1993; Smith and Strom 1993; Reznik et al. 1997)

2.3.3 Activities associated with host localization

Efficiency of hymenopterous parasitoids increases with increasing searching ability of females, which in turn depends on the distance from which females perceive their hosts (i.e. reactive distance) (Pak et al. 1991; Bruins et al. 1994) and of the activities inherent to searching and ovipositing such as walking, resting, jumping and flying. Bruins et al. (1994) reported that reactive distance of *T. brassicae* varied between 3.67 and 4.01 mm. There have been intensive studies on the walking behavior of *Trichogramma*, mostly for use as a quality index in mass rearing programmes (Bigler et al. 1988). Bigler et al. (1988) suggested from their studies on variation in locomotion between laboratory strains of *Trichogramma maidis* that travel speed can be used as a parameter to estimate the capacity for host location and the efficiency of a *T. maidis* strain for inundative biological control programs. In contrast, observations on flight by *Trichogramma* are rare (reviewed in Keller et al. 1985; Noldus et al. 1988), and understandably so because the minute size of the insect makes it difficult to observe. Due to the minute size of adult *Trichogramma* (0.5-1 mm) and their inability to fly upwind, movement between potential host habitats and localization of host is thought to be passive rather than active (Keller and Lewis 1985; Keller et al. 1985). Initiation of flight has been found to be affected by ambient temperature (Forsse et al. 1992). Also, they observed that the presence of fresh host eggs caused a reduction in the proportion of female parasitoids that flew and a delay in the time to flight for the females that did fly.

Young females, with a higher number of ovarian eggs, showed greater searching activity than older ones with fewer eggs (Pak et al. 1985). Pak et al. (1985) also studied the interplant dispersal activity of *T. maidis* in a greenhouse by releasing females in one row of cabbage plants and by determining parasitism in a second row of plants at various distances between the two rows. The rate of parasitism decreased exponentially with increasing distances between the two rows.

3. BIOLOGICAL CONTROL OF CRUCIFEROUS PESTS

Parasitoids are natural enemies of many species of herbivores and could efficiently regulate the lepidopteran pest complex of cruciferous crops in Quebec. Seasonal inoculations or inundative releases of selected parasitoid species may maximize the impact of natural enemies in suppressing this pest complex (Biever et al. 1992; Verkerk and Wright 1997). Such practice exists for more than 50 years, and since then there have been more than 3600 purposeful introductions of parasitoids against more than 500 arthropod pests in almost 200 countries and islands around the world (Greathead and Greathead 1992).

3.1 Control of cruciferous pests with parasitoids

3.1.1 Control of T. ni

Identity and importance of *T. ni* predators are poorly known, and most reports are based on limited observation or association. It is generally considered that predators of *T. ni* are the same as those attacking *P. rapae* and *P. xylostella* (Sutherland 1966; Elsey and Rabb 1970).

Naturally occurring parasitoids of *T. ni* are found in North America. Up to 11 species of parasitoids have been reported and most of them are Hymenoptera restricted to three families: Encyrtidae, Ichneumonidae and Trichogrammatidae (Sutherland 1966; Oatman and Platner 1969; Martin et al. 1981). The Encyrtidae *Copidosoma floridanum* (= *truncatellum*) that lays its eggs within those of the host and completes its development when the host attains prepupal stage is regularly reported (Oatman and Platner 1969; Martin et al. 1981; Kok and McAvoy 1989; Godin and Boivin 1998). It is usually considered the most important egg parasitoid of *T. ni* (Harcourt 1963a). In contrast, the Ichneumonidae parasitoids may occur at low numbers and never represent an important mortality factor (Harcourt 1963a; Godin and Boivin 1998). Three species of egg endoparasitoid of the genus *Trichogramma* were found to parasitize eggs of *T. ni* in cruciferous crops: *T. pretiosum*, *T. minutum* and *T. exiguum* (Sutherland 1966; Oatman and Platner 1969; Elsey and Rabb 1970; Chambelin and Kok 1986). In southern North America, levels of parasitism are generally above 10% and as high as 55% (Martin et al. 1981).

3.1.2 Control of P. rapae

The braconid parasitoid *Apanteles glomeratus* (L.) was not successful in controlling *P. rapae* after its introduction to North America in 1883 (Parker et al. 1971). Jones et al. (1987) observed that natural enemies, such as predators, virus disease, and braconid parasitoids can control late-stage larvae of *P. rapae*. However, they reported a high spatial variability in survival of larvae among the different sites they studied, even though they were located in very similar habitats (Jones et al. 1987). Eggs, larvae and pupae of *P. rapae* can be parasitized by a few species of parasitoids but *Trichogramma* species were rarely recovered (Biever et al. 1992; Godin and Boivin 1998).

By applying a new control system in which there is a mass release of both the pest and the parasitoids, Parker et al. (1971, 1972) were able to control populations of *P. rapae* in Missouri. Oviposition by field populations of *P. rapae* have been found to be deterred by an extract of the wild crucifer, *Eryssium cheiranthoides* L. (Dimock and Renwick 1991)

3.1.3 Control of P. xylostella

Verkerk and Wright (1996) review the wide array of strategies that have been used in attempts to control *P. xylostella* over the last 40 years by consolidating the literature on two and three trophic level interactions. The seasonal abundance of diamondback moth and its parasitoids has been studied in various regions of North America (Reid and Bare 1952; Pimentel 1961; Harcourt 1963; Oatman and Platner 1969; Harding 1976; Latheef and Irwin 1983; Kok and McAvoy 1989; Lasota and Kok 1989; Biever et al. 1992).

All stages of the diamondback moth are attacked by numerous parasitoids and predators with parasitoids being the most widely studied. Adults are often attacked by polyphagous predators such as birds and spiders but not much is known about their impact on diamondback moth populations as indicated by the almost total absence of published research on the subject (Muckenfuss and Shepard 1994 ; Verkerk and Wright 1996). Although over 90 parasitoid species attack diamondback moth (Goodwin 1979), only about 60 of them appear to be important. Among these, 6 species attack diamondback moth eggs, 38 attack larvae, and 13 attack pupae (Lim 1986). Larval parasitoids are the most predominant and effective. Many of the effective larval parasitoids belong to two major genera, *Diadegma* and *Cotesia* (*=Apanteles*) (Harcourt 1986; Mitchell et al. 1997); a few *Diadromus* species., which are mostly pupal parasitoids, also exert significant control. Egg parasitoids belonging to the polyphagous genus *Trichogramma* contribute little to natural control and may thus require frequent mass releases. In their study, Godin and Boivin (1998) found no egg parasitoids while larvae and pupae of moth were parasitized by five parasitoid species in southwestern Quebec. Biever et al. (1992) recovered 3 species of parasitoid but no egg parasitoid.

According to Lim (1986), the absence of effective natural enemies, especially parasitoids, is believed to be a major cause of the diamondback moth's pest status in most parts of the world.

3.2 *Trichogramma* egg parasitoids as biological control agents

Egg parasitoids such as *Trichogramma* are particularly valuable because they kill pests before damage occurs. *Trichogramma* spp. have been recognized as biological control agents for more than 100 years and are the most widely used natural enemy for control of lepidopterous pest species on a number of crops worldwide (Wajnberg and Hassan 1994; Smith 1996).

Several species of *Trichogramma* are being regularly used, mostly through inundative releases against at least 28 different herbivorous pest species on some 20 different crops in 16 countries on more than 18 million ha (Ridgway et al. 1981; Voronin and Grinberg 1981; Voegelé 1988; Hassan 1993). In North America, Europe and Russia, large-scale rearing systems for *Trichogramma* spp have been developed using eggs of the Mediteranean flour moth, *Ephestia kuehniella* Zeller and *Sitotroga cerealella* Oliver. Due to the relatively cheap mass rearing on factitious hosts, *Trichogramma* is used in inundative releases more than any other natural enemy species (Stinner 1977). Species of *Trichogramma* are presently reared for commercial purposes in at least 16 different countries, with the Soviet Union and China leading in acreages of application (Coulson et al. 1982; Ridgway and Morrison 1985). In Canada, the use of *Trichogramma* is relatively recent. Since 1995, corn producers have practiced inundative release of these wasps in Quebec against the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) From 3 ha in 1995, the surface area treated with *T. brassicae* increased to 750 ha in 1999 (F. Fournier, pers. comm. 1999). In Nova Scotia *T. pretiosum* were released in fields for suppression of *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae) on sweet corn (Neil and Specht 1990). In Alberta, *T. brassicae* were released to control *O. nubilalis* (Hübner) (Lepidoptera: Pyralidae) (Yu and Byers 1994). Important studies were conducted for the selection of suitable *Trichogramma* parasitoids to control blackheaded fireworm, *Rhopobota naevana* (Hübner) (Lepidoptera: Tortricidae) infesting cranberries (Li et al. 1994), to control oblique-banded leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), in raspberries and blueberries (McGregor et al. 1998) but also to control forest insect pests, in particular, the spruce budworm, *Choristoneura fumiferana*

(Clemens) (Lepidoptera: Tortricidae) (Smith et al. 1986, 1990; Smith and You 1990; Newton 1993).

3.2.1 Variability in host location ability

The effectiveness of *Trichogramma* parasitoids is largely determined by their ability to find hosts (Stinner 1977; van Lenteren et al. 1982). However, variation in host location ability exists and this could be a major source of inconsistent results in biological control with these wasps.

Trichogramma displays both interspecific and intraspecific variations in regard to their preferences for hosts, crops (recognition and acceptance), in their searching behavior (habitat location, host location) and in their tolerance to environmental conditions. Factors influencing the effectiveness of *Trichogramma* in the field were tested by several research workers in laboratory, semi-field and field experiments (Gonzales et al. 1970; Need and Burbutis 1979; van Lenteren et al. 1982; Pak and van Heiningen 1985; van Dijken et al. 1986; Hassan 1989; Smith 1996).

The causes for variation in foraging behavior are currently poorly understood despite a substantial body of theoretical and empirical literature dealing with the subject (Lewis et al. 1990). Most earlier investigations focused on extrinsic factors such as environmental variables as the source of variation in the parasitoid searching behavior (Waage and Hassell 1982). Very limited consideration has been given to intraspecific variation in the genetic composition or behavioral state. More recent studies showed that foraging responses among individuals of a parasitoid population, even to the same set of stimuli, can be quite variable. Further, the behavior of a given female parasitoid is often plastic and can vary considerably depending on the history of that individual (Wardle and Borden 1985; Lewis and Tumlinson 1988; Vet et al. 1990). Therefore, researchers hoping to use parasitoids for biological control of pests must be aware that the results will be a product of the diversity and plasticity of the parasitoid population interacting with environmental parameters of the foraging arena.

3.3.1 Desirable traits of a Trichogramma *control agent*

The selection of the most appropriate *Trichogramma* parasitoid is a difficult process because there exists considerable interspecific variation among the 145 species known, and the taxonomy of the genus is poorly understood (Pinto and Stouthamer 1994). Local species are generally selected for release on the ecological basis that they are better adapted to the climate, habitat, and host conditions (Voegelé 1988; Hassan 1994).

Among the desirable traits of natural enemies listed by some authors for inundative and seasonal inoculative biological control there are searching efficiency and adaptability to various habitats or, in our context of research, to various plant structures. Other traits include: adaptability to climatic extremes, longevity, host specificity, host discrimination, host utilization (ability to kill a host and/or use it for reproduction), mass productibility, reproductive capacity, sex ratio and lack of negative side effects (van Lenteren 1986; Pak 1988b; Noldus 1989a; Smith 1996).

In the Netherlands, selection programs of *Trichogramma* species/strains were undertaken for the control of lepidopteran pests in cruciferous crops (Glas et al. 1981; van Lenteren et al. 1982; Pak and van Heiningen 1985; van Dijken et al. 1986). The effectiveness of inundative releases of four strains of the egg parasite *Trichogramma* to control five lepidopterous species infesting Brussels sprouts in the Netherlands was examined in small-scale field experiments in 1982-1985 (Pak et al. 1989). Strains were selected on the basis of behavioural characteristics investigated in laboratory experiments, i.e. parasitization activity at low temperature and host-species preference. A strain of *Trichogramma evanescens*, with a high activity at 12^0 C, performed best against *Mamestra brassicae* L. (Pak et al. 1989). However, even its highest rate of parasitism (52%) was not judged sufficiently effective. A strain of *T. maidis* performed best against *Pieris brassicae* L. and *Pieris rapae* L., but parasitism remained low (< 30%). *Plutella xylostella* was an abundant species in some years, but parasitism of its eggs was never observed (Pak et al. 1989). Pak et al. (1990) studied the host selection behavior among nine strains of *Trichogramma* from a laboratory collection of geographic strains in order to select strains for inundative biological control of Lepidoptera on cabbage. Results showed that *M. brassicae* was a highly acceptable host species for all strains, whereas acceptability of *P. brassicae* and *P. rapae* was similar within some strains but varied among others. In Germany, Wührer and Hassan (1993) tested 47 strains of *Trichogramma* and two strains of *Trichogrammatoidea* in laboratory for their ability to attack *P. xylostella*. They retained

T. pretiosum because it had a high egg laying capacity (53.7 per female) and *T. cacoeciae* because it had a strong preference for diamondback moth eggs. In the United States, Vásquez et al. (1997) compared the mortality of *P. xylostella* eggs induced by six *Trichogramma* species. *Trichogramma bactrae*, *T. pretiosum*, and *T. minutum* caused the highest mortalities, between 95 and 98% of *P. xylostella* eggs, and as suggested by the authors, the focus of further greenhouse and field studies should be on these species.

None of these investigations considered, in their selection program, the fact that the different varieties of *B. oleracea* exhibit different structures and that plant structure can influence host searching behavior and the resulting host finding success. Furthermore, the structure of plants changes with age, becoming more complex due to an increase in size, heterogeneity and connectivity. *Trichogramma* species must then be an efficient forager on plants of varying structure to be a successful natural enemy.

CHAPTER 3

EFFECT OF PLANT STRUCTURE, HOST DENSITY AND FORAGING DURATION ON HOST FINDING BY *TRICHOGRAMMA EVANESCENS*

DANIEL GINGRAS and GUY BOIVIN

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ABSTRACT

While searching for hosts on plants, female parasitoids meet different plant structures and host densities that will influence their host finding success. In this study, we determined if plant structure, host density and foraging duration influenced the percentage of *Ephestia kuehniella* eggs parasitized by *Trichogramma evanescens*. One female was introduced to forage either 4 or 24 hours on artificial plants of three different structures (simple, intermediate and complex) on which 4 or 16 host eggs were glued. Plant structure influenced rate of parasitism in both foraging durations while host density was found to be significant only when female had 4 hours to forage. Mean rate of parasitism generally decreased with an increase in complexity of plant structure and host density in both foraging durations. Almost twice as many eggs were parasitized on simple plants compared to complex plants for all host densities and foraging durations. Overall, female parasitoids parasitized, on average, less than one egg per hour except when they foraged 4 hours in the presence of 16 eggs. Plant structure therefore affects host encountering success. Physical structure of a plant mediates ecological interactions and is involved in parasitoid-host population dynamics. It operates by increasing costs associated to host finding.

KEY WORDS Plant structure, trophic interactions, artificial plants, *Trichogramma evanescens*, *Ephestia kuehniella*, size, heterogeneity, connectivity, parasitoids

INTRODUCTION

Animals have to search for and locate food and mate within habitats of varying spatial configurations whose characteristics influence trophic interactions (Price et al. 1980, Price 1991, Roland and Taylor 1997). Vegetation structure and density have been shown to influence predator-prey interactions in aquatic organisms (Main 1987, Diehl 1988, Pierce 1988, Heck and Crowder 1991, Greenberg et al. 1995), and birds (Eiserer 1980, Barnard and Thompson 1985, Brodmann et al. 1997). Plant attributes have also been found to alter the capacity of natural enemies to discover herbivorous insects (Uetz 1991). Encounters of host and prey by parasitoids and predators generally decrease with an increase in plant size (Ables et al. 1980, Thorpe 1985), plant complexity (Ramsy 1977, Bond 1983, Carter et al 1984, Kareiva and Sahakian 1990, Grevstad and Klepetka 1992, Mackauer and Völkl 1993, Geitzenauer and Bernays 1996), plant surface area or volume (Knipling and McGuire 1968, Need and Burbutis 1979, Burbutis and Koepke 1981, Kanour and Burbutis 1984, Bigler and Brunetti 1986, Maini and Burgio 1990).

Plant structure can be described by three variables: size, heterogeneity and connectivity (Andow and Prokrym 1990, Bell et al. 1991). Size refers to the height of the plant while heterogeneity refers to the relative abundance, per unit area or per unit volume, of the different structural components such as leaves, flowers, buds, fruits but also to components, within the same plant, that have different morphologies (glabrous vs hairy leaves, male vs female flowers). Connectivity refers to the absolute abundance, per unit area or per unit volume, of connections between individual structural components. It is thus possible, using these three variables, to quantify the structure of a plant. A simple plant structure will have low values of size, heterogeneity and connectivity and the opposite holds for a complex plant structure.

In their study on the effect of plant structure on searching efficiency of parasitoids, Andow and Prokrym (1990), reported that *Trichogramma nubilale* (Hymenoptera: Trichogrammatidae) parasitized 2.9 more *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae) eggs and found them 2.4 times faster when foraging on simple artificial paper wax models than on complex ones. Lukianchuk and Smith (1997) observed that *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) found significantly more egg clusters of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and were quicker at finding them on simple artificial paper models and on natural foliage surfaces than on the most complex paper or foliage surfaces.

Complex plant structure affects movement of parasitoids, hence reducing host encounter rate and fitness define here . *Trichogramma* parasitoids search for eggs in environments where plant structure but also host abundance and distribution vary temporally and spatially, which can affect host finding success. However, the effect of an increase in complexity of plant structure may be diminished by either an increase in host density or foraging duration by female.

Trichogramma evanescens Westwood (Hymenoptera: Trichogrammatidae) is an egg endoparasitoid of several lepidopteran species and, as such, has to search for patchily distributed host eggs located on plants of varying physical structure, either because they belong to different species or are at different phenological stages. Because the microhabitats exploited by generalist species such as *Trichogramma* are expected to vary, and because searching efficiency is a major fitness component in such short-lived species, we hypothesized that plant structure affects host finding by *T. evanescens* female. Such an impact is likely because *Trichogramma* females locate hosts mostly by walking on plants (Bigler et al. 1988).

In this paper, we determined if plant structure, host density and foraging duration influenced the percentage of *Ephestia* eggs parasitized by female *T. evanescens* on artificial plants of three different structures.

MATERIAL AND METHODS

Females *T.evanescens* used in all experiments were reared on cold-killed *E. kuehniella* eggs at 24 ± 1° C, 50 ± 5% r.h. and 16L:8D. Females were less than 6 hours old, mated, unfed and naive.

In order to reduce variations in color, texture, morphology and chemical volatile, artificial plastic plants were used to determine the effect of plant structure, host density and foraging duration on the rate of parasitism. All plants were of the same height (= 18 cm) and heterogeneity (= 0), but were of three different connectivity levels (simple= 0.2 connection per cm^2; intermediate= 1.5 connection per cm^2; complex= 7 connections per

cm^2) and surface area, which was measured with an area meter apparatus, model 3100 from Li-Cor Inc. (simple= 4.9 cm^2, intermediate= 14.0 cm^2, complex= 20.2 cm^2) (Fig. 3.1). The simple and intermediate plant structures were created by cutting parts of the complex structure.

Four or sixteen eggs of *E. kuehniella* were glued with mucilage Elmer's ® glue on each plant. Eggs were glued, always at the same location, on either sides of the leaf. The plants, fixed on a styrofoam plate, were covered by a 1 L transparent plastic cylinder and one female *T. evanescens* was introduced by the top and deposited with a fine brush at the tip of the plant. The top of the cylinder was then covered with a piece of polyethylene film. The experimental set-up were placed in an incubator at 24° C, 16:8 hours L:D. After either 4 or 24 hours, the female was removed from the cylinder which was left under the same conditions for six days. After that period, parasitized eggs that had turned black were counted. Each combination of level of plant structure and host density was replicated 35 times for the 4 hours experiment and 46 times for the 24 hours experiment.

The experimental set-up consisted in a two-way factorial design, with plant structure and host density as main factors. Foraging durations were tested in separate experiments. Treatment combinations (plant structure x host density) were allocated according to a randomized complete block design. There was no block effect ($P= 0.5096$). Only replicates where at least one egg was parasitized were kept for statistical analysis. Prior to analysis, % parasitism was transformed by arcsine square root (Sokal and Rohlf 1981). An ANOVA was performed to determine the effect of treatment on host finding success and a

Tukey test was applied when the model was significant. SAS software, version 6.12 (SAS Institute 1997), was used for all statistical analysis.

RESULTS

Plant structure affected significantly the rate of parasitism in both the 4 and 24 hours foraging durations (Table 3.1). A significant effect of host density was found when the female had 4 hours to forage but this effect was not significant for the 24 hours duration (Table 3.1). The interaction between plant structure and host density was significant for 24 hours indicating that on plants of identical structure, host density did influence the proportion of host parasitized. The interaction was not significant for the 4 hours duration.

Mean rate of parasitism decreased with an increase in connectivity for both 4 and 24 hours foraging durations. For all plant structure and host density combinations, mean rate of parasitism was higher when females had more time to forage, reaching over 90% in the 24 hours period. Almost twice as many eggs were parasitized on simple plants compared to complex plants for both host density and foraging duration values, except for 24 hours and 16 eggs (Fig. 3.2, A and B).

Mean number of eggs found and parasitized was significantly different between plants of different structure and host density for both foraging durations (Fig. 3.2, A and B). For identical foraging duration and host density, mean rate of parasitism differed significantly between the three plant structures. For identical foraging duration and plant structure, mean rate of parasitism differed between host densities on simple plant only.

The difference in mean rate of parasitism between the 4 and 24 hours foraging durations for identical host density and plant structure is rather small despite the fact that the female had six times longer to forage. After only 4 hours of foraging, females discovered more then 50% of the eggs, except on complex plants. When mean number of eggs parasitized, was divided by the foraging duration, we found that the female parasitoid found and parasitized, on average, less than one egg per hour except when it foraged 4 hours in presence of 16 eggs where it parasitized between 1.5 and 2.5 eggs per hour. Female's rate of egg discovery per hour is between 1.3 and 1.8 times higher on simple plant structure than on complex plant structure for all host density and foraging durations.

DISCUSSION

Female parasitoids need to find suitable hosts to reproduce and, because of the direct link between successful host finding and offspring production, we expect parasitoid foraging behavior to be strongly influenced by natural selection (Cook and Hubbard 1977). In parasitoid foraging studies, it is generally assumed that offspring produced per unit of foraging time is the currency in which fitness gains are expressed (van Alphen and Vet 1986). Female *T. evanescens* search for eggs in environments where not only the abundance and distribution of eggs vary both temporally and spatially but also the physical structure of the macro and micro habitats. A female parasitoid must face two decisions while engaged in the host location process (Vinson 1998): where to forage and how much time to invest in a given microhabitat. Those decisions may have significant consequences on foraging gains and oviposition rates.

Our experiment indicates that female parasitoids foraging randomly, with little or no chemical cues, on a plant with a simple structure register greater foraging gains, leave more descendants and thus have a greater fitness than a female foraging on a plant with a complex structure. Plant structure affects rate of egg discovery, which is higher on simple plants and decreases as plant increases in complexity. Furthermore, level of parasitism increased when host egg density increased, which can be seen as an effect of functional response. The physical arrangement of simple plant models favor orthokinetic movements and rates of egg encounter which lead to high rates of parasitism. When connections are added, orthokinetic movements become more difficult and overlap of search paths may occur more frequently. Also, greater surface area has to be searched, which take more time. This may explain lower rates of parasitism on complex plant.

Our results also show that foraging duration affects finding success and that the structure of the plant becomes less important when a female has more time to search for hosts. The rate of parasitism was not found to be proportional to foraging duration. When the female was given 24 hours to forage, which is really 16 hours of foraging because low or no searching activity occurred during the scotophase (8 hours) (Allemand et al. 1994), instead of 4 hours, the increase in the rate of parasitism was between 1.1 and 1.6, which is less than the increase in time. Relatively high rates of parasitism were reached within 4 hours of foraging by the female. After that period of time, not many eggs were found. Therefore, most of the eggs are found within the first few hours following the release of the female on the plant. Foraging for a longer period of time did not appear to be a good decision since foraging gains would not be much greater. An increase in plant complexity did not increase patch time allocation in this species.

Mackauer and Völkl (1993) defined searching efficiency as the number of eggs parasitized per unit of search time. In our study, the female parasitized less than one egg per hour, although some eggs may have been found but not parasitized because they were rejected. On artificial plants, a female forages randomly because little or no chemical cues are emitted from cold-killed hosts and this could explain such low rates of egg discovery per unit of foraging time. As demonstrated by Lewis et al. (1971), *T. evanescens* detects more easily the presence of hosts in the presence of moth scales which act as a source of kairomones. Lewis et al. (1975) demonstrated that kairomones increased rates of parasitization by *Trichogramma* spp. in the field and greenhouse. Kairomones elicited a more intensified search pattern, but did not act as an attractant. To discover less than one egg per hour, the female must not have explored or moved much on the plant. Also, it appears that young females (0-8h old) are relatively inactive, and only start to move continuously after 24-36h (Marston and Ertle 1973).

In this study we defined and quantified the variables describing the structure of a plant and utilization of artificial plants enabled us to study the effect of plant physical structure alone. Our results are in accordance with other studies (Bond 1983; Carter et al. 1984;

Andow and Prokrym 1990; Mackauer and Völkl 1993; Frazer and McGregor 1994; Geitzenauer and Bernays 1996; Lukianchuk and Smith 1997) where artificial and / or natural plants were utilized and which found that plant structure influenced host finding success by parasitoids. Foraging success of *Trichogramma* spp. can be influenced by other plant parts or plant characteristics such as plant height (Monteith 1960; Ables et al. 1980; Thorpe 1985) and plant surface area (Knipling and McGuire 1968; Need and Burbutis 1979; Burbutis and Koepke 1981; Kanour and Burbutis 1984; Bigler and Brunetti 1986; Maini and Burgio 1990). The problem with these studies is that they compared parasitism values obtained from plants of different ages for which they measured plant surface. Not only plant surface area increases with age but also plant complexity. Therefore, the reduction of parasitism the latter authors attributed to an increase of surface area may rather be attributed to an increase of plant complexity or an interaction of both variables.

For this study we wanted to use artificial models that share some characteristics with natural plants and we wanted to avoid the use of models made with paper, wood or rods. With the plant model used in this study, it was impossible to vary the connectivity without changing the surface area. We are then conscious that surface area varied between the three plant structures and that we must be prudent in the interpretation of the results obtained here. The problem could have been resolved by using modular plants having thin branches that can be added imbeded into each other. Thin branches minimize the effect of an increase in plant surface area.

In this experiment we showed that variation in plant structure affects host-finding success but the density of hosts present on the plant and the foraging duration may diminish the effect of plant structure. Physical characteristics of plants can thus mediate ecological interactions and influence parasitoid-host population dynamics. Foraging on complex plant structure increases costs associated to host finding. Females searching more efficiently will find more hosts and will have a greater fitness value. Because searching efficiency is a major component of fitness in short-lived species, searching behavior is submitted to strong selection. Models of optimization should then take into account that foraging behavior is affected by plant structure.

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Fig. 3.1 Representation of the three artificial plants used in this experiment

(A) simple (B) intermediate (C) complex

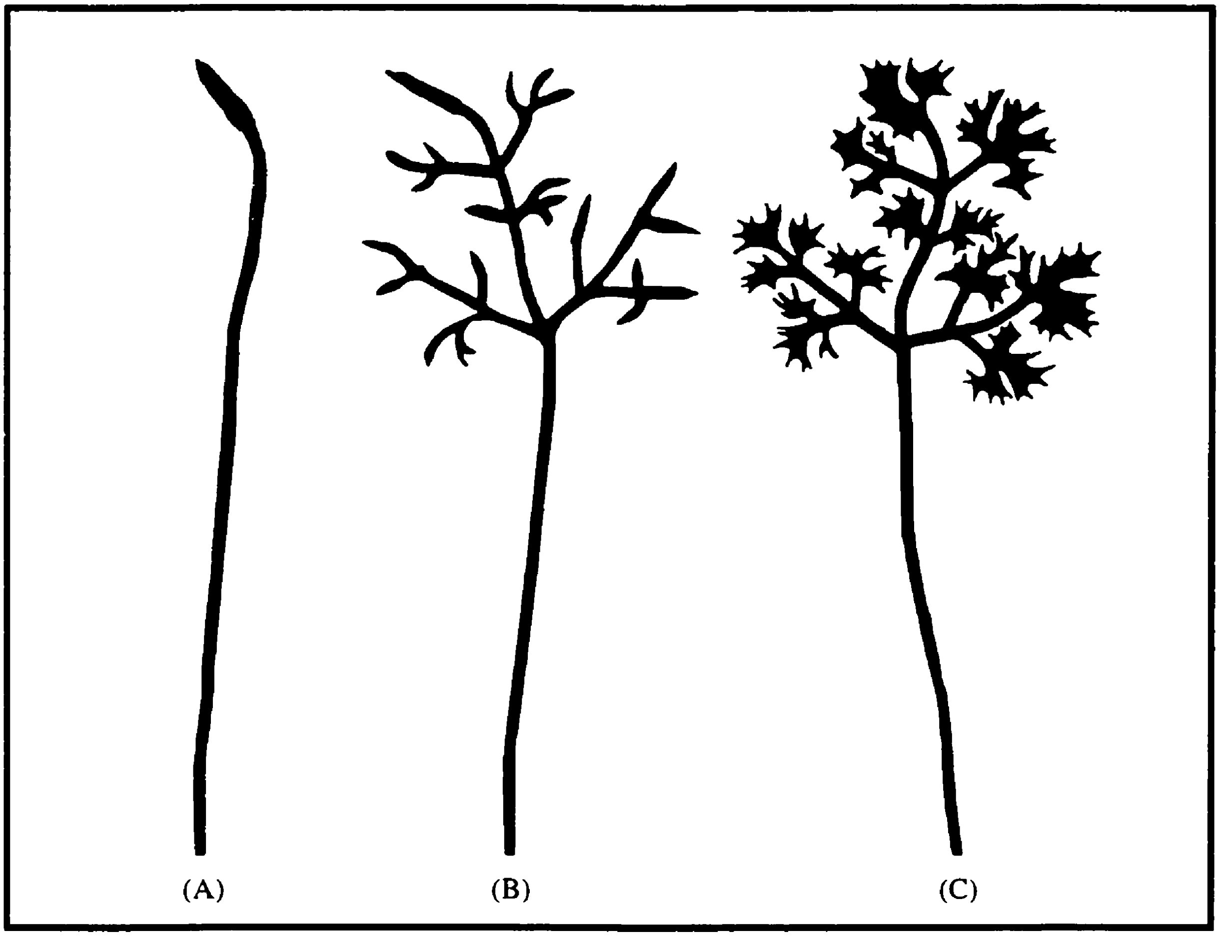
(A)
(B)
(C)

Fig. 3.2 Effect of plant structure and host density on the mean rate of parasitism by female *Trichogramma evanescens* after a foraging duration of (A) 4 hours or (B) 24 hours on artificial plants hosting *Ephestia kuehniella* eggs. Different letters indicate significant difference in mean values related to plant structure for letters within columns and related to host density for letters above columns

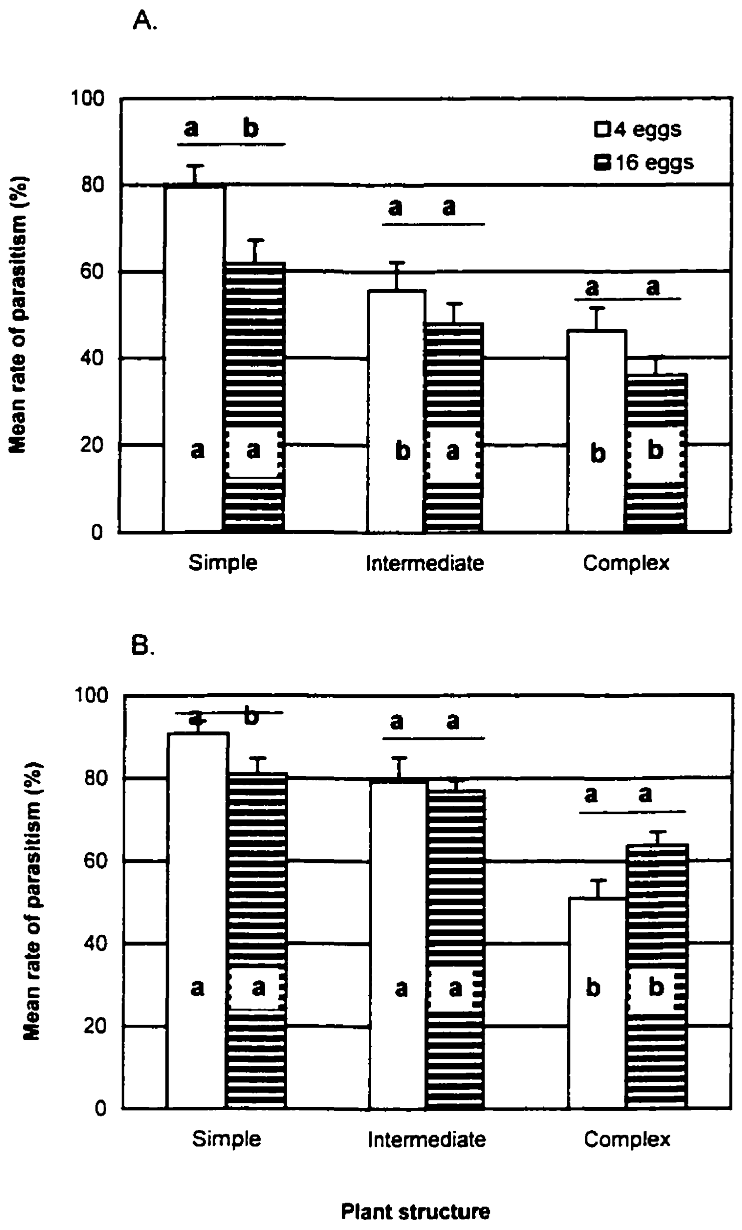
A.
Mean rate of parasitism (%)
100
80
60
40
20
0
□4 eggs
▤16 eggs
a b
a a
a a
a a
b a
b b
Simple
Intermediate
Complex
B.
Mean rate of parasitism (%)
100
80
60
40
20
0
a b
a a
a a
a a
a a
b b
Simple
Intermediate
Complex
Plant structure

Table 3.1. ANOVA on the rate of parasitism according to plant structure and *density of E. kuehniella* host eggs after foraging durations of 4 and 24 hours by female *T. evanescens*

	4 hours			24 hours		
Source of Variation	df	F- ratio	p - value	df	F- ratio	p - value
Model	5	9,63	0.0001 ***	5	13,19	0.0001 ***
Block	4	2,19	0.0687 n.s.	4	1,21	0.2653 n.s.
Plant structure (Ps)	2	16,90	0.0001 ***	2	27,09	0.0001 ***
Host density (Hd)	1	12,55	0.0005 ***	1	3,50	0.0633 n.s.
Ps * Hd	2	0,90	0.4074 n.s.	2	4,14	0.0178 **

CONNECTING STATEMENTS

In chapter 3, we observed a decrease in the number of *E. kuehniella* eggs found and parasitized by *T. evanescens* females with an increase in complexity of plant structure of artificial plants. However, the effect of plant structure was diminished when host density and foraging duration increased. In nature, parasitoids are not only submitted to variations of connectivity but also to variations of size and heterogeneity of the environment in which they search for hosts.

Based on results obtained in chapter 3, we were interested in examining the effect of different combinations of size, heterogeneity and connectivity, components of plant structure on host finding success. One or some component(s) may have more effect than other(s) on parasitism. Also, we wished to develop and validate a predictive model of parasitism for *Trichogramma* parasitoids based on host plant structure. To reach these objectives, we quantified and varied size, heterogeneity and connectivity of artificial plants by keeping plant surface area, host density and foraging duration constant.

CHAPTER 4

MODELLING THE IMPACT OF PLANT STRUCTURE ON HOST FINDING BEHAVIOR OF PARASITOIDS

DANIEL GINGRAS, PIERRE DUTILLEUL and GUY BOIVIN

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ABSTRACT

For many parasitoid species, the final step of host location occurs on plants whose structure varies in time and space, altering the capacity of parasitoids to exploit hosts. Plant structure can be defined by its size, heterogeneity and connectivity. We tested the hypothesis that these three components and all possible interactions affect the level of parasitism of *Trichogramma evanescens* and that parasitism can be predicted if the structure of a plant is known. We quantified and varied the structure of three dimensional artificial plants to determine which component(s) of plant structure best explain variability of parasitism and to develop a model that predict parasitism by *Trichogramma* parasitoids. This model was then validated on three natural tritrophic systems. The experiment with artificial plants revealed that plant structure affected host finding success, which was high on plants with a simple structure and low on plants with a complex structure. A response surface regression showed that only the linear and quadratic terms of connectivity were highly significant, indicating that connectivity best explained the variability in the rate of parasitism obtained. Observed values of parasitism from experiments with three natural tritrophic systems fitted well predicted values of parasitism generated by the model, indicating that parasitism can be predicted if heterogeneity and connectivity of a plant are known. Consequences of these results in regard to population dynamics, evolution and biological control are discussed.

Key-words: Tritrophic interactions; model, cabbage, broccoli, Brussels sprouts, cabbage looper, *Trichoplusia ni, Ephestia kuehniella, Trichogramma evanescens*.

INTRODUCTION

Parasitoids evolved in a multitrophic context (Hassell and Waage 1984; Vet and Dicke 1992) and forage at multiple scales (ecosystem, macro-habitat, micro-habitat, patch) but for many parasitoid species the final step of host location takes place on plants whose structure varies in time and space. The definition of plant structure has, until now, often been subjective and many terms have been used to refer to it (Bell et al. 1991). Various models of plant structure have been proposed, some based essentially on structural and phenological characteristics and patterns of growth, but these remain highly descriptive (Ross 1981; Lawton 1983; Tomlinson 1987 and references therein; Cremers and Edelin 1995; Price et al. 1995). Others aimed to quantify structural components such as branch length and branch angles (Honda 1971; Leopold 1971; McMahon 1975; McMahon and Kronauer 1976; Bell et al. 1979; Honda et al. 1981, 1982; Honjo 1995). Fractal geometry has also been used to quantify the structure of habitats and plants (Williamson and Lawton 1991; Foroutan-pour et al. 1999).

Andow and Prokrym (1990) and Bell et al. (1991) recognized three components to plant structure: size, heterogeneity and connectivity. These three components define to a large extent the potential searching area and could be the most relevant to foraging parasitoids. Previous studies on the effect of plant structure on foraging behavior of parasitoids and predators have focused on a single component. An increase in plant size was found to decrease significantly the percentage of parasitism by different species of *Trichogramma* parasitoids for various host species (Ables et al. 1980; Thorpe 1985). An increase in connectivity was found to reduce host finding success of *Trichogramma* species (Andow and Prokrym 1990; Lukianchuk and Smith 1997; Romeis et al. 1998), aphidiid wasps (Mackauer and Völkl 1993), predatory wasps (Geitzenauer and Bernays 1996), and coccinellids (Grevstad and Klepetka 1992; Frazer and McGregor 1994).

Polyphagous species of predators and parasitoids often forage on a variety of plant structures used by their preys/hosts. For example, parasitoid species of the genus *Trichogramma* exploit eggs of several lepidopteran species on a large variety of plants, from herbaceous to large trees (Suverkropp 1997). In nature, size, heterogeneity and connectivity of plants vary and interact and therefore affect host finding success of parasitoids.

The objectives of this study were (1) to determine the impact of plant structure, defined by different combinations of size, heterogeneity and connectivity, on host finding by *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae); (2) to identify the plant structure component(s) that best explain variability in parasitism; (3) to develop a model of parasitism by using artificial plants and (4) to validate this model on natural tritrophic systems.

MATERIAL AND METHODS

Three-dimensional artificial plants, on which *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs were placed, were used to study the effect of plant structure on host finding behavior by *T. evanescens*. The use of artificial plants eliminates the variability of chemical (e.g. volatiles) and physical (e.g. color, texture) characteristics found in natural plants. The artificial plants consisted of a main stem, two secondary stems, branches, leaves and buds (Fig. 4.1). The three stems were made of wire covered with green plastic. Branches were in flexible green plastic, whereas leaves were in silk and buds were plastic green push pins. Size, heterogeneity and connectivity of artificial plants were varied and quantified.

Size (S) was defined by the height of the plant, and was quantified by measuring, in cm, the main stem. To vary the size, the main stem was cut at four different heights: $S_1 = 15$; $S_2 = 30$; $S_3 = 45$; $S_4 = 60$ cm. Heterogeneity (H) was defined by the relative abundance, per unit area of structural components. To vary the heterogeneity, we used a constant number of 45 leaves for all plant structure but placed 2, 6, 12 or 45 buds along the main and secondary stems and on branches. Heterogeneity was calculated following the formula of the Shannon-Wiener's index of biodiversity (Barbour et al. 1987), which is, $[- \sum X_i / X_o \times (\ln X_i / X_o)]$. In that equation, X_i denoted the number of leaves and the number of buds, while X_o correspond to the sum of leaves and buds. Four values of heterogeneity were thus obtained: $H_1 = 0.1$; $H_2 = 0.3$; $H_3 = 0.5$; $H_4 = 0.7$. Connectivity (C) was defined by the absolute abundance, per unit area, of connections between plant parts. To vary the connectivity, plants were cut to obtain four values: $C_1 = 3$; $C_2 = 24$; $C_3 = 45$; $C_4 = 66$.

These variations of size, heterogeneity and connectivity gave 64 different plant structures that were used as treatments. The simplest plant structure consisted of the main stem, 15 cm high, two secondary stems, 45 leaves, 2 buds and 3 connections between plant parts (Fig. 4.1). The most complex plant structure consisted of the main stem, 60 cm high, two secondary stems, 45 leaves, 45 buds and 66 connections between plant parts (Fig. 4.1). Because the surface of stems compared to the surface of leaves remained constant, the total surface area of plants varied little between treatments. The minimum and maximum values of surface area were respectively 412 and 552 cm^2. Thus, a simple plant structure has low values of size, heterogeneity and connectivity, and the reverse applies to a complex plant structure.

For all experiments we used females *T. evanescens* reared on cold-killed *E. kuehniella* eggs at 24 ± 1° C, 50 ± 5% r.h. and 16L:8D. Females were less than 6 hours old, mated, unfed and naive.

Sixteen cold-killed eggs of *E. kuehniella* were individually glued with mucilage Elmer's glue ® on the margin of 16 leaves per plant structure. The same leaves were used for each replicate. The main stem of artificial plants was then inserted in a circular wooden base of 18 cm in diameter and three *T. evanescens* were introduced with a fine brush on one leaf located at the base of the plant. Plants were then covered individually with a closed plastic cylinder, 18 cm in diameter. In order to keep the volumes of the plant and cylinder relatively proportional between treatments, the heights of cylinders were the same as the enclosed plants. A constant humidity level of 40% r. h. was maintained inside the cylinder by using a piece of cotton soaked in saturated salt water. The experimental set-up was then placed in an incubator at 24°C (16L:8D) for six days. Parasitized *E. kuehniella* eggs turned black six days after parasitization under our experimental conditions. Because we were interested in the eggs parasitized during the first foraging day (24 hours), parasitized eggs were counted on the seventh day and only these were considered in this experiment.

Experimental design and statistical analysis

The three factors (S, H, C) had four levels each, resulting in 64 treatment combinations (4S x 4H x 4C) that were replicated 12 times. Due to time and space constraints, only 32 treatment combinations could be tested simultaneously. The experimental set-up consisted in a hierarchical incomplete block design with min-blocks nested within mega-blocks. The definition of these incomplete blocks satisfied the orthogonality condition (Mead 1988). A first randomization occurred among the three pairs of complete blocks or mega-blocks whereas the second randomization was carried out between the two halves of each complete block or mini-blocks. Thus, a mega-block was formed by all 64 treatment combinations, whereas a mini-block was formed by only 32 treatment combinations. Preliminary analyses showed that the mean rate of parasitism was statistically different between mini-blocks within a complete block. These differences were related to treatment effects, but there was no statistical difference between mega-blocks. Accordingly, we eliminated the mega-block and mini-block structure and grouped the data into complete blocks. Further analyses were performed on five complete blocks, after the removal of one complete block because it was statistically different from the others. A response surface regression was performed on the mean percentage of parasitism calculated over those five complete blocks, after arcsine square root transformation (Sokal and Rohlf 1995). The stability and the robustness of the regression model were assessed using a jackknife procedure (Efron and Tibshirani 1993) in which the percentage of parasitism of each block was predicted by fitting the model to the mean of the four other blocks (see Dutilleul et al. (2000) for a recent application in forest entomology). SAS software, version 6.12 (SAS Institute 1997), was used for all statistical analysis.

To visualize how parasitism varied with heterogeneity and connectivity, we plotted one three-dimensional response surface of the percentage of parasitism against heterogeneity and connectivity and one two-dimensional graphic of the percentage of parasitism as a function of connectivity. The minimum value of heterogeneity and connectivity was zero, whereas the minimum value considered for size was 10 cm. The maximum values of size, heterogeneity and connectivity were 64 cm, 0.9 and 72, respectively.

Model validation

The validation of the model consisted in comparing predicted level of parasitism to values obtained from experiments using *T. evanescens* as parasitoid, *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) as host and either cabbage, broccoli or Brussels sprouts as plant. Seeds of cabbage (*Brassica oleracea* L. variety *capitata*, cultivar 'Green Bartolo'); broccoli

(*Brassica oleracea* variety *italica*, cultivar 'Green valiant'); and Brussels sprouts (*Brassica oleracea* variety *gemmifera*, cultivar 'Hyb Jade E'), were started in individual cells then, after 6 days were transferred into a growth chamber where the temperature was constant at 25^0 C. After a period of 12 days, plants were transplanted into 6 L pots and placed in a greenhouse until they reached 80 days old, calculated from the sowing date. The temperature within the greenhouse varied between 22 and 35^0 C during the day and between 15 and 24^0 C during the night for the duration of experiments. Female *T.evanescens* used in all experiments were reared on cold-killed *E. kuehniella* eggs at 24^0 C and at 16:8. Females were less than 6 hours old, mated unfed and naive. *T. ni* was reared in the laboratory on artificial diet (Shelton et al. 1991) at 23 ± 1° C, 65 ± 5% r.h. and 16L:8D. Adults were maintained in cages. To collect eggs, we first dipped into filtered cabbage juice a strip of polyethylene film that was then introduced in a cage to let the adult females lay their eggs. The polyethylene film was cut into 6 mm^2 pieces and we made sure that each piece contained one freshly (< 12h) laid *T. ni* egg.

Size, heterogeneity and connectivity of cabbage, broccoli and Brussels sprouts plants of 30, 55 and 80 days old were quantified on 21 plants for each age x crucifer combination and mean values were computed (Table 4.1). Size was measured from the highest leaf down to the collar. Heterogeneity and connectivity were quantified as described above. Values of size, heterogeneity and connectivity were then incorporated to the equation of the response surface regression model of parasitism to obtain predicted parasitism values for the three plant varieties and the three ages.

Observed values of parasitism for simple (30 days old), intermediate (55 days old) and complex (80 days old) cabbage, broccoli and Brussels sprouts were obtained experimentally. On each plant, a piece of polyethylene film, containing one *T. ni* egg was glued on each side of 8 different leaves for a total of 16 eggs per plant. On simple plants, 2 or 3 eggs per leaf were glued because they possessed less than 8 leaves. The eggs were all located within 1cm of the leaf margin. Plants were then placed in a cage (85 x 85 x 100 cm) covered with white muslin located within a greenhouse where the temperature varied between 22 and 35 ^{0} C during the day and between 15 and 24 ^{0} C during the night. Twenty-five *T. evanescens* females were introduced on a leaf located at the base of the plant. This number was established following preliminary experiments to obtain a measurable level of parasitism under the prevailing experimental conditions. The plants were left into the cage for 24 hours, after which the 16 eggs of *T. ni* were removed from the plant and incubated. The proportion of parasitized eggs was determined by counting, nine days later, the number of eggs that turned black. This corresponded to the parasitism of the first 24 hours. This experiment was replicated twenty times.

A linear regression of predicted *versus* observed parasitism served to evaluate the performance of the model developed on cabbage, broccoli and Brussels sprouts. Statistical tests using regression procedure were done to determine if there was equality of slopes and if slopes were significantly different from 1. SAS software, version 6.12 (SAS Institute 1997), was used for all statistical analysis.

RESULTS

Parasitism on artificial plants

The response surface regression model had significant linear and quadratic effects and non significant weaker cross-product effects (Table 4.2). A more detailed analysis, term by term, revealed that only the linear and quadratic terms of the connectivity component were highly significant, whereas the cross-product of connectivity and heterogeneity was also significant (Table 4.3). Therefore, the component of plant structure that best explained the variability in the rate of parasitism was connectivity. The equation of the statistical model for the response surface regression is then: Parasitism = 1.115 (0.1032) - 0.01273 (0.0021) C + 0.00454 (0.0022) CxH + $8.60\text{x}10^{-5}$ ($2.22\text{x}10^{-5}$) C^2. Error of the estimate is in parenthesis.

An inverse relationship of parasitism as a function of the linear and quadratic terms of connectivity appeared (Figure 4.2). When we plotted parasitism as a function of heterogeneity and connectivity (Figure 4.3), parasitism tended to decrease with increasing values of heterogeneity and connectivity indicating that female parasitoids were more efficient at finding host eggs when foraging on simple plant structure. The values of parasitism were comprised between the interval of 20% and 60%.

The parameters that were significant in the response surface model showed a good stability in the jackknife procedure. These parameters were the coefficients of the linear and quadratic terms of connectivity and, to a lesser degree, the coefficient of the bilinear term of connectivity and heterogeneity. The connectivity linear coefficient ranged from -0.0141 to -0.0118 with a probability of significance of 0.001 for all values, while the quadratic connectivity coefficient ranged from 0.000075 to 0.000100 with a probability of significance below 0.005 in all cases. The bilinear coefficient of connectivity and heterogeneity, ranged from 0.0035 to 0.0059, with a probability of significance of 0.011 to 0.195. Some of the parameters that were not significant in the original response surface model with all five complete blocks changed sign while others sometimes drastically changed in magnitude, but never became significant in the jackknife procedure, with one exception (i.e., linear effect of size when block 5 was removed). On that basis, we considered that our response surface regression model was stable in the frame of our experiment.

Validation of the model

All three regressions of observed *versus* predicted percentage of parasitism were significant, which gives a predictive power to the expected values generated by the model (d.f.= 1, F= 80.75, P= 0.0001 for cabbage; d.f.= 1, F= 121.47, P= 0.0001 for broccoli; d.f.= 1, F= 30.53, P= 0.0001 for Brussel sprouts) (Fig. 4.4 A, B, C). Slope values varied between 3.4 and 4.1 and all were significantly different from 1 (d.f.= 1, t= 5.73, P= 0.0001 for cabbage; d.f.= 1, t= 6.12, P= 0.0001 for broccoli; d.f.= 1, t= 2.89, P= 0.01 for Brussels sprouts). A test for slopes equality revealed that the three slope's values were not significantly different from each other (d.f.= 2, F= 1.24, P= 0.2976), which permitted merging of the data from the three plant varieties (Fig. 4.5) and lead to a more general model. The regression presented in figure 4.5 was significant (d.f.= 1, F= 98.57, P= 0.0001). The high R^2 values of all regression models indicates that most of the variability is explained by the model. Observed percentage of parasitism of *T. ni* eggs increased with increasing values of expected parasitism and the observed values of parasitism corresponded well to the expected ones over the 40-80 range (Fig. 4.4 A to C and Fig. 4.5).

DISCUSSION

Size, heterogeneity and connectivity define to a large extent the habitats of herbivorous insects and the potential searching area of parasitoids. It was initially thought that all three components may be relevant to foraging *Trichogramma* parasitoids and this is why we varied all three components in our artificial plants. With respect to the scientific objective of determining the plant component that best explained the variability of parasitism, only one of the three main factors and two of the six interactive factors came out significant.

As shown by the results of the response surface regression, connectivity of artificial plants best explained the variability observed in percent parasitism. The important effect of that component of plant structure may be attributable to the fact that *Trichogramma* searches for hosts on plants mostly by walking (Bigler et al. 1988). Plant connectivity could be less important for parasitoids that search by flying or use chemical cues to directly land near their host.

The absence of significant effect of size and heterogeneity under our experimental conditions does not mean that such effects do not exist or are not important to foraging *Trichogramma* parasitoids. An increase in plant size has been found to decrease host discovery in various *Trichogramma* species (Ables et al. 1980; Thorpe 1985; Wang 1997). Differences in plant configurations may explain the results obtained with these studies that used natural plants and natural hosts and our results where we used artificial plants and cold-killed hosts, which emitted no or little chemical cues. Chemical characteristics of plants and hosts modify searching behavior of parasitoids by increasing searching time or by retaining them in a limited area or patch (Lewis et al 1975). Because no or little cues were emitted from the artificial system, arrestment effects did not occur and females may have explored most of the plant. Also, the last cited studies used *T. pretiosum* (Ables et al. 1980); *T.minutum* (Thorpe 1985); *T. ostriniae* (Wang 1997) while we used *T. evanescens* and as observed by Thorpe (1985) some *Trichogramma* species are less influenced by plant size than others.

In our experiment with artificial plants, heterogeneity was created by using different numbers of green push pins, simulating buds, over a constant number of forty five leaves. Host finding success may not be affected by the relative number of plant parts. It would rather be influenced by texture of plant parts (waxy, hairy, smooth leaf surface; plant topography) as revealed by previous studies (Evans 1976; Carter et al. 1984; Juniper and

Southwood 1986; Obrycki 1986; Kareiva and Sahakian 1990; van Lenteren 1995; Sütterlin and van Lenteren 1997).

In their host location process, parasitoid females make choice relatively to where and how long to forage (Stephens and Krebs 1986). These decisions affect the number of hosts encountered and therefore oviposition success. In a study on the proximate mechanisms of parasitoid foraging decisions, Waage (1979), proposed that responsiveness increases with successive encounters with hosts and decrease in the absence of encounters. Based on our results, responsiveness of parasitoids may reach more frequently the lower level of responsiveness when parasitoids forage on complex plant structure. Because plant structure can effectively influence patch residence time (Andow and Prokrym 1990; Weisser 1995), consideration of host plant characteristics by models of optimal foraging may then contribute to a better understanding of host-parasitoid population dynamics.

This study showed that, for similar plant surface area, foraging on complex plant structure decreases host finding or the number of descendants and hence fitness. Complex plant structure likely affects movements associated to host searching, reduces surface area or connections explored per foraging time unit; consequently, it takes more time to explore the whole plant. Plant structure, as defined here can correspond to the ''area of discovery'' of Nicholson and Bailey's (1935) host-parasitoid model, which suggest that the dynamics of the interaction are determined primarily by the densities of the host and the parasitoid, the population growth rate of the host, and the area of search of the parasitoid.

Because instantenous host finding rate of parasitoids is reduced on complex plants, selection pressure for host or prey to occupy these regions preferentially must be strong, and this appears to be true (Askew 1980; Hawkins and Lawton 1987). Because females foraging on simple plants should leave more progenies, we would expect abundance and diversity of parasitoids to be greater on these plants. However, parasitoid diversity has been found to be low on simple plants because they host fewer species of herbivores that serve as alternative hosts and attract fewer polyphagous parasitoid species (Askew 1980; Hawkins and Lawton 1987). Complex plants may provide partial refuge to hosts due to reduced parasitoid efficiency (Smith 1972). Availability of hosts to parasitoid is then affected which in turn has impact on population dynamics (Price et al 1980; Price 1991). Furthermore, more microhabitats are available within complex plants, which permit greater niche diversification and consequently provide a greater measure of enemy free space (Berdegue 1996). A decrease in host finding success with an increase in complexity of plant structure may tend to stabilize parasitoid/host interactions (Smith 1972; Crowley 1978) and allow coexistence of both parasitoids and hosts.

Our validation of the model indicated that connectivity, which had significant effect on artificial plants, can predict, at least qualitatively, the effect of plant structure on host finding by *Trichogramma*. By measuring the characteristics of plant structures on three different varieties of crucifers, we were able to obtain a highly significant relationship between predicted and observed values, indicating that our model has predictive values over a range of plant type. The experimental conditions of the development and the validation of the model differed but the objective of the model is not to predict a precise level of parasitism, which can vary according to the host plant and to the host insect but rather to predict the impact of changes in plant structure on host finding success of *Trichogramma*. If the predicted values of parasitism were mostly between 40 and 80%, the model can, however, predict any parasitism value.

Results from the experiment with natural plants confirm that we can be confident of our approach in defining and quantifying plant structure. Also, the assumption that size, heterogeneity and connectivity are relevant to foraging female *Trichogramma* appear to be partially right. The model developed here may allow comparison of expected host finding success resulting from searching on different quantified plant structures. Modelization of the impact of plant structure on host finding success was achieved, suggesting that we captured the essential of a biological phenomenon associated to host searching by *Trichogramma* parasitoid on a plant. Because the predictions of the impact of changements in plant structure on host finding hold for three tritrophic systems and under different experimental conditions, the model can thus be trusted. We have then good reasons to believe that such predictions may hold in field. This has to be verify. The model can find application to biological control programs by being a tool by helping the planification of the number of parasitoids to be released based on quantification of plant structure. Reliable predictions of natural enemy release rates before the onset of a biological control program can provide practitioners an estimate of the economic feasability of the releases.

Moreover, the broadly comparable structure of very different plant types and the quantitative approach to plant structure used here for studying parasitoid's responses to plant structure appear to provide a promising avenue for comparative research on plant-herbivore-parasitoid interactions. The challenge remains of collecting relevant data on other parasitoid and plant species to test the consistancy of the model developed here. Also, future studies should test how the model behaves under variation of host density and under semi-field or field conditions where other factors influence host searching.

ACKNOWLEDGEMENTS

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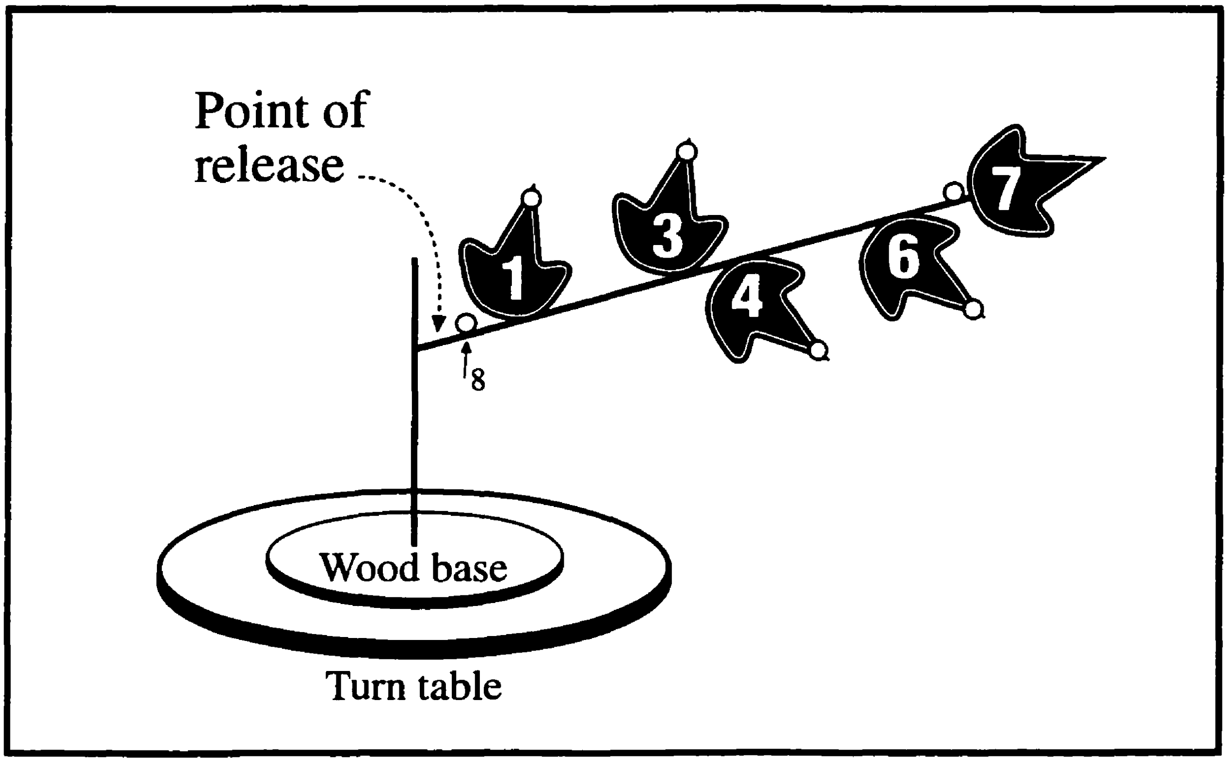
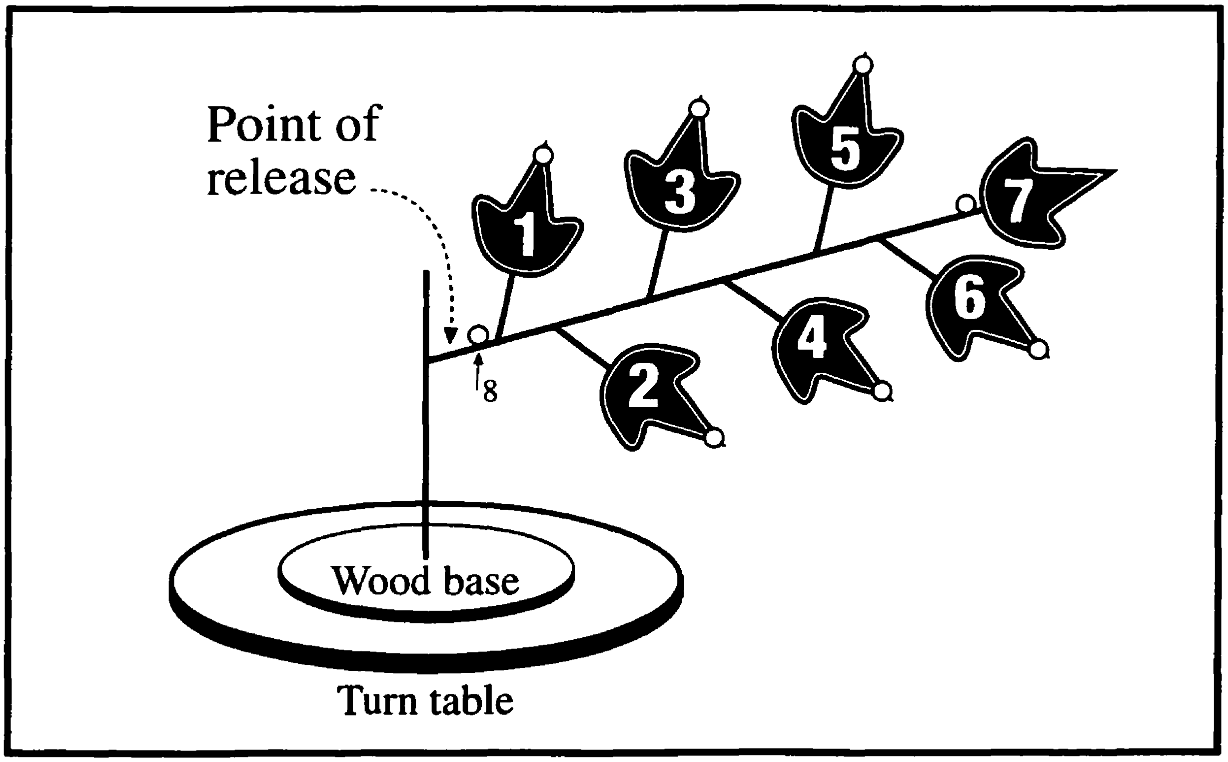
Fig. 4.1 (A) representation of the most simple plant structure; height = 15 cm, heterogeneity = 0.1, connectivity = 3; (B) a detailed view of one branch and leaves ; (C) representation of the most complex plant structure height = 60 cm, heterogeneity = 0.9, connectivity = 66. The square represents buds.


Point of release
1
3
7
6
4
8
Wood base
Turn table

(A)


Point of release
1
3
5
7
2
4
6
8
Wood base
Turn table

(B)

Fig. 4.2. Relationship of the percentage parasitism by *T. evanescens*, as a function of linear and quadratic terms of connectivity

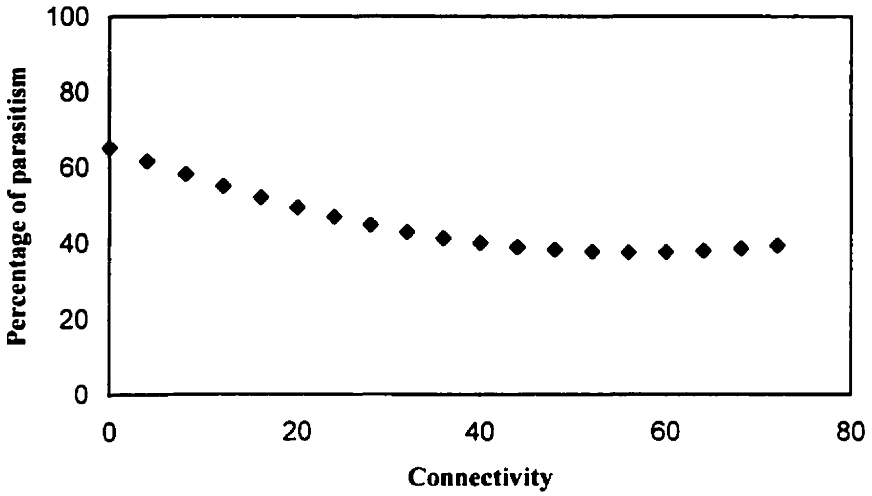
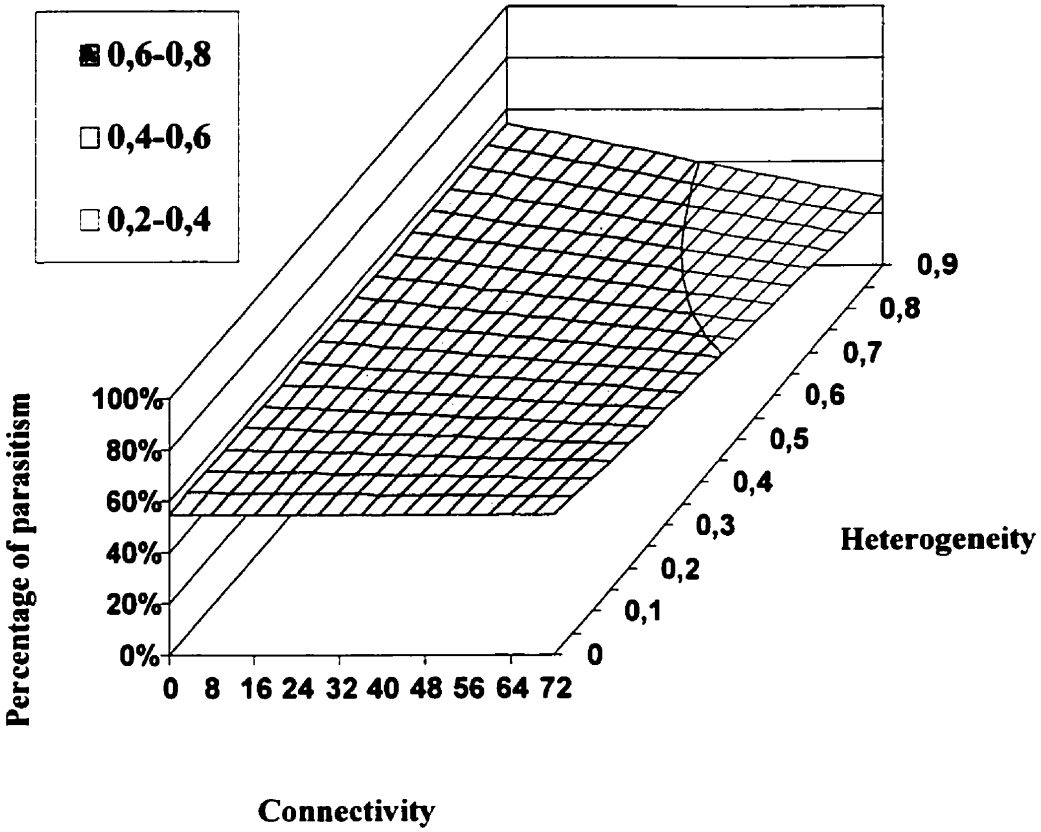

100
80
60
40
20
0
Percentage of parasitism
0
20
40
60
80
Connectivity

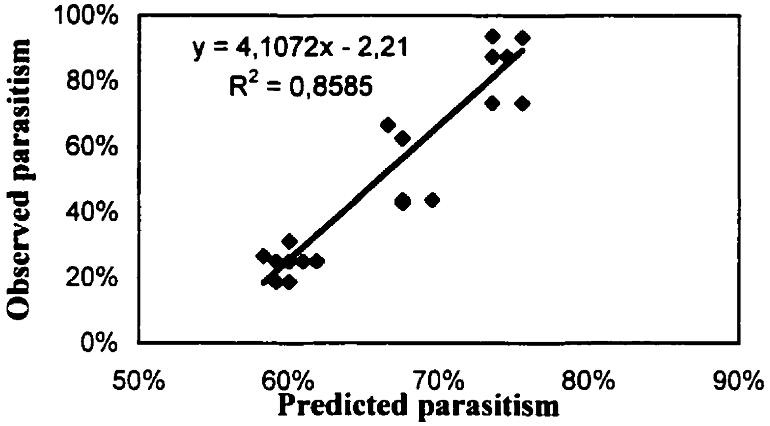
Fig. 4.3. Response surface of the percentage parasitism by *T. evanescens* as a function of heterogeneity and connectivity



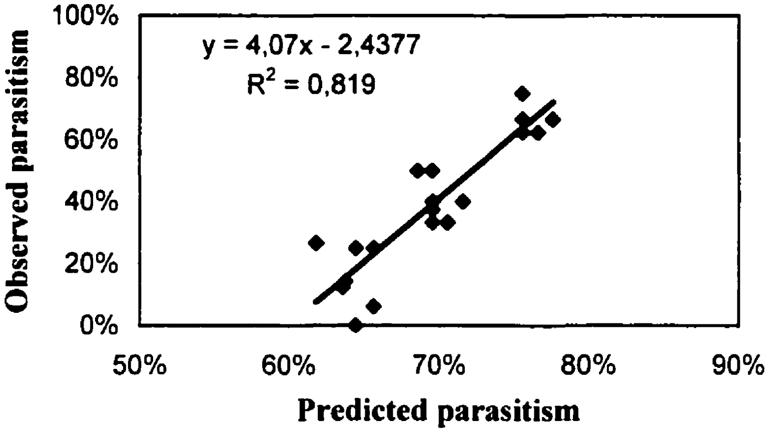
▨ 0,6-0,8
□ 0,4-0,6
□ 0,2-0,4
Percentage of parasitism
100%
80%
60%
40%
20%
0%
0
8
16
24
32
40
48
56
64
72
Connectivity
0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
0,8
0,9
Heterogeneity

Fig. 4.4 Observed values of parasitism of *T. ni* eggs by *T. evanescens* against predicted values provided by the response surface model from an experiment with (A) cabbage plants of 30, 55 and 80 days of age (B) broccoli plants of 30, 55 and 80 days of age (C) Brussels sprouts plants of 30, 55 and 80 days of age

(A)


y = 4,1072x - 2,21
R^2 = 0,8585
Observed parasitism
100%
80%
60%
40%
20%
0%
50%
60%
70%
80%
90%
Predicted parasitism

(B)


y = 4,07x - 2,4377
R^2 = 0,819
Observed parasitism
100%
80%
60%
40%
20%
0%
50%
60%
70%
80%
90%
Predicted parasitism

(C)

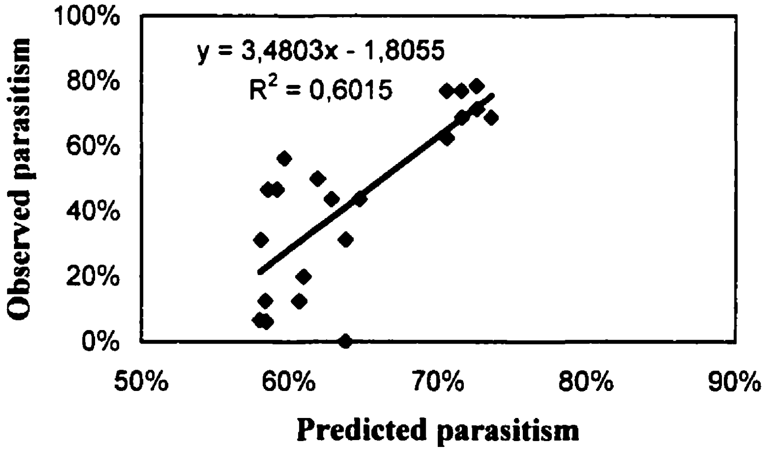

y = 3,4803x - 1,8055
R^2 = 0,6015
Observed parasitism
100%
80%
60%
40%
20%
0%
50%
60%
70%
80%
90%
Predicted parasitism

Fig. 4.5 Observed values of parasitism of *T. ni* eggs by *T. evanescens* against predicted values provided by the response surface model from an experiment with cabbage, broccoli and Brussels sprouts grouped. The solid line represents the simple linear regression of the observed values of parasitism on the expected values

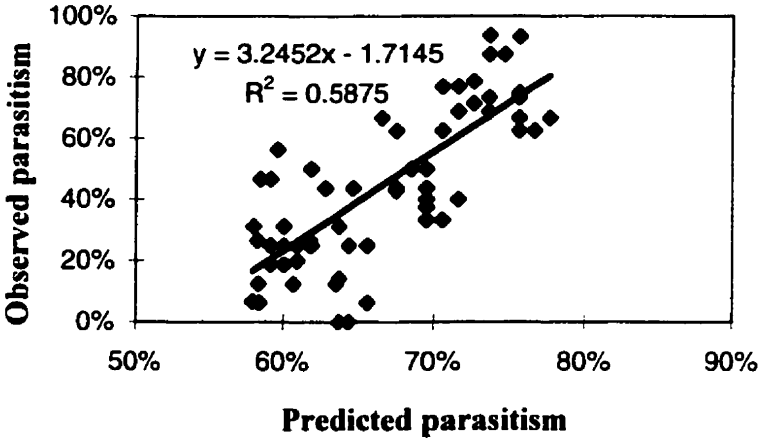

y = 3.2452x - 1.7145
$R^2 = 0.5875$
Observed parasitism
100%
80%
60%
40%
20%
0%
50%
60%
70%
80%
90%
Predicted parasitism

Table 4.1. Mean values (± std) for each component of plant structure and for each plant variety and age

Plant age / Plant variety	Size	Heterogeneity	Connectivity
Age = 30 days after sowing date			
Cabbage (n= 21)	21.7 ± 1.7	0.0 ± 0.0	6.2 ± 0.7
Broccoli (n= 21)	20.2 ± 2.1	0.0 ± 0.0	4.2 ± 0.6
Brussels sprouts (n= 21)	21.9 ± 2.1	0.0 ± 0.0	8.0 ± 1.0
Age = 55 days after sowing date			
Cabbage (n= 21)	35.4 ± 4.8	0.0 ± 0.0	12.5 ± 1.1
Broccoli (n= 21)	41.0 ± 4.3	0.0 ± 0.0	10.5 ± 0.7
Brussels sprouts (n= 21)	40.9 ± 1.8	0.0 ± 0.0	18.6 ± 1.8
Age = 80 days after sowing date			
Cabbage (n= 21)	36.3 ± 2.3	0.0 ± 0.0	21.0 ± 1.2
Broccoli (n= 21)	50.5 ± 9.4	0.1 ± 0.1	17.4 ± 1.8
Brussels sprouts (n= 21)	48.5 ± 6.6	0.7 ± 0.05	35.3 ± 7.1

Table 4.2. Overall analysis of the response surface regression of parasitism of *T. ni* eggs by *T. evanescens* for the experiment with artificial plants

Regression	d.f.	F-ratio	Prob > F
Linear	3	23,99	0,0001
Quadratic	3	5,36	0,0026
Cross-product	3	2,64	0,0589

Table 4.3. Response surface regression analysis of parasitism of *T. ni* eggs by *T. evanescens* for the experiment with artificial plants

Parameter	d.f.	t-value	Prob > |t|
Size (S)	1	-1,084	0,2832
Heterogeneity (H)	1	-0,058	0,9539
Connectivity (C)	**1**	**-6,132**	**0,0001**
(S) * (S)	1	-0,001	0,9992
(H) * (S)	1	1,197	0,2364
(H) * (H)	1	-1,086	0,2822
(C) * (S)	1	1,473	0,1465
(C) * (H)	**1**	**2,075**	**0,0428**
(C) * (C)	**1**	**3,861**	**0,0003**

CONNECTING STATEMENTS

In chapter 4, we observed a decrease in the number of *E. kuehniella* eggs found and parasitized by *T. evanescens* with an increase in heterogeneity and connectivity of artificial plants. We also found that connectivity best explained observed rates of parasitism, suggesting that this component of plant structure may be determinant on foraging behavior of *Trichogramma* females. Moreover, quantification of size, heterogeneity and connectivity permitted the development of a predictive model of parasitism by *T. evanescens* female for quantified plant structure. The model was successfully validated on three tritrophic natural systems. The model did predict changes in the direction and magnitude of parasitism according to variations in plant structure.

Until now, the influence of plant structure on host finding success was studied on artificial plants only and with only one species of *Trichogramma*. The next chapter will examine the effect of three structurally different but closely related natural cruciferous plants on two species of *Trichogramma* foraging for three lepidopteran pests of crucifers.

CHAPTER 5

ECOLOGICAL CONSTRAINTS IMPOSED BY PLANT STRUCTURE ON FORAGING PARASITOIDS

DANIEL GINGRAS, PIERRE DUTILLEUL and GUY BOIVIN

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ABSTRACT

Generalist parasitoids are confronted to different plant structures and to differences in host abundance and distribution within and between plants and these differences may affect host finding success. In this study, we investigated the effect of plant structure on host finding success of two *Trichogramma* species using three species of Lepidoptera and three structurally but closely related crucifers (cabbage, broccoli, Brussels sprouts). We also determined the searching pattern of female parasitoids by characterizing the distribution pattern of parasitized eggs according to leaf side and plant height. Neither species of parasitoid showed a preference for any of the three plant varieties. A repeated measure Anova in time where plant structure defined repeated measures showed significant effects of plant variety, pest species and parasitoid species on parasitism and only the parasitoid x plant structure interaction was significant. Mean percent parasitism generally tended to decrease with an increase of plant structural complexity; being greatest with cabbage, lowest with Brussels sprouts and intermediate with broccoli. On simple plant structures, both wasp species performed well on the three varieties of plants. A doubly repeated measure Anova in space where height of plant and side of leaves defined the repeated measure revealed that females parasitized more eggs at the base of plants and on the inferior side of leaves. This study demonstrates that plant structure can seriously constrain parasitoids while they are foraging for hosts and therefore affects host finding success. These effects have consequences for biological control.

Key words: *Pieris rapae*, *Trichoplusia ni*, *Plutella xylostella*, egg parasitoids, *Trichogramma evanescens*, *Trichogramma pretiosum*, *Brassica oleracea*, cabbage, broccoli, Brussels sprouts, host-parasitoid interactions

INTRODUCTION

In tritrophic systems, the role of the first trophic level in mediating ecological interactions between host and parasitoids has been recognized over the past few years (Takabayashi et al. 1998; Verkerk et al. 1998; De Moraes and Mesher 1999). Plant characteristics may represent a constraint to foraging parasitoids and, as such, impair encountering of hosts by parasitoids. Morphological features of plants such as dense trichomes (Kauffman and Kennedy 1989; van Lenteren et al. 1995), waxy leaves (Carter et al. 1984; Kareiva and Sahakian 1990), leaf surface area (Knipling and McGuire 1968) and complex plant structure (Andow and Prokrym 1990) have all been shown to reduce the effectiveness of predators or parasitoids.

Crucifer crops like cabbage, broccoli, and Brussels sprouts are different varieties of the species *Brassica oleracea* (L.). They possess glabrous and waxy leaves but show very different physical structures defined here by size, heterogeneity and connectivity (Andow and Prokrym 1990; Bell et al. 1991). Cabbage plants are the most simple while Brussels sprouts are the most complex. In North America, these crucifers are mainly attacked by three lepidopetran species, the imported cabbageworm, *Pieris rapae* (L.) (Lepidoptera: Pieridae), the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) and the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) (Harcourt 1957; Pimentel 1961; Harcourt 1963; Sutherland 1966; Shelton et al. 1982; Chagnon et al. 1990; Biever et al. 1992; Godin and Boivin 1998). The diversity, abundance and spatial distribution of these pests vary between plants and within plant parts (Harcourt 1961, 1962; Godin and Boivin 1998) and these variations may increase difficulties associated to localization of hosts by female parasitoids.

The egg parasitoids of the genus *Trichogramma* are considered efficient biological control agents and are widely used commercially for the control of lepidopterous pests in many crops and in several countries (Pak 1992; Li 1994; Smith 1996). Nevertheless, their suitability as biological control agents may vary due to considerable inter- and intraspecific variations in their tolerance to environmental conditions, preferences for hosts, recognition and acceptance of crops and in their searching behavior (habitat location, host location) (van Dijken et al. 1986; Pak 1988a; Hassan 1989). As such, the capacity to search and find hosts on plants of different structures may differ between strains and species of *Trichogramma*. For example, *Trichogramma minutum* (Riley) and *T. pretiosum* (Riley) responded differently to height, a component of plant structure when searching for *Heliothis virescens* (F.) eggs (Thorpe 1985).

Trichogramma evanescens Westwood and *T. pretiosum* Riley (Hymenoptera: Trichogrammatidae) appear promising candidates to control lepidopteran pests of crucifers in Quebec, Canada (Fournier and Boivin 2000). *T. evanescens* is mostly observed on crops and within field habitats (Flanders 1937; Nagarkatti and Nagaraja 1977) whereas *T. pretiosum* can be found in forest habitats additionally to field ones. Based on these differences of habitat exploitation, we expected *T. evanescens* to perform better on plants with simple structure and *T. pretiosum* on plants with complex structure. Moreover, we hypothesized that plant structure accounts for differences in parasitism observed between plant varieties/species for one particular host species. Finally, based on a model predicting the effect of plant structure on host finding success we hypothesized that host parasitism will be greater on plants having a simple structure (Gingras and Boivin 2001). In this study we used cruciferous plants of different sizes, heterogeneities and connectivities to investigate (1) if the two species of *Trichogramma* showed a preference among the three varieties of *Brassica oleracea,* (2) if plant structure influenced host finding success and if plant structure differently affected different species of *Trichogramma*, (3) the exploration pattern of female parasitoids on plants by characterizing the distribution pattern of parasitized eggs according to leaf side and plant height.

MATERIAL AND METHODS

Seeds of cabbage (*Brassica oleracea* L. variety *capitata*, cultivar 'Green Bartolo'); broccoli (*Brassica oleracea* variety *italica*, cultivar 'Green valiant'); and Brussels sprouts (*Brassica oleracea* variety *gemmifera*, cultivar 'Hyb Jade E'), were sown in individual cells and, after 6 days, were transferred into a growth chamber where the temperature was constant at 25 ^{0} C. After a period of 12 days, plants were transplanted in 6 L pots and were then brought in a greenhouse where they continued to grow. The temperature within the greenhouse varied between 22 and 35 ^{0} C during the day and between 15 and 24 ^{0} C during the night.

Females *T.evanescens* and *T. pretiosum* used in all experiments were reared on cold-killed *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs at 24 ± 1° C, 50 ± 5% r.h. and

16L:8D. Females were less than 6 hours old, mated, unfed and naive. The three species of Lepidoptera were reared on artificial diets (Shorey and Hale 1965; Webb and Shelton 1988; Shelton et al. 1991) at 23 ± 1° C, 65 ± 5% r.h. and 16L:8D. Adults were maintained in cages. To collect eggs, a strip of polyethylene film dipped in filtered cabbage juice was introduced in a cage containing adults of one of the Lepidoptera species. Pieces of polyethylene film of approximately 6 mm^2 containing one freshly (< 12h) laid egg were then cut.

All experiments took place within a greenhouse into cages of 85 x 85 x 100 cm covered with white muslin.

Plant preference experiment

In order to determine if females *T. evanescens* and *T. pretiosum* showed preference among the three crucifer varieties, eggs of *P. rapae* were presented to females of each parasitoid species, simultaneously on cabbage, broccoli and Brussels sprouts.

Plants of cabbage, broccoli, and Brussels sprouts of 55 days old were used. On each plant, a piece of polyethylene film, containing one *P. rapae* egg was glued on each side of 8 different leaves for a total of 16 eggs per plant. The eggs were all located within 1cm of the leaf margin. Three plants, one of each variety, were introduced into a cage and placed in a triangle. The leaves of plants did not touch to avoid migration of parasitoids between plants. *Ephestia kuehniella* eggs, glued to a 6.25 cm^2 paper and parasitized by either *T. evanescens* or *T. pretiosum*, were then deposited in the center of the triangle formed by plants. The number of emerging females was estimated for each cage by the emergence from a 1.75 cm^2 paper kept as a reference. Emergence took place within 1 hour after introduction into the cage. The female parasitoids were left in the cage for 24 h, after which the 16 *P. rapae* eggs were removed from the plant and incubated at 24°C (16L:8D). The proportion of parasitized eggs was determined 9 days later by counting the number of *P. rapae* eggs that turned black.

The experimental design consisted in a randomized complete block design with blocks being the time. The experiment was repeated 26 times with *T. evanescens* and 16 times with *T. pretiosum*. Prior to analysis, % parasitism was transformed by arcsine square root to satisfy ANOVA assumptions (Sokal and Rohlf 1995). Also, because the number of females varied between cages, percentages of parasitism were standardized by dividing parasitsm by the number of females. Parasitism was related to the number of females foraging in a cage. Then an ANOVA was performed, using SAS software (SAS Institute Inc. 1997), to determine if there exists a preference under the null hypothesis of equality in mean percent of parasitism between the three plants for both species of *Trichogramma*. All significant analysis were followed by a Tukey multiple comparisons test. Mean values in tables are followed by their standard deviation.

Effect of plant structure

To test the effect of plant structure on host finding success, we used three structurally different but closely related varieties of crucifers (cabbage, broccoli and Brussels sprouts) of three different ages (30, 55 and 80 days from sowing date). The three ages and the three varieties represent an increasing order of plant complexity. For each age, the structure varied between varieties and for each variety, the structure varied with age. Differences in plant structure were much greater between ages than between varieties of identical age (Table 5.1). Plant structure was quantified by determining size, heterogeneity and connectivity. Size was quantified by measuring the height, in cm, from the highest leaf down to the collar. Heterogeneity refers to the relative abundance of structural components, such as leaves and buds. Heterogeneity was calculated by using Shannon-Wiener's index of biodiversity (Barbour et al. 1987), which is, $[- \sum X_i / X_o \times (\ln X_i / X_o)]$. In that equation, X_i denotes the number of leaves or the number of buds, while X_o corresponds to the sum of leaves and buds. Connectivity was quantified by determining the absolute abundance of connections between plant parts.

In these no-choice experiments, we used plants of cabbage, broccoli and Brussels sprouts of either 30, 55 or 80 days old. On each plant, a piece of polyethylene film, containing one egg of *P. rapae, T. ni* or *P. xylostella* was glued on each side of 8 different leaves, for a total of 16 eggs per plant. Four leaves were at the base, and four leaves were at the top of the plant. For plants of 30 days, which had less than 8 leaves, 2 or 3 eggs per leaf were glued. The eggs were all located within 1cm of the leaf margin. Twenty-five females *T.*

evanescens or *T. pretiosum* were introduced on a leaf located at the base of the plant. The female parasitoids were left in the cage for 24 h, after which the 16 host eggs were removed from the plant and incubated at 24° C. The proportion of parasitized eggs was determined 9 days later by counting the number of eggs that turned black. By doing so, we only measure parasitism followed by successful development of the parasitoid immature. Since successful development varies between host eggs, we may under estimate parasitism when parasitoid immature die within the host egg. However, this counting method is simple, fast and currently used.

The experimental design consisted of a randomized complete block design with blocks being time and with a factorial arrangement of treatments. There were three factors, the plant, the pest species and the parasitoid species. Each treatment was replicated twenty times. Because the same plant was used at 30, 55 and 80 days old, we performed a repeated measures ANOVA in time where ages defined the repeated measures (Potvin et al. 1990; von Ende 1993). We were then able to determine if plant structure, pests, parasitoids and all possible interactions had an effect on percentage of parasitism. We reported only the results of the univariate procedure of ANOVA because results of the modified ANOVA and MANOVA were similar. In order to characterize the distribution pattern of parasitized eggs according to leaf side and plant height we performed doubly repeated measures ANOVA in space where leaf side and plant height defined the repeated measures (Dutilleul 1998a, b). Only plants of 55 and 80 days old were retained for these analyses; plants of 30 days old were too small to be divided in basal and apical parts. The latter analysis was performed on means calculated over the three host species and the two species of *Trichogramma*. Such groupment is justified because summation of the number of levels of a factor (i) over the fixed main effects of the factor (a_i) become null, i.e. $\sum$ (i) / (a_i) = a_0 when means are computed (Mead 1988). To satisfy ANOVA assumptions, arcsine transformation to proportional parasitism was applied prior analysis (Sokal and Rohlf 1995) and all statistical analyses used SAS software (SAS Institute 1997).

RESULTS

Experiment on plant preference

The estimated total number of females foraging per cage varied between a minimum of 103 and a maximum of 882 for *T. evanescens* and between a minimum of 172 and a maximum of 972 for *T. pretiosum*. Parasitoid density varied by nearly one order of magnitude. Females of both species of parasitoids foraged and parasitized eggs of *P. rapae* on the three plants. After standardization of percentage of parasitism, the ANOVA revealed that mean percent of parasitism did not differ significantly between the three plants for both *T. evanescens* and *T. pretiosum* (Table 5.2). Therefore, neither species of parasitoid showed a preference between the three *B. oleracea* varieties. The absence of strong preference by these two species for any variety makes them comparable and justifies their utilization to test the effect of plant structure.

Experiment on plant structure

Complexity of plant structure increased with age of plants (Table 5.1). For most ages, Brussels sprouts showed the highest values of size, heterogeneity and connectivity, which make it the most complex structure. Broccoli was the most simple structure when taking into account size, heterogeneity and connectivity values, while cabbage was very often intermediate between these two (Table 5.1). Note that table 5.1 is a duplicate of table 4.1.

The repeated measures analysis of variance revealed strong main effects of both, plant variety and pest species. Significant effects of plant, pest and parasitoid (Table 5.3) indicate that parasitism differed among varieties of plant, pest and parasitoid species. Moreover, the age variable was highly significant. Only the age*parasitoid interaction was significant, indicating that effect of parasitoid species was not constant across plant age classes.

For identical plant age and identical *Trichogramma* species, mean percentages of parasitism decreased in the order cabbage, broccoli and Brussels sprouts in six out of eighteen cases (Table 5.4). The performance of the two wasps foraging on broccoli plants was always similar, except when they foraged for *P. xylostella* on 30 day old plants (Table 5.4). Differences in parasitism between the two wasps were more obvious with cabbage and Brussels sprouts, particularly with 55 days old plants. In total, *T. evanescens* outperformed *T. pretiosum* 8 times and the contrary was seen twice (Table 5.4).

Mean percentage of parasitism of *P. rapae, T.ni* and *P. xylostella* eggs decreased with an increase in age for all three plant varieties (Fig. 1a, b, c). As indicated by standard deviation error bars, there was little variability associated with the mean for 30 days old plants but variability was much greater for 55 and 80 days old plants. The mean percentage of parasitism of 30 days old plants was almost two and three times greater than that of plants of 55 and 80 days old respectively. The mean percentage of parasitism between plant varieties of identical age rarely differed whereas it was always significantly different between ages for the same plant variety (Fig. 1a, b, c).

Concerning the distribution of parasitized eggs, the doubly repeated measures ANOVA in space revealed significant effects of leaf side (df= 1; F= 28.05; p= 0.0001) and plant height (df= 1; F= 353.09; p= 0.0001) on parasitism, indicating significant differences in parasitism between inferior (X ± s.d) (53.81 ± 47.67) and superior leaf sides (49.5 ± 52.27) and between basal (62.16 ± 30.92) and apical plant parts (41.16 ± 43.70). The interaction between those two variables was not significant, indicating that differences in parasitism between leaf sides were similar at the base and at the top of plants.

DISCUSSION

Plant preference

In this choice experiment, females *T. evanescens* or *T. pretiosum* explored all three varieties of crucifers and neither wasp species showed a strong preference for a particular variety. The great variation in the number of female parasitoids between cages and replicates may have not hide a preference effect. A strong preference for one of the three varieties of crucifers by the two *Trichogramma* species would be reflected by high level of parasitism of that variety, because more females will have explored it, whatever the number of parasitoids. However, with hundreds of parasitoids and 48 host eggs, conditions of intense parasitoid competition for hosts likely prevailed. In such a context, only a strong preference/rejection could have been detected. Furthermore, the great difference between female densities surely had an impact on patch time residence and superparasitism, two factors known to influence host exploitation (Godfray 1994). High female density increase superparasitism and decrease patch time residence.

The absence of preference could be explained by their polyphagous nature. *T. evanescens* and *T. pretiosum* are recognized to be generalist species, and as such, exploit host species that may be found on a broad variety of plant species within their respective habitat (Salt 1935, 1938, 1940). The three plant varieties used were of the same age and shared similar values of size, heterogeneity and connectivity as seen in table 5.1. This may contribute to explain an absence of significant difference in mean rates of parasitism between the three plant varieties for both wasp species. Absence of significant difference was also rarely observed for the experiment on the effect of plant structure. Moreover, absence of plant preference can be considered as a quality for natural enemies such as parasitoids when used in biological control programs.

Plant structure

The numerous varieties derived from *Brassica oleracea* are a clear example of the outcome of the process of artificial selection, in which, leaves, stems, roots, axillary buds, or inflorescences have been altered to give rise to strikingly different plant forms and structures (Hawkes 1983). In this experiment, the structure of the three varieties of crucifer studied were a constraint to parasitoids searching for hosts. The two species of parasitoid performed better on broccoli whose structure is the most simple compared to the one of Brussels sprouts when taking into account all three components of plant structure. However, when considering only connectivity, the most critical variable, broccoli showed the lowest values of connectivity but parasitism of host eggs was intermediate while cabbage showed intermediate values of connectivity but host eggs were the most parasitized.

Additionally to the inter-varieties differences in plant structure, the dynamic process of plant growth induces changes in plant structure. Height, heterogeneity and connectivity increased with plant age, decreasing host discovery rates on complex older plants for both species of *Trichogramma*. These findings confirm our hypotheses that percentage of parasitism will be higher on simple plant structure. Other studies found that host discovery by *Trichogramma* decreases with an increase of plant structural complexity (Andow and Prokrym 1990; Lukianchuk and Smith 1997). The increase of variability with plant age can be attributable to intra-specific variation in host location behavior (Waage and Hassell 1982) and to the limited foraging time duration (24 hrs).

Regardless of the host species used, plant structure affects both species of *Trichogramma* but their ability to find host eggs on plants of different structures differed. *T. evanescens* obtained more frequently greater values of parasitism than *T. pretiosum*. However, the hypotheses that, based on differences of habitat exploitation, *T. pretiosum* should outperform *T. evanescens* on complex plants can hardly been confirmed since the number of times there was significant differences was equal between the two species.

The results also showed that for the same host species there exist differences in parasitism between the three cruciferous varieties. Other studies also showed that a host species can experience different attack rates by parasitoids when on different plant species or varieties (Pimentel 1961; Bombosch 1966; Weseloh 1976; Gross and Price 1988; Noldus 1989a; Undayagiri and Welter 2000) or on different parts of the same plant (Weseloh 1974, 1976; Gardner and Dixon 1985). Different hypothesis such as plant odors, plant preference, plant structure were emitted or tested to account for these differences (Gardner 1982; Pimentel 1961). Here we did not test for plant odors but we may assume that differences in the type of volatiles are smaller within than between species (van Etten and Tookey 1979; Simmonds 1979). Therefore, differences of parasitism may not result from plant preferences but rather result from differences in ability and efficiency with which parasitoids search on plants of different structure. Other authors arrived at such a conclusion (Gardner and Dixon 1985; Pimentel 1961; Frazer and McGregor 1994). The results obtained by the latter cited studies and ours may help to gain insight in the causes and consequences of inter and intra variability in host finding success by *Trichogramma* parasitoids either on identical or different plant species.

Patterns of egg deposition vary between the three lepidopteran species which add difficulties to finding hosts but most species lay their eggs under the leaf surface (Harcourt 1961, 1962, 1963; Sutherland 1966). Eggs of the three species of lepidoptera were parasitized by *Trichogramma* females but their distribution pattern varied according to leaf side and plant height. Eggs under the surface of leaves were more frequently found and parasitized than those on top of leaf surface. The concept of elementary unit of foraging (EUF) of Ayal (1987) can possibly explain such a result. The EUF corresponds to a basic component of the environment on which the foraging strategy of an animal has been moulded by natural selection. In this experiment, the plant can represent the EUF on which the searching strategy of female parasitoids may have been moulded but on the natural pattern of egg deposition on plants, explaining why parasitism was greater on the lower leaf surface. Females that developed, by coevolution, such a profitable strategy may encounter more frequently eggs and therefore leave more descendants and have greater fitness, which is the opposite for the pest insects.

The significant difference in the number of eggs parasitized according to plant height can be explained by the point of release as demonstrated by Ables et al. (1980). Female parasitoids were released on a basal leaf and may not had enough time to reach all parts of the plant. Such result have practical implication relatively to the release point of female parasitoids in the field. Thus, *Trichogramma* females did not disperse very much since they concentrated their research for hosts under leaf surface and at the base of the plant, close to the releasing point.

Implications for biological control

Since the effectiveness and success of inundatively used natural enemies are largely determined by their capacity to find hosts (Stinner 1977; van Lenteren et al. 1982), *T. evanescens* appears as an interesting candidate because that species best performed for the different plant structures host species and plant varieties used in this study. Biological control by augmentation requires accurate information on the natural enemy release rate that will yield a predictable response in the pest population and minimize crop damage (Hoy et al. 1991). Biological control program should consider the effect of plant structure while planning releases and adjust dosages in consequence.

This study demonstrates the importance of considering the first trophic level in the planification of inundative release of *Trichogramma* parasitoids for biological control. Such a consideration may provide improved and sustainable development to integrated insect pest management. Moreover, breeding programs of plant resistance to pests may benefit from investigations on the effect of certain plant phenotypes on pest species and their natural enemies.

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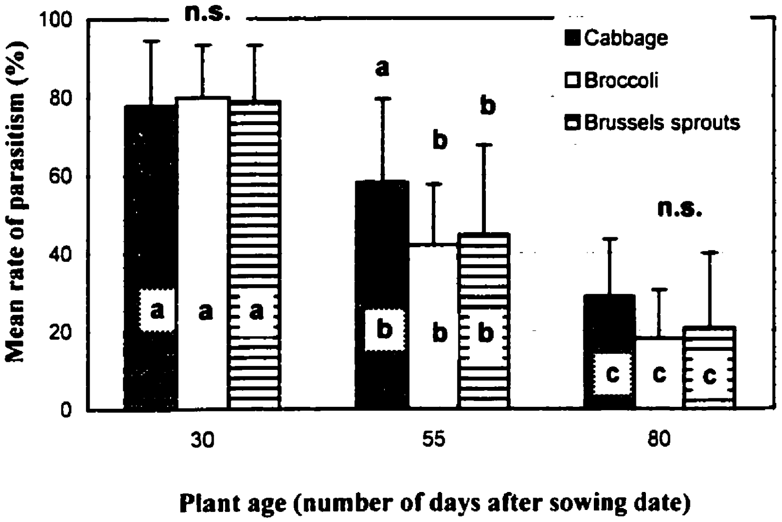
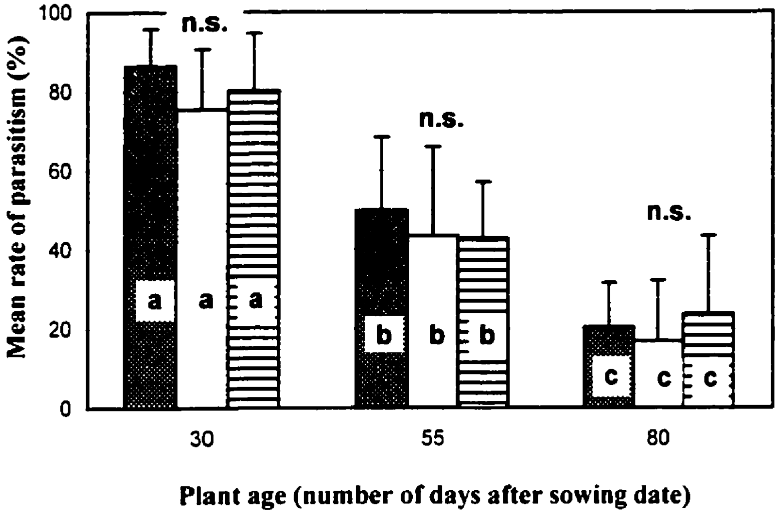
Fig. 5.1 Mean percentage of parasitism of *T. evanescens* as a function of plant structure for (A) *Pieris rapae* (B) *Trichoplusia ni* (C) *Plutella xylostella*. Different letters indicate significant difference in mean values related to plant age for letters within columns and related to plant variety for letters above columns. The bars represents the standard deviation values.

A.


Mean rate of parasitism (%)
0
20
40
60
80
100
n.s.
a
b
b
n.s.
a a a
b b b
c c c
■ Cabbage
□ Broccoli
▤ Brussels sprouts
30
55
80
Plant age (number of days after sowing date)

B.


Mean rate of parasitism (%)
0
20
40
60
80
100
n.s.
n.s.
n.s.
a a a
b b b
c c c
30
55
80
Plant age (number of days after sowing date)

C.

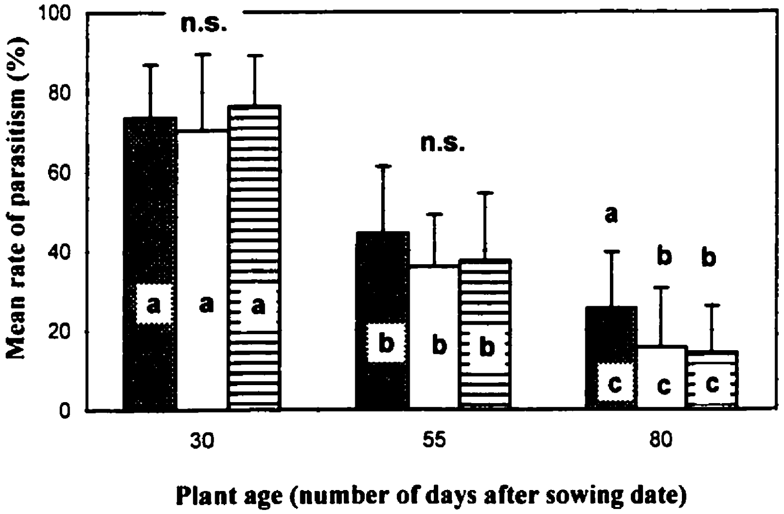

Mean rate of parasitism (%)
0
20
40
60
80
100
n.s.
n.s.
a
b
b
a a a
b b b
c c c
30
55
80
Plant age (number of days after sowing date)

Table 5.1. Mean values (± std) for each component of plant structure and for each plant variety and age

Plant age / Plant variety	Size	Heterogeneity	Connectivity
Age = 30 days after sowing date			
Cabbage (n= 21)	21.7 ± 1.7	0.0 ± 0.0	6.2 ± 0.7
Broccoli (n= 21)	20.2 ± 2.1	0.0 ± 0.0	4.2 ± 0.6
Brussels sprouts (n= 21)	21.9 ± 2.1	0.0 ± 0.0	8.0 ± 1.0
Age = 55 days after sowing date			
Cabbage (n= 21)	35.4 ± 4.8	0.0 ± 0.0	12.5 ± 1.1
Broccoli (n= 21)	41.0 ± 4.3	0.0 ± 0.0	10.5 ± 0.7
Brussels sprouts (n= 21)	40.9 ± 1.8	0.0 ± 0.0	18.6 ± 1.8
Age = 80 days after sowing date			
Cabbage (n= 21)	36.3 ± 2.3	0.0 ± 0.0	21.0 ± 1.2
Broccoli (n= 21)	50.5 ± 9.4	0.1 ± 0.1	17.4 ± 1.8
Brussels sprouts (n= 21)	48.5 ± 6.6	0.7 ± 0.05	35.3 ± 7.1

Table 5.2. Mean percentages of parasitism of *P. rapae* eggs according to the *Trichogramma* species and the *Brassica oleracea* varieties for the experiment on plant preference

Trichogramma sp /Plant var.	n	Mean parasitism (%)	Std
T. evanescens			
Cabbage	26	41.8 a	15.6
Broccoli	26	44.3 a	13.8
Brussels sprouts	26	45.1 a	17.0
T. pretiosum			
Cabbage	16	46.1 a	19.6
Broccoli	16	38.2 a	14.3
Brussels sprouts	16	45.5 a	13.6

Mean followed with identical letters did not significantly differ after a 'Tukey test

Table 5.3. Results from the univariate procedure of repeated measures ANOVA in time of parasitism values of *T. evanescens* and *T. pretiosum* foraging on cabbage, broccoli, and Brussels sprouts of 30, 55 and 80 days old for either *P. rapae*, *T. ni* or *P. xylostella*

Source of variation	df	SS	F value	P > F
Block	2	0,0963	0,65	0,6105
Plant	**2**	**0,6176**	**7,64**	**0,0006**
Pest	**2**	**0,5171**	**6,4**	**0,0019**
Parasitoid (Par)	**1**	**0,4677**	**11,57**	**0,0008**
Plant*Pest	4	0,0557	0,34	0,8478
Plant*Par	2	0,2339	2,89	0,0569
Pest*Par	2	0,0227	0,28	0,7555
Plant*Pest*Par	4	0,1151	0,71	0,5843
Age	**2**	**47,0274**	**822,89**	**0,0001**
Age*Plant	4	0,2089	1,83	0,1218
Age*Pest	4	0,0679	0,59	0,6671
Age*Par	**2**	**0,5691**	**9,96**	**0,0001**
Age*Plant*Pest	8	0,1113	0,49	0,8658
Age*Plant*Par	4	0,1078	0,94	0,4384
Age*Pest*Par	4	0,1177	1,03	0,3911
Age*Plant*Pest*Par	8	0,2423	1,06	0,3895

Table 5. 4. Performance of both *T. evanescens* (Eva) and *T. pretiosum* (Pre) against the three pest species for every plant variety as a function of their structure. The reported values are mean percentages of parasitism.

	Plant variety / Trichogramma species					
Pest species / Plant age	Cabbage Eva	Cabbage Pre	Broccoli Eva	Broccoli Pre	Brussels sprouts Eva	Brussels sprouts Pre
Pieris rapae						
30	77,8	76,7	79,9	79,4	78,8	76,4
55	* 58.1	39,8	42,0	37,3	* 44.6	30,5
80	28,7	25,3	18,0	16,3	20,7	17,7
Trichoplusia ni						
30	86,4	79,2	75,4	77,0	* 80.1	72,5
55	* 49.9	41,3	43,5	38,7	* 42.8	26,5
80	* 20.3	34,7	16,9	15,5	23,7	21,6
Plutella xylostella						
30	73,6	76,8	* 70.4	79,1	76,4	72,1
55	* 44.4	31,1	36,0	31,3	* 37.4	21,8
80	* 25.5	16,6	15,7	16,0	14,3	13,4

* indicates significant differences in mean parasitism between Eva and Pre based on a protected Tukey test.

CONNECTING STATEMENTS

In chapter five, we showed that, in choice tests, neither species of *Trichogramma* had a strong preference for one of the three varieties of *B. oleracea*, which makes them comparable and justify tests for the effect of plant structure. In non-choice tests, we noticed a decrease in host finding success with an increase in plant structural complexity for the two wasp species. However, on simple and intermediate plant structure, *T. evanescens* outperformed *T. pretiosum* more frequently and this was observed with the three lepidopteran host species. Moreover, characterization of the distribution of parasitized eggs according to leaf side and plant height revealed that females concentrated their research under leaf surface and at the base of the plant.

Previous chapters revealed that host finding success of one or two species of parasitoids decreased with an increase in complexity of either artificial or natural plants. In the next chapter, we searched for causes and behavioral mechanisms that could explain results from previous chapters by examining the effect of plant structure on searching strategy and searching efficiency. We demonstrate through an analysis of time, frequency and sequence of activities inherent to searching and ovipositing and plant parts explored that a decrease in host finding with an increase in complexity may result from the searching strategy used to explore a plant.

CHAPTER 6

EFFECT OF PLANT STRUCTURE ON SEARCHING STRATEGY AND SEARCHING EFFICIENCY OF *TRICHOGRAMMA EVANESCENS* WESTWOOD (HYMENOPTERA: TRICHOGRAMMATIDAE)

DANIEL GINGRAS and GUY BOIVIN

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ABSTRACT

When searching for hosts on a plant, female parasitoids use strategies to maximize efficiency. Searching strategies include the type of activities, time budget associated with each activity, time allocated to the different plant parts and exploration sequence of plant parts. Searching efficiency depends on the time taken to find the first egg, the number of eggs found per foraging time unit and the re-encountering frequency of eggs during a foraging period. This study examines the effect of simple and complex plant structures on searching strategy and searching efficiency of the egg parasitoid *Trichogramma evanescens*. Analyses of sequence, frequency and duration of behaviors associated with searching on artificial plants of different complexities were performed. Plant structure affected time associated with activities since time resting was greater on complex than on simple plants. Time spent on the different plant parts such as the limb, the perimeter, the nervures, the limb of inferior and superior leaf sides and the perimeter of the inferior leaf side was significantly greater on complex than simple plant structure. The exploration sequence of plant parts was different from random while the order of exploration of plant parts was independent from plant structure. Plant structure influences searching efficiency. Encountering and re-encountering frequencies of eggs were significantly greater on simple than on complex plant structure. Plant structure had no effect on handling time of eggs. This study demonstrates that plant structure can modulate activities inherent to searching and ovipositing, which in turn affects area searched per foraging time unit and therefore host finding success.

Key words: egg parasitoids, searching efficiency, searching strategy, host finding, movement, area searched, plant structure, activity, orthokinetic, klinotaxis

INTRODUCTION

When searching for hosts on a plant, foraging female parasitoids make strategic decisions such as where and how long to search and whether or not to accept a host once it is discovered. The outcome of these decisions can greatly influence survival and fitness of parasitoids. In trying to understand what determines the decision process of foraging insects, ecologists have increasingly turned to optimal foraging theory, which predicts that a forager should maximize its encounter rate with the most suitable and profitable hosts, and when hosts are abundant, avoid individuals of lesser quality (Stephens and Krebs 1986).

The number of hosts encountered depends on the area searched per time unit (Skellam 1958), handling time and reactive distance (Bruins et al. 1994). It also depends on the type of activities inherent to searching and ovipositing and the time budget of these activities. A female parasitoid that spends most of its time walking has more chance of finding a host than a sessile female. High walking velocity results in a higher probability of encountering hosts or cues that may lead the female to the host (Bieri et al. 1990 in Bigler et al. 1997). Bigler et al. (1988) showed a relationship between walking speed and parasitism of egg masses of *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae) in the field by different laboratory-reared strains of *Trichogramma brassicae* (=maidis) Bezdenco (Hymenoptera: Trichogrammatidae). Age of female parasitoid also appears to affect searching activity since young females, with a higher number of ovarian eggs, showed higher rates of movement than older ones with fewer eggs (Pak et al. 1985).

While most studies on foraging theory and foraging behavior considered host characteristics, primarily host density and distribution, the effect of plant structure on host finding success may be equally important. Plant physical characteristics can impair movement, affect searching times and foraging success of predatory insects (Grevstad and Klepetka 1992) and parasitoids (Andow and Prokrym 1990; Lukianchuk and Smith 1997). However, little is known on the impact of plant spatial structure on searching strategy and searching efficiency.

Searching strategies are implicit in many models that focus on parasitoids foraging behavior (Hassell and Southwood 1978; Weisser 1995 ; Powell et al. 1998). Searching strategies result from genetic, learned and sensory sources of informations available to an insect and have been defined as sets of basic rules of scanning and movement that result in effective host encounter (Bell 1991). We have defined here searching strategies as the time-budget of activities, the time allocated to the different plant parts and the exploration order of plant parts. Searching efficiency includes the time taken to find the first egg, the number of eggs found per foraging time unit and the re-encountering frequency of eggs during a foraging period. Based on the results of Gingras et al. (2001), who found that connectivity is the plant structure component that affects most foraging *Trichogramma*, we hypothetized that plant connectivity affects searching strategies and searching efficiency of female parasitoids. On a simple plant, orthokinetic movement, define as a change in speed or frequency of locomotion, is favored (Visser 1988). On a complex plant that shows numerous connections between plant parts more choices are offered to parasitoids and this may cause females to slow down or even to stop more frequently. Based on this hypothesis, we predict that female parasitoids will be more efficient when searching on simple plant structures than on complex ones. If orthokinetic movements are predominant, we expect that host eggs situated along the twig would be more frequently encountered and parasitized than those situated on leaves because they are located along a relatively straight line while those on leaves require that females take every connection met.

The present study examines under laboratory conditions the effect of plant connectivity on searching strategy and searching efficiency of *Trichogramma evanescens* (Westwood) (Hymenoptera: Trichogrammatidae) foraging randomly on a simple and complex plant structure containing eggs of the host *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae).

MATERIALS AND METHODS

All experiments used female *T. evanescens* reared at 24^0 C, 16L:8D, on cold-killed eggs of the Mediterranean flour moth, *E. kuehniella*. Females were less than 6 hours old, mated, unfed prior to the experiment, naive with respect to the plant and had no previous oviposition experience. *E. kuehniella* cold-killed eggs served as hosts.

Plant structure can be defined by its size (height), heterogeneity (relative number of plant components) and connectivity (absolute number of connections between plant components) (Andow and Prokrym 1990; Bell et al. 1991). Simple and complex plants were used to detect an effect of plant structure on rates of parasitism. The complex plant consisted of a plastic twig, 20 cm long, comprising 7 leaves and 6 connections (petioles) of 1.5 cm long. The twig was supported by a straight segment of 5 cm long, which was inserted in a wood base (Fig. 6.1 a). To obtain the simple plant, we cut all connections and removed leaves number 2 and 5. Thus, simple plants had no connections and only 5 leaves that were directly glued to the twig (Fig. 6.1 b). On each leaf and on both sides (inferior and superior), we distinguished a limb (in black on figure 6.1) and a perimeter, a zone situated within 3mm of leaf margin, represented in white on figure 6.1. On the inferior side, leaves had nervures. One egg of *E. kuehniella* was glued at the tip of each leaf, within the perimeter zone on the superior side, and two eggs were glued on the twig, one at each extremity (Fig. 6.1). The digit on the leaf was also the digit corresponding to the egg (leaf1, egg1, etc...) and egg number eight was closest to the release point, on the twig.

The experiment took place within a cage of 85 x 85 x 100 cm covered with white muslin where the temperature was 25 ± 1° C. A turn table supported the experimental set-up and permited continuous observations of the parasitoid. Additionally to fluorescent light in the room, a fluorescent light, situated at 45 cm over the plant lit the experimental arena and create a light intensity of 1.05 kilolux on the plant. The light intensity was measured with a Li-Cor, model Li-1000-32 data logger apparatus.

One female *Trichogramma* was released on the plant (Fig. 6.1) and was monitored by the same observer by direct observation with the naked eye, for one hour or until it left the plant. Replicates of less than 20 minutes, and those where the parasitoid was lost to sight for more than one minute, were discarded. A new twig was used for each of the 30 replicates per plant structure. The information relative to movements of the female on the plant was classified into four categories: locomotion, substrate, leaf side and leaf number. Locomotion behaviors included walking, resting, and flying. The substrate categories were the twig, limb, perimeter, nervures and eggs. The leaf sides were either inferior or superior. Finally, the leaf number were coded 1 to 6 for the complex plant and 1 to 4 for the simple plant. Observations on searching behavior of the female were recorded on a portable Tandy 1000 compatible IBM computer, using Observer software, version 2.0 (Noldus Information Technology, 1989)

Data treatment and statistical analysis

One way ANOVA was performed to determine if plant structure had an effect on searching strategies and searching efficiency as defined above. Substrate and leaf side categories were combined to refine the analysis of movement of female parasitoids on the plant. These extra-categories were: limb inferior side; limb superior side; within perimeter inferior side; within perimeter superior side. All significant treatments were submitted to multiple pairwise comparisons using a Tukey test. All statistical tests were performed by using SAS software (SAS Institute Inc. 1997). Percentage of time allocated to each element composing the locomotion, substrate and leaf side category were calculated separately for simple and complex plant structure.

Area searched by female parasitoids was estimated by using the relationship $\frac{S}{A} = \frac{N_a}{N}$ (Wiedenmann and O'Neil 1992), where S is the amount of leaf area searched in square centimeters, A is the total amount of leaf area available to search (simple= 43 cm^2; complex= 60 cm^2), N_a is the number of hosts encountered, and N is the number of hosts available to parasitization (8 for complex, 6 for simple plants)

To test if plant structure influenced residence time, the proportion of females that stayed for one hour were compared between plant structures. To test if plant structure influenced flight and leaving frequencies, a 2 x 2 contingency table was constructed. Differences were compared under the null hypothesis of equal frequencies between simple and complex plant structure by using a Chi-square test.

Encountering frequency (EF) corresponded to the number an egg was encountered by a female between the replicates whereas re-encountering frequency (REF) corresponded to the number of time a female came back to an egg previously encountered within the same replicate. Mean and cumulative values of EF and REF were computed over replicates where at least one egg was encountered by females; n= 23 for simple plants, n= 11 for complex plants. Time elapsed between successive discoveries of two different eggs was estimated from replicates where the female encountered at least two different eggs within one replicate; n= 11 for simple plants, n= 1 for complex plants. No analysis of variance was performed to determine if plant structure had an effect on time elapsed between successive discoveries of different eggs because only one case occurred on complex plants.

A sequential analysis of leaves explored by *Trichogramma* females was performed for simple and complex plants (Bekoff 1979; Lehner 1979). By establishing the most probable sequence of units composing a behavior, such analysis can lead to the development of a predictive model of chained units of a behavior. A contingency table presenting in rows the preceding and in columns the following leaf number explored by the female *Trichogramma* was constructed and a chi-square was applied to the contingency table.

RESULTS

The foraging behavior that was typically observed starting from the release point can be described as this: walking along the twig, take a connection (a petiole), when a connection is met, walk rapidly along the latter, explore the leaf by walking in a relatively straight course, interrupted by sharp turns. This type of observation was corroborated by Gardner and van Lenteren (1986). Then the female usually stayed for a long period of time on the leaf. It doesn't move but it may preen itself.

Searching strategy

Plant structure had a significant effect on duration of activities for two out of three activities related to locomotion. Time spent walking by females was significantly higher on simple than on complex plants while time spent resting was significantly lower on simple than on complex plants (Table 6.1). There was no significant differences in flying duration by females when they foraged on simple and complex plants. Flying rarely occurred and when observed, it was to leave the plant. Flying was observed in 13 replicates on simple plants, and in 12 replicates on complex plants.

Time spent walking and resting on the twig by female parasitoids was not significantly different between simple and complex plant structure (Table 6.1). However, time spent walking and resting by females on limb, nervures, and within perimeter was significantly greater when they foraged on complex than on simple plants (Table 6.1). Time spent walking and resting on the inferior side of leaves was significantly greater on complex than on simple plants while the reverse was observed for the superior side of leaves. Furthermore, time spent walking and resting on the limb of inferior and within perimeter of inferior side of leaves by females on complex plants was almost twice the time spent on simple plants. Also there was significant differences between complex and simple plants.

Concerning leaves explored by females, those situated close to the release point were visited more on both simple and complex plants, as revealed by the observed frequencies of following and preceding leaves (Table 6.2). The visit of leaf 1 precede the visit of leaf 3 eighteen times in the sequence of leaves explored by the female on simple plant (Table 6.2). Significant chi-square resulted when applied to the contingency table for simple (X^2

= 52.74; df = 9; $P > 0.05$) and complex plant structures ($X^2 = 39.55$; df = 25; $P > 0.05$). Significant chi-square mean that the exploration of leaves by *Trichogramma* female was not random. Therefore, based on highest observed frequency values presented in table 6.2, the most probable sequence of leaves taken by a female *T. evan*escens will be the first, second, third and fourth leaves encountered on both, simple and complex plants. The foraging strategy was identical.

Searching efficiency

Time spent on leaves without encountering an egg was significantly lower on simple plant structures than on complex ones (Table 6.3). The average time to encounter the first egg was not influenced by plant structure. Time elapsed between successive discoveries of two different eggs was superior on simple plant structure compared to complex plant structure but no statistical difference can be determined since it occurred in only one replicate. The average number of eggs found per hour, which reflects host finding capacity and searching efficiency at the scale studied was 2.08 on simple and 1.09 on complex plants but there was no significant difference. Once a female found an egg, plant structure had no effect on handling time, which was 39 seconds for simple and 38 seconds for complex plant structures. The area searched by female parasitoids was almost three times greater on simple than on complex plants and this difference was significantly different. Plant structure had no effect on residence time on plants by female parasitoids. Females left more frequently before the hour of observation was completed on simple plant structure (frequency= 14) compared to complex ones (frequency= 11) but these differences were not statistically significant ($X^2 = 0.62$, NS).

All eggs were encountered on simple plant structures but not on complex ones as revealed by encountering frequency values (Table 6.4). On both simple and complex plant structures, egg7 was the less frequently encountered despite being situated on the twig, thus along a relatively straight line trajectory from the point of release. Females had a strong tendency to come back to an egg previously encountered as indicated by high cumulative and mean re-encountering frequency values. Cumulative and mean re-encountering frequency of an egg were higher on simple than on complex plant structure and a female came back between two and four times to the same egg on simple plant structure and four times for the only replicate where it occurred on complex plant structure (Table 6.4).

DISCUSSION

Searching within the host habitat in the absence of cues from hosts or plants is assumed to be random for most parasitoids (Waage 1979, Vinson 1984a). In this experiment, it was assumed that females foraged randomly because dead host eggs were used and that no or little cues emanated from them. In such a context, the number of eggs encountered will depend on the area searched per time unit (Skellam 1958). Indeed, on the simple plant structure, females explored greater surface areas per foraging time unit than when they foraged on complex ones as revealed by the estimated area searched.

However, searching has a cost as it reduces time allocated to oviposition and eating and increases risks associated with predation (Stephens and Krebs 1986). Female parasitoids should aim to reduce the area searched per time unit through the use of searching strategies that make them efficient forager.

Searching strategy

The area searched per time unit depends on the type of activity and the time budget of activities. For sedentary hosts, like eggs, female parasitoids must adopt a strategy that maximizes movement, either by walking or flying to cover most of the plant structure of a plant. Walking time was greater on simple than on complex plants which explained that more plant parts were explored on simple plants. Flight was not observed frequently and when it was observed, it was to leave the plant but was rarely used to move from one plant part to another. Yet, dispersal by female *Trichogramma* to other plants or plant parts increases their chances of finding hosts or host cues. Observations on flight by *Trichogramma* are rare (reviewed in Keller et al. 1985; Noldus et al. 1988; Forsse et al. 1992), and understandably so because the minute size of the insect makes it difficult to observe. *Trichogramma* females covered most plant parts but while on leaves, females spent 31 percent of their time on the limb, a result that can partially explain the low encountering frequency of eggs since the latter were located within the perimeter. Such a result is different from the observations of Suverkropp (1997), who observed that *T. brassicae* (=maidis) females spent 18 to 24 percent of their time on the leaf following veins and edges and those of Noldus et al. (1991) who observed that *T. evanescens* spent a significant amount of time on the leaf edge of Brussels sprouts. Moreover, females spent, whatever the plant structure was, 62 percent of their time on the inferior rather than on superior side of leaves. Similar observations were made with *T. brassicae* which spends 1 to 10 percent of total walking time on the upper surface of corn leaves following the central vein and 10 to 20 percent on the lower surface (Gass 1988 in Bigler et al. 1997).

Searching efficiency

Searching efficiency is linked to host finding capacity, which is defined by the number of hosts found per unit of time (Pak 1997). At the plant scale studied, female parasitoids found almost twice as many eggs on simple (2.08 per hour) than on complex plant structures (1.09 per hour) although this difference was not significant. We were expecting that plant structure would influence host finding capacity but even if we observed a trend in the duration, the variability in *Trichogramma* behavior prevented us to get significant results. Females encountered eggs more frequently on simple plants because the spatial organization of these may have favored orthokinetic movements and high walking velocity which increase probability of encountering hosts or cues (Bieri et al. 1990 in Bigler et al. 1997). An increase in connectivity increases the number of pathways and direction possibilities a foraging female can take to find an egg. Females may stop more frequently or reduce walking speed prior taking one pathway or another. Thus, orthokinetic movements become more difficult and overlap of search paths may occur more frequently on complex plant structure. The more frequent stopping and the responsivness principle of Waage (1979) may explain longer resting period of female parasitoids on complex plants. In a study on the proximate mechanism of parasitoid foraging behavior, Waage (1979) proposed that responsiveness increases with successive encounters with hosts and diminishes in the absence of encounters. Responsiveness of parasitoids may thus decrease more frequently to the lower level of responsiveness when foraging on complex plant structure. Therefore, for identical host densities per plant, parasitoids may perceive complex plants as patches of lower quality because more time is needed to discover hosts. If total residence time on the plant was not affected by plant structure, females left more frequently before the hour was completed simple plants than complex ones. Factors that influence patch residence time include richness and quality of the patch, probability of survival of the female and travel risks (Stephens and Krebs 1986; Bell 1991). On simple plant structures, richness and quality decreased more rapidly, which may explain why females left more frequently before the hour of observation was completed.

It was expected that eggs situated along the twig (egg 7 and 8) , thus along a straight line, would be more frequently encountered due to orthokinetic movement predominance. As revealed by the observed frequencies of leaves explored, klinotaxis movements, define as a variation in turning rate, were predominant, which explains that leaves close to the releasing point were visited first. Such behavior also explained why egg7, either on simple or on complex plants was rarely encountered. We would have expect that time before encountering the first egg will be influence by plant structure. The similar spatial configuration and egg localization for the simple and complex plant models and the searching strategy consisting in exploring the first connection met could explain that no difference were obtained. When the female encountered an egg she oviposited in it and plant structure did not influence handling time. Handling time may rather be under control of physiological process. After ovipositing, parasitoids, including *T. evanescens*, often innately make an intensive investigation of the area adjacent to the host, an observation supported by previous studies (Laing 1937; Jackson 1966; Hokyo and Kiritani 1966). Moreover, females had a strong tendency to come back more than once on the egg they just parasitized, a behavior that reduces searching efficiency. Typical egg laying behavior was observed again sometimes, meaning that the egg was presumably superparistized. Rosenheim and Mangel (1994) stated that a parasitoid with an imperfect ability to discriminate between unparasitized hosts and hosts that it has attacked earlier within the same patch, experiences a risk of self-superparasitism when attacking multiple hosts within a single patch. Self-superparasitism can incur costs in the form of lost time and eggs. Early patch leaving can be favored as a means of avoiding the costs of self-superparasitism.

Complexity of plants can therefore be a complicating factor in foraging activities. Because in nature few host species are found on a uniform, featureless surface, parasitoids going through the host location process will often require more than a single strategic decision. In complex plants, the discovery of a host represents the outcome of an hierarchical series of decisions. These decisions can sometimes have evolutionary consequences for parasitoids such as a change in abundance, diversity and the number of descendants and hence fitness value.

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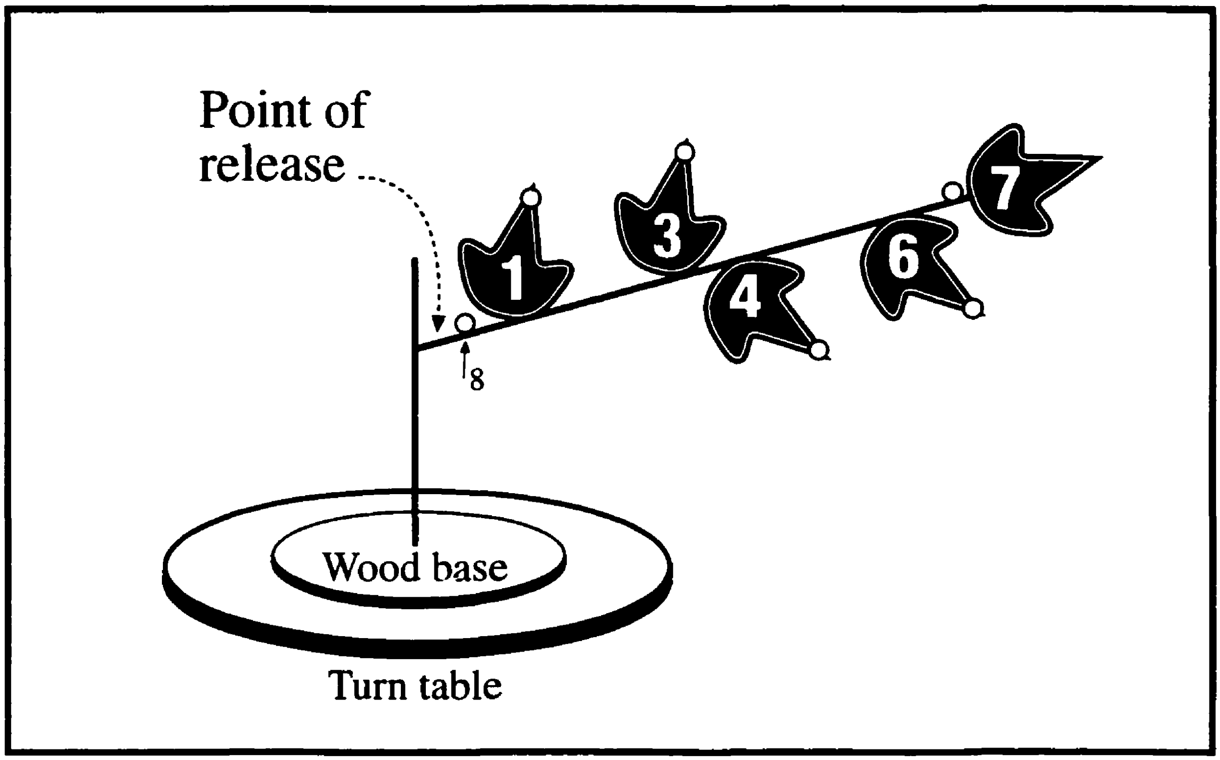
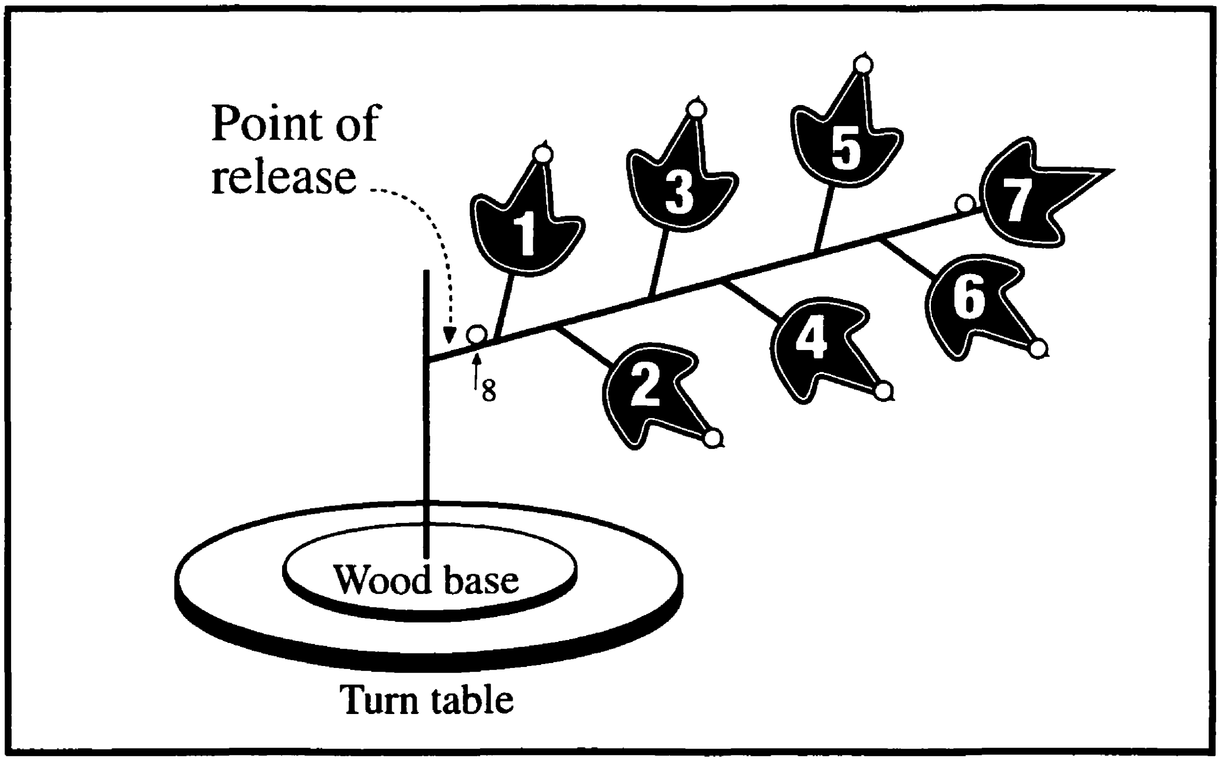
Fig. 6.1. (A) Representation of a part of the experimental set-up with the complex plant structure treatment. The complex plant consisted of a plastic twig 20 cm long comprising 7 leaves and 6 petioles 1.5 cm long. The twig was supported by a straight shaft 5 cm long inserted in a wood base.

(B) The simple plant was identical to the complex one, except leaves number 2 and 5 and all petioles were removed. The white circles represent the eggs. The digits on leaves correspond to leaf and egg numbers.


Point of release
1
3
4
6
7
8
Wood base
Turn table

(A)


Point of release
1
2
3
4
5
6
7
8
Wood base
Turn table

(B)

Table 6.1 Results of pairwise comparisons from Tukey tests following significant analysis of variance and the percentage of time (%) spends by females for the locomotion and substrate categories. Time variables are reported in seconds.

Category	Plant Structure	n	Time spent (in seconds)		
			Mean ± Std		%
Locomotion					
walking	Simple	30	1405.11 ± 824.87	a	64,6
	Complex	30	723.90 ± 470.84	b	35,5
resting	Simple	30	672.16 ± 474.07	a	30,9
	Complex	30	1201.22 ± 593.22	b	59,0
flying	Simple	13	96.93 ± 79.88	a	4,5
	Complex	12	110.68 ± 125.39	a	5,4
Substrate					
twig	Simple	30	484.94 ± 432.68	a	19,2
	Complex	30	221.26 ± 299.53	a	6,1
within perimeter	Simple	30	885.22 ± 540.47	a	35,1
	Complex	29	1142.11 ± 923.24	b	31,3
nervures	Simple	24	93.41 ± 97.94	a	3,7
	Complex	22	624.99 ± 901.02	b	17,1
limb	Simple	30	763.37 ± 902.58	a	30,3
	Complex	28	1123.44 ± 1019.14	b	30,8
eggs	Simple	27	294.68 ± 146.06	a	11,7
	Complex	12	539.57 ± 458.29	b	14,7

Table 6.1 (cont'd) Results of pairwise comparisons from Tukey tests following significant analysis of variance and the percentage of time (%) spends by females for the leaf side categories. Time variables are reported in seconds.

Category	Plant Structure	n	Time spent (in seconds) Mean ± Std		%
Leaf side					
inferior	Simple	30	1570.88 ± 1097.03	a	55,5
	Complex	30	2348.88 ± 1358.46	b	73,9
superior	Simple	29	1259.18 ± 921.76	a	44,5
	Complex	26	829.47 ± 808.47	b	26,1
limb inferior side	Simple	29	672.50 ± 566.35	a	39,4
	Complex	29	1046.40 ± 894.21	a	43,9
limb superior side	Simple	26	130.73 ± 86.58	a	7,6
	Complex	25	140.15 ± 62.79	a	5,9
within perimeter inferior side	Simple	30	351.4 ± 278.53	a	20,6
	Complex	28	782.5 ± 584.23	b	32,8
within perimeter superior side	Simple	29	552.2 ± 464.66	a	32,4
	Complex	26	413.8 ± 377.31	a	17,4

Table 6.2 Contingency tables of leaves explored by female *T. evanescens* that either precede or follow another leaf for each plant structure. Reported values are observed frequency.
(---) = non existent data

		Simple plant structure						Complex plant structure					
	Leaf's Number	Following leaf						Following leaf					
		1	***2***	***3***	***4***	***5***	***6***	***1***	***2***	***3***	***4***	***5***	***6***
	1	0	---	18	4	---	1	0	9	0	0	1	0
Preceding	***2***	---	---	---	---	---	---	2	0	1	1	0	0
leaf	***3***	5	---	0	7	---	2	0	0	0	2	0	0
	4	2	---	4	0	---	6	0	0	1	0	0	0
	5	---	---	---	---	---	---	0	0	0	0	0	1
	6	1	---	1	2	---	0	0	0	0	0	0	0

Table 6.3 Results of pairwise comparisons from Tukey tests following significant analysis of variance of time variables associated with presence and activities of females on simple and complex plant structures.

Activity	Plant Structure	n	Time spent (in seconds) Mean ± Std	
on a leaf without encountering an egg	Simple	2792	29.96 ± 68.29	a
	Complex	1839	50.03 ± 123.56	b
before encountering the first egg	Simple	23	411.71 ± 482.98	a
	Complex	11	377.66 ± 448.96	a
between successive discoveries of two eggs	Simple	11	982.92 ± 377.31	
	Complex	1	436.67	
Handling time	Simple	827	38.62 ± 56.77	a
	Complex	337	38.09 ± 57.72	a
Area searched (cm2)	Simple	23	21.30 ± 13.9	a
	Complex	11	8.20 ± 2.26	b
on plant (total residence time)	Simple	30	2 739.74 ± 1 105.91	a
	Complex	30	3 005.58 ± 970.23	a

Table 6.4. Cumulative encountering frequency (EF) and cumulative and mean re-encountering frequency (REF) of eggs for replicates where at least one egg was found. (----) = non existent data; n.a.= not applicable; n.s.= non significant; * significant at 0.05; ** significant at 0.01; *** significant at 0.001 after application of chi-square

	Simple (n= 23)		Complex (n= 11)	
Egg Number	EF	REF	EF	REF
1	17	53; 3.1	0 ***	n.a
2	----	----	6	24; 4.0
3	9	40; 4.4	0 **	n.a
4	6	16; 4.0	1 *	11; 11**n.s.**
5	----	----	0	n.a
6	6	16; 4.0	0 *	n.a
7	1	0; 0	1	0; 0 **n.s.**
8	8	16; 2.0	4	6; 1.5 **

Chapter 7

SUMMARY AND CONCLUSION

Parasitoids must search for and locate food, mate and hosts within habitats and plants of varying spatial configurations whose characteristics influence trophic interactions (Price et al. 1980, Price 1991, Roland and Taylor 1997). The subject of this research was to determine the effect of plant structure on searching behavior and host finding by *Trichogramma* parasitoids. The four main objectives were: (a) to quantify plant structure and to determine if it affects host finding success (b) to develop and validate a model of parasitism using artificial plants; (c) to compare the rate of parasitism between two species of *Trichogramma* foraging on cruciferous plants of different structures and to examine the distribution pattern of parasitized eggs of hosts/pests by *Trichogramma* according to height and leaf side of cruciferous plants; (d) to examine the influence of plant structure on searching strategy and searching efficiency of *Trichogramma* female parasitoids. Major contributions in relation with the objectives of this study were detailed in preceding chapters. This study permits to gain a better understanding of how and why plant structures influences *Trichogramma* females foraging for hosts.

The structure of a plant was defined by its size, heterogeneity and connectivity. Size corresponded to the height of the plant, and was quantified by measuring, in cm, the main stem, from the highest leaf to the collar. Heterogeneity corresponded to the relative abundance, per unit area or per unit volume, of structural components, such as leaves, buds, flowers and fruits, but also to components within the plant, with different characteristics (glabrous versus hairiness leaves) or morphologies (male versus female flowers). Heterogeneity was calculated by using the formula of Shannon-Wiener's index of biodiversity (Barbour et al. 1987), which is, $[- \sum \; X_i / X_o \;\; x \;\; (\ln X_i / X_o)]$. In that equation, X_i denotes the abundance of each structural component (number of leaves or number of buds, etc ...) while X_0 corresponds to the sum of the abundance of all structural components (number of leaves + number of buds, etc ...). Connectivity corresponded to the absolute abundance, per unit area or per unit volume, of connections between plant parts.

It was assumed that these three components of plant structure can represent to a large extent the potential searching area, or searching niche of parasitoids. It was initially believed that size, heterogeneity and connectivity and all possible interactions influence searching behavior and alter the capacity of *Trichogramma* females to discover hosts.

This study showed that when *Trichogramma* females foraged under different combinations of size, heterogeneity and connectivity values, connectivity best explained the variability of parasitism obtained and therefore was found to be the major component of plant structure influencing host finding success. None of the double or triple interactions between size, heterogeneity and connectivity was found significant. Such results clearly revealed that plant structure influences host-parasitoid interactions. Nevertheless, greater foraging duration and host density attenuated the effect of plant structure.

A model of parasitism that incorporates the three components of plant structure was developed. The statistical model of regression was based on experiments with artificial plants for which the structure was quantified and used different combinations of size, heterogeneity and connectivity and where plant surface area was similar. Application of the model was tested with three natural cruciferous plants and one pest species, *T. ni*. The model worked well and allowed predictions of parasitism by *Trichogramma* foraging on plants for which the size varied between 10 and 64, the heterogeneity varied between 0 and 0.9 and the connectivity varied between 0 and 72. The model appeared robust and stable as revealed by a jackknife procedure, which then permits extrapolation to plants exhibiting superior values of heterogeneity and connectivity than those used to develop the model. Validation studies of the model's predictions by using natural cruciferous plants of different structures provided excellent results. The model did track quite well the direction of the negative relationship between parasitism and plant structure but also the magnitude of parasitism as a function of plant structure. Neverthless, the assumption that size, heterogeneity and connectivity are relevant to foraging *Trichogramma* females revealed partially correct. Modelization of the impact of plant structure on host finding success was achieved, suggesting that we captured the essential of a biological phenomenon associated to host searching by *Trichogramma* parasitoid on a plant. The model developed here may allow comparison of expected host finding success resulting from searching on different plant structure. The model can also find application to biological control programs. Such a model can help to plan the number of parasitoids to be released. Reliable predictions of natural enemy release rates before the onset of a biological control program can provide practitioners an estimate of the economic feasability of the releases. However, future studies should aim to test the generality of the model developed here to other species of parasitoids and plants. Also, we should test how the model behaves to variation in host density.

This study also showed that plant structure differently affects different species of *Trichogramma* regardless of the pest species foraged. Indeed, when *T. evanescens* and *T. pretiosum* searched for eggs of either *P. rapae*, *T. ni*, *P. xylostella* on natural cruciferous plants of different structures, likely cabbage, broccoli and Brussel sprouts of three ages 30, 55 and 80 days from sowing date, *T. evanescens* outperformed *T. pretiosum*. Percentages of parasitism, by the twenty five *Trichogramma* females, were high on simple plant structure of 30 days old but never exceeded 86% while they remained low on intermediate and complex plant structure. Introduction of higher number of individuals females may increase parasitism. When *Trichogramma* females were given the choice to forage on the three varieties, neither species of parasitoid showed a strong preference for one plant variety or plant structure. Absence of preference for a particular plant variety or plant structure can be view as a quality for a natural enemy. In addition to foraging on plants of different structures, the diversity, abundance and distribution of host eggs may vary between plants and within plant parts. When host eggs were placed at different height and on both side of leaves, it was found that plant height and leaf side had significant effect on the distribution of parasitized eggs. More eggs were parasitized at the base of the plant, a result attributed to the release point of *Trichogramma* female which was on a basal leaf. Such a result revealed a small capacity of dispersion of females within a plant within a 24 hours foraging duration. Females may thus explored the plant by walking rather than by jumping and flying. Also, more eggs were parasitized on inferior side of leaves, suggesting that females spent more time searching under leaf surface. Such a result have practical and theoretical implications. From a theoretical point of view, it implies that herbivores with such a spatial distribution of their eggs will leave less descendants and have lower fitness. From a practical point of view, it will influence release point of *Trichogramma* parasitoid but also selection program. We may select parasitoid having a strategy of exploration of plant that corresponds to the pattern of distribution of herbivores.

Searching behavior of parasitoids is of crucial importance in understanding the population dynamics of host-parasitoid systems because of the direct link between successful searching and parasitism (Comins and Hassell 1979). Searching is costly and has to be reduces to minimum. Females that present best adaptations or that use strategies minimizing costs associated to searching may find more hosts have greatest fitness value and may be favored by evolution.

Plant structure was found to influence and interfere with searching strategy and searching efficiency. Searching strategies includes the type of activities and time allocated to each activity, time allocated to the different plant parts and the sequence of exploration of plant parts. Searching efficiency considers the time taken to find the first egg, the number of eggs found per foraging time unit and the re-encountering frequency of eggs during a foraging period. We discovered that klinotaxis movement was predominant when *Trichogramma* females encounter a leaf when she forages randomly, i.e. in the absence of chemical cues from hosts and plants. Probabilities that leaves situated in the vicinity of the landing or releasing point will be visited first are very high. If a female takes every leaf met, it might insure that no one is forgotten but this can be costly. When a female *Trichogramma* explored a leaf, she spent most of her time within the perimeter when compared to the limb and nervures. Females foraging on simple plant structure explored more plant parts and discovered more eggs. However, on both plant structures females had a strong tendency to come back on an egg they just encountered, which decreased searching efficiency. Females rest more when they foraged on complex plant structure because it requires them to stop more often and for longer period of time to take a decision when they encounter a leaf.

This study clearly demonstrates that plants can affect bottom-up interactions by modifying the capacity of parasitoids to exploit and kill hosts. The structure of plants influenced oviposition success of parasitoid by modulating female's searching behavior. Foraging on complex plant affects movements associated to search, reduces surface area explored per foraging time unit and significantly increases searching time. Consequently, it decreases probability of host finding because it takes longer to explore all the plant. It then appears advantageous to forage on simple plant structure from an optimal foraging and evolutionary perspectives. Models of optimal foraging that takes host plant characteristics may be more realistic and may contribute to a better understanding of host-parasitoid population dynamics. Because host finding is reduced on complex plant structure, selection pressure for host or prey to occupy these regions preferentially must be strong. Also, more complex plants may provide partial refuge to host and therefore affect availability of hosts to parasitoids which in turn has impact on population dynamics (Gardner and Dixon 1985; Price and Clancy 186; Price 1988 all in Weisser 1995). Furthermore, microhabitat possibilities are much higher within complex plants, which permits greater niche diversification and consequently provides a greater measure of enemy free space (Gross and Price 1988, Berdegue et al. 1996).

Additionally, this study demonstrates the importance of considering the first trophic level in the planification of inundative release of *Trichogramma* parasitoids for biological control. Such a consideration may provide improved and sustainable development to integrated insect pest management. Moreover, breeding programs of plant resistance to pests may benefit from investigations on the effects of certain plant phenotypes on pest species and their natural enemies.

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